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MICROSCOPICAL SCIENCE.

EDITED BY

SIR RAY LANKESTER, K.C.B., 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 LINCÉI OF ROME, AND OF THE AMERICAN ACADEMY OF ARTS AND SCIENCES OF BOSTON; ASSOCIATE OF THE ROYAL ACADEMY OF BELGIUM; HONORARY MEMBER OF THE NEW YORK ACADEMY OF SCIENCES; AND OF THE CAMBRIDGE PHILOSOPHICAL SOCIETY, AND OF THE ROYAL PHYSICAL SOCIETY OF EDINBURGH, AND OF THE BIOLOGICAL SOCIETY OF PARIS, AND OF THE CALIFORNIA ACADEMY OF SCIENCES OF SAN FRANCISCO; AND OF THE ROYAL ZOOLOGICAL AND MALACOLOGICAL SOCIETY OF BELGIUM; CORRESPONDING MEMBER OF THE SENKENBERG ACADEMY OF FRANKFURT-A-M; 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 PRESIDENT OF THE BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE; LATE FULLERIAN PROFESSOR OF PHYSIOLOGY IN THE ROYAL INSTITUTION OF GREAT BRITAIN; LATE LINACRE PROFESSOR OF COMPARATIVE ANATOMY AND FELLOW OF MERTON COLLEGE, OXFORD.

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CONTENTS.

CONTENTS OF No. 201, N.S., FEBRUARY, 1907.

MEMOIRS :	PAGE
On the Parietal Sense-organs and Associated Structures in the New Zealand Lamprey (<i>Geotria australis</i>). By ARTHUR DENDY, D.Sc., F.L.S., F.Z.S., Professor of Zoology in King's College (University of London). (With Plates 1 and 2)	1
Studies in Spicule Formation. V.—The Scleroblastic Development of the Spicules in Ophiuroidea and Echinoidea, and in the Genera <i>Antedon</i> and <i>Synapta</i> . By W. WOODLAND, Demonstrator of Zoology, King's College, London. (With Plates 3 and 4)	31
Studies in Spicule Formation. VI.—The Scleroblastic Development of the Spicules in some Mollusca and in one Genus of Colonial Ascidians. By W. WOODLAND, Demonstrator of Zoology, King's College, London. (With Plate 5)	45
A Preliminary Consideration as to the possible Factors concerned in the Production of the various Forms of Spicules. By W. WOODLAND, Demonstrator of Zoology, King's College, London	55
On <i>Neurosporidium cephalodisci</i> , n. g., n. sp., a Sporozoön from the Nervous System of <i>Cephalodiscus nigrescens</i> . By W. G. RIDWOOD, D.Sc., Lecturer in Biology at St. Mary's Medical School, University of London; and H. B. FANTHAM, B.Sc., A.R.C.S., University College, London; Demonstrator in Biology at St. Mary's Medical School. (With Plates 6 and 7)	81
Gametogenesis and Fertilisation in <i>Nematus ribesii</i> . By L. DONCASTER, M.A., late Mackinnon Student of the Royal Society; Lecturer in Zoology in the University of Birmingham. (With Plate 8)	101
The Molluscan Radula: its Chemical Composition, and some Points in its Development. By IGERNA B. J. SOLLAS. (With Plate 9)	115
Observations on Tooth-Development in <i>Ornithorhynchus</i> . By J. T. WILSON, Professor of Anatomy, University of Sydney, N.S.W., and J. P. HILL, Jodrell Professor of Zoology, University College, London. (With Plates 10—12)	137

CONTENTS OF No. 202, N.S., MAY, 1907.

MEMOIRS :	PAGE
The Origin and Nature of the Green Cells of <i>Convoluta roscoffensis</i> . By FREDERICK KEEBLE, M.A., Sc.D., University College, Reading, and F. W. GAMBLE, D.Sc., Manchester University. (With Plates 13 and 14)	167
On the Development of the Plumes in Buds of <i>Cephalodiscus</i> . By W. G. RIDWOOD, D.Sc., Lecturer on Biology at St. Mary's Medical School, University of London. (With 11 Text-figures)	221
On the Structure of <i>Ænigma ænigmatica</i> , Chemnitz; a Contribution to our Knowledge of the Anomiacea. By GILBERT C. BOURNE, M.A., D.Sc., F.L.S., Fellow of Merton College; Linacre Professor of Comparative Anatomy in the University of Oxford. (With Plates 15—17, and 2 Text-figures)	253
On the Chromatin Masses of <i>Piroplasma bigeminum</i> (<i>Babesia bovis</i>), the Parasite of Texas Cattle-Fever. By H. B. FANTHAM, B.Sc.Lond., A.R.C.S., University College, London, and St. Mary's Hospital Medical School. (With Plate 18, and 44 Text-figures)	297
The Skin, Hair, and Reproductive Organs of <i>Notoryctes</i> . Contributions to our Knowledge of the Anatomy of <i>Notoryctes typhlops</i> , Stirling.—Parts IV and V. By GEORGINA SWEET, D.Sc., Melbourne University. (With Plates 19 and 20, and a Text-figure)	325
<i>Parorchis acanthus</i> , the Type of a new Genus of Trematodes. By WILLIAM NICOLL, M.A., B.Sc., Gatty Marine Laboratory, St. Andrews. (With Plate 21)	345

CONTENTS OF No. 203, N.S., AUGUST, 1907.

MEMOIRS :	
The Chætognatha, or Primitive Mollusca. With a Bibliography. By R. T. GÜNTHER, F.L.S., F.R.G.S., Fellow of Magdalen College, Oxford. (With 10 Text-figures)	357
The Structure, Development, and Bionomics of the House-fly, <i>Musca domestica</i> , Linn.—Part I. The Anatomy of the Fly. By C. GORDON HEWITT, M.Sc., Lecturer in Economic Zoology, University of Manchester. (With Plates 22—26)	395
<i>Trichomastix serpentis</i> , n.sp. By C. CLIFFORD DOBELL, B.A., Scholar of Trinity College, Cambridge. (With Plate 27, and 2 Text-figures)	449

CONTENTS.

V

	PAGE
Notes on Common Species of Trochus. By H. J. FLEURE and MURIEL M. GETTINGS, University College, Aberystwyth. (With Plate 28)	459
Note on the Formation of the Skeleton in the Madreporaria. By MARIA M. OGILVIE GORDON, D.Sc.(London), Ph.D.(Munich), F.L.S.	473
Studies in Spicule Formation. VII.—The Scleroblastic Development of the Plate-and-Anchor Spicules of Synapta, and of the Wheel Spicules of the Auricularia Larva. By W. WOODLAND, The Zoological Laboratory, King's College, London. (With Plates 29 and 30, and 6 Text-figures)	483

CONTENTS OF No. 204, N.S., NOVEMBER, 1907.

MEMOIRS:

The Development of the Head-muscles in <i>Gallus domesticus</i> , and the Morphology of the Head-muscles in the Sauropsida. By F. H. EDGEWORTH, M.B., D.Sc., Professor of Medicine, University College, Bristol. (With 39 Text-figures)	511
The Development of <i>Ophiothrix fragilis</i> . By E. W. MACBRIDE, M.A., D.Sc., F.R.S., Professor of Zoology in McGill University, Montreal. (With Plates 31—36, and 4 Text-figures)	557
On the Segmentation of the Head of Diplopoda. By MARGARET ROBINSON, University College, London. (With Plate 37, and 6 Text-figures)	607
The Fixation of the Cypris Larva of <i>Sacculina carcini</i> (Thompson) upon its Host, <i>Carcinus mænas</i> . By GEOFFREY SMITH, M.A., New College, Oxford. (With 6 Text-figures)	625
Physiological Degeneration in <i>Opalina</i> . By C. CLIFFORD DOBELL, B.A., Scholar of Trinity College, Cambridge. (With Plate 38, and 2 Text-figures)	633
Some Facts in the Later Development of the Frog, <i>Rana temporaria</i> . Part I.—The Segments of the Occipital Region of the Skull. By AGNES I. M. ELLIOT, B.Sc., Associate of Newnham College, Cambridge. (With Plates 39 and 40)	647

TITLE, INDEX, AND CONTENTS.

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Gametogenesis and Fertilisation in <i>Nematus ribesii</i> . By L. DONCASTER, M.A., late Mackinnon Student of the Royal Society; Lecturer in Zoology in the University of Birmingham. (With Plate 8)	101
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On the Parietal Sense-organs and Associated Structures in the New Zealand Lamprey (*Geotria australis*).

By

Arthur Dendy, D.Sc., F.L.S., F.Z.S.,

Professor of Zoology in King's College (University of London).

With Plates 1 and 2.

(A) INTRODUCTORY REMARKS.

SOME years ago, whilst residing in New Zealand, I had the good fortune to obtain a plentiful supply of living specimens of the New Zealand freshwater Lamprey, *Geotria australis*. These specimens were all in what is known as the "Velasia" stage of their development, none of them being sexually mature, and all of them without the characteristic gular pouch of the adult.¹ They were, however, of considerable size, averaging nearly a couple of feet in length, and the organs which form the subject of the present memoir were probably already fully developed. For the purposes of this investigation a considerable number of specimens were hardened and preserved in a perfectly fresh condition by means of various re-agents, of which absolute alcohol, Zenker's fluid, and Flemming's solution yielded the most satisfactory results. In some cases the head was simply cut off and hardened in toto, while in others it was partially dissected in the fresh state before being placed in the hardening re-agent.

Eight series of sections, longitudinal and transverse, were

¹ For further particulars as to these specimens and the species to which they belong vide Dendy and Olliver (1).

cut by the paraffin method, some through the entire head, and some through the brain, after removal from the cranium. Most of the material was stained in bulk with Ehrlich's hæmatoxylin, and some of the sections were counter-stained on the slide by means of acid fuchsin or eosin.

Sections of material fixed in absolute alcohol were found to be particularly valuable for demonstrating the arrangement of the pigment in the "pineal eye," this pigment, as is well known, being soluble in acids, and, therefore, often entirely absent from material treated with strongly acid hardening re-agents; such as Flemming's solution or Zenker's fluid.

I first observed the very well-developed "pineal eye" of the New Zealand Lamprey in a *Geotria Ammocœte* which had been preserved in chrom-osmic solution by my late colleague Professor T. J. Parker, and given to me for investigation by his successor at the Otago University Museum, Professor W. B. Benham. The present investigation, however, was largely stimulated by the remarkable results obtained by Studnička in his researches on the minute histology of the parietal sense-organs of the European Lampreys (*Petromyzon*), and I am glad to be able to a large extent to confirm these results, and perhaps even to still further extend our knowledge of these remarkable structures. My work has been greatly facilitated by the recent publication of Studnička's admirable monograph on "Die Parietalorgane" in Oppel's 'Lehrbuch der Vergleichenden mikroskopischen Anatomie der Wirbeltiere' (2), which renders detailed discussion of the writings of earlier investigators superfluous.

(B) TOPOGRAPHICAL ANATOMY OF THE FORE-BRAIN AND ITS DERIVATIVES.

In its general characters the brain of *Geotria* in the "Velasia" stage agrees closely with that of the adult *Petromyzon*. Externally perhaps the most striking difference consists in the very distinct lobulation of the surface of the large olfactory lobes (fig. 1, *O.L.*), while internally the division

of the cavity of the saccus vasculosus into right and left halves by a well-developed longitudinal septum (fig. 2, *Sept.*) deserves mention.

The thalamencephalon has, in its anterior part, a thin membranous roof which rises upwards in a prominent dome. This dome lies immediately behind and between the olfactory lobes, and the thin roof bends down in front to form the lamina terminalis (fig. 2, *L.T.*). On this thin dome-shaped roof of the third ventricle lie the organs with which we are more immediately concerned, the pineal or parietal sense-organs. There are in the Lampreys, as is well known, two of these sense-organs, and in the genus *Petromyzon* one lies beneath the other, the upper one being by very much the better developed of the two, and being commonly spoken of as the "pineal eye." In the terminology of Studnička the upper one is described as the "pineal organ," and the lower one as the "parapineal organ." According to the view adopted by myself (3), and long since maintained by Gaskell (4), these two sense-organs are really members of a pair which have become displaced, the upper and better developed representing the right "parietal eye," and the lower the left one. This view is supported in a very interesting manner by the arrangement of the two organs in *Geotria*. It will be seen from figs. 1 and 2 that the larger and better developed of the two does not lie above but behind the smaller and less well-developed organ, so that both are distinctly visible when the brain is viewed from above. Moreover, I find in all cases where the organs have been carefully examined in situ, that the anterior one lies a little to the left side of the posterior, the latter being approximately median in position. This clear indication of the paired origin of the parietal sense-organs affords a close parallel to the condition described by myself in embryos of *Sphenodon* (3). In both cases there is an anterior parietal organ lying immediately in front of and a little to the left of a posterior one, but in *Sphenodon*, curiously enough, it is the left (anterior) organ which becomes well developed as the apparently unpaired "pineal eye" of

the adult, while in *Geotria* and *Petromyzon* it is the right (posterior) member of the pair which becomes dominant.

In *Geotria* the posterior (right) parietal sense-organ is seen to be connected with an opaque-looking band of tissue (fig. 1, *P.S.*), which runs backwards to the posterior commissure. This is the pineal stalk, including the pineal nerve (cf. fig. 6), and representing the hinder part of the original outgrowth of the brain, whose anterior part forms the "pineal eye." Owing, doubtless, to the enormous development of the right habenular ganglion (fig. 1, *G.H.R.*), the pineal stalk is pushed somewhat to the left side. Posteriorly, the pineal nerve is connected, as will be shown later on, both with the right habenular ganglion and with the posterior commissure. The left habenular ganglion is, as in *Petromyzon*, very much smaller than the right, and is divided into anterior and posterior portions. The posterior portion (fig. 2, *G.H.L.*) lies in immediate contact with the right habenular ganglion, with which it is connected by a transverse band of fibres, the *commissura habenularis superior* of Studnička's terminology. The anterior portion (fig. 2, *G.H.A.*) lies immediately beneath the left (anterior) parietal sense organ (parapineal organ), and is connected with the posterior portion by means of a stout band of nerve-cells and fibres underlying the pineal stalk and constituting the *tractus habenularis* of Studnička (fig. 2, *T.H.*). (In fig. 2 both right and left ganglia habenulæ are shown for diagrammatic purposes, but it would not really be possible to see both in a strictly median sagittal section such as is supposed to be represented.)

Almost immediately behind the right habenular ganglion, but separated from it by a well-marked recess (the *recessus infrapinealis*), lies the posterior commissure (figs. 1 and 2, *C.P.*). In a longitudinal section of the brain (fig. 2) the posterior commissure is cut transversely, and appears as a somewhat oval body projecting downwards and backwards into the brain-cavity at the posterior dorsal limit of the third ventricle. On the antero-ventral face of the posterior commissure lies a conspicuous longitudinal groove (fig. 2, *Ep.G.*)

lined by an epithelium composed of very much elongated columnar cells. This groove is evidently formed by union of the pair of grooves which I described in the *Ammocete* in 1902 (5); it has since been termed by Sargent (6) the "ependymal groove." In the "*Velasia*," though the two grooves are closely approximated, they still show clear indications of their double origin (fig. 3, *Ep. G.*). The endymal groove is continued forwards into the recessus infra-pinealis, and thence for a short distance beneath and to the left of the right habenular ganglion, gradually losing the special character of its epithelium.

According to Sargent (6) the epithelium of the endymal groove serves for the support and attachment of the anterior branches of Reissner's fibre on their way from the optic reflex cells to the brain cavity, in which the main fibre lies freely. From my own observations on *Geotria* I have come to the conclusion that the anterior constituent fibrils of Reissner's fibre (fig. 2, *R.F.*) do leave the brain substance in the endymal groove as described by Sargent, but this discovery by no means disproves the view which I previously (5) put forward with regard to the function of the endymal grooves in the *Ammocete*. Reissner's fibre itself is very conspicuous in *Geotria*, as shown in fig. 2, *R.F.*, but further discussion of this part of the subject may be conveniently left until later on.

(c) THE PINEAL ORGAN (RIGHT PARIETAL EYE).

General form and structure.—The structure of this organ (fig. 7) is in its general features very similar to that described by Studnička for the European lampreys. It consists of a hollow vesicle, about half a millimetre in maximum diameter, and having a very characteristic shape, not unlike that of a pear, with the pineal stalk representing the stalk of the pear. The upper surface of the optic vesicle, turned towards the light, is perfectly circular in outline and very much flattened like a button (fig. 4), while the lower surface is strongly convex, especially posteriorly, where the wall of

the vesicle gradually passes into the pineal stalk. The upper and outer wall of the vesicle is formed by the unpigmented, transparent "pellucida," while the lower and inner wall is formed by the "retina," under which term we may include both the retinal epithelium and the layer of ganglion cells and nerve fibres which underlies it (fig. 7). The retinal epithelium has a characteristic opaque white appearance owing to the abundant granules of white pigment imbedded in the pigment cells, and this, seen through the transparent pellucida, gives the whole organ its characteristic chalky appearance even when seen with the naked eye or under a simple lens. The line of junction of the pellucida with the retina, all round the circular margin of the upper surface of the organ, is very sharply defined; the wall of the optic vesicle is here thinner than in any other part, and the edge of the pigmented retinal epithelium appears from above as a distinct, opaque, white margin to the pellucida (fig. 4).

The thickest part of the wall of the optic vesicle lies posteriorly just where it joins the stalk. The whole organ is doubtless, as in *Petromyzon*, developed from the enlarged distal extremity of a hollow pineal outgrowth, the proximal portion (stalk) of which becomes solid and gives rise to the pineal nerve. The original cavity of this outgrowth persists distally as the cavity of the optic vesicle, and a smaller portion of it persists in the thickness of the wall of the optic vesicle, just in front of the point of entrance of the pineal nerve, and forms the "atrium" of Studnička. In *Petromyzon*, according to this author, the atrium communicates freely with the main cavity of the optic vesicle, but yet shows a tendency by enlargement to form an independent cavity. In *Geotria* I have not been able to detect any communication between the atrium and the main cavity of the optic vesicle; they appear to be completely separated from one another. The atrium (fig. 7 *At.*) usually appears both in longitudinal and transverse sections as a small oval or almost circular cavity lined by columnar cells. In one series of longitudinal section there are indications of one or two

subsidiary atrial cavities lying behind the principal one, and doubtless representing a further remnant of the original lumen of the pineal outgrowth; another series of sections shows that this appearance may be due to curvature of the atrium, whereby its lumen may be seen twice in the same section.

The proper cavity of the optic vesicle is well developed and usually of the shape shown in longitudinal section in fig. 7, with a funnel-shaped depression in the middle of the lower surface, probably indicating the original connection with the atrium. The funnel-shaped depression may, however, be almost, if not quite, unrecognisable (fig. 2). The peculiar network of protoplasmic strands which occupies the cavity of the optic vesicle will be described later on.

The whole organ is enclosed externally in a thin and ill-defined layer of fibrous connective tissue, which may be regarded, for the most part at any rate, as an extension of the pia mater.

Histology of the pellucida (fig. 7, *Pell.*).—The outer surface of the pellucida is smooth and even, but its inner surface is produced into large, irregular villi or processes which project into the cavity of the optic vesicle and are connected by thin strands of tissue with the retina (fig. 7, *P.St.*). The pellucida is composed, for the most part at any rate, of a single layer of columnar cells, which are enormously elongated to form the projecting villi. These cells contain conspicuous oval nuclei (fig. 7, *N.C.C.*) situate near their inner ends. Between the villi the inner surface of the pellucida, though uneven, is smooth, but at the inner ends of the villi the cells appear drawn out into threads, which go to form the strands of tissue connecting the pellucida with the retina. The columnar cells themselves appear to contain but little cytoplasm, which is only slightly granular and stains lightly with Ehrlich's hæmatoxylin and with acid fuchsin. Their outlines are well defined, but with a characteristic wavy appearance, which I attribute to shrinkage. Amongst these columnar cells, in the outer part of the pellucida, one finds a

number of almost spherical nuclei of doubtful significance, but resembling the nuclei of the ganglion cells of the retina. At various levels in the pellucida one also finds a small number of very darkly staining, small, irregular nuclei, having a shrivelled appearance, and closely resembling the "connective tissue" nuclei found in the interior of the optic vesicle and in the nervous layer of the retina.

Histology of the retina.—The retina, as already indicated, may be divided into two perfectly distinct layers, the epithelial layer, composed of pigment cells and sense cells, and the layer of ganglion cells and nerve fibres which lies behind it, and which we may call, in short, the nervous layer. Both these layers increase greatly in thickness as they recede from the pellucida, and around the atrium the nervous layer becomes so strongly developed as to form a veritable ganglion (fig. 7).

The epithelial layer of the retina (figs. 5 and 7) is composed of the same two kinds of elements as have been recognised by Studnička in *Petromyzon*—viz. sensory cells and pigment cells. The former (fig. 5, *R.S.C.*) are greatly elongated, slender rods whose inner ends project into the cavity of the optic vesicle and terminate in irregularly rounded, swollen knobs, while their outer ends branch into fibrils which lose themselves in the fibrillar network of the nervous layer. These rods have large oval nuclei (fig. 5, *N.S.C.*) situated towards their inner ends, and causing a fusiform swelling in the rod itself. The end-knobs of the rod (fig. 5, *S.C.K.*), and the rods themselves (apart from the nucleus), are only lightly stained with Ehrlich's hæmatoxylin, but take up acid fuchsin with great avidity, whereby they are rendered very conspicuous. Studnička describes the end-knobs in *Petromyzon* as being differentiated into inner and outer portions, but I have not succeeded in detecting any such differentiation in the case of *Geotria*, the knobs appearing to be practically homogeneous. The adhering ends of the protoplasmic strands (fig. 5, *P.St.*) which connect the sense-cells with the pellucida, may, however, spread out on the knobs, and thus give rise to the appearance

of an outer layer which stains less deeply with acid fuchsin. Possibly this is the explanation of the appearance described by Studnička. In other respects the sensory cells appear to be quite identical with those of *Petromyzon*.

The intervals between the sensory cells are filled by the pigment cells, which Studnička has, no doubt correctly, identified with the ependymal cells of the general internal lining of the brain cavities. The pigment cells (figs. 5, 7) are broadest at their inner ends, next to the lumen of the optic vesicle, and taper gradually outwards till their slender, thread-like outer extremities, which may apparently branch, are lost in the fibrillar network of the nervous layer, along with the outer ends of the sensory cells.

So far the pigment cells agree fairly well with those of *Petromyzon*, as described by Studnička (2), but there is one very important difference. In *Petromyzon* it appears that they terminate at their inner extremities in a smooth surface, through which the knobbed ends of the sense-cells project, while, according to Studnička's latest account, they themselves have no differentiated inner segments or knobs at all. In *Geotria*, on the other hand, the inner end of each pigment cell (fig. 5, *I.S.P.C.*) is very distinctly segmented off, and separated from the outer and principal portion of the cell (*O.S.P.C.*) by what looks like a limiting membrane (*L.M.*). In depigmented sections, as shown on the left-hand side of fig. 5, this "limiting membrane" is very conspicuous, and appears, at first sight, to form the inner surface of the retina; it has a characteristic dotted or beaded appearance. Careful observation shows, however, that even in depigmented sections the remains of the inner ends of the pigment cells, as well as the projecting knobs of the sense cells, may be clearly recognised as the inner side of the "limiting membrane," though not nearly so conspicuous as in sections in which the pigment is preserved, as shown on the right-hand side of fig. 5.

Thus in *Geotria* the pigment cells as well as the sense-cells are provided with differentiated, knobbed inner

extremities, but the knobs of the latter project further into the cavity of the eye than those of the former. The knobs of the pigment cells are much broader than the sense-cells at the same level, so that they form almost a continuous layer inside the limiting membrane, penetrated by the slender rods of the sense cells on their way to the sense cell knobs (fig. 5, *S.C.K.*) in the cavity of the optic vesicle.

The nuclei of the pigment cells (fig. 5, *N.P.C.*) are situated towards the outer extremities of the outer segments, at about the same level as the nuclei of the sensory cells, from which they may be distinguished by their somewhat smaller size and less dense-looking protoplasm. The pigment granules (composed of phosphate of lime?) are minute spherical bodies evenly distributed throughout the inner segment and the greater part of the outer segment, but not, so far as my observations show, occurring in the slender outermost portion of the pigment cell beyond the nucleus.

Examination of Studnička's earlier figures (7, Pl. III, figs. 6, 7, 8) suggests that in *Petromyzon* also the pigment cells may have differentiated knobbed inner extremities. The idea that only one kind of cell is present in the retinal epithelium, as shown in these figures, is doubtless, as Studnička himself has since pointed out, erroneous. According to his earlier observations, however, all the epithelial cells have knobbed (but unpigmented) extremities, and it appears just possible that he has abandoned too much of his previous results in making the necessary correction. In spite of the precision of his later account, it might be worth while to re-investigate this point in the light of our knowledge of *Geotria*, in which the segmentation of the pigment cells is so obvious as to leave no room for doubt.

The nervous layer of the retina consists of ganglion cells, nerve fibres, and connective-tissue cells. The ganglion cells (figs. 5, 7, *G.C.*) are very conspicuous on account of their large spherical nuclei, surrounded by only a small quantity of cytoplasm. The cytoplasm is often scarcely recognisable, while at other times it is more distinct and exhibits a multi-

polar character. The nuclei contains a few well-defined, darkly-staining chromatin granules. In the thinner parts of the retina (fig. 5) the ganglion cells are comparatively few in number, and occur chiefly towards the outside, just within the connective-tissue capsule of the eye. In the neighbourhood of the atrium, however, they are accumulated in large numbers, as already stated (fig. 7, *G.C.*).

The nerve fibres are extremely delicate and form a network (together with connective-tissue fibres ?) in which the ganglion cells are embedded (fig. 5, *N.F.N.*). It is probable that there is a special layer of nerve fibres between the ganglion cells and the connective-tissue sheath (fig. 7, *C.T.S.*), but I have not found it possible to distinguish it clearly from the latter.

The connective-tissue cells of the retina are distinguished by their elongated and very darkly-staining nuclei (fig. 5, *C.T.N.*'), resembling those found in the connective-tissue sheath; they seem to indicate the presence of connective-tissue fibres, running more or less vertically through the retina.

Irregular masses of pigment granules, similar to those found in the pigment cells of the epithelial layer, occasionally occur in the nervous layer, but these can hardly be regarded as essential constituents of this layer.

Histology of the wall of the atrium.—The atrium is lined by a single layer of columnar ependymal cells, none of which contain pigment, and I have not been able to demonstrate the existence of sensory cells in this region.

Contents of the optic vesicle.—Much discussion has taken place as to the nature of the irregular network which so constantly appears in the interior of the pineal eye (fig. 7, *P.St.*). The researches of Studnička leave no room for doubt that it is a normal constituent of the organ and not merely an artifact, although probably it undergoes much alteration during the processes of hardening and in the preparation of sections. It is probably partly due to coagulation of the albuminous contents of the optic vesicle, but it is also undoubtedly in part cellular in nature. As Studnička has shown in *Petromyzon*, the columnar cells of which the pellucida is composed are

connected with the sensory cells of the retina by delicate strands of tissue which traverse the lumen of the optic vesicle. This is very evident in the case of *Geotria* also, as indicated on the left-hand side of fig. 7. This figure, however, represents a section of an eye which has been somewhat abnormally distended in the processes of preparation, and in which, consequently, most of the delicate connecting strands have been ruptured; in other cases, where the pellucida has not become artificially arched outwards, all the projections of its inner surface are connected in this manner with the retina, and the general direction of the connecting strands is vertical. The strands themselves appear to be formed by outgrowth of the inner ends of the long columnar cells of the pellucida, which become attached to the knobs of the retinal sense cells. In all cases which I have observed, however, they appear to branch and form an irregular network (fig. 7), which may be partially due to artificial entanglement. Entangled, as it were, in the meshes of this network, one finds numerous small nuclei, which often stain very darkly and exhibit a characteristic shrivelled appearance as if undergoing degeneration. Sometimes, also, one finds an irregular mass of almost homogeneous material with nuclei adhering to its surface—probably identical with the “syncytial mass” described and figured by Studnička in *Petromyzon fluviatilis*, but, in my opinion, an artifact due to coagulation and entanglement. Such a mass is shown in the middle of the optic vesicle in fig. 7. Studnička regards the “plasmatischen Netze und Syncytien” as representing the remains of a “corpus vitreum,” but this appears to be a mere question of terminology, and it is extremely doubtful whether it is desirable to apply the term “corpus vitreum” to such very definite structures as the protoplasmic strands which connect the pellucida with the retina, although it is quite possible that these may be imbedded in a “corpus vitreum” during life. It seems probable that the function of these connecting threads may be to afford support to the freely projecting knobs of the sense cells by attaching them to the pellucida.

(D) THE PINEAL NERVE AND ITS CONNECTIONS.

It is well known that in *Petromyzon* the so-called "pineal outgrowth" arises immediately in front of the posterior commissure, and grows forward above the roof of the fore-brain in the form of an elongated hollow sac, whose distal extremity enlarges and becomes modified in structure to form the pineal or parietal eye, while the proximal portion, or "stalk," becomes solid, and by histological differentiation is, in part at any rate, converted into the pineal nerve.

In *Geotria*, as in *Petromyzon*, the original point of connection of the pineal stalk with the brain is clearly indicated by the depression between the posterior commissure and the right habenular ganglion known as the recessus infrapinealis, as shown in figs. 2 (*R.I.P.*) and 6. At this spot the epithelium of the ependymal groove, in sections, is usually pulled out and separated from the rest of the ependymal epithelium owing to the inevitable contraction in preparation, while remaining closely adherent to the pineal stalk above it, to which it is intimately attached by fibres which appear to belong to the pineal nerve. This connection of the epithelium of the ependymal groove with the pineal nerve has not, so far as I am aware, been hitherto observed, and appears to me to be a matter of considerable interest, though it must not be forgotten that some at any rate of the connecting fibres may be merely connective tissue.

The pineal stalk in *Geotria* is not, as a whole, very sharply defined, but merges on either side in the mass of arachnoid tissue which lies outside the brain. It thus appears much more definite in longitudinal than in transverse sections, forming a solid cord, apparently of loose connective tissue (figs. 1, 6, *P.S.*), in which the pineal nerve itself is imbedded. This nerve consists of a bundle of numerous very slender, non-medullated fibres, containing elongated nuclei, and indistinguishable from those of higher vertebrates, as represented, for example, in fig. 138 of Schäfer's 'Essentials

of Histology' (ed. vi). This bundle of fibres may easily be traced from the nervous layer of the retina of the pineal eye, in which it distinctly originates, to the surface of the brain immediately behind the right habenular ganglion and above the posterior commissure. The latter part of its course, as seen in a series of longitudinal sections, is shown in fig. 6. Shortly before reaching the brain it divides into several short branches. One of these (*P.N.* 1) is directly connected with the epithelium of the ependymal groove in the manner already described. Another, or perhaps several small bunches of fibres (*P.N.* 2), comes off more posteriorly, and its fibres probably pass into the posterior commissure (*C.P.*), and apparently through this to the inner surface of the ependymal groove (*Ep.G.*). In all my sections, however, a small shrinkage cavity (fig. 6, *S.C.*) is developed just above the posterior commissure, and the fibres of this branch of the pineal nerve are probably thereby ruptured, so that they appear to terminate abruptly above the shrinkage cavity, while from the lower surface of this cavity very delicate (nerve?) fibres run obliquely across the posterior commissure to the inner surface of the ependymal groove. Another branch (*P.N.* 3) of the pineal nerve comes off more anteriorly than either of those yet mentioned, and, curving forwards between the right habenular ganglion and the ependymal epithelium, joins the Meynert's bundle of the right side, and then, curving upwards with the latter, forms a band of fibres which can easily be traced into the middle of the habenular ganglion, as shown in fig. 6.

It thus appears that the pineal nerve is connected (1) with the epithelium of the ependymal groove (both directly and possibly also by fibres which pass through the posterior commissure), (2) with the right habenular ganglion, and (3) with the right bundle of Meynert. The connection with the habenular ganglion was long since maintained by Gaskell (4), but has since been doubted by Studnička (2), who maintains that, whereas the "parapineal organ" is connected with the left habenular ganglion and the superior (haben-

ular) commissure, the pineal organ itself is connected with the posterior commissure. Studnička makes use of this apparent discrepancy as an argument against the theory of the paired origin of the parietal sense organs. We shall have occasion to discuss this question somewhat more in detail at a later stage.

According to the observations recorded above the connection of the pineal nerve with the posterior commissure, about the existence of which there can be very little doubt, may be due simply to the fact that some of the nerve fibres traverse this commissure in order to reach the epithelium of the ependymal groove. Curiously enough, the existence of this remarkable structure—the ependymal groove—appears to have been hitherto ignored by those authors who have investigated the pineal organs, and, conversely, those who have dealt with the ependymal groove have entirely neglected its relations to the pineal nerve.

In my memoir on the subject, published in 1902 (5), I described a pair of these grooves in the *Ammocœtes* both of *Geotria* and *Petromyzon*, and, believing that I had detected cilia on the long columnar cells with which they are lined, I termed them "ciliated grooves," and suggested that they might serve to promote the circulation of the fluid in the brain cavities, especially in relation to a highly vascular vertical fold of the choroid plexus, which in the *Ammocœte* hangs down, gill-like, into the brain cavity in the immediate neighbourhood of the grooves in question. Sargent (6) in his remarkable memoir on the optic reflex apparatus of vertebrates, criticises this view, maintaining that what I had interpreted as cilia are really constituent fibrils of Reissner's fibre, and that the ependymal groove functions merely as an attachment plate for these fibrils, which supports them as they leave the brain on their way to join the main fibre lying freely in the brain cavity. It does not seem to me that these two views are incompatible with one another, and I find it difficult to believe that such a remarkable and well-developed structure as the ependymal groove should be required solely for the

function which Sargent assigns to it. The question of ciliation must be left for future investigation to settle, but Sargent has evidently misunderstood my observations on this subject, for he makes me say that the cilia of the grooves are longer than those of the ventricular walls generally, whereas I both described and figured them as being much shorter. What I described as cilia are, therefore, probably not the same structures as Sargent describes as constituents of Reissner's fibre, though I now believe myself that they may possibly indicate merely a striated margin of the columnar epithelium. My recent observations show, however, that in the *Velasia* stage of *Geotria* it is possible to make out extremely fine threads (fig. 6, *R.F.*'') projecting from the epithelium of the ependymal groove, much longer than the supposed cilia, and these are in all probability the nerve fibrils described by Sargent. Sargent maintains, as already stated, that these fibrils are connected with, and in fact go to make up, the fibre of Reissner, and he regards the whole system as a short circuit for optic reflexes. He has found Reissner's fibre, with similar relations, throughout the entire vertebrate phylum, and brings forward experimental as well as histological evidence in support of his views.

In *Geotria* Reissner's fibre (fig. 2 and 6, *R.F.*) is conspicuously developed, and has in most respects the same relations as described by Sargent in *Petromyzon*. It appears to originate in the immediate neighbourhood of the ependymal groove, beneath the posterior commissure, and is made up of a number of branches (fig. 6, *R.F.*', *R.F.*'') which can be traced close up to the columnar epithelium of the groove.¹ Though I have not been able actually to demonstrate the connection between this epithelium and Reissner's fibre, I see no reason to doubt the correctness of Sargent's statement as to the existence of such a connection by means of the delicate fibrils which emerge from the epithelium.

¹ The two grooves in *Geotria* are so closely approximated as to form practically a single groove (fig. 3, *Ep.G.*), the form of which, however, clearly indicates its double origin.

These fibrils are so extremely slender that it is almost too much to hope for to find unbroken continuity, especially when we remember that the coagulation of the fluid in the brain-cavity and the shrinkage in preparation must tend to cause rupture. In fig. 6 a great tuft of extremely fine branches (*R.F.'*) is shown coming off from Reissner's fibre beneath the hinder part of the posterior commissure, whilst more anteriorly the main fibre divides into two approximately equal branches (*R.F. ''*), and at *R.F. '''* delicate fibrils are seen emerging from the ependymal epithelium. These appearances strongly confirm the observations of Sargent as to the connection of Reissner's fibre with the ependymal groove. As to the origin of the constituent fibrils from optic reflex cells within the substance of the brain, however, I am not able to make any definite statement. In fig. 3 I have shown the existence of a group of large nerve-cells (*N.C.*) situated in the anterior lateral part of the tectum opticum on either side of the posterior commissure. These obviously correspond to two of the groups of optic reflex cells described by Sargent in *Petromyzon*, and represented in his fig. 7, but I have not seen any connection of these cells with the ependymal groove, such as he figures. This, however, by no means proves that no such connection exists, and it must be remembered that my material was not specially prepared for the purpose of tracing nerve fibres.

I have, however, already shown that fibres from the pineal nerve are connected with the ependymal epithelium on its inner aspect, while Sargent has shown that branches of Reissner's fibre are connected with the same epithelium on its outer aspect. One is tempted to conclude, therefore, that the pineal eye is connected with Reissner's fibre through the pineal nerve, and thus linked up with the optic reflex system. This conclusion obviously involves one in what appears at first sight to be a very serious difficulty. It must be remembered that the pineal nerve is apparently a sensory nerve, while Reissner's fibre is a motor nerve, and a direct connection between the two, without the intervention of ganglion cells in

the central nervous system is, to say the least of it, extremely improbable. This difficulty may be overcome, however, by supposing that the "reflex cells" required are situated in the great ganglionic swelling which surrounds the atrium of the pineal eye, and which, of course, is actually developed as an outgrowth of the central nervous system.

I do not wish to press this suggestion too far, however, in the present state of our knowledge, nor is it necessary to do so in order to link up the pineal eye with the optic reflex apparatus, for Sargent has shown that some of the constituents of Reissner's fibre issue from the base of the right habenular ganglion. Now this ganglion is undoubtedly connected with the pineal eye through the pineal nerve, as I have already indicated, and it is extremely probable that we have here reflex cells which transmit stimuli received through the pineal nerve to Reissner's fibre.

From the region of the posterior commissure it is quite easy to trace Reissner's fibre backwards through the iter and the fourth ventricle, into the canalis centralis of the spinal cord, as shown in fig. 2. It is worth noticing that, as it passes beneath the cerebellum, it does not become imbedded in the roof of the brain as takes place in adult *Petromyzon*, but remains free throughout its course. This free condition is also found in the young *Petromyzon*, so that it is not unlikely that in *Geotria* also Reissner's fibre may become imbedded in the growing tissue of the cerebellum with advancing age. I have not followed it backwards beyond the commencement of the spinal cord.

(E) THE PARAPINEAL ORGAN (LEFT PARIETAL EYE) AND ITS RELATIONS TO THE BRAIN.

The parapineal organ, or left parietal eye (figs. 2, 8, *L.P.E.*), is, as already pointed out by Studnička for *Petromyzon*, essentially similar in structure to its larger and more perfectly developed fellow of the primitive right side. Its position, in front of and a little to the left of the "pineal organ," has already

been sufficiently described. Perhaps the most remarkable difference which it exhibits as compared with its fellow consists in the manner in which it is connected with the brain, the organ itself lying immediately upon the anterior division of the left habenular ganglion (figs. 2, 8, *G.H.A.*), while its apparent nerve, the tractus habenularis of Studnička (figs. 2, 8, *T.H.*), is the long-drawn-out portion of the left habenular ganglion which connects the anterior and posterior portions of the latter. There is, therefore, strictly speaking, no proper nerve to the left parietal eye, which remains seated immediately upon the brain, though no doubt the tractus habenularis functions as such.

The parapineal organ of *Geotria* is a hollow sac, much smaller than the pineal organ and of different shape, flattened dorso-ventrally and elongated transversely (figs. 1, 4, *L.P.E.*). It may be slightly, but distinctly, constricted in the middle into right and left halves, or it may be more irregular in outline, as shown in fig. 4. The attachment to the anterior division of the left habenular ganglion, though broad, does not include by any means the whole of the ventral surface, so that the parapineal organ is marked off from the ganglion by a deep constriction, deeper in front and at the sides than it is posteriorly. The outer surface of the organ is covered with a thin sheath of connective tissue (fig. 8, *C.T.S.*) continuous with the pia mater of the brain.

The wall of the parapineal organ may be divided into pellucida and retina exactly as in the case of the pineal eye itself, but the distinction between the two is not nearly so well marked. The pellucida (fig. 8, *Pell.*) consists of a layer of columnar cells, the inner surface of which is in places drawn out into irregular processes projecting into the cavity of the organ exactly as in the case of the pineal eye, only in a less perfectly developed condition. Outside these columnar cells are numerous spherical nuclei, probably indicating a layer of ganglion cells similar to those found in the retina. In the pineal organ such nuclei are almost absent from the pellucida, and in *Petromyzon* Studnička describes the pellucida of

the parapineal organ as consisting of only a single layer of cells.

The pellucida passes quite gradually into the retina, which consists of an epithelial layer of columnar cells facing the cavity of the organ and backed by a nervous layer of ganglion cells and nerve fibres. Thus the retina has a close general similarity to that of the pineal organ, from which, however, it differs strikingly in the entire absence of pigment. According to Studnička the retinal epithelium of the parapineal organ in *Petromyzon* consists of sensory cells and ordinary ependymal cells, but I have not been able to distinguish clearly between the two in *Geotria*. As in *Petromyzon*, the characteristic knob-like projections of the retinal cells into the cavity of the organ, which are so conspicuous in the pineal eye, are not to be found in the parapineal.

In the interior of the parapineal organ we find, exactly as in the pineal, a network of delicate threads connecting the pellucida with the retina (fig. 8). Here again this network appears to be formed by outgrowth of the columnar cells of the pellucida, and contains small nuclei scattered in it.

The nervous layer of the retina must be considered in connection with the underlying anterior division of the left habenular ganglion (fig. 8, *G.H.A.*). This consists of a central mass of finely granular or punctate matter devoid of nuclei, but partially surrounded by nerve cells, as shown in longitudinal section in fig. 8. In transverse sections (fig. 9) the central mass is seen to extend laterally in a pair of horizontal, wing-like projections, beneath which the nerve cells are accumulated. From the upper surface of the central mass stout bands or tracts of fibres are given off, which curve upwards amongst the ganglion cells of the retina, and sometimes appear to extend even into the pellucida. These fibrous bands can, in part at any rate, be traced directly backwards into the tractus habenularis, as shown in fig. 8.

The anterior division of the left habenular ganglion passes backwards quite gradually into the tractus habenularis.

The latter exhibits a very characteristic crescentic form in transverse section, with the horns of the crescent, which are directly continuous with the wing-like outgrowths of the punctate substance in the anterior enlargement, turned upwards. The upper part of the crescent is composed chiefly of longitudinal nerve fibres (compare fig. 8) cut across, while the lower part is occupied by a somewhat thinner layer of nerve cells covered by the ependymal epithelium.

(F) ACCESSORY STRUCTURES OVERLYING THE PARIETAL SENSE ORGANS.

The parietal sense organs lie in the cranial cavity immediately beneath the connective tissue wall of the cranium, between the nasal and occipital cartilages, as shown in fig. 2. The membranous wall of the cranium (fig. 2, *C.T.C.*), composed of very dense fibrous connective tissue, thins out somewhat, and is slightly arched upwards in this region, and the upper surfaces of the sense organs are closely pressed against it. Immediately above this there is a thick mass of very much modified connective tissue forming the principal part of the so-called cornea of Studnička (fig. 2, *C.T.P.*). This mass of connective tissue is a well-defined structure both in the Lampreys and in *Sphenodon*, where it occupies the parietal foramen, and it seems desirable to distinguish it by a special name. I therefore propose to call it the "parietal plug." In *Geotria* it consists of a somewhat basin-shaped mass of fibrous tissue, in which the fibres run almost vertically, but converging somewhat below, where the plug is narrower than it is above. The fibres are arranged in dense, multi-nucleate bands, which branch and anastomose freely with one another to form a network with lacunar meshes. Probably these meshes are occupied in life by a gelatinous material, of which traces are still recognisable. The upper ends of the fibrous bands of which the plug is composed are closely attached to the under surface of the corium or dermis. This layer does not appear to undergo any special modification as it passes

over the plug, except such as I have to mention shortly in regard to the pigment. Above the corium comes the epidermis, which again exhibits a perfectly normal structure.

When the dorsal surface of the head is examined carefully, a small, light-coloured patch is visible a short distance behind the nostril. This patch is somewhat elongated and nearly oval in outline (perhaps, rather, key-hole shaped), and constitutes the well-known "Scheitelfleck" of German authors. It lies immediately above the parietal plug, and owes its pale colour to the fact that the pigment, elsewhere so abundantly developed in the integument, is here almost entirely absent. Elsewhere we find the pigment cells arranged in two layers, an outer and an inner. The outer one (fig. 2, *Pig.*¹) lies in the corium at a very short distance beneath the epidermis, and is but feebly developed, consisting of a sparse layer of much-branched cells. This layer is still more feebly developed in the region of the "Scheitelfleck," but not entirely absent. The inner layer of pigment (fig. 2, *Pig.*²) lies immediately beneath the corium, which it separates from the underlying looser connective tissue. It is very much denser than the outer layer, and is completely absent beneath the "Scheitelfleck," terminating abruptly on reaching the upper margin of the parietal plug.

From the foregoing account it will be evident that the light-transmitting tissues which overlie the parietal organs have essentially the same structure and arrangement as in *Petromyzon*, but, if we may judge from Studnička's figures, the parietal plug is better defined in *Geotria*.

(g) GENERAL CONSIDERATIONS.

The function of the parietal sense organs.—In considering the question of function, one must distinguish sharply between the right and the left parietal sense organs. The former is a well-developed "pineal eye," containing the essential structural elements which one is accustomed to associate with a light-perceiving organ, and, in common with

Studnička, I find it impossible to believe that, in the Lampreys, it is not at the present day functional. It exhibits, in my opinion, no sign of degeneration; sense cells, pigment cells, and ganglion cells are all present in a high degree of perfection, and the retina is connected with the brain by a well-developed nerve. The enormous development of the right habenular ganglion and of the right bundle of Meynert, with which the pineal nerve is connected, also clearly indicate functional activity on the part of the pineal eye. Perhaps the most striking evidence in favour of this view, however, is afforded by the modification of the overlying tissues to form a light-transmitting apparatus. It is one of the fundamental axioms of biology that disuse leads to degeneration, and we may safely assume that a high degree of structural differentiation implies a corresponding degree of functional activity.

Everything points to the fact that the function of the pineal organ is that of light perception, and therefore we are justified in speaking of it as an "eye." Its structure, however, especially in the Lampreys, differs in important particulars from that of any other eye known to us. In the Lampreys, at any rate, it is not, as Studnička has already pointed out, a cameral eye, and we cannot suppose it to be capable of forming an image. There is nothing which we are justified in regarding as a lens, and the peculiar nature and arrangement of the "white" pigment is calculated to reflect the rays of light in every direction, and thereby prevent the formation of an image, even if the necessary dioptric apparatus were present.

On the other hand, it may well be that the brilliant white pigment, by reflecting the light rays upon the knobs of the sense cells, may thereby serve to intensify the light stimulus and render the whole organ extremely sensitive to the variations in the intensity of the illumination to which it is exposed.¹ Such sensitiveness might be of great value in giving timely warning of the approach of enemies from

¹ This view of the function of the knobs of the sense cells is totally opposed to that of Studnička (12), who regards them as so many independent lenses, each serving to focus the light upon its own particular sense cell.

above, before they come within range of the paired eyes, and this I consider in all probability to be the function of this organ. If the connection of the pineal eye with Reissner's fibre, which I have suggested above, really exists, we may further conclude that the efficiency of the organ is greatly increased by the "short circuiting" of the optic reflexes in the same manner as has been described by Sargent in the case of the ordinary paired eyes.

As regards the parapineal organ, or left parietal eye, it is more difficult to express an opinion. Here we have, in the absence of pigment and of the projecting knobs of the sense cells, evidence either that the organ has never attained so high a degree of organisation as its fellow, or that it has suffered degeneration, and similar evidence is afforded by the much smaller size of the left habenular ganglion and the left bundle of Meynert. The fact that it usually lies concealed beneath the pineal eye also points to loss of function as a light-perceiving organ; and it is interesting to note that in this respect the genus *Geotria*, in which both organs are exposed to the light, one in front of the other, appears to be in a less degenerate condition than *Petromyzon*. The retention of the well-developed connection of the parapineal organ with the left habenular ganglion, however, seems to indicate that it is still in some degree functional. It is difficult to understand why one member of the original pair should tend to degenerate any more than the other, but the degeneration itself may be connected with a possible greater efficiency of a strictly median organ in appreciating what is taking place immediately above the animal.

The paired origin of the parietal sense organs.—The idea of a median, unpaired, Cyclopean eye on the top of the head of the primitive Vertebrate ancestors has so struck the popular imagination and become so firmly rooted even in scientific literature that it is extremely difficult to gain general acceptance for the modification of this somewhat crude notion necessitated by modern research. Yet the necessity for such modification confronts us at almost every

point of view, morphological, embryological, and even palæontological. In this connection we cannot content ourselves with the consideration of any one Vertebrate group, but must seek for evidence from as wide a field as possible. I discussed the problem at some length in my memoir (3) "On the Development of the Parietal Eye and Adjacent Organs in *Sphenodon* (Hatteria)," published in this 'Journal' in 1899, with special reference to the Tuatara. Since that date the embryological investigations of Cameron have afforded striking confirmation of the views then adopted, and my researches on the New Zealand Lamprey, described in the present memoir, strongly confirm me in the opinion that the so-called pineal and parapineal organs represent the right and left members of a primitive pair.

The evidence derived from the study of *Geotria* may be summarised as follows :

(1) The parapineal organ, in its position to the left of the pineal, still shows evidence of its primitive paired character.

(2) The structure of the pineal and parapineal organs is essentially identical, although the former is much more highly developed than the latter.

(3) The connection of each of the two sense organs with the corresponding member of the habenular ganglion pair need no longer be questioned.

(4) The marked asymmetry in point of size of the two habenular ganglia, and of the two bundles of Meynert, corresponds exactly to the unequal development of the two parietal sense organs with which they are connected, and leaves no doubt as to the paired character of the whole system.

The embryological investigations of Cameron (8, 9) confirm strongly the general results obtained by Hill, Locy, and myself. In *Amphibia* and in the chick Cameron shows that the "epiphysis" is in origin a bilateral structure, just as Hill had shown for *Teleosteans*, Locy for *Elasmobranchs*, and the present writer for *Sphenodon*. In some cases, including man, however, Cameron (10) maintains that there is a decussation of the nerve fibres at the base of the epiphysis, each lateral

half of the "pineal body" being supplied by fibres which come from the habenular ganglion of the opposite side. This may be true in certain types, but as Cameron himself recognises, there is no evidence for any such decussation in the Lampreys, where each of the parietal sense organs is undoubtedly innervated from the habenular ganglion of its own side.

By no means the least interesting evidence in favour of the paired origin of the parietal sense organs is that afforded by the study of fossil fishes, the history of which affords a curious illustration of the influence of what we may call the "Cyclopean theory" of the pineal eye. The following quotation from Bashford Dean's work (11) on 'Fishes, Living and Fossil' will serve to make this clear, and at the same time to indicate the character of the palæontological evidence in question: "The evidence, however, that the median opening in the head shields of ancient fishes actually enclosed a pineal eye is now felt by the present writer to be more than questionable. The remarkable pineal funnel of the Devonian *Dinichthys* (fig. 134) is evidently to be compared with the median foramen of *Ctenodus* and *Palædaphus* (= 'Sirenoids,' p. 122); but this can no longer be looked upon as having possessed an optic function, and thus practically renders worthless all the evidence of a median eye presented by fossil fishes. It certainly appeared that in the characters of the pineal foramen of *Dinichthys* there existed strong grounds for believing that a median visual organ was present. . . . But the function of this pineal foramen, unfortunately for speculation, could not have been optical. It occurs in a fish (*Titanichthys*) closely related to *Dinichthys*, and, as the writer has recently found, is of a distinctly paired character, its visceral and outer openings bearing grooves and ridges which demonstrate that the pineal structures must not only have been paired, but must have entered the opening in a way which precludes the admission of the epiphysis. . . . It must, for the present, be concluded, accordingly, that the pineal

structures of the true fishes do not tend to confirm the theory that the epiphysis of the ancestral vertebrates was connected with a median unpaired eye."

If we once recognise the paired origin of the parietal sense organs, the fact that a paired pineal foramen occurs in the ancient *Titanichthys* need cause us no surprise.

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EXPLANATION OF PLATES 1 & 2,

Illustrating Professor Arthur Dendy's paper “On the Parietal Sense-organs and Associated Structures in the New Zealand Lamprey (*Geotria australis*).

EXPLANATION OF LETTERING.

At. Atrium of pineal organ. *C.* Cerebellum. *C.H.* Cerebral hemisphere. *C.H.S.* Commissura habenularis (=C. superior). *C.M.* Corpus mammillare (= lobus infundibuli). *C.P.* Posterior commissure. *C.T.* Connective tissue. *C.T.C.* Connective tissue wall of cranium. *C.T.N.* Nuclei of connective tissue cells. *C.T.N'*. The same in nervous layer of retina of pineal organ. *C.T.P.* Parietal plug of modified connective tissue overlying the parietal sense organs. *C.T.S.* Connective-tissue sheath. *Ep.E.* Ependymal epithelium. *Ep.G.* Ependymal groove. *Epid.* Epidermis. *G.C.* Ganglion cells. *G.H.A.* Anterior division of left habenular ganglion. *G.H.L.* Posterior division of left habenular ganglion. *G.H.R.* Right habenular ganglion. *Inf.* Infundibulum. *I.S.P.C.* Inner segments of pigment cells. *L.M.* “Limiting membrane.” *L.M.B.* Left bundle of Meynert. *L.P.E.* Parapineal organ (= left parietal eye). *L.T.* Lamina terminalis. *Med.* Medulla oblongata. *Musc.* Muscle. *Na.C.* Nasal cartilage. *N.C.* Nerve cells. *N.C.C.* Nuclei of columnar cells of pellucida. *N.F.N.* Network of nerve fibres, etc. *Not.* Notochord. *N.P.C.* Nuclei of pigment cells. *N.S.C.* Nuclei of sense cells. *Oc.C.* Occipital cartilage. *O.Ch.* Optic chiasma. *O.L.* Olfactory lobe. *O.N.* Olfactory nerve. *O.S.P.C.* Outer segments of pigment cells. *Par.C.* Parachordal cartilage. *P.B.* Pituitary body. *Pell.* Pellucida. *Pig¹*, *Pig²*. Outer and inner pigment layers of integument. *Pl.Ch.* Choroid plexuses. *P.M.* Pia mater. *P.N.* Pineal nerve. *P.N¹⁻³*. Branches of pineal nerve. *P.S.* Pineal stalk. *P.St.* Protoplasmic strands in interior of pineal organ. *Ret.* Retina. *R.F.* Reissner's fibre. *R.F¹⁻³*. Constituent branches of Reissner's fibre. *R.I.P.* Recessus infrapinealis. *R.M.B.* Right bundle of Meynert. *R.P.* Recessus præ-opticus (= R. chiasmaticus). *R.P.E.* Pineal organ (= right parietal eye). *R.P.O.* Recessus post-opticus. *R.S.C.* Retinal sense-cells. *S.C.* Shrinkage cavity above posterior commissure. *S.C.K.* Terminal knobs of sensory cells of retina. *Sept.* Longitudinal septum

dividing the saccus vasculosus into right and left halves. *Sp.C.* Spinal cord. *T.H.* Tractus habenularis. *Thal.* Thalamencephalon. *T.O.* Tectum opticum. *V*³. Third ventricle. *V*⁴. Fourth ventricle. *X.* Point where the bundles of Meynert reach the base of the brain.

(All the figures refer to *Geotria australis* [Velasia].)

PLATE 1.

FIG. 1.—Brain; dorsal view after removal of the choroid plexuses of the mid- and hind-brain. $\times 10$.

FIG. 2.—Sagittal section through the brain, with the surrounding cranium and overlying structures (slightly diagrammatic, and with the arachnoid tissue omitted).

FIG. 3.—Transverse section of the brain in the region of the posterior commissure, showing the ependymal groove, bundles of Meynert, etc. Drawn under Zeiss A, oc. 2.

PLATE 2.

FIG. 4.—The pineal and parapineal organs (right and left parietal eyes), as seen from above under a dissecting lens.

FIG. 5.—Diagram showing the structure of the retina of the pineal organ (right parietal eye).

FIG. 6.—Longitudinal vertical section through the recessus infrapinealis, showing the relation of the pineal nerve to the posterior commissure, the right habenular ganglion, the right bundle of Meynert, the ependymal groove, and Reissner's fibre (slightly diagrammatic).

FIG. 7.—Sagittal section of the pineal organ (right parietal eye), drawn under Zeiss D, ocular 2 (slightly diagrammatic).

FIG. 8.—Longitudinal vertical section through the parapineal organ (left parietal eye) and the anterior portion of the left habenular ganglion. Drawn under Zeiss D, oc. 2 (slightly diagrammatic).

FIG. 9.—Transverse section through the hinder part of the anterior portion of the left habenular ganglion, immediately behind the parapineal organ. Drawn under Zeiss D, oc. 2.

Studies in Spicule Formation.

V.—The Scleroblastic Development of the Spicules in Ophiuroidea and Echinoidea, and in the Genera *Antedon* and *Synapta*.

By

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With Plates 3 and 4.

INTRODUCTORY.

THE greater part of the material utilised in this inquiry was obtained from the Marine Biological Laboratory at Plymouth, and consisted of two common examples of the Ophiuroidea, viz. the small *Amphiura elegans* (Amphiuridæ) and the much larger *Ophiothrix fragilis* (Ophiothricidæ), and one of the Echinoidea—*Echinus esculentus*. Recently-metamorphosed specimens of this latter were mostly obtained from the artificially-reared plutei referred to in Study III (13), and were about a score in number; however, one or two slightly larger specimens were also secured from dredgings. Most of my results have been obtained from observations on the viviparous *Amphiura elegans*. In this Ophiurid the bursæ, as is well known, function as brood-chambers, and, in consequence, young animals are very easy to obtain in every stage of development. The methods of preparation adopted were as follows:—A hundred or more

living *Amphiurus* being obtained, the disc of each animal was cracked, so as to permit the free access of the fixing, staining, and preserving reagents to the young animals contained within the bursæ, and these were then fixed with 1 per cent. osmic acid, stained with picro-carmin, and preserved in 90 per cent. alcohol in the manner already described in previous studies. Some of my best slides, however, have been obtained by merely fixing the live *Amphiurus* in absolute alcohol, subsequently staining for a fortnight or more in a saturated solution of safranin in absolute alcohol and washing out in absolute alcohol for a month or so after (if the alcohol be warmed less time will suffice). In many cases I also employed lichtgrün as a plasma stain, but, if employed, the solution in absolute alcohol must either be very weak, or, if a saturated solution, the *Amphiurus* must only be immersed in it for a minute or so, otherwise the tissues become opaque. Lichtgrün, when successfully employed, undoubtedly gives the best results; it is, however, quite possible to work without it, though in this case it is more difficult to be quite certain on occasion as to whether a particular cell belongs to a particular spicule or not. When the *Amphiurus* have been stained the discs are opened and the young ones extracted; these are then transferred to xylol, and finally mounted in balsam. In general only the youngest *Amphiurus* (about 0.5 mm. in diameter; see fig. 1) yield satisfactory results, though now and again it is possible to observe young spicules in the arms of the older *Amphiurus*. It is surprising how very few really satisfactory young *Amphiurus* are obtainable from numerous parents: from at least five or six hundred adults I have only managed to secure about a score of young ones showing the origin of spicules in an unmistakable manner.

The above-described methods of preparation were also employed in the examination of the specimens of *Ophiothrix* and *Echinus*.

My *Antedon* material consisted of very young specimens which, after having their discs opened, were prepared by the osmic and picro-carmin method. The imperforate thin plate

spicules (which do not seem to have been previously described) are lodged in the hypostroma of the integument, and in consequence the portions of integument to be mounted must, when freed from the viscera and musculature, be placed inside upwards on the slide.

My *Synapta* material, consisting of *S. hispida* and *S. digitata*, was obtained from Naples, and I believe was simply fixed and preserved in alcohol. I stained portions of the body-wall by the safranin and lichtgrün method and obtained good results.

In many cases, especially of the older spicules of *Amphiura*, it is difficult to decide upon the exact number of cells in connection with a spicule, and such must be simply passed over. The figures provided in the accompanying plate were all drawn from spicules about which there existed no doubt whatever as to the number of scleroblasts attached, and these were only obtainable by careful and persistent searching.

THE ORIGIN OF THE SPICULES IN *AMPHIURA ELEGANS*.

In *Cucumariidæ* (13) the plate-spicule originates as an elongated granule (thick-set needle) with its length disposed at right angles to the line joining the masses of the two scleroblasts usually concerned in its deposition; in some cases, however, four scleroblasts are concerned in the deposition, two being situated on each side of the needle. In *Amphiura elegans*, on the other hand, the plate-spicule originates in the same manner as the small superficial spicules of the *Cucumariidæ* do, viz. as an approximately spherical granule contained within a single cell (fig. 2). This difference of origin between the holothurian and the ophiuroid large plate-spicules can be correlated with other more general differences affecting the manner of lime secretion as it occurs in the two groups, and these I shall shortly indicate. For the present it suffices to point out that the needle and the granule correspond in form in both cases with the general

space disposition of the scleroblasts concerned in their individual formation: the elongated needle is deposited by a binucleated mass of scleroplasm with maximum and minimum diameters in the plane of the body-wall, and the spherical granule by a scleroblast whose corresponding diameters are all approximately equal. The granule in *A. elegans* next assumes a flat three-cornered shape whilst still contained by the single scleroblast, and the three corners of the triangle thus formed then elongate to form a young triradiate spicule (fig. 3). Shortly after this stage is reached the nucleus of the scleroblast divides and two scleroblasts in consequence appear in connection with the young triradiate (fig. 4). Approaching nuclear division in the scleroblast is always denoted by large size and faint coloration by picrocarmine (fig. 5*a*, *e.g.*); as is well known picrocarmine is a stain which never renders the details of karyokinesis visible. The three arms of the spicule next show signs of bifurcation at the extremities (figs. 5, 6), and, indeed, the whole of the further development of the spicule (when this is not situated at the extreme edges or ends of the arms, in which position certain parts elongate greatly in a distal direction) consists, as in Cucumariidæ, of a series of bifurcations resulting in a more or less circular perforated plate (fig. 7), but it is noticeable that the attached scleroblasts differ from those of Cucumariidæ in their much greater number. The fact that abnormally-large nuclei are so often met with in these scleroblasts renders it exceedingly probable that they are all (thirty or forty in the case of the larger plates) derived from the original mother-scleroblast, although it is evidently impossible to make a decisive statement to that effect. This repeated division of the mother-scleroblast might, indeed, be attributed to the necessity for the proximity of nuclear substance to the constantly-increasing area of deposition (the well-known experiments of Verworn on *Polystomella* proving enucleated portions of protoplasm to be incapable of secreting a shell pointing the argument) were it not that no such subdivision occurs in the case of other spicules of equal size

(Study IV). Why in Ophiuroids (and all other echinoderms save holothurians) there should thus exist this definite relation between the number of attached scleroblasts and the size of the spicule, and not in holothurians I am quite unable to say. (As a remarkable illustration of this fact compare the stool spicules of *Thyone* [Study IV, fig. 55] with the young spines of *Ophiothrix* [fig. 13]).

Not all of the spicules are derived from an original tri-radiate structure; occasionally the spherical granule elongates to form a rod which bifurcates at its extremities, as in *Cucumariidæ*; occasionally also the young spicule assumes a more or less irregular form, and in *Ophiothrix* I have observed four- and six-rayed young spicules (figs. 10 *b* and *c*), but all these are very rare. The tri-radiate form of the young spicule is by far the most usual. It must also be mentioned that the largest of the perforated plates figured is small when compared with most of the spicules present in the young embryos of fig. 1, and of course the plates of the adult *Amphiura* are still larger by comparison.

The scleroblasts in *Amphiura* are spherical cells which assume a subspherical form when attached to the spicule; the nucleus is relatively large, and if stained with picrocarmine shows a distinct nucleolus. The cytoplasm is faintly granular. Nuclei vary in size to some extent even in the same individual, and, as already stated, become very large previous to division. In specimens fixed with absolute alcohol the nuclei appear somewhat contracted.

THE SPICULES IN *OPHIOTHRIX FRAGILIS* AND *ECHINUS ESCULENTUS*.

My material for ascertaining the mode of spicule formation in these two genera being very limited I have merely attempted to confirm the supposition that the process is here identical with that in *Amphiura elegans*, and this I have had no difficulty in doing. My *Ophiothrix* material consisted of several young specimens with discs about 1 mm. in

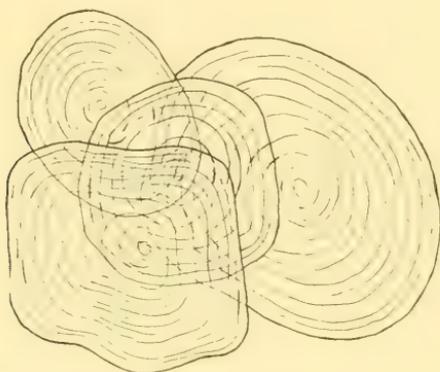
diameter. Most of the plates in the young *Ophiothrix* are extremely thick, and with comparatively few and small perforations (fig. 11); however, the ordinary thinner plates are also present. The spicule originates in the same manner as that already described (figs. 8—10). The young forms represented by figs. 10 *b* and *c* are, as already mentioned, unusual. Fig. 10 *c* indeed reminds one as regards shape of a young stage in the formation of an auricularian wheel. Fig. 13 represents a young spine which develops in much the same way as the stool of *Thyone*, save of course that the basal plate originates as a granule in one cell, and that the number of scleroblasts concerned in its deposition is much greater. It is quite possible that not all the cells represented in this figure are scleroblasts, some possibly belonging to the surrounding membrane, and it is clearly impossible to distinguish between them; it is quite safe to say, however, that most of them are scleroblasts.

In *Echinus esculentus* (and *miliaris*) also the development of the spicule follows on the same lines (figs. 14—18).

THE PLATE-SPICULES IN *ANTEDON BIFIDA*.

The majority of the spicules situated in the region of the disc of *Antedon* lie towards the inner side of the soft integument, and are of two kinds—the imperforate thin “glasplättchen” of comparatively wide diameter and concentrically marked (see accompanying text-figure), and the ordinary perforated plates, which, however, often assume a very irregular shape, and occasionally become mere branching structures. All transitions (fig. 21) are to be found between the “glasplättchen,” which are very unechinoderm in appearance, and the ordinary perforated plates; the former, however, are much the more common, and exist in great numbers. Since the development of the perforate plates and branching spicules is quite normal, i.e. the same as that already described for *Amphiura*, *Ophiothrix*, and *Echinus*, I shall merely describe the simple development of the imperforate

spicules. These plates each originate as a spherical granule contained within a single cell (fig. 19), and this granule gradually becomes larger (fig. 20), and early assumes the plate-like form (fig. 22). There is thus no rod or triradiate stage—so common in the development of echinoderm spicules—in the growth of these plates, and the plate is never of the perforate type. The nucleus of the original mother-scleroblast divides at an early stage of growth of the spicule and the number of nuclei present at different stages of growth (a



Full-sized imperforate plates of *Antedon bifida* \times cir. 270 diameters. These plates are very thin, easily fractured, show concentric lines (presumably of growth), and are to be found in large numbers in the hypostroma of the integument.

score or more on fully-developed plates) is strictly proportional to the size attained by the spicule. These large thin squamose spicules of *Antedon* do not seem to be generally known, although they exist in great numbers. They are very easily decalcified in virtue of their extreme thinness, and, as I have already mentioned, are very unlike echinoderm spicules (so much so that I at first mistook them for artefacts). As Dr. Bather has very kindly pointed out to me, similar, but much thicker, imperforate plates (with no concentric markings) have been described, among others, by Ludwig in Holothurians (4), Ophiuroids (5) (also Mortenson [8]), and Asteroids (*Astropectinidæ*) (6).

I may also mention that the dark granules, so conspicuous in the scleroblasts of many Cucumariidæ, are also present in the scleroblasts of *Antedon*; indeed, they are usually dark without any additional staining with lichtgrün.

THE SMALL PLATE-SPICULES OF *SYNAPTA HISPIDA* AND
S. DIGITATA.

In addition to the plate-and-anchor spicules of *S. inhaerens* I have examined the similar (though easily distinguishable) spicules of *S. hispida* and *S. digitata*, and, as might have been expected, I find that the disposition of the scleroplasm concerned in their formation is essentially the same as that which I described in Study IV. Through lack of material I have not as yet been able to ascertain the disposition of the scleroplasm in the early stages of development, though I hope to do so shortly.

Besides these conspicuous plate-and-anchor spicules there exist in *S. hispida* and *S. digitata* (though not in *S. inhaerens*) small elongate plate-spicules, containing in the former species a single perforation in their centre, and the development of these perforate plates of *S. hispida* is remarkable. I may mention that these small plates are particularly numerous in the region of the muscle-bands, though they are also to be found in the intervening spaces.

The plate in both species arises quite normally as a more or less spherical granule in the centre of a single scleroblast (fig. 23), and, as in *Antedon*, this gradually becomes elongated (fig. 24) and plate-like, but, differing from *Antedon*, the nucleus of the scleroblast remains single throughout the entire development. In *S. digitata* these plate-spicules cease growth at the stage depicted in fig. 30, which, it will be noticed, is identical with the stage of development represented by fig. 24 of the plate-spicule of *S. hispida*. In this latter species the plate elongates considerably, and expands laterally to some extent except in the vicinity of the nucleus,

the result being that the nucleus becomes lodged in a depression to one side of the plate (fig. 25). The further development of the spicule consists of the enclosure of the nucleus by the extension of the calcite, as shown in figs. 26—28. Two arms of calcareous matter extend round the nucleus on either side, meet, and finally fuse, and the adult plate, in consequence, contains a central perforation, in which the nucleus is imprisoned (fig. 28). Occasionally, when the perforation is larger than usual, a secondary ingrowth of calcite occurs (fig. 28*b*); occasionally also the two arms of calcite first overlap each other instead of fusing immediately (figs. 27*a* and 27*b*), and sometimes, but rarely, secondary pairs of arms are formed, which tend to emulate the first pair in their direction of growth (fig. 28*a*). This last feature (as also that represented by fig. 29) proves, however much it may appear to the contrary, that the presence of the nucleus is not the only stimulus giving rise to the peculiar mode of extension of the calcite just described. In fact, here, as in the cases described in the foregoing parts of this paper and elsewhere, the nucleus, with its associated mass of cytoplasm, probably has very little to do with the direction of growth of the calcite—with the form of the spicule—and must largely be discounted as a factor in the production of spicular forms.

THEORETICAL CONSIDERATIONS AND PREVIOUS WORK.

From the results described above, and from the figures of young spicules in the various classes of echinoderms provided by Agassiz (1), Ludwig (7), Seeliger (9), Fewkes (2, 3), Théel (11, 12), and many others, we may assume what has, indeed, been already implied, viz. that in Ophiuroidea, Asteroidea, Echinoidea, and Crinoidea, the typical mode of scleroblastic development of the spicules is that described above for *Amphiura elegans*, i. e. the spicule originates as a triradiate structure contained within a single cell. From the figures of these and other authors, on the other hand, we may also assume that the typical mode of development of

the plates of Holothuroidea is that described by me for the Cucumariidæ (Study IV), viz. the origin of the elongated calcareous needle between two or four cells, its growth to form a rod, the bifurcation of the extremities of this rod, and so on. Up to the present I only know of one exception to this rule, Semon (10) describing and figuring most distinctly the triradiate mode of origin of certain spicules in the holothurian *Chiridota venusta*. But this, and possibly a few other exceptions, do not invalidate the general rule, and, as before mentioned, this difference of origin between most echinoderm spicules and the spicules of holothurians can be correlated with a general difference which exists between the modes of skeleton formation in the two groups, i. e. this rule can be justified by a reason for its existence. The quantity of lime respectively secreted by most echinoderms and by holothurians differs greatly—in the former group the stroma is packed with a calcareous stereom, whereas in most individuals of the latter the skeleton is only represented by isolated spicules—and correlated with this difference is (a) the fact that in the former group every scleroblast gives rise to a spicule, whereas in the latter at least two scleroblasts have to co-operate for the same purpose, and (b) the equally cogent fact that in most echinoderms scleroblasts multiply very rapidly (shown by the number of scleroblasts per spicule), whereas in holothurians they multiply very slowly. In other words, the difference in the origin of the spicule in the two groups is correlated with the amount of the skeleton present—with the skeleton-producing capacity.

Previous work on the subject of the present paper, so far as I have been able to discover, has been very small in amount. In fact, the only paper that I know of describing the origin of the spherical granule and the young triradiate in a single scleroblast is that of Semon (10) on the holothurian *Chiridota* just referred to. Semon also figures very distinctly the young triradiate with two scleroblasts (similar to fig. 4). At the same time Semon represents the triangle-

shaped spicule as a tetrahedron, and, curiously enough, represents this tetrahedron as forming the distinct centre or basis of the older spicules, secondary calcareous matter, so to speak, prolonging the solid angles of the tetrahedron. As I have stated in Study III, I believe this tetrahedron structure to be quite imaginary, and I certainly cannot credit without more evidence its persistence as the visible basis of older spicules.

Fewkes (2), describing the metamorphosis of *Echinarachnius parma*, says that "the first limestone formation which was observed is a trifold spicule in the wall of the body of the growing sea-urchin. In its very first form this trifold spicule is spherical in contour. Later it assumes a trifold shape, and seems to be enclosed in a transparent sac, the outer wall of which is believed to be formed of epiblast, the calcareous body being formed possibly in mesoblast"! Fewkes also adds that he does not know whether these trifold bodies develop into the plates or not.

Théel, in his papers on *Echinus miliaris* (12) and *Echinocyamus pusillus* (11), quite correctly describes the development of the spicules, and also states his opinion that "they first originate from cells which have wandered in between the tissues," but he gives no details of the scleroblastic development.

Ludwig, Fewkes, and others provide numerous figures showing the young triradiate spicules and older stages, but from not employing suitable staining reagents they entirely overlooked the scleroblasts in connection with the young spicules.

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EXPLANATION OF PLATES 3 AND 4,

Illustrating Mr. W. Woodland's "Studies in Spicule Formation" (V).

Fig. 1 drawn natural size; Figs. 2, 3, 8, 9, 14, 15, and 23 \times 1280 diameters; all other figures \times 640 diameters. All figures (save Fig. 1) drawn with the camera lucida.

PLATE 3.

Development of the plate spicules of *Amphiura elegans* (Figs. 1—7), *Ophiothrix fragilis* (Figs. 8—13), and *Echinus esculentus* (Figs. 14—18).

FIG. 1.—Young *Amphiura* in which the spicules were observed. (Several specimens of *Amphiura* were considerably smaller than those here depicted.)

FIG. 2.—The origin of the plate-spicule in *Amphiura elegans* as a spherical granule contained within a single scleroblast.

FIG. 3.—The triangular and subsequently triradiate forms assumed by the initial granule within the scleroblast.

FIG. 4.—Young (usually) trifid spicules with two scleroblasts attached (derived from division of the mother scleroblast).

FIG. 5.—Older stage of the triradiate. In *a* one nucleus is dividing; in *b* one nucleus has divided, three scleroblasts being present.

FIG. 6.—The bifurcation of the arms of the triradiate. The number of scleroblasts has increased, and nuclei are seen to be about to divide.

FIG. 7.—Older spicules with numerous scleroblasts.

FIGS. 8—12 represent stages in the formation of the usually thick plates (with small perforations) of *Ophiothrix fragilis*. In Fig. 10, *b* and *c* represent young stages of unusual shape. Fig. 12 is a young spicule of the thin plate variety.

FIG. 13 represents one of the lateral trifid spines of *O. fragilis*. The numerous scleroblasts in connection with the spicule is striking when compared with the two scleroblasts associated with the somewhat similar "stool" spicule of *Thyone* (Study IV, pl. 34).

FIGS. 14—18 represent young stages in the formation of the plates in *Echinus esculentus* similar to those above.

PLATE 4.

Development of the imperforate plate-spicules of *Antedon bifida* (Figs. 19—22), and of the plate-spicules of *Synapta hispida* (Figs. 23—29), and *S. digitata* (Fig. 30).

FIGS. 19—22.—The development of the imperforate plate-spicule of *Antedon bifida*. Fig. 21 represents spicules which show some affinity in their form to the ordinary perforate plate-spicules of echinoderms. The size attained by the adult thin perforate plate may be realised by comparing the present figures of young plates ($\times 640$) with the text-figure showing adult plates ($\times 270$).

FIGS. 23—30 represent the development of the curious plate-spicules of *Synapta hispida* and *S. digitata*, which are very small in comparison with the plate-and-anchor spicules. Fig. 24, representing a young stage in the development of the plates of *S. hispida*, shows forms very similar to those of Fig. 30, which represents adult spicules of *S. digitata*. Figs. 25—28 illustrate the enclosure of the nucleus within the central perforation. Fig. 29 shows plates in which the central aperture is considerably larger than the average.

Studies in Spicule Formation.

VI.—The Scleroblastic Development of the Spicules in some Mollusca and in one Genus of Colonial Ascidians.

By

W. Woodland,

Demonstrator of Zoology, King's College, London.

With Plate 5.

SPICULES IN NUDIBRANCH MOLLUSCA.

The nudibranchs examined, all obtained from Plymouth, consisted of three genera—*Goniodoris castanea*, *Archidoris tuberculata*, and *Lamellidoris bilamellata*. Most of my specimens were simply fixed and preserved in absolute alcohol, and were, in consequence, much contracted; others were fixed with 1 per cent. osmic acid, opened to give admission to the stain, and stained as usual with picro-carmin (three hours), and I received them in this condition. In both cases I divided the animals horizontally into halves (with scissors), and in each instance scraped away with a scalpel the viscera and most of the musculature of the body-wall, until the portion of integument left looked to some extent translucent (whether unstained or stained). I usually found that the integument of the foot showed the spicules better than that of the dorsum. The unstained portions of integument (alcohol specimens) I stained in a saturated solution of

safranin (nigrosin is also good) in absolute alcohol for a fortnight, washed thoroughly in warm absolute for a day or so, stained for a few minutes in a saturated solution of lichtgrün in absolute alcohol, again washed well in absolute, cleared in xylol and finally mounted in balsam. In most cases the picrocarmine specimens became too dark if also stained with lichtgrün.

The spicules of these three genera are all somewhat irregular monaxons with a concentric structure when viewed in section, and containing a larger or smaller amount of organic matter, but they differ slightly both in size and shape according to the genus. In *Archidoris* the spicules are much more slender than in the other two genera, and do not attain such a great length; moreover, they do not possess a sudden thickening situated midway in the length of the spicule like those of the other two genera, and the nucleus of the scleroblast is much smaller both relatively to the size of the spicule and absolutely, is spherical instead of oval and possesses a distinct nucleolus. The spicules of both *Goniodoris* and *Lamellidoris* are, as just mentioned, thickened at the middle and in the latter genus this midway thickening (which is not so prominent as in *Goniodoris*) often possesses one or two large spikes. The spicules of *Lamellidoris* attain a much greater size than those of *Goniodoris*, and are much smoother in general outline. Corroded spicules exhibit straight-sided regular outlines at the exposed edges, whence we may suppose that nudibranch spicules, like most other calcareous spicules, are essentially aggregates of calcite crystals.

The nearest approach to the spherical concretion stage of the spicule which I have observed is that represented in fig. 1. Without doubt the spicule originates, as in every other case of simple spicules, in this form, but unfortunately my specimens are not young enough to show this, although the stages figured afford sufficient proof that this is the case. The granule becomes a rod and the rod assumes the form of the adult monaxon, growing over its entire surface (but, of course, chiefly at the extremities) by the deposition of cal-

careous matter derived from the scleroblast cytoplasm which entirely surrounds the spicule. The median portion of the adult spicule is formed first (evident when this is thickened), the tapering extremities growing out from this. The scleroblast, i. e. single nucleus, never divides, so that the spicules are purely unicellular products, and in most cases the body of the scleroblast (the small mass of protoplasm immediately surrounding the nucleus) is constantly situated midway in the length of the spicule, i. e. in the vicinity of the thickening in those spicules possessing this feature.

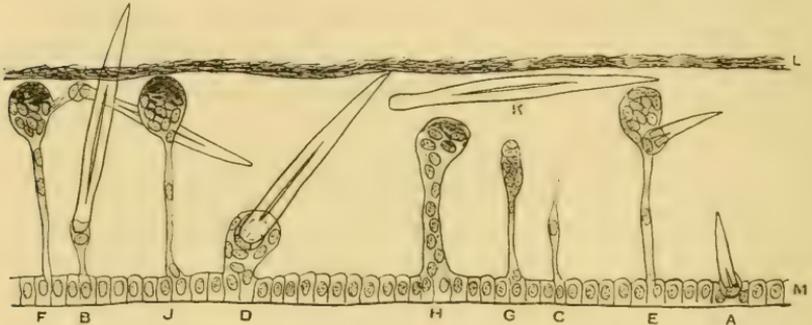
Spicules in Aplacophorous Mollusca.

My material consisted of specimens of *Proneomenia aglaopheniæ* and *Dondersia banyulensis*, specially prepared at Plymouth by the osmic acid and picro-carminic method which gave excellent results.

So much has been previously written and so many good figures provided in connection with the development of these characteristic calcareous spicules of the Aplacophora that my sole excuse for re-considering the subject is the uncertainty which still prevails as to whether these spicules are unicellular (Thiele [8], Wiren [9]) or multicellular (Heuser [3], Hubrecht [4], Kowalevsky and Marion [5], Pruvot [7]) in growth. There is also a misapprehension to correct, which is that the spicules embedded in the cuticle of *Proneomenia* are "in relation internally with epithelial papillæ" (Sedgwick's 'Text-book of Zoology,' p. 353, likewise Pelsener's 'Mollusca' in Lankester's 'Treatise on Zoology').

To state the results of my direct observations and inquiries as briefly as possible, I may say that it is now quite certain that the spicules of the Aplacophora, like those of the Polyplacophora (Pelsener), all arise individually in a single cell of the hypodermis (figs. 7—13). It is also certain that the spicules in *Proneomenia* (and other Aplacophora which possess an integument of similar type) do not in the majority of cases bear any relation to the hypodermal papillæ, and that

when they do the relation is purely an accidental one. This occasional accidental relation apparently originates thus: the young spicules arise each in a single cell situated in the hypodermis, and in the majority of cases the portion of the hypodermis containing this spiculiferous cell remains in its ordinary position, but if, as sometimes happens (say in 20 per



Semi-diagrammatic drawing of the hypodermis and cuticle of *Pro-neomenia aglaopheniæ*, illustrating the "carrying up" of the spiculiferous cells and spicules by the hypodermal papillæ. The figure ($\times 400$ diam.) is composed of drawings of the actual objects brought together into one field. In A the spicule is in its normal position; in B the scleroblast is being detached from the hypodermis; in C the scleroblast has lost its spicule, this lying free as at K; in D a scleroblast with a well-grown spicule has been caught up in the young papillary elevation; in E a fairly young spicule has been carried up some distance by a papilla; in F the spicule has similarly been carried up, but is older; G and H represent young papillæ; J a full grown papilla, with its pigmented swollen extremity lying just below the outer limit of the cuticle. It must be understood that normally the papillæ and spicules are quite distinct, not being in any way associated. L represents débris on the exterior of the thick cuticle; M is the hypodermis.

cent. of cases), it becomes raised up into a papilla, the spiculiferous cell is inevitably carried up with it, and then the spicule superficially appears to be a product of the papilla (see the accompanying text-figure). The majority of the spicules are quite separate from the papillæ, but in those cases in which they are associated the purely accidental nature of this association is proved by the various positions which the spicule assume relative to the papilla, these illustrating the various stages of "carrying up" referred to above.

The spicules of *Proneomenia*, from their first appearance each as a small needle contained within a single cell (figs. 7, 8), grow in a more or less vertical direction, and soon burst through the cell-membrane (fig. 9). From this stage onward the further growth of the spicule is confined to the basal extremity (so resembling the growth of a hair), which alone is enveloped by the cell-substance (fig. 10). An axial cavity appears in the spicule substance before the spicule is half grown, but closes up proximally before the full size is attained. Many of the scleroblasts attached to the larger spicules become more or less withdrawn from the hypodermis (text-figure, B, C), and subsequently also lose connection with the spicules themselves, which, perhaps owing to contractions of the integument, often come to lie, when full grown, near the outer limit of the cuticle (κ in text-figure).

In *Dondersia* the monaxon spicule, instead of being vertical in position as in *Proneomenia*, is disposed more or less horizontally from the first (figs. 11, 12) in correspondence with the thin cuticle (thin as compared with that of *Proneomenia*, being little more than the thickness of the hypodermis). These spicules also when adult become separated from the hypodermis, and lose their scleroblasts.

I may add that the figures and statements of Heuscher and Hubrecht, affirming the multicellular papillary origin of the spicules of *Proneomenia*, are misleading, though superficially they appear to be correct. They have solely resulted from insufficient attention being paid to detail, and, bearing in mind the facts described above, it will easily be seen how the mistake has arisen.

It may also be mentioned that the hypodermis contains many gland-cells with mucilaginous contents, which have been, on at least one occasion, figured as young spicules! Needless to say the two are readily distinguishable.

With reference to the mode of growth of these spicules of the Aplacophora, I may here point out that the production of a straight "finished" mineral structure by terminal accretion due solely to the activity of a single scleroblasts

under undisturbed conditions is here clearly demonstrated; there is in these aplacophore spicules no question whatever as to whether or no the symmetrical form is due to crystallisation or the like. And, bearing this example in mind, it further cannot be denied that the straightest rays of the triradiates of clathrinid *Calcarea* in all probability owe their symmetry to the same cause. Crystalline matter is deposited by the terminal cell in a similar manner in both cases, and, so long as the conditions remain undisturbed, the growth of the spicule must continue in a straight line. "Bio-crystallisation" and the rest are here at least superfluous. On the other hand, introduce disturbed conditions and, as might be expected, the more irregular spicules of *Leucosoleniidae* and *Sycons* are the result. Further, granting placid conditions, bring three cells into such close apposition that their inner surfaces become adpressed into the outline of a triradiate and let each of these cells divide centripetally, the distal cell in each case producing, in relation with the fixed proximal cell, a straight monaxon,¹ and personally I can see no reason why cumbrous hypotheses should be invented in order to explain why the three contained angles of such a triradiate spicule (found in most calcarea) should in almost all cases be equal. Surely the equiangularity is the direct result of the apposition of the three scleroblasts, as I have previously contended [10].

No such explanations, however, apply to the young triradiate "stars" of most echinoderms described in the last Study, which, however, differ, like most other spicules, from the calcareous spicules of sponges and *Aplacophora* in that they are entirely invested by the formative protoplasm.

THE SPICULES IN THE ASCIDIAN GENUS *LEPTOCLINUM*.

I have examined three species of *Leptoclinum*—*L. commune*, *L. maculosum*, and *L. sp.*— all obtained from

¹ This monaxon is, under these conditions, pointed at both ends. In *Aplacophora* each spicule, corresponding to its one-celled basal origin, is truncated at its proximal end.

Naples and preserved in alcohol. In all cases I embedded portions of the colony in paraffin wax and cut thin free-hand sections with a razor; I then stained these sections in the manner described below. The spicules are identical in the three species. Staining methods which do not differentiate the nuclei very prominently do not give good results for ascertaining the number of scleroblasts in connection with the adult stellate spicules, chiefly because the mass of the spicule more or less effectually hides all objects situated underneath, and the conical protuberances largely obscure objects situated to the side. The use of picro-carmin would doubtless give better results, but I was unable to employ this stain. The only effectual method which I employed was the ordinary borax-carmin method, the differentiation with acid-alcohol dissolving the spicules sufficiently to render the scleroplasm apparent. This method merely revealed one scleroblast (nucleus) in connection with each spicule, the layer of scleroplasm closely investing the entire spicule and following all its outlines, and the large nucleus being situated in a mound of protoplasm at the periphery (figs. 17, 18). The stellate spicule originates in a scleroblast as a spherical granule (fig. 14; these early stages are quite visible in the non-decalcified safranin and lichtgrün-stained preparations) which later acquires spines on its surface as it increases in size (fig. 16), and which spines ultimately become the conical processes of the adult spicule.

Previous literature dealing with the scleroblastic development of Ascidian spicules is very small in amount (see Herdman [2] for the literature relating to Ascidian spicules up to 1885, since which year, I believe, no literature on the present subject has appeared). Loewig and Kölliker [6] in 1846 provided very poor figures of the stellate "cellules incrustées" in *Didemnum*, and illustrated one of these "cellules" partly decalcified, showing the cell-wall spherical in outline. Giard [1] in 1872 figured stellate spicules of *Eucœlium* and *Didemnum* also situated each within a circle which is supposed to represent the cell-wall; he figured as

well several small spicules situated within one cell. No nuclei are shown in any of these figures. In the first place I may say that I do not remember ever having observed more than one spicule contained within a single cell; and secondly, that the above authors are greatly mistaken in supposing the outline of the scleroblast containing the adult spicule to be spherical. A scleroblast containing a spicule only remains spherical when the contained spicule is small compared with the original size of the cell (microscleres of some siliceous sponges and the young stages of larger spicules e.g.); when as in *Leptoclinium* or *Didemnum*, the spicule is many times larger than the original scleroblast, this latter necessarily becomes distended and envelops the entire spicule with a thin layer of scleroplasma, which of course assumes the shape of the spicule. If the spicules are too hastily decalcified it is quite conceivable that the evolution of gas, being coincident with the disappearance of the mass of the spicule, inflates the scleroblast, and thus causes it to artificially assume a spherical shape; indeed, I have some evidence that this is liable to occur. Decalcification by means of the acid-alcohol used in the borax-carminé method is very slow, and the scleroplasma retains its stellate form, though the spicule has largely or wholly disappeared.

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EXPLANATION OF PLATE 5,

Illustrating Mr. W. Woodland’s “Studies in Spicule Formation” (VI).

Figs. 1 and 2, $\times 640$; Figs. 3–6, $\times 192$; Figs. 7–18, $\times 1280$. All drawings were made with the camera lucida.

FIG. 1.—Young stages in the development of the monaxon spicules of *Goniodoris castanea*. The thick median portion is the first to be formed.

FIG. 2.—Apparently an abnormal spicule of *G. castanea* possessing two thickened club-like extremities and two nuclei, and so somewhat resembling a young aleyonarian spicule.

FIGS. 3 and 4.—Older spicules of *G. castanea*, each entirely enveloped in cell-substance and with one nucleus.

FIG. 5.—Small monaxon spicules of *Archidoris tuberculata*. These spicules possess no central thickening and have small spherical nuclei.

FIG. 6.—Small (young) spicule of *Lamellidoris bilamellata*; large type of spicule, and usually possessing a median spine.

FIGS. 7 and 8.—Young spicules of *Proneomenia aglaopheniæ*.

FIGS. 9 and 10.—Older spicules of the same.

FIGS. 11 and 12.—Young spicules of *Dondersia banyulensis*.

FIG. 13.—Older spicule of the same.

FIGS. 14–18.—Stages in development of the stellate spicules of *Leptoclinum commune*.

**A Preliminary Consideration as to the possible
Factors concerned in the Production of
the various Forms of Spicules.**

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So many misconceptions prevailing as to the essential nature of spicules, and in consequence as to the causes which can possibly give rise to the multitudinous and remarkable forms which spicules assume, a brief consideration of the subject seems to be called for. Such at least will serve to remove sundry erroneous ideas which are continually being put forward in explanation of these phenomena and will also point out the direction in which further investigation is necessary for their better comprehension.

SPICULES DEFINED.

Recalling to mind among the more obvious features of spicules that these bodies (*a*) always possess a form other than the spherical (otherwise being termed concretions), (*b*) always possess curved surfaces and never plane facets (so being distinguished from crystals whether these be simple or aggregate), and (*c*) that, although always intra-cellular in origin and usually so during their whole subsequent development, yet certain spicules (calcareous spicules of sponges, plutei and aplacophore Mollusca, and some siliceous sponge spicules) are extra-cellularly produced during the later part of their

growth,¹ we may define a spicule as a hard, crystalline or colloidal deposit, of more or less extended and often definite and complex form, always possessing curved surfaces and never plane facets, formed initially within a cell or a cell-fusion, and whose subsequent growth, which may be intra- or extra-cellular, is due either solely to the activity of the mother-cell or cells and its or their division-products if formed, or also partly to the activity of cells not derived from the original mother-cell or cells. Spicules which originate as a single deposit in the interior either of a single cell or of two or more cells which are more or less fused (and by a "cell" we mean a nucleus associated with a mass of protoplasm whose limits may or may not be distinguishable from other similar cells, thus considering a syncytium as a collection of cells) and whose subsequent growth is solely associated with this cell or these cells or its or their division-products are termed simple spicules (spicules of *Spongilla* and apparently most other Monaxonida, many tetraxonid spicules, radiolarian spicules, alcyonarian spicules, monaxons of calcareous sponges, many holothurian spicules, etc.); simple spicules which arise in juxtaposition, and which subsequently become fused so as to form a complex whole of a higher order of individuality (e. g. triradiates of calcareous sponges, the rosettes of the *Esperia* larva and possibly the plate-and-anchor spicules of *Synaptidæ*) give rise to what may be termed aggregate spicules; finally, spicules whose later growth is in part effected by adventitious

¹ By intra-cellular growth we mean that the deposit is enclosed on all sides by the substance of the cell, as is the case in most spicules. In *Calcareo*, however, the apical actinoblasts, e. g. of the calcareous triradiates, are cylindrical structures enclosing the rays at their apical extremity and a certain portion of their length, and it is therefore clear that the deposits which lengthen the rays are not intra-cellularly produced as above defined, since they are not entirely surrounded by the cell; and from this cylindrical disposition of the scleroblast to the simple adhesion of the cell to the surface of the spicule there is obviously every transition.

cells, i. e. cells not derived from the original mother cell or cells concerned in their production (spicules of the pluteus larva, lithistid desmas, etc.) are termed secondary spicules.

ARE THE FORMS OF SPICULES INHERITED ?

In considering the possible factors giving rise to the various forms which spicules assume, it will facilitate matters, more especially as showing in what direction the factors are to be found, if it can possibly be decided first of all whether or no the forms of spicules are inheritable. But previously to discussing this particular subject we must have clear ideas as to what exactly we mean when we decide that any particular skeletal or other structure is inherited. Heredity must after all be regarded as a complex of physical causes identical in nature with those which produce purely inorganic phenomena, and, mentally, at any rate, we must carefully distinguish these physical causes constituting heredity from the physical (ontogenetic) causes giving rise to non-inheritable organic structures, though this is admittedly usually very difficult to do in fact. No one will deny that the form of a bone, tooth, nail, valve of a diatom or other similar non-living part of an organism is inherited, but in asserting this we evidently must mean that the form of the protoplasmic mould which produces the bone, tooth, nail, or valve is the part inheritable, since the non-living matter composing these structures being unable to reproduce itself obviously cannot inherit properties. Evidently then to assert that the form of a siliceous or calcareous spicule is inherited implies that the disposition of the scleroblasts associated with any given type of spicule is that which is inherited, and that the spicule itself, like the bone or diatom-valve, is simply deposited in a mould already formed for it by the scleroblasts. That is, the disposition of the scleroplasm on this hypothesis determines the form of the spicule and not vice versâ.¹

¹ "I must rather maintain that the form of the sponge spicules is determined by the organic matrix in and from which they originate, and that the

Now the only direct way in which to prove or disprove this supposition that spicular forms are inherited is to rear spicule-bearing organisms in water deprived of the material necessary for the formation of the skeleton. If we do this we shall then, if the supposition be correct,¹ find that the scleroblasts in an echinoderm, e. g. will form moulds of the plate or more complicated spicules, differing only from those formed under normal conditions in that they do not contain spicules. Is there any evidence to prove or disprove this? The only experiments conducted on the lines just described that I know of are those which were performed by Maas (13, 14) in 1904 on calcareous sponges, and by Herbst (9) in 1892 on echinoplutei. Unfortunately, these experiments were but of little value so far as they relate to the present problem since the spicules of *Calcarea* and plutei are not wholly enclosed by cell-substance (the hypothetical moulds) as most other spicules are, but simply increase in length and thickness in the same way as a hair or horny fibre does (Study VI), and the dispositions of the cells² associated with the formation of these spicules (which dispositions were produced in these experiments) are not such as to be solely related to the forms of the spicule but are brought about by other causes (Studies I and III). Up to the present then we possess no experimental evidence that the form of the spicule is determined by a scleroblastic mould,³ and the direct proof or disproof of

formative forces are in no essential way different from those which are everywhere exhibited in the shaping of the living organism and its parts." (Schulze [23]; similarly Maas [15]).

¹ And if the altered chemical conditions of existence have no pathological effect on the development of the organism—which substantially appears to be the case according to the experiments of Maas and Herbst, referred to below.

² Herbst says nothing about a triradiate mould being formed in one of the constituent cells of each lateral cluster; but then, of course, he did not look for one.

³ Even in calcareous sponges, in which a kind of "mould" is stated to normally occur (Minchin [17], Woodland [Study I]), this in all probability is but a preliminary deposit of horny matter, which later forms the spicule-sheath (Minchin). The instances of cell "moulds" described by Chun

this supposition that the forms of spicules are inherited is still a desideratum.

Although, therefore, we cannot as yet state decisively that the forms of spicules are not inherited, yet there exist certain facts which appear to justify us in provisionally making this statement.¹ In the first place the results of recent inquiries in experimental embryology prove that for any given cell (blastomere) of a growing organism to be able to produce a living part of the adult organism adapted in form to the other parts (an organ), this must be connected with the other cells. The part which a given blastomere plays in the formation of the adult organism is a function of its constant position relative to the other blastomeres, i. e. a function of its localised connection with the other blastomeres. Detach a blastomere from its fellows, and it either gives rise to a complete though diminutive organism or becomes a wandering-cell or a germ-cell; in no case does it give rise to a part of the adult organism adapted to the general economy in structure and relative position, i. e. an organ.² And so far

(Auricularia wheels) and Théel (spicules of *Elasipoda*) were, without doubt, merely cases of decalcification, as I have elsewhere pointed out (Study IV).

¹ In what follows the general tone of the argument would imply that I altogether reject as unwarranted the hypothesis of the inheritance of spicular forms. This is not the case. I have taken up, somewhat vigorously perhaps, the opposite position to that held by most zoologists who have worked at spicules solely in order to see what can be made of that position, and, rather to my surprise, I find that there is a good deal to be made of it. For example, in connection with my main argument, based upon the results of experimental embryology, if it should turn out that the forms of spicules are inheritable, it seems to me that, in view of these additional facts, the problem of heredity will be rendered more complex than it already is. I do not reject as unwarranted the inheritance hypothesis in the case of spicules, for the obvious reason that at present we are so ignorant as to what heredity can and cannot do. Nor, without definite proof to the contrary, is it justifiable to summarily reject the convictions of those who have worked for years on siliceous sponges e.g. I think it right, however, that the objections to the inheritance hypothesis here elaborated should be published (see Note at end).

² Occasionally, as in *Ctenophora*, detached blastomeres, in virtue of once having been joined to the other blastomeres in a definite position, produce

as it is legitimate to assert such a thing it is impossible that it should; an ovum is a mechanism which evolves into an organism—"crystallises out" so to speak—and any portion which separates off necessarily remains uninfluenced by the "crystallising out"¹ process. The whole fabric of Weismannism is based upon this assumption, and whether the superstructure be sound or not no one doubts but that the assumption is valid. Moreover, all histological researches of recent years confirm the supposition that, apart from the various classes of wandering-cells, the whole organism is a syncytium. Now the theory of the inheritance of spicule forms asserts that most scleroblasts (not all, since many spicules are not in any way adapted to the architecture of the organism) are unique in this respect, being able, though having lost connection with the rest of the organism, to give rise to a complicated structure (an organ—the spicule-mould) adapted in shape and function by inheritance to that part of the organism in which it happens to be situated. Take, as a concrete example, one of the complicated siliceous hexactines of hexactinellid sponges so marvellously adapted to fit in, so to speak, with the architecture of the sponge-wall which it inhabits. The hexactine, like other spicules, has undoubtedly originated as a simple deposit within one or more scleroblasts (see Ijima² [11]), and separately that part of the organism which they would have done if undetached. But this capacity would be of no use in the case of a wandering cell which, as the term implies, is constantly changing its position, since the organ produced would not be in relation with the rest of the organism—in Ctenophora it would be futile e. g. for a detached blastomere to produce say an eighth of an embryo in one of the tentacles (see Wilson's "The Cell," chap. ix).

¹ This likening of the developmental process to crystallisation is a truer analogy than might be supposed. In the formation of complex aggregate crystals (the familiar fronds on the window-pane, e. g.) the co-ordination of the multitudinous simple crystals to form one pattern is only possible by mutual contact between the constituent crystals; the least separation leads to the production of another centre of crystallisation.

² I am at present working on hexactinellid spicules, and can amply confirm the statements of Ijima and Schulze ('Hexactinellida,' "Expedition auf

these scleroblasts are wandering-cells. Now the theory of the inheritance of spicule forms asserts at the very least that this scleroblast or scleroblast-fusion inherits the property of becoming enormously distended in size, of so distending as to assume externally the form of a spicule adapted in its main features to its immediate environment, and of correspondingly forming an internal mould necessarily identical in shape in every detail with the spicule to be deposited, which shape, as just implied, varies according to the position in the organism which the wandering-scleroblast happens to occupy at the time of deposition.¹ And these wandering-cells termed scleroblasts are supposed to inherit the property of undergoing all these changes (in the main definitely related to the architecture of the rest of the organism and complex in the extreme) without having any connection with the other component cells of the body—an assumption which, if my above remarks are true, is quite unwarranted, to say the least. This hypothesis of the inheritance of the forms of spicules, in fact, either contradicts the ascertained truths of experimental embryology or implies that scleroblasts are not wandering-cells.

It is true that in the development of *Balanoglossus*, annelids and other animals, masses of cells are stated to be often completely detached from the rest of the embryo, and that these sometimes undergo changes of form related to the surrounding tissues whilst so detached, but it will invariably be found that either these changes of shape are simple mechanical adaptations to the architecture of the organism (formation of coelomic sacs, etc.), or are due to the fact that these cells have quite recently been connected with the rest of the organism, the changes of shape being adapted to the general scheme of development because the mass of cells has

dem Dampfer 'Valdivia,' Bd. iv, 1904) that these spicules are completely invested by scleroplasm.

¹ A scleroblast can be supposed, on the hypothesis, to inherit the property of giving rise to spicules of different shapes, according to its localisation in the organism, on the principle of the "equipotentiality" of blastomeres.

retained its relative position in the organism (formation of evaginations of the separate enteron in *Balanoglossus* to meet the stomodæal and proctodæal invaginations of the epiblast, etc.). Including these apparently anomalous cases, it is one of the most certain facts in Biology that if a detached cell (or cells) is to produce an organ of the adult organism, it must become connected with its fellows.¹ And as I have pointed out above, the hypothesis of the inheritance of spicule forms denies this.

The above argument, however, strictly speaking, solely applies to spicules adapted in form to the architecture of the organism (most of the large complicated spicules), but many spicules exist which exhibit no such relationship (microscleres of siliceous sponges, stellate spicules of Ascidians, many holothurian and aleyonarian spicules, etc.) But even in this case the scleroblasts will be unique amongst wandering-cells if they produce by heredity an internal spicule-mould (often also, as just stated, undergoing distension and external change of form), i.e. if they assume a complicated structure.² Apart from this very hypothetical case of the scleroblasts I know of no instance of wandering-cells becoming complicated in form at all. Spermatozoa are cells separated off from the rest of the organism, and which become very complex in structure, but this complexity (definitely related to a function) arises in the cell before it is detached, and is analogous to the production of the ciliated cells of a *Vorticella* or of medusæ from a hydrozoan colony;

¹ Prof. Dendy has kindly brought to my notice the following striking illustration of my argument. Lefevre ("Jour. Morph.," 1898), in a paper on "Budding in *Perophora*," states that wandering cells (free blood-cells) produce the heart, neural ganglion, gonads, and some other organs of the buds, but in order to do this they must become closely attached to the organism. As I have argued in the text, cells which remain free either become wandering cells or, as shown, e.g. by the rediæ and cercariæ of the common *Distomum*, produce miniature organisms.

² The mere idea of a wandering cell possessing the capacity to form an internal mould of the complexity required for the production of a hexactinellid floricate or onychaster, e.g. seems absurd.

in other words, the complexity is a part of the continuous development of the organism.

Only in the case of such organisms as Radiolaria, in which the scleroplasm, i.e. the portion of the body-substance in which the deposition of spicule-substance occurs, is continuous with the rest of the organism, do the above arguments fail to apply. Granting this we may still ask what is the evidence that in Radiolaria the forms of the spicules are inherited? And the answer from most quarters will be: the same class of evidence which leads us to state that the forms and patterns of the siliceous valves of diatoms, e.g. are inherited. The siliceous valves of diatoms and the siliceous spicules of Radiolaria are both cytoplasmic secretions, and surely what applies to one applies to the other. In replying to this criticism, a distinction must first be drawn between the general region of deposition of the silica and the particular distribution of the silica within that region. The general shapes of the diatom valve and the radiolarian shell (or region containing the spicules in those Radiolaria and higher organisms which possess no shell) indicate the former and are undoubtedly inherited—are determined, like the form of bone, by the disposition of the scleroplasm, i.e. by a mould. But, on the other hand, it is altogether another question as to whether the forms of the individual spicules or the patterns on the radiolarian shell or diatom valve are inherited.¹ Anyone who questions the inheritance of these patterns is at once met with the answer that particular patterns are constantly reproduced by particular organisms, and that therefore they must be inherited (!)—an answer altogether beside the mark. Given a colloid of the same composition under similar conditions, and if it produces a particular pattern on one occasion for purely physical reasons (ontogenetic causes) it will on another, just as calcite crystals are always produced whenever the required conditions are conformed to. Previous to the experiments of Herbst, the arms

¹ The patterns on dinoflagellate and desmid plates, coats of pollen-grains and seeds, and scales of ganoid fishes may also perhaps be included here.

of plutei were supposed to be inherited for just this same reason, viz. because echinoid larvæ always had them; nevertheless, as Herbst has shown, they are not inherited, since they merely result from the elongation of the spicular rods. It is indeed more than possible that the patterns on diatom valves, radiolarian shells, and the rest are purely physical products—ontogenetically determined—and that physicists will some day be able to reproduce these patterns under artificial conditions as Rainey did in the case of certain organic calcareous structures. The perforations in diatom valves and radiolarian shells it is true undoubtedly serve a physiological necessity (since were there no perforations, the imprisoned organisms would die) and the organism is thus evidently able to control to some extent the deposition of silica, but physiological necessities ipso facto never yet produced ornamentations of no use or only incidentally of use to the organism, and until the contrary is proved we are fully justified in assuming for the reasons given and about to be given that the forms of radiolarian and all other spicules are not inherited.¹

But there is a still more satisfactory answer with which to reply to the criticism that radiolarian (and other) spicules, being, like cuticular products, cytoplasmic secretions, therefore owe their forms to the same cause, and this is that, as all observers have agreed, spicules as a class do differ in several very important respects from every other class of cell-deposits. One of these differences is that, excepting crystals, spicules are the only deposits assuming definite

¹ Or only inherited in the sense that their form is determined by the colloidal nature (see below) and architecture of the organism, which properties of course are inherited. Assuming that the forms of spicules are "inherited" in this sense is very different to assuming either (A) that every individual scleroblast is guided by some unimaginable means to precisely that position in the organism in which the spicule, which it is alone capable of producing (by a power which no other class of cells is known to be capable of, i. e. by forming an intracellular mould), is adapted to the economy of the organism; or (B) that every individual scleroblast is capable (in addition to possessing the unique power just mentioned) of producing by heredity

(other than spherical or approximately spherical) forms which arise in the interior of cells: all other definitely-shaped deposits arise on the exterior of cells, and individually owe their (inherited) form as a whole (though not necessarily their patterns and the like) to that of the cytoplasmic surface (mould) which produces them. As before stated, there is no evidence that the forms of spicules are determined by internal moulds; on the contrary, evidence exists which leads us to suppose that such moulds cannot exist.

Another and indeed the principal feature distinguishing spicules from other cell-deposits is their general nature which, as all authorities agree, is closely allied to that of crystals and similar bodies. The geometrical symmetry of the forms of many spicules, and their physical nature, are both characteristics pointing to the close affinity which exists between spicules and crystals, or bodies allied to crystals, such as those which I have below termed "crystallomorphs." It has already been shown how the forms of spicules differ from those of crystals, but it has not yet been shown how very similar, both as regards condition of formation, physical properties, and variety, symmetry and complexity of form, spicules are to crystallomorphs. The one feature in which spicules differ from crystal-like bodies is their, in many cases, obvious adaptation of form to the architecture of the organism—the feature which of course has led to the supposition that their forms are inherited—but as I shall show later, this feature can be otherwise accounted for. The assertion that, despite the facts, spicules have no affinity with crystalline bodies, but are more closely allied to utter dissimilar various forms of spicules, according to its position in the organism, whilst quite uninfluenced by the rest of the organism (since it is unconnected with the rest of the organism). To say that the scleroblast is influenced by "heredity" at a distance is merely to assert what I assert, viz. that direct ontogenetic causes determine the form of the spicule and not heredity. A scleroblast in all probability no more produces a spicule-mould, in the sense that a nephroblast produces a nephridium, than a cell of adipose tissue swells out by heredity to produce a spherical oil-drop.

though often also symmetrical structures like bones, feathers, and the like is the assertion necessarily implied in the supposition that the forms of spicules are inherited.

Another very important difference distinguishing spicules from other cell-deposits relates to their disposition in the organism. Apart from the fact already sufficiently insisted upon that all hard deposits save spicules (not those of Radiolaria) arise in cells connected with the rest of the organism, there is the additional significant fact that all (except concretions and crystals) these non-spicular deposits (bones, teeth, nails, hairs, scales, feathers, etc.) are, on account of the connection of the secreting cells with the rest of the organism, usually laid down (obviously by inheritance) only in those particular parts of the organism where they are required, the appendicular skeleton, e. g. being formed in the axes of limbs where the greatest stresses exist, dermal bones protect particular viscera in particular places, nails occur on the terminal phalanges where most contact occurs, and so on. Spicules, on the other hand, are not limited in their distribution in this manner, but tend to occur wherever the purely physical conditions permit the wandering cells to secrete them (calcareous spicules, e. g. cannot occur in the vicinity of digestive or other organs where acid solutions abound), and their local adaptations in form to the architecture of the organism are, there is good reason to believe, determined by purely physical causes which influence the scleroblasts during the development of the spicule.

In view, then, of the above arguments, which, collectively, are, in my opinion, of considerable weight, and, in the absence of direct proof to the contrary, we are, I think, for the present justified in declining to entertain the hypothesis of the inheritance of spicule forms. The hypothesis of the inheritance of spicule forms, it is true, does not necessarily imply that such forms have arisen by the process known as natural selection; natural selection, as even the most ardent selectionists admit, is obviously incompetent to account for the complex symmetry of spicule forms. The hypothesis

simply implies that the variations of spicule forms are non-controlled, and that they persist without reference to the economy of the organism. My objections to this hypothesis are, in short, that the implied capacity of the protoplasm to form the requisite spicule moulds is shown, in all probability, to be non-existent by the facts both of experimental embryology and cell physiology, and that the resources of physical science can provide examples of structures much more closely allied to spicules than any class of bodies known to be organically produced, i. e. by inheritance.

FACTORS POSSIBLY CONCERNED IN THE PRODUCTION OF SPICULE FORMS.

Assuming the above conclusion to be valid, we are thus at liberty to consider purely physical factors as being fully competent to account for the varied forms which spicules assume. Before enumerating possible factors it will be as well to state definitely that we do not consider crystallisation, in the strict sense of the term,¹ to be one of these. To put the argument in brief, we may reiterate that no crystal possesses curved surfaces, and, since no spicule exists without them, therefore no spicule is a crystal. But, apart from this, we may point out that all spicules composed of crystalline matter consist of calcite, and that the various crystalline forms of calcite being strictly limited in number (none of which bear the slightest resemblance to any form of calcareous spicule) and all modifications of one type, it is evidently impossible to refer the multitudinous and widely-different forms of calcareous spicules to any such factor as the crystalline properties of their substance. In fact, the

¹ It is important, in view of the loose application of the term, that I should explain that by a crystal I mean solely a mass of matter which has assumed, on solidification from a dissolved or fused condition, a form bounded by plane surfaces referable to one of the six systems recognised by crystallographers. Crystals, as thus defined, may be simple (most compact crystals) or aggregate (snow-crystals, e. g.).

crystalline nature of calcite, as a factor in determining form, seems to exhaust itself, in the case of calcareous spicules, in producing the individual crystals, of which these spicules are composed; certainly there is no reason to suppose that it has any influence on the form of the aggregate (see Maas [15]). With regard to siliceous spicules, hydrated silica (opal), so far as I know, has never been observed to assume a crystalline form, although some of the siliceous concretions figured by Maas (16) are angular in outline.

Several authors have contended for the recognition of certain calcareous spicules as crystals on the ground that, like true calcite crystals, they behave optically (with polarised light) as "crystal individuals" (Bidder [1] and others). I have not here the space to discuss such a large subject, but I may point out that the value of this supposed criterion of a crystal is easily shown, in the case of spicules at least, to be naught by the fact that, according to this criterion, the shape of a simple spicule like one of those of *Alcyonium* is not due to crystallisation (though undoubtedly a spicule individual), whereas the shape of a compound quadriradiate spicule of *Calcarea* is (though this spicule is composed of four spicule-individuals secondarily joined together to form a system).

The above arguments, it must be admitted, are quite valid, but they are only entirely valid provided that the term crystal retains the definition I have above supplied. Many facts, however, point to the conclusion that the form of a crystal is as much a function of the medium in which the crystal is deposited as of the properties of the crystal substance itself, and since, as is well known, a given crystalline substance will, in the presence of different media, give rise to the most varied forms (these forming, however, a continuous series on account of numerous transitional forms—facets giving place to curved surfaces among other changes), it becomes questionable as to whether we are justified in restricting the term crystal to the old meaning. Now the media most potent in these effects on crystalline and

amorphous deposits are undoubtedly colloidal media,¹ and, since complex shapes produced by colloidal media are almost always characterised by the possession of curved surfaces, and thus, though connected by all transitional forms with those of simple crystals, constitute a class of bodies possessing common characters, I propose that, to distinguish them from crystals as above defined, they shall be termed "crystallomorphs."² Whether crystallomorphs are always modified aggregate crystals (as in many cases they are) or sometimes equivalent, as regards the order of crystal individuality, to a simple crystal is not at all clear from published accounts on the subject. If, then, we distinguish crystallomorphs from crystals as just suggested, we are still justified in stating that spicules are not crystals; whether spicules are crystallomorphs is a question I must discuss later.

Three factors in the production of spicule forms are conceivable: (*a*) The gross mechanical factor, or the shaping of a structure due either to actual contact with surrounding objects (contact which, in this case, would affect the shape of the spicule by influencing the scleroblasts depositing it and not the spicule itself, which is a rigid structure) or to the configuration of the secreting substance; (*b*) the influence at a distance—*actio in distans*—of different parts of the organism on the scleroplasm; and (*c*) the factor which produces crystallomorphs. These three factors I will discuss as briefly as possible.

Factor (*a*) has been employed by several authors in the interpretation of spicule forms with, however, but varying success. The well-known line-of-least resistance theory of

¹ So far as I know, the influence of other crystalline substances in solution on developing crystals is solely to give rise to very complex aggregate crystals—not possessing curved surfaces (see Lehmann [12]). It is very probable, however, that the presence of such crystalloid solutions greatly facilitates the colloids in the production of complex crystallomorphs, probably being largely instrumental in giving rise to variety of form.

² I originally proposed (British Association, York, 1906) the term "colloidomorph," but this is evidently defective. "Crystallomorph" is somewhat awkward, but it seems to me preferable to Rainey's "coalescence body."

Sollas and the alveolar theory of Dreyer are notable examples. With reference to the latter, the inability of the theory to account for the well-ascertained fact that all spicules arise from approximately spherical concretions, and that comparatively very few ever assume the tetraxon form, releases us from the necessity of considering it.¹ The theory of Sollas, on the other hand, logical enough in its premises, and possibly providing an explanation of some forms of spicules, most lamentably fails when confronted, e. g. with the complicated siliceous spicules of many sponges and the calcareous spicules of many holothurians. Sollas's theory, however, if properly understood and applied, accounts for a good many of the facts. Consider, e. g. the significant fact that the forms of spicules in general are constantly adapted as regards their space dimensions to their position in the organism containing them; flat spicules, e. g. are generally found in situations limited by two more or less definite parallel surfaces (triradiates of *Calcarea*, plate-spicules of holothurians, etc.), and spicules of three dimensions are always found either in situations far removed from limiting surfaces (most alcyonarian spicules, asters of siliceous sponges, and some colonial ascidians, etc.), or with their parts disposed in relation to these (quadriradiates of *Calcarea*, the hexactine macrosclere and its modifications in hexactinellids, etc.). Moreover, definiteness of form of the spicule generally, if not always, exhibits some correspondence with the definiteness of its immediate environment. Most symmetrical spicules occur in regions of the organism which are symmetrically disposed with regard to the spicule, and irregularly-formed spicules, on the other hand, are generally found in situations characterised by the absence of definite architecture. Such correspondences, to be found both in spicules contained in different organisms and in different parts of the same organism, must be due to the effects of either factor (*a*) or factor (*b*) on the

¹ Dreyer's similar explanations referring the forms of radiolarian shells to an alveolar conformation of the scleroplasm have no facts whatever to support them. There is no evidence of this particular alveolar conformation.

spicules, or, rather, on the scleroblasts which deposit them, and this seems still more probable when we remember that scleroblasts are constituted of a soft and highly complex sensitive substance which must be readily influenced by mechanical and other forces, which are in all cases transmitted through the gelatinous matrix surrounding the spicules.

Further, in considering mechanical factors as applied to biological phenomena, "mechanists" are too apt to forget that the substance of organisms is after all living, in other words, possesses among other features the capacity of "spontaneously" altering its configuration within certain limits. For example it is quite possible that the spinous processes and elongated and branching forms of many spicules are attributable to the pseudopodial activity of the scleroplasm resulting from physiological requirements, and it is certain, as I have elsewhere stated, that the perforate character of most echinoderm plate-spicules and of radiolarian shells is due to the necessity for communication across the area occupied by the spicule or skeleton, and is probably determined ontogenetically in each case, though exactly how it is difficult to say. But at present I have not the space to do more than suggest this form of activity of the protoplasm as a possibly important factor in the production of spicule forms.

I may finally remark that even the crystal-like symmetry of some (certainly not of most) spicules (in Calcareous Mollusca) can be referred to purely mechanical conditions,¹ as I have pointed out in Studies I and VI. However, although I believe that many individual features of spicules can be attributed to purely mechanical causes, yet it is quite evident that factor (*a*) in all its aspects is but a sub-

¹ The fact stated by Sollas [25] with reference to the spicules of calcareous sponges (aggregates of calcite crystals), viz. that "the position of the rhombohedra relative to the surface of the spicules is very similar to that which may be observed in rhombohedra of calcite filling up a cavity within a limestone rock, or inside the chamber of an ammonite," is suggestive. Sollas adds that "we must suppose that the deposition of calcite within the spicule-sheath occurs according to just the same laws which are followed in the purely mineral world."

subsidiary one when the more complicated forms of spicules are concerned.

Concerning factor (*b*) this has been so little considered that, though in all probability it is a very important one from our present standpoint it is impossible to discuss it at any length. It is probable that factor (*b*) (in conjunction with factor [*a*]) has a lot to do with producing that adaptation of the form of the spicule to the architecture of the organism (occasionally it is the reverse) which is often so conspicuous. Crystallomorphs plainly exhibit this feature—the presence of an adjacent though separate object to one side, e. g. clearly modifying the shape of the crystallomorph on that side. Moreover, the organism seems to exert a decided influence on the disposition of the spicules in such cases as in certain Radiolaria, e. g. and therefore similar influences may be at work in more complicated organisms. However, we possess no data as yet in connection with this subject, and beyond making the preceding suggestions it is impossible to say anything about it.

Concerning factor (*c*) there exist, as already stated, a sufficient number of facts which seem to me to indicate that we must rely in the main on this factor (in conjunction with factors (*a*) and (*b*)) for our future comprehension of spicule forms. It is significant that, of colloidal media, albumen was found by Ord and others to be the most effective in the production of what he terms “coalescence bodies,” and what I venture to term crystallomorphs. To gain an adequate idea as to the nature of these bodies it is necessary to refer to the works of Rainey (20, 21), Harting (8), Ord (18, 19), Vogelsang (26), Slack (24), Lehmann (12), Bowman (2), and others on the subject, but for present purposes a few statements descriptive of the nature of some of the simpler crystallomorphs will suffice.¹ Thus Ord, employing albumen and other colloidal media, states that “triple phosphate being

It is perhaps a fact of some significance that the most definite and complex forms of calcareous spicules are those containing least organic (horny) substance in their composition.

used, the stalactitic crystals were found turned into rounded rods, bulging at many points into beads and variously bent, twisted, and interwoven, so as to bear some resemblance to the form in which mineral matter is deposited in the skeletons of some of the Echinodermata. Other phosphates (phosphate of calcium) were found in irregular, elongated, curved, and branching masses." Calcium carbonate is deposited in albumen in the form of small spheres covered with curved and pointed spines; calcium oxalate in gelatin forms mulberry masses, spheres, dumb-bells, feathered octahedra with curved pinnæ, etc., and uric acid is still more protean. The curious ball- and cone-shaped deposits which corrosive sublimate forms in balsam are known to everybody. And, as Bowman, Vogelsang, and others have shown, some of the forms assumed in colloidal media by common salt, santonin, salol, pyrogallol, antipyrin and other substances are marvellous in their complexity and beauty. And it must not be forgotten that all these forms are determined by colloidal media alone. As stated above, crystalloid media have an immense influence in producing the most varied complex aggregate crystals, and if, in addition to these, there are present, as is the case in living organisms, colloidal media of highly complex and variable constitution, the possibility of the development of elaborate crystallomorph forms is obvious. It is true that these crystallomorphs are all composed of so-called crystalloid matter, whereas the greater number of spicules are composed of colloid matter (mainly opal), and, as I have before stated, I am not aware of any experimental or other evidence that colloids are capable of assuming crystallomorphic forms. However, it is well known that typical colloids like egg- and serum-albumen and certain globulin proteids are easily capable of ordinary crystallisation and in consequence we have some reason to suppose that colloid crystallomorphs are at least possible. Applying our present scanty knowledge of crystallomorphs to the subject of spicules, the researches of Gautier (7) and others have made known to us that the protoplasms, so to speak, even of

different species of animals in all cases appreciably differ in chemical constitution from each other,¹ and even supposing that the substance of all calcareous spicules, e. g. is constant in composition, the different colloidal natures of the various organisms in which spicules are found are in all probability amply sufficient, in conjunction with factors (*a*) and (*b*), and the different crystalloid media present to account for the various spicule forms encountered.²

As showing in some degree the complexity of this subject of the causes determining the forms of spicules I may instance the extraordinary nature of even the common process of simple crystallisation, as ascertained by Frankenheim, Vogelsang, and several other observers. Vogelsang observed that the first visible stage in the formation of certain crystals (sulphur crystallising from carbon bisulphide solution, e. g.) was the appearance of liquid globules, which subsequently aggregate to form small solid isotropic spheres which he termed globulites, these again arranging themselves in definite patterns successively coalesce to form still higher aggregates (crystallites, margarites, etc.) until finally the crystalloids (the "integrant molecules" or crystal particles) are produced.³ It would be interesting to know whether the

¹ Also the well-known fact that different tissues and regions of the substance of the same organism differ widely in chemical constitution must be remembered, this possibly largely accounting for the different forms of spicules found in different parts of the same animal (Chiridota and other Holothurians, Hexactinellids, etc.); moreover, in some cases, as Maas (16) has shown in *Tethya*, e. g. the scleroblasts differ among themselves as regards total size, size of nucleus, character of cytoplasm, etc., and this consequently introduces another cause for the diversity of form of spicules contained in the same organism.

² Arguments for the inheritance of spicule forms, based upon the numerous transitional forms connecting together spicules of different types, are of little value, since all crystallomorphs exhibit exactly the same phenomenon on a very large scale, and, as is well known, all transitions exist even between true crystals of very different shapes. Moreover, in the case of spicules, there exist more causes to produce these transitional forms than in the case of crystals or crystallomorphs.

³ Dr. Rosenheim [22] has brought to my notice certain incidental

alveolar structure of calcareous and siliceous spicules described by Bütschli (3) has anything to do with the globulites of crystal formation.

Whatever may be the value of the above suggestions, one fact is certain, viz. that prolonged investigation (largely experimental) on the entire subject is absolutely essential before we can hope to interpret in at all an exact manner the shapes of spicules.

NOTE.

One important and, indeed, vital source of evidence respecting the inheritance or otherwise of spicule forms, which I have left almost unconsidered in the text, lies in the answer we must give to the question as to whether modifications of spicule forms, evidently due to ontogenetic causes, ever appear in the development of the spicule apart from these causes. Obviously, if this can be shown to be a fact in a single instance, then the inheritance of this particular spicule form is proved, and that of others rendered probable, though how the arguments I have advanced above in opposition to this are to be met I fail to see. Only one such apparent example of the inheritance of spicule forms, which might be cited in evidence against my position occurs to me just before returning the corrected proofs of this essay, and this example is taken from a paper I have myself published. In the first of my *Studies in Spicule Formation* (*Sycon* sponges) I have suggested that the elongation of the "posterior" ray in certain *Sycon* triradiates is directly due to its vertical basal position, the stresses incident upon the sponge being largely borne by the longitudinal element of the skeleton, especially at the sponge base, and the actinoblasts of the basal ray being thus stimulated to secrete more actively. Now it so happens that in *Sycon ciliata* and some other sponges the superior size of one of the rays is apparent in the observations of his own on the formation of choline crystals, in which similar phenomena are described.

development of the triradiate even before the three minute needles secreted by the sextet have united to form an aggregate or compound spicule (Study I, fig. 32), and therefore long before any stresses in the sponge-wall could possibly have produced any such modification. Therefore, if this larger ray of the young triradiate is the large "posterior" ray of the adult spicule, it seems to me that the inheritance of this particular form of spicule is proved. But the fact is that this larger ray of the young triradiate does not, by any means, always become the "posterior" ray of the adult spicule, though it often does (probably for the reasons assigned in Study I, pp. 263—265). Observations of the *Sycon* oscular rim show that the young triradiates are disposed very irregularly, and only exceptionally does the large ray point exactly towards the base of the sponge. As I have stated in Study I "the large ray is often found pointing as much as 80° from the downward vertical line," and more recent observations prove that the large ray may not uncommonly lie in exactly the opposite direction to that in which the majority of the basal rays of the adult spicules lie; in short, the large ray of the young triradiates may point in any direction, whereas that of the adult triradiates generally points basally. These facts seem to me to show that the large ray of the young *Sycon* triradiate is not the inherited large "posterior" ray of the adult spicule, since, were it so, the cause which is capable of thus reproducing in the young spicule a structural characteristic only directly producible in the adult spicule should be equally capable of determining the appropriate disposition of that structural characteristic.

As an alternative to the inheritance hypothesis I can only suggest that the above feature of the *Sycon* triradiate is of the same order of form-modifications as the clubbed extremities of the triradiates of *Clathrina clathrus*, the gastral ray spikelets of *C. cerebrum*, etc.—modifications produced ontogenetically without reference to the economy of the organism, and possibly mechanical or crystallomorphic in nature. Assuming this to be the explanation of the large

ray of young *Sycon* triradiates it is noteworthy that this feature has been made use of to some extent by the organism, since if the triradiates are rotated sufficiently (Study I, pp. 263—265) the large basally-directed rays afford extra support and enable the sponge to assume a more erect posture. Thus the shapes of spicules are not only in part determined by the form of the organism, but the contrary is also the case.

This large ray of the young *Sycon* triradiate is the only instance apparently affording support to the theory of the inheritance of spicule forms which I can at present call to mind. But even if later evidence definitely proves the inheritance of spicule forms to be a fact I shall not regret having published a discussion which at the least illustrates the complexity of the whole subject and suggests inquiry in more than one direction.

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On *Neurosporidium cephalodisci*, n. g., n. sp.,
a Sporozoön from the Nervous System of
Cephalodiscus nigrescens.

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With Plates 6 and 7.

INTRODUCTION.

The organism described in this paper occurs in the nervous layer of the ectoderm of *Cephalodiscus* (*Idiothecia*) *nigrescens*, Lank., a large and massive form of *Cephalodiscus* dredged by the "Discovery" on January 13th, 1902, in 100 fathoms, off Coulmann Island, near Victoria Land, in the Antarctic Ocean.

The specific name *nigrescens* was given to this *Cephalodiscus* by Prof. Ray Lankester,¹ the polypides being to the naked eye of a sooty black colour ; a detailed description of the polypides and the tubarium or "house" of *Cephalodiscus nigrescens* is given in the "Reports of the 'Discovery' Expedition," published by the British Museum.

The Sporozoön that occurs in *Cephalodiscus nigres-*

¹ 'Proc. Roy. Soc.,' 1905, pp. 400—402.

cens is found in relation with the nervous, deeper layer of the ectoderm. It does not always occur actually within that layer, since it may bulge outwards into the more superficial cells of the ectoderm, or may project inwards into the underlying tissues, but the new organisms seem always to be situated in the nerve tissue before they begin to enlarge and sporulate. On account of this peculiarity we apply to the organism the generic name *Neurosporidium*. *Cephalodiscus* being the host in which the parasite has been encountered, we base the specific name upon that. The organism is thus denominated *Neurosporidium cephalodisci*.

No unicellular parasites of *Cephalodiscus* have up to the present been described. In *Cephalodiscus gilchristi* there is a Copepod Crustacean occurring parasitically in the stomach of a large proportion of the polypides¹; this parasite is closely allied to the Copepods that occur in the gut of Tunicates, and belong to the family Ascidicolidæ of Giesbrecht.

Masterman² has drawn attention to "cyst-like structures" occurring near the anus of *Cephalodiscus dodecalophus*, which he is disposed to regard as larvæ of *Cephalodiscus*, but which from their position are more likely to be parasites. They are of comparatively large size, and judging by the large size of the cells as drawn in the figure, they are more probably the young of some metazoon parasite than cysts of Sporozoa.

M'Intosh³ marks by the symbol *bp.* a series of three spherical bodies occurring in the deeper part of the nervous layer of the ectoderm, which might at first glance be taken for Sporozoa. Their position is one commonly occupied by the parasite which forms the subject of the present paper, and their size and general shape carry the resemblance with

¹ Ridewood, W. G., "Cephalodiscus gilchristi," 'Marine Invest. South Africa,' iv, 1906, p. 181.

² 'Trans. Roy. Soc. Edinb.,' 1898, p. 513, last paragraph; also pl. 5, fig. 86.

³ "'Challenger' Reports,' part 62, vol. xx, 1887, pl. 6, fig. 3.

Neurosporidium still further; but it is to be noticed that the central, uninucleate cells that one would take to be the spores, are enclosed within a wall composed of a single layer of regular cells, and not by a structureless cyst or capsule. In the explanation of the figure, the parts marked *bp.* are said to be "sections apparently of the shield pores in their progress outwards," but it is highly improbable that the proboscis canals could, in a section taken at right angles to the buccal shield, be so cut as to present a circular outline, and even then the presence of the third body is difficult to explain, for the proboscis canals are short, and with firm walls of closely-set epithelial cells, and could not, even in the most contorted polypides, be cut across twice in the same section.

In view of the close relationship which is admitted to exist between *Balanoglossus* and *Cephalodiscus* it is worthy of mention in this connection that Spengel¹ has found in the cœlom of a species of *Balanoglossus* (*Ptychodera minuta*) masses of small, uniform, nucleated cells which he is inclined to regard as of a parasitic nature. Caullery and Mesnil² agree with Spengel that these bodies are parasitic organisms, and they refer them provisionally to the new order of the class Sporozoa to which they have given the name Haplosporidia, the order in which we propose to place the *Neurosporidium* of *Cephalodiscus*.

MATERIAL AND METHODS.

The specimens of *Cephalodiscus nigrescens* obtained by the "Discovery" were fixed, some in a 5 per cent. solution of formalin, some in Perenyi's fluid, and some in picric acid solution. Serial sections of the polypides were cut for the

¹ 'Fauna and Flora des Golfes von Neapel,' Monogr. 18, 1893, pp. 661, 662; pl. 2, figs. 19, 20; pl. 3, figs. 50, 51; pl. 4, figs. 60, 61, 76, 79, 80; pl. 5, fig. 105.

² "Haplosporidies," 'Arch. de Zool. Exp. et Gén.,' iv, 3, 1905, p. 164, and pl. 13, fig. 125.

purpose of investigating the anatomical structure of this new species of *Cephalodiscus*, and these sections, and a few additional ones cut more recently, were utilised for the study of the Sporozoön. The sections were 5μ , 6μ , and 7.5μ in thickness. The majority of them were stained with Ehrlich's hæmatoxylin and eosin, the others with hæmatoxylin and orange G, or Mayer's hæmalum, or borax carmine. The structural details of the parasite were most satisfactorily shown by the sections that were cut from material fixed in 5 per cent. formalin and stained with hæmatoxylin and orange G, or hæmatoxylin and eosin. The parasites were studied with a Zeiss 3 mm. apochromatic homogeneous immersion objective, used in combination with compensating oculars 4 and 8, and occasionally 12. In some of the stages they are of large size (pl. 6, figs. 1 and 2, *Nsp.*), and can be readily recognised with a $\frac{2}{3}$ inch objective.

OCCURRENCE OF THE PARASITE.

The infection appears always to commence in the nervous tissue, and although the parasites when at their largest may project beyond the nerve layer into other tissues (pl. 6, fig. 1, *Nsp.*), they remain in relation with nerve tissue at one part of their surface. The parts of the nervous system in which the parasite has been found are the central nerve mass (pl. 6, fig. 2, *Nsp.*), the nerve layer of the dorsal wall of the buccal shield (practically a continuation of the central tract), the nerve layer of the ventral wall of the shield (pl. 6, fig. 1, *Nsp.*), and the lateral nerve tracts near the collar pores. The parasite has not been found in the nerve tracts of the plumes, nor in those of the stolon.

The occurrence of *Neurosporidium* in the nerve tracts of *Cephalodiscus* is not the first instance of a Sporozoön infecting nerve tissues, although infection of such tissues is not common. Three instances are known of *Neosporidia*

infecting the nervous system of Teleostean fishes. Pfeiffer¹ in 1892 discovered a Myxosporidian in the cranial and spinal nerves of the Grayling (*Thymallus vulgaris*), the cysts lying between the medullary sheath of the nerves and the sheath of Schwann; Schuberg and Schröder² in 1905 described a similar, but not identical Myxosporidian, which they named *Myxobolus neurobius*, in the nerves of the Brook Trout (*Salmo fario*); and in 1898 Doflein³ described a Microsporidian, *Glugea lophii* (now known as *Nosema lophii*), occurring in the ganglion cells of the central nervous system of the Angler (*Lophius piscatorius*).

The proportion of infected specimens of *C. nigrescens* is considerable. Out of a total of twenty polypides examined, *Neurosporidium* was found to occur in eight.

Spherical or slightly oval spores of *Neurosporidium* appear to set up a local degeneration in the nervous, deeper layer of the external epithelium of *Cephalodiscus*, and in the oval spaces so formed the parasites grow and sporulate, and become surrounded by an ill-defined, somewhat irregularly alveolar capsule (pl. 7, figs. 9, 12, 15, *cps.*). This capsule is not of the nature of an ordinary cyst, secreted by the parasite peripherally, but would appear rather to be formed by the surrounding tissue of the host, much in the same way as is the enveloping membrane of the *Sarcosporidia*.

The capsule is not in all cases clearly evident. Some of the parasites, appearing as groups of spherical bodies when examined under a Zeiss A or D objective, nearly fill the spaces in the host tissue in which they lie, while others are more or less closely surrounded by a capsule (pl. 7, fig. 12, *cps.*), which itself is separated from the wall of the host tissue by a space. The space (fig. 12, *cav.*) is bounded externally by a thin layer of the same material (fig. 12, *cps*¹.) lining the

¹ Pfeiffer, L., 'Untersuchungen über den Krebs; die Zellerkrankungen und die Geschwulstbildungen durch Sporozoen,' 1893, p. 75.

² Schuberg, A., and Schröder, O., 'Archiv für Protistenkunde,' iv, 1, 1905, pp. 50 et seq.

³ Doflein, F., 'Zool. Jahrb.,' Anat., xi, 1898, p. 332.

host tissue. The capsule and this latter layer are refringent, and yellowish-brown in colour; they seem to be chitinous, but special tests were not applied.

These chitinoid layers are not always present, and even when they are to be recognised, they are so indefinite and irregular that we prefer not to use the word cyst at all, but to employ a more indefinite term, such as capsule. Probably the layers in question are produced by the host tissue in an attempt, unsuccessful as it appears, to isolate the parasite from organic connection with itself, or else they consist of broken-down nerve tissue which has taken a more or less spherical shape in accommodating itself to the space between the parasite and the still healthy part of the host tissue.

In some of the cavities a coagulum is to be noticed surrounding the parasite (fig. 7, *coag.*, also figs. 5 and 6). The coagulum is probably directly connected in its origin with the degeneration of the host cells, and the production of the capsule.

STRUCTURE AND LIFE-CYCLE OF NEUROSPORIDIUM.

The parasites in their smallest phase occur as little round or oval cells (free spores or amæbulæ), about 3μ (2 to 4μ) in diameter (see fig. 3), each with a single nucleus. These are found lying in or among the nerve cells of the host, having apparently entered this tissue by infiltration. It is not possible, with the material at our disposal, to say definitely if there is an intracellular phase of the parasite within a nervous epithelial cell at the beginning of the life-history of the parasite. Nuclear division takes place in the amæbula, and young trophozoites, with two or three nuclei in a somewhat irregular mass of protoplasm (fig. 3, *b*, *c*, *d*) are of common occurrence, often lying in a space among the nervous tissue cells of the host. Figures of nuclear division (fig. 3, *c*) are very rarely and imperfectly seen in our material.

The young trophozoites are usually about 5μ in diameter. The protoplasm of the trophozoite at this stage is granular

and easily stained, while in the one-cell or oval corpuscle stage the protoplasm is hyaline and only slightly granular, and does not stain deeply. Several young trophozoites may occur in one space (fig. 6).

Further nuclear division rapidly takes place, and multinucleate trophozoites, spherical, ovoid, or cylindrical in shape, are found with their many nuclei embedded in a more or less rounded or slightly lobulated mass of deeply-staining granular protoplasm (figs. 8 and 9). The multinucleate trophozoites correspond with the "plasmodium" stage of the Haplosporidia of Caullery and Mesnil,¹ though they are not markedly irregular or amœboid in outline. These trophozoites are 30 μ to 40 μ long, and 25 μ to 35 μ broad.

A "plasmodium" may be formed by the nuclear division and growth of the oval corpuscle or amœbula that constitutes the earliest stage of the parasite. It may also be formed by the fusion and growth of several young trophozoites (fig. 7), as can be seen by careful examination of a series of sections passing through a cavity containing several parasites at this stage. Such a fusion or concrescence approximates very closely to the formation of a true plasmodium.

In the multinucleate trophozoite phase the protoplasm of the parasite is not surrounded by a cuticle, and Neurosporidium hereby differs from such a type as *Bertramia*, which it otherwise much resembles at this stage. The protoplasm is opaque, and crowded with closely distributed granules. Around each nucleus, however, is usually an area of clear protoplasm (fig. 8). Some of the nuclei are rather large, 1 μ to 1.5 μ , or even 2 μ in diameter, but no definite nucleolus or karyosome is visible; the chromatin of these nuclei seems to be evenly distributed. No refringent granules such as occur in *Bertramia asperspora*² were noticed in the deeply-staining protoplasm. It is to be noted, however, that while

¹ Caullery, M., and Mesnil, F., "Recherches sur les Haplosporidies," 'Arch. Zool. Exp. et Gén.,' iv, 1905, pp. 101—181, three plates.

² Minchin, E. A., "Sporozoa," Lankester's 'Treatise on Zoology,' part 1, fasc. 2, 1903, p. 310.

our *Neurosporidium* consists of preserved material only, *Bertramia* was described from living specimens.¹

In the larger and older multinucleate trophozoites the nuclei and their surrounding zones of clear cytoplasm are distinctly seen (fig. 9), marking the beginning of the segregation of the plasmodial mass into pansporoblasts.

The term pansporoblast was first employed by Gurley² in connection with the Myxosporidia (in the wider sense, including Microsporidia). A pansporoblast ('sphère primitive' of Thélohan) may be described as a spore-mother-cell differentiated within the substance of the trophozoite, destined to give rise to sporoblasts, from which spores are developed. (The term sporoblast is applied to cells which develop each into a single spore, and the term pansporoblast is applied to a cell which by division gives rise to sporoblasts.) In the Myxosporidia the differentiation takes place by the concentration of cytoplasm around one of the nuclei of the endoplasm, and as a rule the protoplasm becomes more transparent. The pansporoblasts of a trophozoite may be many or few, or even a single one. A greater or less amount of the protoplasm of the trophozoite usually remains over, together with a number of residuary nuclei of the endoplasm, although in some Microsporidia, e. g., *Thelohania* and *Pleistophora*, the whole trophozoite becomes converted into a single pansporoblast. In the typical Myxosporidia each pansporoblast gives rise ultimately to two spores; in the Microsporidia, on the other hand, each pansporoblast may give rise to four, eight, or a larger number of spores.³

The term pansporoblast may be extended in its application to the Haplosporidia, for although in *Bertramia* the

¹ See also Warren, E., "On *Bertramia kirkmani*," 'Annals Natal Gov. Mus.,' vol. i, London, 1906, pp. 7—17.

² Gurley, R. R., "On the Classification of the Myxosporidia," 'Bull. U. S. Fish. Comm. Rept. for 1891,' xi (1893), pp. 407—420: see his footnote on p. 408.

³ Except perhaps in *Nosema pulvis*. See Perez, Ch., "Microsporidies parasites des crabes d'Arcachon," 'Soc. Sci. d'Arcachon, Stat. Biol.,' ann. viii, Paris, 1905, pp. 15—36.

trophozoite divides into a number of bodies, each of which becomes a single spore, yet in the case of *Haplosporidium scolopli* and *H. marchouxi*¹ the bodies into which the trophozoite divides undergo each a division into four spores.² The "bodies" in question are thus pansporoblasts in the sense described above.

In *Neurosporidium* a definite segregation of the cytoplasm of the trophozoite around the nuclei marks the beginning of the formation of pansporoblasts (figs. 7, 10, and 11). The general shape of the trophozoite at this stage is ovoid (fig. 11), or sometimes nearly sausage-like (fig. 10). The pansporoblasts are uninucleate cells with hyaline protoplasm, lying embedded in the opaque, granular protoplasm of the trophozoite. They are irregularly, but fairly closely packed in this granular mass, sometimes slightly more crowded together at the periphery of the trophozoite than in the centre (fig. 10). They are spherical or oval in shape, and 3μ or 4μ in diameter.

The pansporoblasts in some trophozoites are very quickly formed, the phase of nuclear division being rapidly passed through, or even omitted, since this stage may be reached, in true "plasmodial" manner, by the fusion of several young trophozoites, all lying within a common cavity in the host-tissue, as already noted. By careful examination of series of sections, what appears to be the fusion of such masses of young trophozoites can be seen, and a stage in the process is shown in fig. 7.

The cavity in the host-tissue enclosing the parasite increases in size as the growth of the parasite proceeds; in all probability the parasite causes progressive degeneration of the neighbouring host tissue.

Up to this point the development of *Neurosporidium* has been simple, in many respects closely following that of a typical *Haplosporidian*, such as *Bertramia*. The mode of

¹ Caullery and Mesnil, loc. cit., p. 114 and p. 117.

² In *Haplosporidia* there is no visible distinction between sporoblast and spore; the former passes directly into the latter.

sporulation, however, is distinctive, and of considerable importance in determining the systematic position of the parasite.

All the pansporoblasts in a trophozoite of *Neurosporidium* are formed, by internal segmentation, at the same time. They then commence to increase in size, growing at the expense of the protoplasmic ground mass of the trophozoite. Each pansporoblast becomes less well-marked in outline; its protoplasm is still rather homogeneous (non-granular), but deeply staining, and its centrally-placed nucleus divides and discharges chromidia throughout the cytoplasm of the pansporoblast. Each pansporoblast thus becomes a spore-morula, for the division of its nucleus into groups of chromidia, or small portions of chromatin (daughter-nuclei), results in the formation of many small unicellular sporoblasts, distributed not only peripherally, but throughout the substance of the pansporoblast, as may be seen by careful focussing.

The division of the nucleus of the pansporoblast is not of the serial karyokinetic type, first into two, then into four, and so on, but is a kind of simultaneous "multiple fission" of the reproductive chromatin into groups of chromatin forming the nuclei of the many sporoblasts, each of which soon becomes a spore with little or no further differentiation. The vegetative chromatin of the nucleus of the pansporoblast remains in the centre of the spore-morula; it is a pale-staining body, and cannot be recognised until the spores have scattered (see fig. 14, *r.n.*).

Each spore is about 1μ or 2μ in diameter, and stains deeply; its small nucleus is surrounded by hyaline protoplasm. From a full-grown trophozoite a very large number of spores is formed, since the trophozoite gives rise to many pansporoblasts, and each pansporoblast grows and divides into many spores. A full-grown trophozoite containing spore-morulae in sporulation is more or less spherical, and measures about 40μ to 70μ in diameter (figs. 12 and 13). The spore-morulae are spherical, and 10μ to 15μ in diameter.

The uninucleate spores, which are small gymnospires or amæbulæ, gradually pass out of the cavity around the parent organism by more or less irregular apertures in the capsule, and so invade the surrounding tissue of the host, and start fresh infections in other parts of the nervous system of the *Cephalodiscus*.

Each spore on becoming free grows to about 2, 3, or 4 μ in diameter, and division of its nucleus begins, while its cytoplasm becomes granular and a little more opaque. In this way young trophozoites are formed, such as those described in the first paragraph of this section.

All the cytoplasm of the spore-morula is not used up in the formation of spores, and there is also nuclear material left over. These residuary masses are clearly shown in our preparations, and their fate may here be considered in some detail. When the spores have left the parent spore-morula, the latter has a fairly definite contour, rather more distinct than during sporulation. The protoplasm is granular, and there is usually a pale, centrally-placed nucleus. The protoplasm stains feebly, and the granules in it are distinct. The residual nucleus is pale in colour, and reddish or brownish-red after staining with hæmatoxylin, and it thus stands in marked contrast with the deeply purple nuclei of the spores in the same preparation. In a few spore-morulæ examined little or no residual nuclear matter was found, even after the examination of all the sections into which they had been cut.

One or two rather more deeply staining dots may be seen in the pale residual nucleus in some, rather rare, cases (fig. 15). The residual nuclear matter is, as already stated, the remains of the vegetative chromatin of the spore-morula, the reproductive or generative chromatin being distributed in the nuclei of the spores. Deeply staining granules of chromatin are seen in capsules at this stage, sometimes in two or three groups (fig. 15, *chr.*), but usually aggregated towards the centre of the mass (fig. 17, *chr.*). These are groups of chromidia, or small masses of chromatin, of about

half the diameter of the nuclei of the spores. Some of them are surrounded by a clear area of cytoplasm, and may form spores, but many would appear to be residual, and not destined to give rise to spores.

The actual transition between the stage of sporulation in a spore-morula and that of residual matter is depicted in fig. 14. The capsule, of which a small part only is shown, is full of ripe spore morulæ, and these are in process of division into uninucleate spores; one of them, however, is slightly in advance of the rest, and has already divided into spores, which have scattered, leaving the remains of the spore-morula, pale in colour, with granular protoplasm and remains of nucleus. The outline of this relic (fig. 14, *r. sp. m.*) in the capsule is more clearly seen than that of its neighbours; its protoplasm is of a light brownish tint, and its nuclear residue of a brownish pink, after staining with hæmatoxylin, which imparts a characteristic purple coloration to the neighbouring sporulating masses, forming a clear and striking contrast.

The remains of spore-morulæ, after sporulation, undergo degeneration in old capsules (fig. 16). Their outlines become less distinct, and vacuoles appear in the now coalescing mass of protoplasm (fig. 17). The residual nuclei become less clear, but chromidia may still be seen near the centre of the degenerating mass (fig. 17). Some of the spores, formed from the spore-morulæ before they degenerated, may for a time be seen in the neighbouring tissue and in the cavity in which the parent capsule lies (fig. 16).

While the above account of the life-history of *Neurosporidium* suggests how the infection may spread from one part of the host to another, by the amæbulæ and young trophozoites migrating along the course of the nerve tracts, we have no evidence to offer which can explain the spread of the parasite to new hosts. In *Bertramia asperspora*, of Rotifers, the mode of spread has been watched by Bertram; the spores have no power of independent movement, but are disseminated passively on the death and disin-

tegration of the body of the host.¹ Possibly the same may happen in the present instance.

SYSTEMATIC POSITION OF NEUROSPORIDIUM.

In determining the systematic position of *Neurosporidium cephalodisci* the following features in the life-history, so far as we have been able to trace it, should be kept in view:

1. From a capsule lying in a cavity in the nervous system of *Cephalodiscus* gymnosporos or amæbulæ are liberated, as rounded masses of clear, naked protoplasm, 2 to 4 μ in diameter, each with a centrally placed nucleus.

2. The next stage is one in which a small multinucleate trophozoite lies in a small cavity in the nervous system. This stage is reached, either by the rapid growth and nuclear division of a single amæbula, or by the coalescence of several amæbulæ (plasmodium).

3. Nuclear division of the trophozoite continues, and the size increases, until the body is about 30 to 50 μ in diameter, and ovoid in shape. The general protoplasm is granular and opaque, but there is a clear zone of protoplasm around many of the large nuclei.

4. The large ovoid trophozoite segments into pansporoblasts, each a single cell, 3 to 5 μ in diameter, consisting of a large nucleus surrounded by clear cytoplasm.

5. The nucleus of each pansporoblast divides into many daughter-nuclei, and the pansporoblast enlarges and becomes a spore-morula, 10 to 15 μ in diameter. Each daughter-nucleus, with its small mass of clear cytoplasm, becomes a sporoblast, and then a spore. The spores are generally distributed throughout the spore-morulæ.

6. The spores ultimately separate, and pass out into the adjacent parts of the nervous system of the host. The remains of the spore-morulæ, consisting of granular protoplasm, pale-staining remains of nuclei, and some free chromidia, undergo gradual degeneration.

¹ Caullery and Mesnil, loc. cit., p. 136.

7. Around the parasite in its various stages (except the amœbula stage) is an ill-defined capsule, partly alveolar, and probably secreted by the cells of the host.

The systematic position of *Neurosporidium* may be readily determined by reference to the characters summarised above. The somewhat irregular form of the trophozoite and its possession of several nuclei; the appearance of pansporoblasts, denoting the commencement of spore-formation, at an early stage in the growth of the parasite; the division of the pansporoblast to form many spores; and the intercellular (histozoic) habitat of the parasite; all these are characteristic of the Neosporidia.

Further, on account of the small, simple uninucleate spores, without polar capsules, the increase in the number of nuclei during the trophic stage, and the segmentation of the full-grown trophozoite into ovoid pansporoblasts, we place the parasite among the Haplosporidia.

The division of the pansporoblast into many spores is a feature which does not occur in the life-history of the typical Haplosporidian. In *Haplosporidium scolopli* and *H. marchouxi* the number of spores formed from each pansporoblast is four; in *Bertramia* it is one. In *Rhinosporidium kinealyi*, however, a parasite from the nasal mucous membrane of man, recently described by one of us (Fantham)¹ in collaboration with Prof. Minchin, and also by Beattie,² the portion of the life-history in question finds a fairly close parallel.

In *Rhinosporidium* there is a multinucleate trophozoite phase, followed by segmentation into many closely packed pansporoblasts lying within a thin peripheral layer of un-

¹ Minchin, E. A., and Fantham, H. B., "*Rhinosporidium kinealyi*, n. g., n. sp.: a Sporozoön from the Mucous Membrane of the Septum Nasi of Man," *Quart. Journ. Micr. Sci.*, n. s., xlix, 3, 1905, pp. 521—532, two plates.

² Beattie, J. M., "*Rhinosporidium kinealyi*: a Sporozoön from the Nasal Mucous Membrane," *Journ. Path. and Bact.*, xi, 3, 1906, pp. 270—275, two plates.

differentiated protoplasm; each pansporoblast becomes a spore-morula, dividing into about a dozen small, round, uninucleate spores. In these respects *Rhinosporidium* resembles *Neurosporidium*. In *Rhinosporidium*, however, there is a well-defined cyst secreted by the parasite, and the outline of the spore-morula is rather more distinct, there being a definite membrane round each, and the outline of each spore in a full-grown spore-morula is also well marked. This slight difference in the definiteness of contours is of little importance as compared with the segmentation of pansporoblasts into many spores, which is in marked contrast with what occurs in the typical *Haplosporidia*.

Neurosporidium differs also from *Rhinosporidium* in that the spore-formation from pansporoblasts is not progressive, as it is in the latter. In *Rhinosporidium* the pansporoblasts, within a large cyst, are disposed in three zones, merging the one into the other, namely, a peripheral zone of uninucleate pansporoblasts just within a thin layer of undifferentiated protoplasm, an intermediate zone of pansporoblasts with two or three nuclei each, in which zone spore-formation is proceeding, and a central mass in which each pansporoblast, now a spore-morula, contains about a dozen uninucleate spores. In other words, spore-formation proceeds in a centrifugal direction within the cyst, the central pansporoblasts completing their sporulation before the peripheral ones begin to divide.

In *Neurosporidium*, on the other hand, there is an interval between the simultaneous formation of the uninucleate pansporoblasts and their segmentation, and the segmentation of the spore-morulæ occurs simultaneously throughout the same capsule.

As pointed out by Minchin and Fantham,¹ *Rhinosporidium*, in the successive formation of its pansporoblasts, resembles Schewiakoff's parasite of the *Cyclopidae*,² to which

¹ *Loc. cit.*, p. 529.

² Schewiakoff, W., "Ueber einige ekto- und ento-parasitische Protozoen der Cyclopiden," *Bull. Soc. Imp. Nat. Moscou*, n. s., vii, 1893, pp. 1—29, pl. 1.

Caullery and Mesnil¹ have recently given the generic name *Scheviakovella*.

The degree of importance to be attached to the mode of sporulation, whether simultaneous or successive within the same cyst, is for future investigations to decide; for the present, our opinion is that the production of many spores by each pansporoblast instead of a few spores or a single spore, a feature possessed in common by *Rhinosporidium* and *Neurosporidium*, is of more importance than the former feature, in which the two genera differ.

After due consideration of the various points above set forth, we definitely place *Neurosporidium* and *Rhinosporidium* in the order Haplosporidia, extending the order to include these forms. Further, we divide the extended order Haplosporidia into two sections; (1) the **Oligosporulea** (nom. nov.) for forms like *Haplosporidium*, *Bertramia* and *Cœlosporidium*, in which each pansporoblast produces only a small number of spores or a single spore, and (2), the **Polysporulea** (nom. nov.) for forms like *Rhinosporidium* and *Neurosporidium*, in which the pansporoblast gives rise to many spores, either successively or almost simultaneously.

Some protozoologists express doubt as to the homogeneity of the Haplosporidia as a group, and not altogether without reason. But *Cœlosporidium*, *Bertramia*, and *Haplosporidium* form a well-defined section, and we have shown above the relation of *Neurosporidium* and *Rhinosporidium* to this section. The precise limits of the order Haplosporidia are difficult to define because of the elementary structure and simple developmental cycle of the organisms which that order includes.

The Haplosporidia show points of resemblance with the Mycetozoa and with the Rhizopoda. The possibility, however, must not be overlooked that while some of the forms are truly primitive, others may be spuriously so, and may owe their simplicity to degradation from a higher stock, a

¹ Loc. cit., p. 156.

process which in so many groups of animals is known to result from indulgence in a parasitic mode of life.

For the present the Neosporidia may be divided as follows :

1. Cnidosporidia (Doflein), with polar capsules.

Myxosporidia, e.g. *Myxobolus*, *Sphærospora*.

Microsporidia, e.g. *Glugea*, *Pleistophora*.

Actinomyxidia, e.g. *Hexactinomyxon*.

? Sarcosporidia, e.g. *Sarcocystis*.

2. Haplosporidia (Caulley and Mesnil), simpler forms than the above, without polar capsules.

Oligosporulea, e.g. *Bertramia*, *Haplosporidium*.

Polysporulea, e.g. *Rhinosporidium*, *Neurosporidium*.

SUMMARY.

The parasite begins its life-cycle as a round or oval gymnosporid or amœbula in the nervous system of the host (figs. 3 and 4).

The amœbulæ cause a degeneration of the nerve-tissue immediately around them, and come to lie within cavities, one amœbula in a cavity, or several (fig. 5).

The amœbula becomes a multinucleate trophozoite, either by enlarging and undergoing nuclear division, or by coalescence with other amœbulæ or young trophozoites, or by a combination of both processes (figs. 6, 7, 8, 9).

The capsule surrounding the parasite is ill-defined, and is probably formed by the host (fig. 12, etc., *cps.*).

The trophozoite segments into uninucleate pansporoblasts (figs. 10, 11), each of which enlarges and becomes a spore-morula.

The spore-morula gives rise to many small spores (figs. 12, 13), and after the liberation of these, there remains a mass of granular protoplasm, with residual nuclei (figs. 15, 16, 17).

The infection probably spreads through the nervous system of the host by the migration of the amœbulæ and trophozoites. The mode of cross-infection from host to host is not known.

It is proposed to divide the Haplosporidia into the Poly-

sporulea (pansporoblasts giving rise to a number of spores) and the Oligosporulea (pansporoblasts giving rise to a few spores or to a single spore each).

The systematic position of *Neurosporidium* is as follows:

Phylum.—Protozoa.

Class.—Sporozoa.

Sub-class.—NEOSPORIDIA.

Order.—Haplosporidia.

Section.—Polysporulea (nov. sec.).

Genus.—*Neurosporidium* (nov. gen.).

Species.—*cephalodisci* (nov. sp.).

EXPLANATION OF PLATES 6 AND 7,

Illustrating Dr. Ridewood and Mr. Fantham's paper on *Neurosporidium cephalodisci*, n. g., n. sp., a Sporozoön from the Nervous System of *Cephalodiscus nigrescens*."

PLATE 6.

For the two photomicrographs from which figs. 1 and 2 were drawn we are indebted to Dr. N. H. Alcock, Lecturer on Physiology in St. Mary's Hospital Medical School, and to him we hereby tender our best thanks. The photographs were taken by monochromatic light obtained by a Thorpe's grating, and Zeiss's apochromatic objectives were used.

FIG. 1.—Section of a polypide of *Cephalodiscus nigrescens* taken transversely to the length of the body, and passing behind the pedicle of the buccal shield and behind the mouth. $\times 120$. *Nsp.* *Neurosporidium* in the thick ventral wall of the buccal shield. *ph.* Wall of pharynx. *t.c.* Trunk cœlom. *m.* Muscles of the ventral body-wall.

FIG. 2.—Section of a polypide of *Cephalodiscus nigrescens* taken transversely to the length of the body, and passing through the central nerve mass and the notochord. $\times 100$. *Nsp.* *Neurosporidium* in the central nerve mass. *s.* Septum between the right and left collar cavities. *no.* Notochord. *p.c.* Proboscis cavity, or cavity of the buccal shield. *b.s.* Thick ventral wall of the buccal shield. *lph.* Base of the lophophore, cut obliquely. *a.* Ectoderm around the anus, cut tangentially.

PLATE 7.

All the figures on Plate 7 were outlined with camera lucida (Abbé), using apochromatic objective 3 mm. homogeneous immersion (Zeiss) and compensating oculars 4 and 8.

All the figures of this plate, except fig. 16, are enlarged 1000 diameters.

SIGNIFICANCE OF THE LETTERING.

cav. The cavity in the host tissue in which the parasite lies; it often contains a coagulum (*coag.*). *chr.* Chromidia or daughter-nuclei resulting from the division of the nucleus of the spore-morula in the formation of spores, principally those daughter-nuclei remaining over in old and degenerating capsules. *cps.* Capsule, an ill-defined, sometimes alveolated membrane around the parasite; it is not a true cyst, and is apparently formed by the tissue of the host. *cps'*. A similar layer lining the healthy tissue of the host. *nu.* Nucleus (figs. 4 to 9), or daughter-nucleus (figs. 12 to 14). *p'spbl.* Pansporoblast. *r.n.* Residual nucleus of the spore-morula. *r.sp.m.* Residual cytoplasm of the spore-morula. *vac.* Vacuole.

FIG. 3.—A number of free spores. *a.* An amæbula, with one nucleus. *b* and *d.* Later stage, young trophozoites with two and three nuclei respectively. *c* shows nuclear division, rather indistinctly, but as well as could be expected from the material to hand.

FIG. 4.—Deeply staining spore (amæbula) lying in a definite cavity in the nervous tissue of the host.

FIG. 5.—Several such spores in a cavity, surrounded by a thin envelope (*cps'*).

FIG. 6.—Several young trophozoites lying in a cavity.

FIG. 7.—Young trophozoites coalesced into a plasmodium, lying in a cavity, with another young trophozoite, which examination of the series of sections shows to be continuous with it. A coagulum is also seen, probably resulting from the remains of the host cells. This specimen is in the stage of pansporoblast formation. Although smaller than those shown in figs. 8 and 9, it is in a later stage of the life cycle.

FIG. 8.—Multinucleate trophozoite or "plasmodium."

FIG. 9.—Multinucleate trophozoite, surrounded by a capsule, lying in a cavity in the host tissue.

FIG. 10.—Elongate trophozoite, with oval pansporoblasts lying in deeply staining granular protoplasm. There is also a small trophozoite by the side, lying in the same cavity.

FIG. 11.—Ovoid trophozoite, full of pansporoblasts.

FIG. 12.—Several spore-morulae (full-grown, segmenting pansporoblasts), which are dividing into spores. The parasite is seen surrounded ill-

defined capsule (*eps.*), somewhat alveolar in character, and apparently formed by degenerating host tissue; it lies in a cavity in the host tissue, which latter is lined by a membrane (*eps'*).

FIG. 13.—Typical capsule, full of spore-morulæ, each dividing into many spores.

FIG. 14.—Part of a capsule containing spore-morulæ, one of which, having shed its spores, shows a residual granular mass of cytoplasm, and a pale, ill-defined nucleus. The difference in the staining reaction of this remnant of the spore-morula from that of its sporulating neighbours is very striking.

FIG. 15.—Capsule containing residua of spore-morulæ. Groups of nuclear granules (chromidia), some of which may perhaps form spores, while others will degenerate, are scattered about, chiefly in the centre. These chromatic dots are smaller than the daughter-nuclei shown in fig. 13, each of which latter becomes the nucleus of a spore. Some of these smaller nuclei (*chr.*) are surrounded by a layer of clear, refractive protoplasm.

FIG. 16.—Further stage in degeneration of residua of spore-morulæ. These consist, at this stage, chiefly of residual granular protoplasm, which is becoming vacuolated. At the periphery of the cavity, now ill-defined, in which the large mass lies, are seen some of the spores and young trophozoites formed from these spore-morulæ. $\times 500$.

FIG. 17.—Still further degeneration of residual matter of the spore-morulæ, showing vacuolated protoplasm and remains of nuclei (*r.n.*) of some of the spore-morulæ. Some chromidia are grouped in the centre, but these were probably not destined to form spores.

Gametogenesis and Fertilisation in *Nematus ribesii*.

By

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With Plate 8.

In a previous paper¹ I gave an account of the maturation and behaviour of the polar nuclei in several species of sawflies which develop parthenogenetically.

In all these species there were two maturation divisions, giving rise to an egg nucleus and three polar nuclei, and in some cases fusion took place between the second polar nucleus and the inner half of the first. The egg nucleus sank into the yolk and began to divide to form the embryo, while the polar nuclei in all cases ultimately disintegrated. Since whenever the chromosomes were clearly visible their number appeared to be eight, both in the maturation mitoses and in the later divisions in body-cells, it was concluded that no reduction in the ordinary sense took place. But if fertilisation ever takes place by conjugation of male and female pronuclei, an obvious difficulty arises with regard to the chromosome number in fertilised eggs, and since the process of fertilisation had not been thoroughly examined at the time when the paper referred to was written, it was necessary to leave the question open in the hope of finding a satisfactory answer later. This paper gives an account of

¹ 'Quart Journ. Micr. Sci.,' vol. 49, 1906, p. 561.

the work done on the fertilised egg in *Nematus ribesii* and on the gametogenesis in that and other species.

The methods used were generally the same as before, but it was found that, in searching for male pronuclei in the eggs of impregnated females, thionin or gentian violet were more satisfactory stains than iron hæmatoxylin, since they stain nucleus and cytoplasm but leave the yolk uncoloured. In the work on spermatogenesis and the development of the ovarian egg, osmic fixatives (e. g. Flemming's fluid) were largely used in addition to sublimate.

THE FERTILISED EGG IN *N. RIBESII*.

In some animals, e. g. the bee, the fertilised egg is easily distinguished from the virgin by the presence of sperm asters in the yolk, but in the sawflies nothing of the kind can be found, and over 200 eggs had to be cut and examined before it became certain that conjugation of male and female pronuclei takes place. In very young eggs I had occasionally found minute rod-like bodies in the peripheral protoplasm near the anterior end, which are probably the heads of spermatozoa, and in somewhat later eggs bodies which appeared to be degenerating nuclei sometimes appear in a similar position. In eggs laid by impregnated females there are frequently in the yolk in front of the polar region more or less numerous small radiating patches of protoplasm which sometimes appear to contain indistinct nuclei, but protoplasmic masses not certainly distinguishable from these are found also in virgin eggs, although with less regularity. In eggs which are probably fertilised there are also frequently lines of protoplasm running inward from the edge of the egg near the point where the spermatozoa had been found. But in no case have I been able to recognise with complete certainty the male pronucleus before the maturation divisions of the egg are completed, and after that stage nuclei found in the yolk may always be derived from the

egg-nucleus itself. It is never possible, therefore, to say with certainty that a given egg is fertilised or not.

But after much time spent in vainly trying to follow the entrance of the spermatozoon and its conversion into the male pronucleus, I at last was able to observe the conjugation of the sperm-nucleus with that of the egg, and so to prove that true fertilisation does take place (fig. 1). It occurs immediately after the maturation divisions; the three polar nuclei lie near the edge of the egg (two of them in the same section as the egg and sperm nuclei), and the fusion of the two inner polar nuclei has not yet taken place. The male and female pronuclei are in contact, the male being distinctly smaller than the female, but in another egg in which the same stage is seen the two are of about equal size. The subsequent stages of the conjugation and division of the zygote nucleus have not been observed, but the section represented in fig. 1 leaves no reasonable doubt that normal conjugation takes place. It therefore became necessary to reconsider my previous conclusions with regard to the number of chromosomes, since never more than eight have been found in either fertilised or virgin eggs. I was thus led to work out the spermatogenesis, and to the fresh work on the maturation divisions to be described later.

In my previous paper I mentioned that the behaviour of the polar nuclei appeared to be slightly different in fertilised and in virgin eggs, and subsequent work has confirmed this.

In the virgin egg of *N. ribesii* the two inner polar nuclei fuse and give rise to a group of chromosomes, which is generally clearly double, with eight in each half. The two halves of the group do not lie far apart, and commonly remain without much change for some time. But in the majority of eggs from impregnated females the chromosome groups derived from the two inner polar nuclei lie completely and sometimes widely separated, as if the conjugation between the nuclei had been much less complete than in virgin eggs (figs. 2, 3, and 4). Further, in virgin eggs the polar chromosomes usually do not divide, at least for some

time, but in fertilised eggs they frequently divide comparatively early, giving groups containing as many as sixteen chromosomes rather irregularly arranged in the "polar protoplasm." That this difference in behaviour is really connected with fertilisation is made probable by the fact that it rarely, if ever, occurs in eggs which are certainly virgin, but in the eggs laid by impregnated females it is frequent. Further, in several eggs laid by impregnated females the polar nuclei follow the typical virgin arrangement, and in these the little rayed protoplasm masses in the yolk, characteristic of fertilised eggs, are absent; but other eggs laid by the same female have the fertilised type of polar chromosomes, and in these the rayed protoplasm patches are also present.

It appears, therefore, that the fertilisation of the egg nucleus, or the presence of spermatozoa in the egg, in some way influences the behaviour of the polar nuclei.

SPERMATOGENESIS.

When it had been shown that normal fertilization could take place in *N. ribesii*, it became necessary to re-examine the maturation divisions in order to make certain about the chromosome number, which I asserted in the previous paper to be eight both in the maturation and in the somatic mitoses, and also apparently in fertilized eggs. The maturation of the egg begins immediately after it is laid, so that it is very difficult to get good preparations of the early stages, and I therefore decided to examine the matter first in the development of the spermatozoa.

In very young male pupæ, shortly after the larval skin is cast in the cocoon, the testes consist of compact groups of cells at the sides of the alimentary canal. These cells (spermatogonia) have relatively large nuclei containing a conspicuous nucleolus (plasmosome) and eight or about eight chromatin masses apparently attached to the nuclear membrane (fig. 5). Division figures are scarce, but when found

they show clearly about eight rather large chromosomes in the equatorial plate, which split so that eight travel towards each centrosome (fig. 6 *a, b*). At a later stage the testis becomes larger, and consists of lobes or compartments in each of which all the cells are in about the same stage. By the time the colours of the mature fly are beginning to appear the testis contains nothing but spermatids and nearly mature spermatozoa, but when the pupa is still white all stages from spermatogonia to spermatids are found in different lobes, often in the same section.

In the nucleus before the first maturation divisions the chromatin consists of a number of irregular masses (apparently about eight, but they are always rather indistinct). Shortly afterwards it becomes condensed into four more concentrated masses, each of which frequently appears double or quadruple (fig. 7 *a, b, c*). A spindle is then formed, and the four chromatic masses become tightly packed together in the equatorial plate, which is much smaller than in the spermatogonial divisions. There are conspicuous centrosomes. The chromosomes in the spindle are so tightly packed together that it is difficult to be certain of their number, but a comparison of many mitoses leaves little doubt that there are four, each of which is bivalent (fig. 8 *a, b*). The mitosis appears to be of the heterotype form, resembling the figures found by Moore in the cockroach¹ except that the chromosomes are fewer and very much smaller (fig. 9 *a, b*). They are, however, appreciably larger than the chromosomes in the maturation mitoses of the egg.

The second maturation division is easily distinguished from the first by the fact that the spindle is of about half the diameter; the chromosomes are usually even more tightly packed, so as frequently to appear as a single body, but in clearer cases there is little doubt that there are four (fig. 10 *a, b, c*). At the telophase a vesicular spermatid nucleus is formed, with the chromatin arranged round the edge

¹ 'Quart. Journ. Micr. Sci.,' vol. 48, 1905, pp. 489 and 571.

giving it a characteristic appearance (fig. 11). This becomes converted into the head of the spermatozoon.

It must be concluded therefore that in the male the normal somatic number of chromosomes is eight; that four "gemini" appear in the prophase of the first maturation division, and that finally four chromosomes are distributed by heterotype and homotype divisions to each spermatid nucleus.

There is no trace of the "polar body" formation described by Meves in the spermatogenesis of the bee.²

OÖGENESIS.

In the larva before it casts its skin within the cocoon the ovaries are much like the testes of the male, but larger, with bigger nuclei. The ovary is enclosed in a cellular sheath, and some ovarian cells are already larger than others; these will form the eggs, while the more numerous smaller cells give rise to the nutritive and probably to the follicle-cells. All the nuclei at this stage contain about eight chromatin masses and one to three nucleoli (fig. 12). In the young pupa the egg tubes are already differentiated, and in a longitudinal section of a tube the changes in the nucleus can easily be followed. At the apex of the tube the nuclei are like those in the larval ovary; below this zone the chromatin becomes distributed through the nucleus as fine dots, which are often aggregated together in one part, as in a sort of synapsis (fig. 13). The egg nucleus then enlarges considerably, and the chromatin appears as an irregular thread; at this stage two or three nucleoli are generally conspicuous (fig. 14). After this stage yolk begins to be deposited, and before the egg is ripe the nucleus, which has been very large, dwindles so that in nearly ripe eggs I have been totally unable to find it.

In the larval ovary mitoses may be found in the ovarian cells and in the sheath; those actually in the ovary appear to have eight chromosomes (fig. 15 *a, b*). But in the sheath in

² 'Anat. Anzeiger,' xxiv, 1903, p. 29.

all the mitoses observed the number is more than eight; usually it seems to be sixteen, but in some cases the figure suggests more than sixteen very small chromosomes (fig. 16 *a-f*). Wilson¹ has described spindles with double the somatic number in the ovary-sheath of Hemiptera, and regards them as abnormal, but the figures seen in *N. ribesii* certainly suggest that the eight chromosomes in the primitive germ-cells are compound, composed of a greater number of smaller units, possibly more than sixteen.

In the pupal ovary the egg-cells are already definitely formed, and do not divide further, but merely undergo the usual growth with deposition of yolk. The follicle cells are now quite small, and an occasional mitotic figure is visible; these are rarely clearly defined, but appear to have eight chromosomes. When the egg has reached its full size the follicle cells become degenerate, with obscure dark-staining nuclei. Groups of similar degenerating cells are found here and there in the larval ovary.

The fact that the chromosome number in the ovary, and probably in the follicle cells, is smaller than that found in the sheath is of considerable interest.

In addition to the case described by Wilson, and referred to above, the same kind of thing has been observed by Petrunkevitch in the bee,² in which he found the unreduced number to be sixteen in the egg, but sixty-four in the blastoderm, and it is still more conspicuous in *Ascaris*, which, according to Boveri, has a large number of very small chromosomes in the somatic cells, but only four in all the cells on the "germ-track," from the fertilised egg up to the maturation divisions of the germ cells.³ These facts suggest that it may happen not infrequently that the chromosomes in cells of the germ-track may be compound, and consist of a number of smaller units which become separated in somatic cells. But

¹ "Studies on Chromosomes," iii, 'Journ. Exp. Zoo.,' vol. iii, No 1, 1906.

² 'Zool. Jahrb.,' vol. xiv, 1901, Anat. und Ontog., p. 573.

³ Boveri, 'Ergebnisse über die Konstitution der Chromatischen Substanz des Zellkerns.' (Fischer, Jena.)

even if this is found to be a phenomenon of general occurrence it does not necessarily affect the hypothesis of the individuality of the chromosomes in any essential point.

CHROMOSOMES IN THE MATURATION DIVISIONS OF THE EGG.

It has now been shown that in the spermatogonial and oogonial divisions there are eight chromosomes, and that in the spermatocytes these are reduced to four in the normal heterotype manner. These facts led me to re-investigate the maturation divisions of the egg, since in my previous paper (*loc. cit.*) I gave evidence that in both first and second polar mitoses the number was eight.

The chromosomes in the maturation of the egg are much less easy to observe than in the spermatogenesis, for there are difficulties of technique to be overcome, and the egg has to be preserved at exactly the right moment. But after cutting some hundreds of eggs I have been able to convince myself that while there are two types of maturation. In some eggs no reduction takes place, and eight chromosomes pass into each of the four nuclei produced by the polar mitosis. In other eggs four double chromosomes are found in the equatorial plate of the second maturation division, and these separate into their component halves sending four into each daughter-nucleus (figs. 17—21).

I have never obtained a section of the first polar mitosis in which it is quite certain that there are four "gemini," although some figures strongly suggest this; but at the close of the first division, when the chromosomes are arranging themselves to form the equatorial plate of the second mitosis, four double chromosomes are sometimes clearly visible (figs. 20, 21). I have also several preparations which show only four when the second polar mitosis is already begun. A comparison of figs. 17 and 19, 18 and 20, respectively, will show the difference between the reducing and equational types of maturation.

It must therefore be concluded that in some eggs pairing

(synapsis) of chromosomes takes place before the maturation divisions, resulting in the separation of complete chromosomes at one of the mitoses, while in other eggs no pairing takes place, and each chromosome undergoes two equational divisions. In connection with this it is noticeable that in the eggs having the equational type the eight chromosomes are about half the size of the four seen in reduced eggs.

I have found the reduced type in eggs from both virgin and impregnated females, so that the view which first suggested itself, viz. that reduction only takes place in eggs which contain spermatozoa, is not tenable.

Re-examination of my sections of *Pœcilosoma luteolum* confirms me in the belief that in that species, which yields females from virgin eggs, and is normally not fertilised, there are two equational divisions in all the eggs of which I have suitable preparations.

In the developing egg ^{of *Nematus*:} the somatic mitoses of fertilised eggs appear always to have eight chromosomes; a larger number has never been found. This is what would be expected if only eggs which undergo reduction are capable of fertilisation.

In virgin eggs commonly eight are found, but in some cases the equatorial plate seems to have four only, showing that reduced eggs when not fertilised can develop as far as the blastoderm stage (fig. 22). The number of eggs which die before hatching varies, in some batches being very small, in others more considerable; it is possible that the reduced eggs are those which fail to develop to larvæ. Since, however, it has been shown by the mitoses in the ovary sheath that the chromosomes are possibly compound, it may happen that reduced eggs which are not fertilised restore the normal number of chromosomes by division of the compound chromosomes, as was asserted by Petrunkevitch (loc. cit.) with regard to the bee.

The conclusion that the eggs of one species may either undergo reduction, or may retain the full number of chromo-

somes, although in each case there are two polar mitoses, is of considerable interest. I know of nothing quite parallel with it hitherto observed in animals, but I think it not unlikely that in the two generations of the Gallflies, one of which is bisexual and the other purely female, a similar state of things may be found to exist. That there may be two types of egg, one of which is reduced and requires fertilisation, and the other not reduced and parthenogenetic, is of course not infrequent, but in such cases the eggs generally have obvious external differences, and the unreduced form has only one polar body. A condition more nearly resembling that found in *N. ribesii* has been observed by Rosenberg in *Hieracium*,¹ in which the egg-cell in some flowers on a head is reduced and can be fertilised, in others on the same head not reduced and parthenogenetic. But here again the number of maturation divisions is probably not the same in the two cases. In the bee, according to Petrunkevitch, all the eggs are reduced, but if not fertilised, the somatic number of chromosomes is restored automatically.

The conclusions here reached may make it necessary to reconsider the provisional hypothesis of sex-segregation sketched in my previous paper, but until further facts are obtained in other species it seems premature to discuss the bearing of my results on the problem of the determination of sex. I have not found it possible, owing to the minute size of the chromosomes, to determine whether anything comparable with Wilson's "heterotropic" chromosome exists in *Nematus*. In some figures (e. g. the group represented in fig. 18) only seven chromosomes are visible instead of eight, but when they are so minute it is always possible that two are superposed and not distinguishable apart.

In conclusion I take this opportunity of expressing my gratitude to Mr. J. E. S. Moore for allowing me to compare some of my preparations with his, and for valuable help in elucidating my sections.

[NOTE.—In a series of eggs all laid by one insect on one

¹ Brit. Ass., York, 1906. Discussion on Fertilisation, Sects. D and K.

day the polar mitoses are abnormal. The most extreme case (fig. 23) shows the "polar protoplasm," full of dots arranged roughly in lines like iron-filings in a magnetic field. At each pole of the figure is a group of more conspicuous stained bodies which may be chromosomes. Some of the other eggs show a somewhat similar appearance on a smaller scale, and in others nothing is clearly distinguishable in the polar protoplasm. In all the eggs the peripheral protoplasm is narrower than usual, and in the most markedly abnormal eggs it is practically absent. I have occasionally found appearances of the same kind, but much less pronounced, in eggs laid by other insects, but have not sufficient cases to be able to throw any light on their meaning.]

SUMMARY.

1. True fertilisation (conjugation of male and female pronuclei) may take place in *N. ribesii*, and the behaviour of the polar nuclei is slightly different in fertilised and virgin eggs.

2. In the spermatogenesis there are eight chromosomes in spermatogonial divisions; four "gemini" appear at the beginning of the meiotic phase, and by heterotype and homotype mitoses distribute four chromosomes to each spermatid.

3. In the oogenesis eight chromosomes appear in oogonial mitoses, but in divisions of nuclei in the ovary sheath more than eight are found, suggesting that the chromosomes of the germ-cells are compound.

4. In the polar mitoses of the egg two types of maturation are found. In some eggs there are successive equational divisions so that the egg nucleus and each of the three polar nuclei contains eight chromosomes. In other eggs normal reduction takes place, separating entire chromosomes from one another, and only four are found in each of the daughter nuclei.

5. It is probable that only such reduced eggs are capable

of fertilisation, but when unfertilised they may continue to develop at least as far as the blastoderm stage.

Birmingham University;
November, 1906.

EXPLANATION OF PLATE 8,

Illustrating Mr. L. Doncaster's paper on "Gametogenesis and Fertilisation in *Nematus ribesii*."

All figures are drawn with an oil-immersion lens, but are not exactly on the same scale. Those illustrating spermatogenesis are more highly magnified than the remainder. All represent *Nematus ribesii* except figs. 12, 13, 14.

FIG. 1.—Conjugation of male and female pronuclei. Three polar nuclei near the edge of the egg.

FIGS. 2, 3, 4.—"Polar protoplasm" of fertilised eggs showing chromosome groups derived from polar nuclei.

FIG. 5.—Nucleus of spermatogonium.

FIG. 6.—Spermatogonial mitoses. (A) Metaphase, side view; (B) Equatorial plate.

FIG. 7, A, B, C.—Spermatocyte: three prophases of heterotype mitosis. (A) Showing 8 chromosomes; (B and C) Pairing to form 4 double chromatin masses.

FIG. 8.—Heterotype mitosis, equatorial plate. (A) Pole view; (B) Side view.

FIG. 9, A, B.—Heterotype anaphases.

FIG. 10.—Homotype. (A) Pole view of equatorial plate; (B, C) Anaphase, side view.

FIG. 11.—Spermatid.

FIG. 12.—Young oogonium, *N. lacteus*.

FIGS. 13, 14.—Stages of growth of oogonium, *N. lacteus* pupa.

FIG. 15.—Oogonial mitoses, larval ovary. (A) Pole view; (B) Side view.

FIG. 16, A—F.—Mitosis in ovary sheath with more than 8 chromosomes. (A) Equatorial plate, pole view; (B, C) Similar stage seen from side and obliquely; (D, E, F) Anaphases.

FIG. 17.—Second polar mitoses, equational type, with 8 chromosomes.

FIG. 18.—Second polar mitosis, equatorial plate in pole view, with 7 chromosomes, some preparing to divide.

FIG. 19.—Second polar mitoses, metaphase; reduced type, with 4 chromosomes.

FIG. 20.—Equatorial plate of reduced type, showing 4 double chromosomes.

FIG. 21.—Stage between first and second maturation divisions, reduced type, with 4 double chromosomes each end.

FIG. 22.—Two blastoderm mitoses, each with 4 chromosomes and conspicuous centrosomes.

FIG. 23.—Abnormal polar mitosis.

The Molluscan Radula: its Chemical Composition,
and Some Points in its Development.

By

Igerna B. J. Sollas.

With Plate 9.

HISTORY.

THE molluscan radula, or dental ribbon, has been the subject of research for at least a century and a half. Aristotle (3), though he speaks of teeth in *Limax*, alludes apparently to the ridges on the jaw, and there is no evidence that he knew of the existence of the radula: but it is interesting to find that the great naturalist was well aware of the fact that whelks bore holes in shells with the proboscis, although he cannot have fully understood the process. Poli made a jest of the tale as a fable, but Osler re-affirmed it in 1832 without knowing of previous work, and is now credited with having been the first to observe this interesting habit.

Swainmerdam is the discoverer of the radula: he gives a description of both the radula and jaw of the snail (*Helix aspersa*), in Dutch and Latin, in his 'Biblia Naturæ,' Leyden, published posthumously. His death, as we are told by Boerhaave in the Life of the author prefixed to this work, occurred in 1680. The work is now too antiquated to possess more than an historical interest.

In 1757 Adanson (1) described radulæ from various gastropods of Senegal: the teeth are "infinitely small, hardly

visible, though sometimes perceptible to the touch. Looked at with the microscope . . . the pointed ends of the teeth are turned towards the stomach like those of the tongue of the lion or cat." Adanson observed the regular arrangement of the teeth, and in some cases counted them, finding 20,000 teeth in 200 longitudinal rows in a bulimoid land-snail which the natives call "Kambeul," and 200 in 10 longitudinal rows in *Patella*.

Poli (13), in 1791, was the first to give a clear figure of a radula in his magnificent work 'Testacea utriusque Siciliae.'

Troschel (22), in 1836, first established the radula as an organ of great systematic importance. Curiously enough, in the same year van Beneden, in a paper written in 1835, and not quoted in the literature of this subject, points out the possible value of the radula in determining the reality of doubtful species. Troschel's work attracted the interest of zoologists to the radula; after an interval, in which Lebert, Allman, and particularly Lovén, worked along the new lines, Troschel published his 'Gebiss der Schnecken' (1856-1863)—a general and masterly work now well known. His interest was not restricted to the form of the teeth, but extended to their chemical composition. Though Troschel was the first to make the suggestion—thrown out apparently as a shrewd guess—that growth takes place at the posterior end of the radula to make good the waste going on in front, yet he did not follow it up by closer study, nor did he investigate the development of the organ. It has been one of the chief problems of later workers, but they have arrived at somewhat conflicting results. By combining a study of the chemical composition with that of the development some of the difficulties which have arisen may be removed.

CHEMICAL COMPOSITION.

In 1845 Hancock and Embleton (6), in a study of the anatomy of *Eolis*, state that the radular teeth consist of silica. They base their conclusion on the partly mistaken observation

that the teeth do not dissolve in either acetic or nitric acid, while hydrofluoric acid corrodes them. No particulars of their experiments are given. The same authors investigated the teeth of *Buccinum* and came to the same conclusion.

In 1852 Leuckart (9), being interested in the distribution of chitin in the animal kingdom, examined, among many other objects, the radulæ of Gastropoda and Cephalopoda, and pronounced them to be chitin. He emphasised the fact that his identification of chitin rested entirely on two characters—one its resistance to caustic alkali, the other its solubility in boiling nitric acid. He adds: "It is possible that in this sense chitin is a collective conception, and that many special modifications will be discovered later. Perhaps we may conclude this from the varying behaviour of chitin when treated with alkali," and he expresses a wish that chemists would investigate the matter. About the same time Bergh (4), without knowing of Leuckart's paper, confuted Hancock and Embleton's view, and demonstrated the absence of silica in three species of Prosobranchiate Gastropods. Bergh's is the first exact investigation; we are indebted to Troschel for a German translation of an extract of his paper, which is written in Danish. Bergh showed that in *Buccinum antiquorum* (*Triton nodiferum*), and in *Strombus gibberula* most concentrated acids bring about corrosion of the radula in the cold, and eventually complete solution on boiling, while dilute hydrofluoric acid does not alter the teeth in form, but renders them more transparent. Incinerated ribbons of *Marsenia perspicua* gave no silica. The radula of *Buccinum antiquorum* gave the reactions of iron and calcium phosphate.

Troschel was dissatisfied with what he considered the contradiction in the results of Leuckart and Bergh,¹ and therefore undertook with Bergemann experiments which combined and reconciled the results of both these investigators. *Helix*,

¹ This remark seems hardly fair to Leuckart, who nowhere states that chitin is the sole constituent of the radula and is not interested in the ash; of Bergh's paper I have only read the extract given by Troschel.

Patella, and Dolium were chosen for study, attention being directed both to the jaw and radula. The radula of these three forms was found to behave in a similar manner and consists of an organic constituent, chitin, together with the inorganic constituents, iron, calcium, carbonic, and phosphoric acid. It was further shown that the radula of *Helix nemoralis* contains 5 per cent. of ash, that of *Dolium galea* 6 per cent.

Koehler's paper, published (7) in the same year as Troschel's 'Das Gebiss der Schnecken,' deserves a word of mention, since this observer also affirmed the presence of both an organic and an inorganic constituent, and suspected the occurrence of calcium.

Later workers continue to make divergent statements in describing the chemical composition of the radula. Sollas (19) in 1885, when studying the nature of the silica in organisms generally, made use of measurements of the refractive index and specific gravity; he concluded that in the molluscan radula silica was present and that, as in so many organisms, it was in the form of opal (silica hydrate), but he does not mention the species on which his observations were made. Bloch (5) and others speak of the radula as chitin, but their views do not appear to be based on original observation. Similarly some modern text-books refer to this organ as composed of chitin or conchiolin, others speak of it as siliceous. Huxley and Ray Lankester, with more caution, do not commit themselves on this point. It thus seemed worth while to look once more for definite evidence of the presence or absence of silica and of chitin in the radula. It will conduce to brevity if I state at once the general results I have obtained. I find that in all the odontophorous Mollusca the radula has an organic basis of chitin; the Docoglossa are unique among Mollusca in the composition of their teeth, of which the most important constituent is silica hydrate or opal. All the other groups, including the Rhipidoglossa, form a second type in which the radular chitin is hardened superficially by deposits containing calcium, iron, and phosphoric acid, which, together

perhaps with an additional organic substance, form that outer covering so long known as the enamel layer but hitherto unexplained. I have not been able to confirm Troschel's statement that carbonic acid is present, and though I have made repeated attempts, I have failed to determine whether magnesium is one of the mineral constituents. These points, therefore, must still be left to chemists. The Chitonidæ present us with a deviation from the second type and stand alone among the forms I have examined. In this family ferric oxide is the most important mineral constituent and is the cause of the dark colour of the teeth.

With the partial exception of *Helix aspersa*, the ash of the radula preserves the form of the teeth.

In the first type of radula or that of the *Docoglossa* the mineral matter may form as much as 27 per cent. of the whole ribbon, this is the case in *Patella vulgata*; while in the second type it may contribute only 2·4 per cent., as in *Helix aspersa*, though in this species it sometimes rises to 3·3 per cent.; in *Dolium galea* it amounts, according to Troschel's analysis, to 6 per cent.

It is interesting to find that the *Docoglossa*, which are so well-defined a group in other respects, differ so widely, not only from the *Pectinibranchiata*, but also from the *Rhipidoglossa* in the composition of the radula.

We may commence our more detailed account with the *Docoglossa*, and first with

PATELLA.

If the radular ribbon of *Patella* is boiled in strong nitric acid, the organic parts are completely dissolved, and there sinks to the bottom of the test-tube a coarse-grained insoluble residue. Microscopic examination shows that this consists of the dark red brown cusps or free biting ends of the lateral teeth (fig. 1), together with some thin, colourless, transparent pieces; some of these latter are free, some remain attached to the cusps, showing that they are the skeleton of the basal

part of the lateral teeth. This last point may be confirmed by performing the solution in the cold, when all the hard parts are left in their natural relative positions. Prolonged boiling with nitro-hydrochloric acid or hydrochloric acid and potassium chlorate in an open test-tube failed completely to remove the dark red-brown colour of the cusps; to accomplish this the teeth must be placed with one of these solvents in a sealed glass tube and heated in a water bath for some days. After this treatment some of the cusps become completely freed from their iron content and appear perfectly colourless and transparent; others, however, after digestion for an entire week retain some of their red-brown colour.

The specific gravity of the teeth thus prepared was ascertained by means of a diffusion column,¹ in which the majority were found to float in a dense zone at a level corresponding with a specific gravity of 1·98, though numerous examples ranged on each side of this to 1·87 on the one hand and 2·08 on the other. This variation in specific gravity corresponds with a difference in the degree of hydration of the silica: experiments made on colloid silica show that when this is prepared from water glass it possesses a specific gravity of 1·86 and contains 16·3 per cent. of water; when obtained from silicon fluoride, its specific gravity is 1·98, and the water amounts to 9·85 per cent.; while sponge spicules with a specific gravity of 2·04 contain 7 per cent. of water. Thus it would appear that the water in the siliceous basis of the Patella teeth varies in amount from 7 to 16 per cent. with a mean of 9·85 per cent.

It is interesting to observe in this connection that the association of water and silica in the silica hydrates occurs without any change of volume in either of the constituents, and thus the proportion of water in the hydrate can be directly calculated from the specific gravity. In the following table the results of such a calculation are given for a

¹ For an account of this method see Sollas, W. J., "Physical Characters of Calcareous and Siliceous Sponge Spicules," 'Proc. Roy. Soc. Dublin,' vol. iv, p. 378, 1885.

small number of cases, the specific gravity of amorphous silicon dioxide being taken as 2.21.

SiO_2 , H_2O , water 23 per cent., sp. gr. 1.73 (SiO_2)₃, (H_2O)₂, water 16.6 per cent., sp. gr. 1.85.

(SiO_2)₂, H_2O , water 13 per cent., sp. gr. 1.9; (SiO_2)₃ H_2O water 9.1 per cent., sp. gr. 2.0.

(SiO_2)₄, H_2O , water 7 per cent., sp. gr. 2.04.

Under the microscope the teeth which have been treated as described above present a faint brown granular appearance when seen by transmitted light and a bright milky white by reflected light: in this behaviour, which results from the abundant presence of minute pores, they resemble common opal. Between crossed Nicols they exhibit faint but evident double refraction with undulose extinction; the same character is sometimes presented by mineral opal, and is attributed to internal stress, the existence of which in the teeth is suggested by their liability to spring apart along fractures parallel to their length.

The refractive index of the siliceous residue of the teeth was determined by Becke's method and found to be closely approximate to 1.45; the fluids used were mixtures, one of 10 parts, the other 20 parts of Price's glycerine, to 1 part of water. Taking the refractive indices of these mixtures to be 1.449 and 1.454, the above result is obtained.

Teeth from which the iron has not been completely extracted are distinguished by a relatively high specific gravity; under the microscope the ferric hydrate or oxide presents a blood-red colour by transmitted light and marked double refraction.

The ribbon of *Patella*, when soaked for some hours in strong hydrofluoric acid, becomes colourless or nearly so throughout, but the forms of the teeth are perfectly preserved. If it is next boiled in nitric acid it dissolves completely, as might be expected, since the siliceous matter has already been removed by the hydrofluoric acid. On incineration the ribbon retains its form with a surprising completeness. The ash proves to contain, in addition to silica already

mentioned, a noticeable quantity of calcium, iron, magnesium, and phosphoric acid.

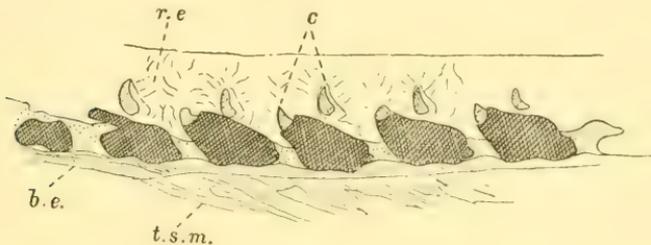
The radula after treatment with hydrofluoric acid proves very resistant to prolonged boiling in caustic potash (5 per cent. to 40 per cent.); this suggests that it is composed of chitin. With a view to testing this, radulæ were treated with hydrofluoric acid, boiled for a considerable time in a 5 per cent. solution of caustic potash, which was frequently changed, and were finally extracted with water, absolute alcohol, and ether. The specific gravity of the organic portion of the radula which remained after these processes was the same as that of chitin obtained from the carapace of *Astacus*, and subjected to the same treatment—viz. 1.40. The specific gravity was determined by a diffusion column formed of chloroform and absolute alcohol. I find that chitin from various sources has a specific gravity differing but slightly, if at all, from that of *Astacus*, and I hope to publish details on this subject shortly. The refractive index of this organic basis of the radula was found to lie between the same limits as that of chitin, viz. 1.550 and 1.557.

There is an interest of a general nature attaching to the fact that the radula of molluscs has a chitinous basis, since Bloch has brought forward special arguments (5) to prove that this membrane is not produced by the direct metamorphosis of the formative cells, but by secretion. Bloch is able to cite a number of workers who share his view, and he refutes the arguments of Wiren, the only supporter of Trinchese in the suggestion that the radula is formed by direct metamorphosis of cell protoplasm. Chatin, on the other hand, in 1892, maintained that the chitin of Libellulid larvæ is formed by direct transformation of the chitinogenous cells.

After soaking in hydrofluoric acid the entire ribbon and teeth stain with borax carmine and other preparations, though with differences in intensity in the various parts. When the fresh radula is treated with the ordinary stains this is not the case, as may be seen from fig. 8. The ribbon in this figure has been stained first with Bethé's stain and afterwards

with borax carmine. Bethe's stain acts readily on those parts which resist borax carmine and remain unstained in ribbons treated with borax carmine only.¹

The action of hydrofluoric acid renders it possible to make sections of the radula; these reveal the organic matter as a solid basis having the form of the complete structures. With the ordinary stains the cusps are less deeply coloured than the roots of the lateral teeth (text-fig. 1), with Bethe's stain the bases of the roots of these teeth and parts of the marginals



TEXT-FIGURE 1.—A longitudinal section of the radula of *Patella vulgata* after treatment with strong hydrofluoric acid and staining with iron hæmatoxylin. *t. s. m.* Tensor superior muscle. *b. e.* Basal epithelium. *c.* Organic basis of the cusps. *r. e.* Roofing epithelium.

¹ Bethe's method: the object is placed in a 10 per cent. aqueous solution of anilin hydrochloride to which one drop of fuming hydrochloric acid is added for every 10 c.c. of water. After washing thoroughly the object is transferred to a 10 per cent. solution of potassium bichromate. It will be seen that at the young end of the *Patella* ribbon each row of teeth is uniformly coloured by the carmine stain, the youngest teeth are paler than those immediately in front of them. In front, again, of the darker red teeth the roots of the laterals and centrals (inner laterals) are coloured green by Bethe's stain, their cusps being still red like the marginals. Finally, as we pass forwards, the cusps become golden-yellow and then red-brown owing to the presence of iron oxide, and the innermost marginal bears a band of green. The contrast in staining properties between the marginals and remaining teeth is very striking. The basal membrane itself is red, but in the preparation it has been removed as far as possible, as it obscures the differentiation in specimens mounted whole. These differences in staining properties are to be found with greater or less deviations in the radula of other odontophorous molluses.

alone are coloured; so that in this case there is a curious reversal of the usual relative behaviour of these stains. I have not found any other case in which Bethe's stain will colour in section a structure which has an affinity for the ordinary staining reagents, though this is commonly the case in working with material in bulk.

The above description refers to *Patella vulgata*; it is equally applicable to *P. pellucida*, as well as to a number of other species of *Patella* sent me by the kindness of Professor Mitsukuri, and probably to all species of *Patella*. To Professor Mitsukuri I am also indebted for specimens of *Nacella*, the teeth of which, isolated by boiling in fuming nitric acid, are shown in Fig. 6. The marginals, as in *Patella* and all *Docoglossa* examined, do not contain a siliceous skeleton; they dissolve completely in nitric acid and are consequently not represented in the figure.

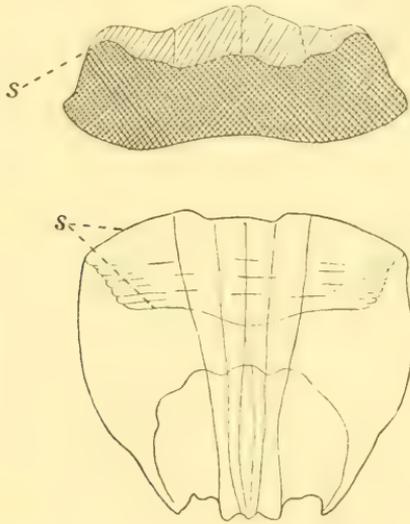
CRYPTOBRANCHIA.

My thanks are due to Mr. Rathbun, of the United States National Museum, for specimens of *Cryptobranchia concentrica*. The lateral and central teeth possess a skeleton of siliceous pieces which are fused in each row into a single plate, but it is possible in this plate to detect outlines which seem to mark the limits of once separate teeth. Apparently there were three laterals and one central, the central tooth being much reduced. In the darkly coloured cusps the line of demarcation between the outer and inner laterals is clear, but the two inner laterals of each side are closely fused together (text-fig. 2).

ACMAEA.

Specimens of *Acmaea virginea* were obtained from Plymouth. I owe to the kindness of Dr. Harmer examples of *Acmaea saccharina*, as well as of many other molluscs. Marginals being absent in this genus the greater part of the radular substance is silica; the solution of the organic matter

sets free from the portion corresponding to each row of teeth a pair of substantial siliceous plates of rectangular outline when seen en face. On each of these are seated the red-brown cusps of the lateral teeth. The cusps are detached from the plates by further boiling. The basal pieces of each



TEXT-FIGURE 2.—A siliceous basal plate and the cusps carried by it, isolated from the radula of *Cryptobranchia concentrica* by the action of boiling nitric acid. *ss'* Surfaces of attachment of the cusps and plate to one another.

side of the radula, though free from those of the other side, are closely fused among themselves, leaving no trace of the outlines of separate pieces.

The teeth, after treatment with nitric acid, have a refractive index which closely approaches that of chloroform; 1.4 being a little lower in the case of *A. virginea*, and a little higher in that of *A. saccharina*, there is a corresponding difference in the value of the specific gravity, which was found to be 2.1 in the latter and 2.0 in the former species. It is open to doubt, however, whether this difference is constant, for the examples investigated were far from numerous.

LEPETA.

I take this opportunity of thanking the Rev. Professor Gwatkin and Dr. O. Nordgaard for examples of this genus.

Lepeta coeca.—The siliceous basal pieces of each row are united into a single piece as in *C. concentrica*, and as in that species the basal plate is divided by longitudinal lines into six areas, whereas the coloured cusps appear to be formed by the union of four pieces. In this case, however, the double basal piece belongs to the outer cusps.

RHIPIDOGLOSSA.

Classified according to the chemical composition of the radula, these forms belong to the second type mentioned above (p. 118), in which the proportion of mineral matter in the radula is small and does not include silica.

In all the forms belonging to the second type which I have investigated the teeth can be isolated from the membrane by cold nitric or hydrochloric acid. Teeth of *Trochus ziziphinus* freed in this way and washed were found to have a refractive index lower than 1.557, and higher than 1.550. The same is true of all the radulæ of this type. The refractive index of chitin lies between the same limits.

Fig. 12 shows the result of staining the radula of *Trochus ziziphinus* with hæmatoxylin; it is necessary to isolate the teeth by teasing, as the membrane stains darkly and the teeth are so close-set that it is impossible to make out details in the radula stained intact. All the teeth take the stain at their roots; in the series of marginals the length which takes the stain increases as we pass outwards along a transverse row, while, finally, on the outer side of the marginals is a paddle-shaped piece made up of five flat pieces, placed edge to edge (the flabelliform teeth). This piece stains completely, and contrasts with the teeth lying to its inner side. This contrast is most marked in the younger parts of the radula. The

marginals pass by many gradations of form into the lateral teeth. These outermost pieces, on the other hand, present a sudden change in the series of marginals, being longer than their neighbours, and agreeing in their staining properties with the marginals of *Patella*. In fact, the reactions to stains almost tempt us to suggest that the teeth generally regarded as marginals are multiplied laterals, and that the marginals are represented by these deeply staining teeth at the outermost edge of each row.

Haliotis and *Emarginula* give somewhat similar results.

TÆNIOGLOSSA.

Littorina littorea furnished the chief and most convenient material for the investigation of this group. The radulæ were dissected out from a thousand individuals of the species, and dried at 100° C.; they weighed 0·430 gm., and afforded, on incineration, 0·0158 gm. of ash, or 3·7 per cent. This was found to contain iron, calcium, and magnesium. A second experiment was made on the ribbons of several thousand individuals, from which 0·0508 ash was obtained; this yielded 0·0083 of phosphoric acid, or rather, of P_2O_5 , corresponding to 16·3 per cent. or to 35·6 of calcium phosphate. A contrast between the staining reactions of the marginals and the central and lateral teeth exists here as in the case of *Patella*, though it is less marked (fig. 9), and the teeth run through the same stages as regards staining capacity during their growth as in that genus, but in the long radula sheath it is noticeable that the teeth retain for a long time their affinity for the common stains, and only quite near to the mouth-cavity become green under the action of Bethe's stain. Their great hardness is discovered when an attempt is made to cut sections of the buccal mass. Previous treatment with nitric acid is necessary for success in this process.

RHACHIGLOSSA.

In *Buccinum undatum*, as there are no marginal teeth, the ribbon, doubly stained by the above method, does not become marked out, as in previous cases, into a green median region, bordered on each side by a red marginal band; none of the teeth in the fully developed parts of the radula are coloured by protoplasmic stains, but all take Bethe's stain. It is noticeable that the teeth are very rapidly matured—a necessary property in a tongue which must be quickly worn out. In a specimen of *Purpura lapillus* I found every one of the teeth in the mouth-cavity with cusps broken off. It would seem as though the molluscan radula had become adapted to hard work in two distinct ways: in the one case it becomes very resistant through a long-continued process of development—witness the long radula sac—in the other a less strong radula is rapidly worn out and as speedily renewed.

PULMONATA.

Helix aspersa.—A remarkable discrepancy was found to occur in the behaviour of the radula of this species when incinerated on different occasions. Several lots containing from twenty to one hundred and fifty radulae were heated, in some cases with an ordinary Bunsen burner in a platinum crucible, in other cases with a Herapath. These experiments were all made in winter-time, and all gave the following result: the radula at first preserved its form in ash, but soon fused with continued heating: it gave an exceedingly minute fusible drop, which solidified to an enamel-like glass, yellow when hot, white when cold. In winter, also, the ash was always found to contain some silica: the ash from some 150 ribbons was boiled in fuming nitric acid, in this reagent it proved only partially soluble; the insoluble residue was

washed in water and returned to the platinum spoon and again heated: it was then infusible and was presumably silica. In a quantitative analysis the amount of silica was found to be about one third of the total ash. In one case, in the month of April, 500 radulæ were incinerated with a Bunsen and the ash afterwards ignited in a Herapath with a dissimilar result from the preceding; no fusion of the ash occurred, and in the analysis which followed no silica was found. On the other hand, the presence of a large quantity of phosphoric acid was indicated: the ash, which weighed 0·0101 grm., yielded 0·0036 grm. of P_2O_5 , or no less than 35·6 per cent.

DEVELOPMENT.

The history of the study of the development of the radula has been so excellently set forth by Rössler that on this point I cannot do better than refer to his paper (15).

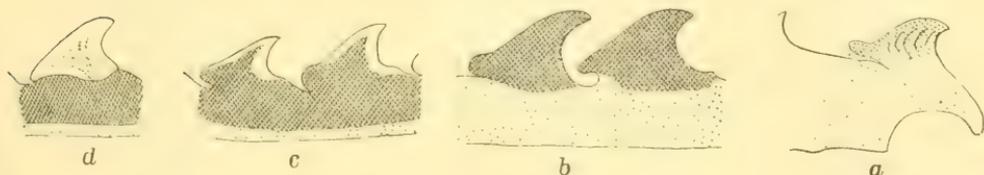
It has already been stated that there is a certain amount of disagreement among those who have studied the growth of the radula. The radula passes backwards into the radular sac, which is the seat of its renewal; it exhibits a continuous forward movement due to growth from behind taking place simultaneously with wear of the front end which is in use. The radular sac is an evagination of the wall of the foregut; it consists of a basal epithelium and a roofing epithelium; these two epithelia pass into each other by way of a heap of cells at the posterior end of the sac. In front of this cell aggregate, immediately behind and below the last formed tooth, are a set of cells, the odontoblasts, which may be few and large or more numerous and small. The roofing epithelium extends between the young teeth at the posterior end of the sac, fitting closely into the spaces between them; it, in many cases, secretes a cuticular substance in the anterior portion of the sac which likewise fits into the spaces between the teeth, so that the teeth lie in pits of this secretion. All

are agreed that the odontoblasts give origin to the teeth, but some would have it that the roofing epithelium secretes a kind of enamel or hard outer layer which is spread over the surface of the tooth originally laid down by the odontoblast. Among those who take this view are Rössler (15), Rücker (14), Sharp (18), and Bloch (5). Bloch describes the enamel layer as consisting of specially hard cuticular substance. Two writers, more recently—Rottmann and Schnabel—deny the presence of any such enamel, and maintain that the teeth are laid down from the first in their definitive form and size, and that the roofing epithelium contributes no substance whatever to the radula, and is not to be regarded as secretory. There is further difference of opinion as to the exact method by which the forward movement is brought about, and on other points.

The facts which have been already stated in dealing with the Docoglossan and other radulæ place beyond all doubt the importance of the roofing cells of the radular sac in all cases; in the case of *Patella* the lateral teeth as first formed are soft and colourless, and it is only those situated at a considerable distance from the odontoblasts which betray by their yellow colour the presence of iron oxide, and this must have come from the roofing cells. The other changes in the maturing teeth all point to the secretory nature of the roofing epithelium, a function which is strongly suggested *à priori* by the conspicuous accuracy with which the cells of this epithelium fit in between the teeth, leaving not the minutest portion of tooth or membrane surface untouched. Schnabel and Rottmann were working with material other than Docoglossa, namely with Gastropoda and Cephalopoda. Therefore it will be worth while to consider at any rate one of these cases in greater detail. That some considerable changes do take place in the teeth after their first formation is already clear, but the changes as seen in microscopic sections are interesting and afford some further light.

The young teeth, as we know from dissection are soft; they take protoplasmic stains but slightly, and agree in this with the

part of the membrane on which they are seated. They show lamination, the laminae running parallel to the matrix cell (Rössler's). Soon, as we pass forwards along the ribbon, there is a sharp contrast in staining properties between the young teeth and the underlying part of the basal membrane (text-fig. 3), but this contrast is not maintained, the increased



TEXT-FIGURE 3.—Longitudinal sections of the radula of *Helix aspersa*, stained with hæmatoxylin, showing four stages in the development of the teeth. *a*, the youngest tooth of the radula; *d*, a tooth from the mouth-cavity; *b* and *c*, intermediate stages. (Drawn with Zeiss camera lucida, Zeiss obj. D., eyepiece 4.)

staining power spreading downwards through the thickness of the membrane but always leaving a thin layer which preserves the original resistance to stains. At the same time that the membrane darkens the teeth, which nearer the origin were uniformly darkly stained, acquire a lighter periphery, the core remaining darkly stained; finally the surface layers become quite colourless and only some scattered small spheres of darkly staining matter remain in the interior. These spheres are arranged with a certain definiteness in rows which converge towards the posterior basal part of the tooth. Such are the appearances seen in sections treated with a protoplasmic stain only, whether hæmatoxylin, safranin, borax carmine, or carmalum. If we first apply Bethe's stain and then safranin or carmalum, the whole section will be coloured pink with the exception of the cores of the adult teeth, which are green, and the surface layers of the same, which are colourless. These outer layers are Sharp and Rössler's enamel. Rössler made the interesting note that they are not doubly refracting, while the other parts of the

radula are so. But they are evidently not formed, as Rössler suggested, as a special secretion apposed to the outer surface of the tooth as first formed, but rather by intussusception.

With regard to other doubtful points, Rössler assumed that the same odontoblasts secrete all the teeth that are produced during life; but later writers have thought that these cells must be periodically replaced, for, as Bloch points out, "unless we suppose the odontogenous cells to be replaced from time to time we cannot understand how larger teeth are formed with the growth of the animal, for it is unlikely that cells continually engaged in active secretion can also grow" (p. 381). The new odontoblasts, according to Bloch, are formed out of the same cell complex from which the upper epithelium is renovated. This assumption, he thinks, contains no improbability "since the upper epithelium and the odontoblasts have the same task, namely, the secretion of chitin." But from what has preceded it is clear that the functions of the roofing epithelium and of the odontoblasts are very different; this need not, however, impair Bloch's main argument that the odontoblasts are replaced; there is no reason why a single cell complex should not give off chitin-secreting cells on the one hand and cells secreting mineral matter on the other. The single apical cell of the stems and roots of various plants does more than this.

Again, Rössler considered that the radula must glide forward to a certain extent over the basal epithelium, which he said is delayed by the action of the retractor muscle (tensor superior muscle of Amandrut). Though I do not agree with his view, Rössler, in alluding to the action of the muscle, seems to me to have touched on an important problem which has been quite neglected by some authors. To this I shall return presently. Bloch objects to Rössler's view: "Ich kann mir nicht denken wie die Basalplatte, die die Zähnen trägt, und nach den Präparaten mit ihrem Epithel in innigen Verbindung zu sein scheint, über diesen Zellschicht hinweggleite. Da ist nur eine Möglichkeit nämlich die Zelle bewegen sich mit der Basalplatte nach vorn." In the paragraph which follows he

seems to say that the two structures keep pace in the younger end of the radular sheath, but that later the epithelium may be delayed. This admission amounts to granting that the epithelium throughout its length cannot keep pace with the membrane except by stretching—if this word can be permitted to stand for the conversion of the high columnar cells of young basal epithelium into lower cells. For if equal increments of epithelium and of membrane are added in the radular sac, then clearly there will be no gliding of one structure over the other in any part of their length: they will keep pace with each other. If, however, the increments of epithelium are less, then cells of the epithelium must “stretch” or increase in volume in order to keep pace with the overlying membrane. It is highly probable that the increments of epithelium are less than those of the membrane, for in the mouth-cavity the epithelium is generally lower than in the sac; in *Helix aspersa* one cell in the basal epithelium in the mouth-cavity covers as great a length of membrane as four cells in the sac. Consequently if we were to assume that equal lengths of epithelium and of basal membrane were added in the sac, this would involve a considerable relative movement of the epithelium and membrane in the mouth-cavity, the epithelium moving more quickly than the membrane, and this is wholly unlikely.

That the odontoblasts are replaced by fresh cells derived from the cell aggregate at the extremity of the radular sac seems most probable and is, I think, the view which has gained acceptance; at the same time, investigators differ among themselves as to whether the replacement is gradual, so that each group of odontoblasts secretes several teeth before it passes on into the basal epithelium, or, more sudden, each odontoblast group only secreting once before it is relieved by recruits. On full consideration this view will be found to involve a somewhat remarkable life-history for the odontoblasts. Starting from the indifferent cell mass from which they arise by cell-division, they become elongated and form a set of cells which possess as a whole a definite and constant shape. They secrete chitin first for the teeth, next for the basal membrane

and are then described as exhausted. But they now pass on and become the youngest cells of the basal epithelium, shortening till they are of a uniform height with their neighbours. They then travel forward, adhering to the basal membrane and gradually shorten still further. As they continue their course they encounter, as they leave the radular sheath to enter the mouth-cavity, the superior tensor muscle of Amaudrut (2). Now they have to play a new and arduous rôle: they must adhere to the radular membrane on the one hand and make connection with the tensor muscle on the other, and their tensile strength must be at least as great as that of the pull from the muscle. Strange to say, they next become liberated from the muscle again and pass forwards, now as a low epithelium, until they encounter another muscle, the inferior tensor, when some of them become connected with it. After this they once more move forwards to form part of the walls of a groove (the sublingual groove of Rössler) which is the natural outcome of the mode of growth of the radula and which allows of the free play of the buccal cartilages in eating.

In conclusion I wish to express my indebtedness to Professor Sollas for much suggestion and help in carrying out the work contained in this paper, particularly in connection with the observations on the relation between the specific gravity of silica hydrate and its degree of hydration.

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EXPLANATION OF PLATE 9,

Illustrating Miss Igera B. J. Sollas’ paper on “The Molluscan
Radula: its Chemical Composition, and Some Points in
its Development.”

FIG. 1.—Lateral teeth of *Patella vulgata*, isolated by nitric acid. 1, Two inner laterals (with broken cusps), showing the overlap of the basal pieces; 2, an outer lateral; 3, an inner lateral; 4 and 5, basal pieces from which the cusps have become detached.

FIG. 2.—Lateral teeth of *Patella vulgata* which have been subjected to the action of nitro-hydrochloric acid in a sealed tube.

FIG. 3.—A portion of the ash of an incinerated radula of *Patella vulgata*.

FIG. 4.—*a*, A siliceous basal plate from the radula of *Acmaea virginea* bearing three cusps, isolated by nitric acid. *b*, *c*, The same less magnified and showing the three cusps separated from the basal piece, by further action of nitric acid, and adhering together.

FIG. 5.—A siliceous basal plate from the radula of *Acmaea saccharina* bearing cusps.

FIG. 6.—Lateral teeth of *Nacella* sp. *a*, Outer; *b*, inner lateral tooth. The portion *a''* becomes isolated (by boiling acid) from *a*, leaving *a'*.

FIG. 7.—A lateral tooth of *Chiton* sp. isolated from the radula by the action of strong cold nitric acid, showing the hollow chitinous basal portion and the brown cusp which contains iron oxide.

FIG. 8.—Portions of the radula of *Patella vulgata* in order of succession (*a—d*) from the radula sac to the mouth cavity. Bethe's stain and carmalum.

FIG. 9.—Portions of the radula of *Littorina littorea* in order of succession (*a—d*) from the radula sac to the mouth-cavity. Bethe's stain and eosin.

FIG. 10.—Portions of the radula of *Buccinum undatum* in order of succession (*a—d*) from the radula sac to the mouth-cavity. Bethe's stain and eosin.

FIG. 11.—A rough sketch of a strip of the radula of *Trochus ziziphinus* including the marginals and laterals of three rows of one side. Stained with carmalum.

FIG. 12.—Isolated teeth teased out of a radula of *Trochus ziziphinus* which had been previously stained with hæmatoxylin.

FIG. 13.—Eight teeth from two rows of the radula of *Helix aspersa*, including two centrals. Bethe's stain and eosin; surface view.

FIG. 14.—Two teeth and the underlying basal membrane of *Helix aspersa* in longitudinal section. Bethe's stain and safranin.

FIG. 15.—Three teeth and the underlying basal membrane of *Helix aspersa* in longitudinal section. Stained with safranin only.

Observations on Tooth-Development in Ornithorhynchus.

By

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With Plates 10—12.

(1) INTRODUCTION.

SINCE the appearance of Professor E. B. Poulton's first announcement (1) of his discovery of true teeth of mammalian type in a mammary-fœtal specimen of *Ornithorhynchus*, several papers have appeared dealing with the dentition of this animal. Only one of these (2), embodying a more detailed account, by Poulton himself, of his own investigations, concerns the developing teeth prior to their eruption. The extreme difficulty of procuring the necessary material has hitherto stood in the way of further research in this direction. The stage investigated by Poulton is accordingly the only one in which, so far as we are aware, there is any record of the non-erupted and immature dentition.

The observations herein set forth concern two specimens of mammary fœtus of *Ornithorhynchus*. One of these is practically identical in its grade of development with Poulton's

stage, or perhaps very slightly younger; the other belongs to a distinctly earlier period.

In this communication we do not propose to enter into any detailed discussion of the original literature dealing with the dentition of *Ornithorhynchus*. This, indeed, consists merely of Poulton's two papers (1, 2), and of papers by Mr. Oldfield Thomas (4) and Professor Charles Stewart (5), on the characters and condition of the erupted teeth in the adolescent animal. Such points as require attention will receive special notice in the course of the paper.

The material which Professor Poulton had at his disposal seems to have represented three distinct specimens from the collection of Professor W. K. Parker, but of these two were represented only in a fragmentary way. All the specimens would seem to have belonged to the same period of development.

In the stage described by him Poulton found that there were present in the upper jaw "three considerably developed and large teeth." These were already multicuspidate, with the principal cusps calcified. In addition to these, and immediately behind and to the inner side of the posterior one, he found another tooth-rudiment in a very early stage of development.

In the lower jaw he found corresponding tooth-structures, except that corresponding to the anterior tooth of the upper jaw. The actual presence or absence of the lower opponent of the latter could not be definitely ascertained owing to imperfection of the material, but its existence was inferred as highly probable. The presence of four teeth was thus regarded as established, with certainty in the upper jaw, and with great probability in the lower.

Subsequently Oldfield Thomas (4) and later Charles Stewart (5) described the appearance of the fully-developed teeth in the half-grown animal. From Stewart's description and figures it appears that three teeth are erupted in the lower jaw, of which the anterior two are very large and multicuspidate, whilst the posterior one is very much smaller.

The latter, it is clear, must derive its origin from that small, papillated enamel-organ which Poulton described and figured in his mammary-fœtal stage. The two large anterior teeth in Stewart's specimen are, of course, the same as the two large and partly calcified enamel-organs present in Professor Poulton's specimen. In the upper jaw Stewart found "a small, oval, soft papilliform elevation, which apparently corresponded with the most anterior of the three pairs of teeth found by Mr. Poulton," whilst the second and third very large fœtal teeth were represented by large multicuspidate teeth, like the two anterior teeth in the lower jaw, to which teeth they appear to correspond. On the other hand, in the upper jaw there appears to be no representative, in the adolescent animal, of the enamel-organ representing the fourth tooth stated by Poulton to be present "in a very early stage" or condition of development, and corresponding to the early papillated enamel-organ which he figures from the lower jaw.

Professor Stewart concludes that "the complete dental formula would, as far as at present known, be $\frac{3-3}{3-3}$, as surmised by Mr. Poulton." These last words are surely due to an oversight, for Professor Poulton clearly expresses the surmise that the complete dental formula should read $\frac{4-4}{4-4}$.

Nevertheless, the above analysis of Professor Stewart's contribution indicates that it is far from certain that the actual teeth of the upper jaw in the adolescent animal correspond, each to each, with the three teeth of the lower jaw. It is obviously rather improbable either that the large anterior tooth of the lower jaw should be the genuine opponent of the small anterior tooth of the upper jaw, or that the small posterior tooth of the lower jaw should, similarly, correspond to the very large tooth which is the posterior one in the upper jaw of the adolescent animal. And we have also to take account of Poulton's definite statement that he found behind the second large tooth of the upper jaw a tooth-rudiment in

a very early stage of development, and corresponding with the one he actually figures in the lower jaw.

From an investigation of the dentitional condition of a foetal *Ornithorhynchus* of a stage closely resembling that examined by Mr. Poulton, we are enabled, as we believe, to throw some additional light on the subject under consideration.

This specimen (designated as "Beta" in our collection) measured 250 mm. along the dorsal curvature of the body from the tip of the snout to the tip of the tail, and 107 mm. measured in a straight line from the vertex of the head to the dorsal convexity of the tail. This latter measurement is, however, practically worthless for comparison, since it depends on the acuteness of curvature of the specimen, and this is by no means constant. Poulton's specimen was stated to be 83 mm. long "in the curled-up attitude in which it had been received," fixed by the alcohol. From the published drawing of the specimen (3, Pl. XV *a*) we estimate its dorsal contour line from tip of snout to tip of tail to have been practically the same as our own, viz. 250 mm.

Our specimen was in excellent condition. Its head was removed and divided into two portions slightly to one side of the median plane by an incision which was not quite parallel to the latter. The major (left-hand) portion was imbedded in celloidin and decalcified in nitric alcohol. The smaller (right-hand) portion was also decalcified and imbedded in paraffin. Serial transverse sections from both sides of the head were thus available for examination.

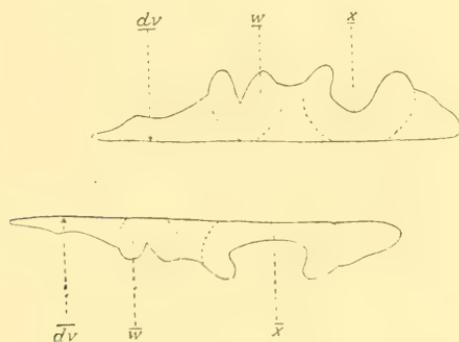
In addition to this specimen "Beta" we have also investigated the dentitional condition of a younger "mammary foetus," whose external characters were minutely described and figured by one of us a number of years ago (9). The dorsal contour-line of this specimen (catalogued as "Delta" in our collection) measured 80 mm. from tip of snout to tip of tail. Both sides of the head of this specimen have been examined in complete transverse sectional series. The sections from one side are thick, and were cut in celloidin and stained in picric-hæmatoxylin. Those from the other

side are thin (11μ) and were cut in paraffin and stained in borax-carminé.

(2) DESCRIPTION OF THE PHASE OF TOOTH-DEVELOPMENT SEEN IN THE YOUNGER SPECIMEN ("Delta").

The phase of tooth-development which we find to be manifested in our younger specimen is one of which no account has hitherto been available for Ornithorhynchus. It is characterised by the presence of a continuous dental lamina

TEXT-FIG. 1.



throughout a considerable extent of both jaws. The lamina begins, in the upper jaw, about 5.3 mm. behind the tip of the snout, and extends backwards for a distance of 2.6 mm. The lamina in the lower jaw begins about half a millimetre in front of the plane of its commencement in the upper jaw, and extends backwards to end at a distance of 2.8 mm. further back.

Text-fig. 1 represents a scheme of the organisation of the dental lamina in the two jaws at a magnification of about 18 diameters. From this it is evident that the lamina in each jaw shows two definite papillated enamel-organs. These are in the early stage at which practically the entire thickness of the lamina is involved in their production, prior to the

emancipation from them of a "residual dental lamina" or "Ersatzleiste" (Pl. 10, figs. 1, 2, 3).

The two enamel-organs in each jaw occupy very unequal lengths of the dental lamina. As shown in text-fig. 1, the anterior of the two enamel-organ ("w") is considerably less elongated antero-posteriorly than the one behind it ("x"). There is also a slight difference in height in favour of the posterior enamel-organ, but there is very little difference between their dimensions measured in the coronal plane—i. e. transversely to the length of the dental lamina. The form of the anterior enamel-organ further shows a difference from that of the posterior one. Its papilla is relatively more elongated and is more pointed than that of the posterior enamel-organ. This characteristic of the anterior tooth-germ is illustrated (for the lower jaw) in fig. 3. In this figure it will be seen that the "residual dental lamina" ("Ersatzleiste") is just beginning to emancipate itself, by further growth, from the medial aspect of the enamel-organ. In front of the anterior enamel-organ, in each jaw, the dental lamina is prolonged for a considerable distance as a narrow ridge of no great vertical height (text-fig. 1). In one place it exhibits a small localised thickening ("dv") lying about midway between the anterior enamel-organ and the plane of the anterior disappearance of the much reduced lamina. Apart from this slight bulbous thickening, the cross-section of this anterior segment of the dental lamina exhibits a slightly flask-shaped, though attenuated form, right up to its point of disappearance anteriorly. It therefore here lacks the broad and shallow character which one is accustomed to associate with the true and original anterior, or rostral, extremity of the dental lamina. Its actual form here is suggestive rather of the suppression of a formerly more extensive anterior segment of the lamina. Nor is this merely a surmise. For, in the course of this attenuated anterior segment of the lamina, lying in front of the more anterior of the two evident enamel-organs, we find a calcified vestigial toothlet. This is present in both jaws in our series of thin

sections from the left side of the head, stained in borax-carminc (Pl. 10, fig. 4). It is also well seen in the upper jaw in the hæmatoxylin-stained series from the opposite side (fig. 5). It is, however, only doubtfully present in the lower jaw of this series. Where it is visible the vestigial denticle in each case projects partly into the mouth-epithelium, partly into the labial aspect of the neck of the slender dental lamina, so that it is partially surrounded by the epithelial cells of these structures, which form a cap for its more superficial portion. Its deeper or root-portion is simply imbedded in the connective tissue. The dentine forms a shell partially enclosing a more irregularly arranged dentinal mass (fig. 4).

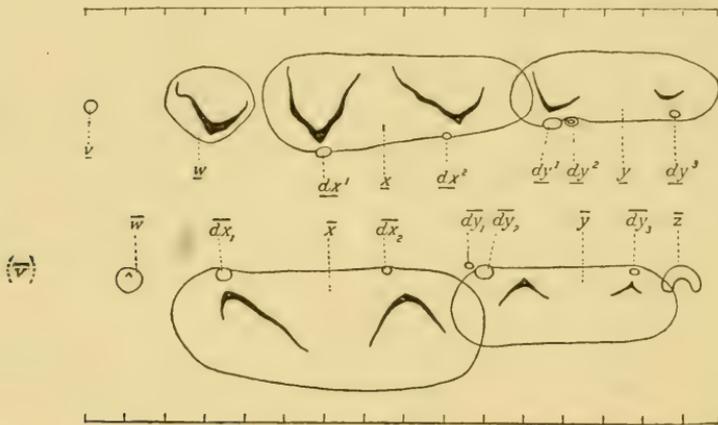
It is in relation with this vestigial tooth that the attenuated anterior segment of the dental lamina shows itself slightly enlarged and swollen ("kolbig") on cross-section. This somewhat bulbous character is not well illustrated in the sections reproduced, since it only reaches its maximum in the sections immediately in front of those figured. In the figures, however, there is some indication of a capsular arrangement of the connective tissue around the lamina. The enlargement of the lamina is too insignificant in amount to permit of its accurate proportional representation in the schematic text-fig. 1.

Behind the large posterior enamel-organ the dental lamina is continued backwards for some little distance as shown in the text-fig. 1. This posterior segment of the lamina is relatively plumper and more massive than the anterior segment, in accord with its more fertile and progressive character; for, as we shall subsequently show, this segment gives rise to one of the large teeth of the later stage. Already the advent of this tooth is heralded by a more localised bulbous swelling, which is illustrated, in the lower jaw in Fig. 2. This swelling shows a slight flattening of its fundus (though not in the section depicted) which represents the commencement of the process of cupping or papilla-formation. In these respects the condition is practically identical in both jaws of this specimen.

(3) DESCRIPTION OF THE PHASE OF TOOTH-DEVELOPMENT SEEN IN THE OLDER SPECIMEN "BETA."

In the specimen of our older stage (text-fig. 2) there are present in each jaw two very large enamel-organs, each with a number of calcified cusps with whose special arrangement we are not now concerned. These enamel-organs are without doubt those of the two large multicuspitate teeth which are

TEXT-FIG. 2.



found in the adolescent animal. Provisionally, and for the present purpose, we designate the more anterior of these as "x" and the posterior as "y" in the case of the upper teeth, and as "x" and "y" in the case of the lower (text-fig. 2¹). In front of "x" in the upper jaw is a very much smaller conical enamel-organ with one main, prominent, well-calcified cusp. This cusp lies above the anterior end of the large tooth "x" in the lower jaw, and we distinguish it by the symbol "w." Some distance in front of the lower tooth "x" we find a calcified tooth of very small dimensions which we regard as the true morphological opponent of "w" in the

¹ In this schematic figure no attempt has been made to indicate the dental lamina. Only the actual tooth-germs are shown. Magnification about 8 diam.

upper jaw, bearing in mind the fact that in both of our stages the entire dental lamina and its derivatives in the lower jaw are uniformly in advance, as regards position, of the corresponding structures in the upper. This small tooth, then, we indicate by the symbol "w." Now, some distance in front of the tooth "w" in the upper jaw the deeper portion of the dental lamina shows a small and rudimentary, but distinctly papillated, enamel-organ. This we designate as "v." It lies a short distance in front of the plane of "w" of the lower jaw. No trace of any lower opponent to "v" is to be found. It is, however, obvious that the anterior region of the dental lamina in *Ornithorhynchus* is that which is most under the influence of those factors which have brought about the special modification of the mouth and jaws. It is, accordingly, in this region that we might expect to meet with evidences of suppression. And since, as we have already pointed out, the dental lamina in the lower jaw is topographically in advance of that of the upper jaw, it is not surprising that no trace of a lower "v" is to be met with in this relatively late stage.

Passing now to the posterior region of the dental lamina of our older specimen, we find that the posterior extremity of the tooth "y" projects behind the plane of its opponent "y." Behind the latter the dental lamina shows a small, but well-developed, papillated enamel-organ, uncalcified, and just beginning to emancipate itself from the residual dental lamina. This enamel-organ we here designate as "z." Opposite to this in the upper jaw we still find the hinder extremity of "y." When the posterior end of the latter is reached, the upper dental lamina continues for a short distance (0.15 mm.) as a thick and bulky structure and then suddenly stops without showing any definite differentiation into an enamel-organ. This terminal thickened portion of the lamina may, however, be regarded as the anlage of a potential upper "z," and Poulton would appear to have found it actually papillated, although it apparently never erupts.

- (4) SYNTHETIC COMPARISON OF THE CONDITIONS MET WITH IN THE STAGE OF TOOTH-DEVELOPMENT LAST DESCRIBED, (a) WITH THE ADOLESCENT CONDITION, AND (b) WITH THE EARLIER "FŒTAL" STAGE.

(a) With the adolescent condition.

When we compare the condition above described with that of the adolescent animal as described by Oldfield Thomas and Stewart, it is evident that in the upper jaw three of the tooth-representatives which we have referred to undergo eruption, viz. our "w," "x," and "y." In the lower jaw also three teeth cut the gum, but these are our "x," "y," and "z."

- (b) With the earlier foetal stage (i.e. our specimen "Delta").

When we now institute a comparison of the condition we have described as occurring in our older stage with that met with in the younger of our two specimens, the conclusions which we are disposed to draw are not those which at first suggest themselves, and which for a time we actually entertained.

It has been shown that in the younger specimen "Delta" there are two well-developed enamel-organs present in each jaw, but these are of widely different dimensions, the posterior being much more elongated than the anterior.

Moreover, the shape of the anterior is different from that of the posterior, its papilla, as seen in coronal section, being more slender and pointed.

In the later foetal stage "Beta," on the other hand, the more anterior ("x" and "x") of the two large enamel-organs in each jaw are considerably more elongated than the posterior ("y" and "y"). For this and other reasons we

are constrained to regard the relatively elongated enamel-organs which are posterior in both jaws of the younger stage as the representatives, not of the teeth "y" and "y," but of "x" and "x" respectively in the jaws of the older stage, "Beta." It will be observed that both in the younger and in the older "x" is substantially more elongated than "x," and is also situated in a somewhat more advanced position.

The above identification of "x" in the younger specimen necessitates the assumption that, between this stage and the older one there has occurred a tolerably rapid growth and differentiation of the posterior portions of the dental laminae of the younger stage (*v.* text-fig. 1). This is precisely what one might expect to take place during the process of differentiation of a molar series; and it has already been shown that, not only is the hinder segment of the dental lamina in the earlier stage relatively plump and well-developed, but that there is actually present in it the early rudiment of an actual posterior tooth. Further, it may be stated that the increase in absolute length of the head which has occurred in the later stage is very considerable, allowing of marked elongation of the posterior region of the jaw and of the molar lamina. And, in fact, comparative measurements show that the total length of the lamina in the older stage is not merely absolutely but relatively greater than in the younger.

Comparison of the two schemes in text-figs. 1 and 2 (which, though schematic, are nevertheless drawn to scale) will show that in the older specimen the enamel-organ "x" is not only larger than its upper opponent, but is actually the largest tooth-rudiment in either of the jaws. On the other hand, the lower anterior enamel organ, "w," in the younger specimen, is appreciably smaller than its upper opponent, "w," and this early inferiority of "w" already foreshadows the marked reduction of "w," as compared with "w," in the older foetal specimen, as well as its eventual entire absence in the adolescent animal.

For these reasons we have no hesitation in identifying the anterior of the two prominent papillated enamel-organs in the upper and lower jaws of our younger specimen with those developing teeth in the older which we have marked as "w" and "w" respectively.

In the course of our description of the older stage we have noted the presence of a small cupped and somewhat imperfectly formed enamel-organ in front of the conical calcified tooth "w." The enamel-organ in question is quite deeply placed and is obviously in series with the more fully developed teeth behind it. We have designated it as "v." Again, in the younger specimen we have shown that there is present, in the precisely corresponding coronal plane, a small and already strongly calcified vestigial tooth (figs. 4 and 5). This actually indents the deep aspect of the mouth epithelium at the labial side of the attachment to the latter of the dental lamina. This, therefore, cannot be the enamel-organ "v" of the older specimen. But we have also seen (p. 143) that, in the immediate neighbourhood of this vestigial tooth, the dental lamina is somewhat enlarged and bulbous. It seems tolerably evident that this bulbous enlargement of the adjacent part of the dental lamina must be the genuine representative of the later enamel-organ ("v") (text-fig. 2). We, therefore, hold that this latter tooth-rudiment, "v," must be regarded as the true morphological successor to the small calcified vestigial tooth which is present in our smaller specimen. The latter vestigial tooth we accordingly indicate by the symbol "dv," as being the deciduous predecessor of the enamel-organ "v" of the later stage.

In the lower jaw of the older specimen no representative of a lower "v" was met with. But in the younger, as we have already stated, there is found, on one side certainly, and more doubtfully on the other, a structure which, in its situation and its relations to other structures, exactly corresponds to the small calcified upper tooth "dv," and thus represents a lower "dv." In the neighbourhood of this vestigial tooth-element the dental lamina is slightly enlarged,

but, as we have just seen, no more mature product of this enlargement appears in the later stage of development. This is doubtless to be accounted for by the advanced position in the jaw occupied by the tooth-germ in question.

If we now take account of the combined conditions set forth in the schematic text-figs. 1 and 2, it will be evident that they tend to establish the view that representatives of five quasi-permanent teeth are developed in each jaw during the phases of tooth-development under consideration. These include the recognised teeth of the adolescent animal. (The most posterior member of the series in the upper jaw ("z") is not indicated in the scheme.) In addition to these there have also been indicated vestigial representatives of deciduous predecessors to the most anterior of these five tooth-elements.

We shall presently adduce evidence to prove that the deciduous vestiges already described are not the sole representatives of an earlier tooth-generation occurring in our specimens. Meanwhile we may observe that the dentional characters above set forth seem to afford evidence of the operation of factors which determine early suppression and abortion of the more anterior segment of the dental lamina and its derivatives in Ornithorhynchus.

(5) CONCERNING THE EPITHELIAL NODULES FORMERLY DESCRIBED BY PROFESSOR POULTON IN CONNECTION WITH THE ENAMEL-ORGANS OF ORNITHORHYNCHUS.

In the course of the account given by Professor Poulton of the developing teeth in Ornithorhynchus, he described a number of epithelial nodules situated "almost immediately over the apex of each calcified cusp of the second and third tooth." He states that "nothing of the kind could be made out in the case of the first upper tooth" (i. e. our tooth "w"). In these nodules he found that "the inner cells appear to be corneous and collected into a dense central mass, between which and the outer fusiform cells is a space containing

loosely packed cells resembling the former in character." Their position was "at the extreme edge of the stellate reticulum," and from his figures they all appear to lie inside the enamel-organ as defined by its outer epithelial layer. "In some cases," he notes, "there was the appearance of an epithelial cylinder extending from the nodule towards, or perhaps reaching the stratum intermedium or enamel cells over the apex of the cusp." Further, there was always a nodule above each of the chief cusps, while they were never found elsewhere.

We have found the same nodular structures to be present in our older specimen "Beta," which nearly corresponds with the specimens which formed the subject of Poulton's investigation, and we are therefore in a position to supplement his account of these interesting structures.

(1) Relation of the nodules to the enamel-organs.—In the first place, we find that, although the nodules may appear to be included within the large enamel-organs, they are really morphologically outside of them. They have originally lain in contact with the exterior of the enamel-organ and have been gradually enveloped through the relatively enormous expansion of the latter. In many sections the inclusion may seem to be complete, and, indeed, is so, so far as such sections are concerned. But when the series of neighbouring sections is carefully examined, we find that an opening or depression in the surface of the enviroing enamel-organ is discoverable, through which the engulfed nodule is still in touch with the connective tissue of the tooth-sac. In certain sections, indeed, the nodule appears to lie in a comparatively shallow recess or bay in the surface of the enamel-organ (Pl. 11, figs. 6 and 7). In other cases it is difficult to be quite certain that such a communication with the exterior as we have described is demonstrable. But in no case is it indubitably absent, and its undoubted presence in other cases is presumptive evidence that the condition in all is originally identical.

We also have observed the appearance of epithelial cylinders

or strands (fig. 13) in relation to the tips of cusps in the vicinity of nodules in the position indicated by Poulton. We find, however, that where they are present their connection with the tip of the cusp is the constant and characteristic relation, whilst their relation to the concentric epithelial nodule, if the latter be present, is inconstant and variable. We find (*a*) that there may be a cylindrical or strand-like prolongation from the tip of a cusp, either inner or outer, without any nodule being present in relation with it; and (*b*) such an epithelial strand is absent in some cases in which a nodule is present in the vicinity of the apex of a cusp. Thus there are no epithelial cylinders in connection with the cusps to which the nodules marked "dx¹" and "dx²" in text-fig. 2 are related. Nor has the presence or absence of the epithelial strand anything to do with the extent to which the nodule is imbedded in or recessed into the main enamel-organ; in the case of "dx¹" the nodule lies largely outside of the enamel-organ in a very shallow bay of its surface-contour (figs. 6 and 7). On the other hand, the nodule "dx²" is largely surrounded by the tissue of the main enamel-organ, but here, again, no epithelial cylinder is present. We therefore believe that the relation of the epithelial cylinder to the nodule is a mere accident of the common relationship which both these structures seem to possess to the prominence of the cusp. But while the relationship between the nodule and the point of the cusp is not necessarily an intimate one, as text-fig. 2 will show, the relationship between the point of the cusp and the epithelial cylinder is fundamental. The cylinder appears to be, in fact, nothing but a prolongation of the inner enamel-epithelium of the apex of the cusp, accompanied by a sheath, composed of those cells of the stratum intermedium which form a more condensed layer in contact with the inner enamel-epithelium. In certain cases it is true that the epithelial strand so constituted does appear to reach and come in contact with the outer shell of the concentric nodule, but this relationship is not an invariable one, and in all probability is of no essential significance. In fig. 13 there is reproduced

a photomicrograph of one of the epithelial strands, in which its real nature as a prolongation of the epithelium of the tip of the cusp is manifest. High-power examination has also shown us, in the case of a few sections through such a strand, that the inner enamel-epithelium is actually prolonged in the core of the cylinder in the form of two closely-applied layers of cells. Between these layers a line, indicative of a potential lumen, is just recognisable, and we regard the cylinder as an abortive prolongation of the apical part of the pulp-cavity, within which pulp-cells have failed to penetrate, or have wholly disappeared, so that neither dentine nor odontoblasts have been differentiated within it. The epithelial cylinders thus represent, in our view, portions of the cusps which have undergone ontogenetic reduction.

(2) Position and arrangement of nodules with reference to cusps.—Poulton states that there was always a nodule over the apex of each of the chief cusps of the two large teeth, while they were never found elsewhere. We likewise find a nodule in relation with each of the two chief cusps of these teeth—i. e., over the series of internal cusps in the upper, and of the external cusps in the lower jaw (text-fig. 2).

The photomicrographs herewith reproduced in figs. 7, 8, and 10 illustrate the topographical relationship of several of the chief cusps to their corresponding nodules as seen in the section-series from one side of our specimen "Beta." In figs. 8 and 10 the nodules appear to lie inside the enamel-organ, whilst in fig. 7, as already stated above, it only partially indents the superficial aspect of the enamel-organ (*cf.* also fig. 6 from opposite side).

It will further appear from an examination of text-fig. 2, which also illustrates the condition met with on the same side of the head, that in the case of the anterior chief cusp of the enamel-organ "y," there are two nodular structures present in the vicinity of the cusp. The more anterior of these is seen in the photomicrograph in fig. 8. It presents the typical characters of the nodules as described by Poulton and as we

find them exemplified in the majority of instances (*cf.* fig. 12). The more posterior of the two nodular structures, however, differs in a highly significant manner from the general type of nodule. Its structural characters are well shown in the photomicrographs in figs. 9 and 11. It will be seen that within a concentrically arranged connective-tissue capsule there is a pale zone, which on high-power examination (fig. 11) shows radially arranged cells identical with those of the internal epithelium of the main enamel-organ as shown in fig. 9. Within this epithelium is a deeply stained ring of dentine, and within this, again, is a connective-tissue core or pulp. We have, therefore, here present all the essential structures of a typical tooth, and there can be no hesitation in regarding it as a vestigial tooth. Yet in its position and relations to the enamel-organ, whose superficial aspect it slightly indents, it exactly resembles the concentric epithelial nodules with which it is in series (*cf.* figs. 8 and 9, and 11 and 12).

In the case of the lower teeth (text-fig. 2) quite similar relations obtain in respect of the presence of epithelial nodules in the vicinity of the principal cusps, which are in this case the external cusp series. Here, again, there are two nodules which are more or less in the neighbourhood of the anterior chief cusp of "y." The more posterior of these is placed in advance of the prominence of the cusp with which it is presumably associated. In fact, it lies just in front of the anterior limit of calcification of the cusp, instead of over its prominence. The second and more anteriorly placed nodule in this region is much smaller and more superficially placed, and it lies wholly in front of the calcified anterior cusp of the tooth "y." Indeed, so far as its position is concerned it is placed rather in a vertical relation to the hinder portion of the enamel-organ of the more anterior tooth "x," though behind the plane of the posterior calcified external cusp of the latter. The idea of its possible relation to the tooth "x" is further supported by the fact that it lies more labially than the larger nodule behind it, which is related to the anterior

cusps of "y," the anterior end of the enamel-organ of "y" overlapping the hinder end of "x" on its mesial aspect.

In other respects the character and position of the nodules in the lower jaw require no further illustration than that which is afforded by text-fig. 2.

That the above arrangements are not merely fortuitous is further testified to by the examination of the section-series from the opposite side of the head of the same specimen. The condition there existing is practically identical with that set forth in text-fig. 2. The only noteworthy deviations from this scheme are: (a) that the well-calcified vestigial toothlet in the upper jaw, illustrated in figs. 9 and 11 (and which we have indicated by the symbol "dy²" in the scheme in text-fig. 2), is on this side represented by a concentric epithelial nodule of precisely similar character to the other nodules with which it is in series, and (b) that the anterior of the two nodules ("dy¹"), which in text-fig. 2 are seen to lie opposite the adjacent ends of the two large lower enamel-organs "x" and "y," is absent on this side. It has already been stated that it is comparatively small and insignificant on the other side, so that its absence on the side now under consideration is the less surprising.

(3) The morphological character of the nodules.—From the foregoing account it will already be apparent that we regard the series of epithelial nodules as constituting a series of vestigial representatives of an earlier tooth generation. That they must be so regarded has already been surmised by Marett-Tims (7) on the basis of Poulton's original account of them. Tims was apparently led to this interpretation of the structure in question through his own observations (6) of the presence of corresponding structures in connection with the dentition of other mammals, structures which seemed to him to demand a like explanation.

The evidences in favour of such an interpretation may be summarised as follows:

(a) The nodules are not fortuitously distributed, but form a more or less regular series both as regards number and posi-

tion in both jaws and on both sides of the jaw in our older specimen "Beta." They would appear to have had a closely similar arrangement in Poulton's specimen.

(b) In our specimen the typical concentric epithelial nodules are in series with the undoubted vestigial toothlet which we designate as "dy²"; and on one side of the upper jaw "dy²" is represented by a typical concentric epithelial nodule.

(c) Some of the typical nodules, though devoid of dentine, show traces of other of the elementary tooth constituents. Thus the nodule which we designate as "dy¹" shows traces of a stellate enamel-epithelium around the condensed concentric laminae of epithelium; and several of the nodules exhibit in their central core a collection of deeply-staining nuclei similar to those which form the obvious pulp of the genuine denticle "dy²" (r. fig. 12, illustrating the structure of a typical nodule).

(d) The nodules appear to be identical in character with those present in various other mammals which Tims has shown to require, in all probability, a like interpretation.

(4) Serial homology of the nodules with the vestigial teeth "dv" and "dv" present in the younger specimen "Delta."—In the course of our description of the younger of our two specimens, "Delta," we have demonstrated the presence (figs. 4 and 5) of a vestigial tooth, "dv," in connection with the segment of the dental lamina lying in front of the more anterior of the two large enamel-organs there present (i. e. our tooth "w"). We have previously given our reasons for regarding this vestigial toothlet, "dv," as belonging to an earlier tooth generation from that to which the more posteriorly placed enamel-organs "w" and "x" belong. It now only remains to indicate that in our opinion the strongly and precociously calcified degenerative toothlet, "dv," is in series with, and homologous to, the nodular vestiges present in the older specimen in relation with the teeth "x" and "y." The successor, "v," of the vestigial "dv" is represented in the younger specimen by a swelling of the dental lamina which,

in the older specimen, has taken shape as the small and still uncalcified enamel organ shown in text-fig. 2 as "v," in front of the calcified tooth "w."

The view of the homology of the small vestigial tooth "dv" of the younger specimen with the nodular series of the older is further strengthened by the fact that its lower opponent, "dv," is in a more advanced stage of involution than upper "dv." It lacks the definite dentinal character of the latter, its structural constituents show concentric lamination, and altogether its appearance is strongly suggestive of the structure of the nodules of the older stage.

(5) Origin of the nodules.—It might have been expected that the possession of a younger stage for comparison with that represented by our specimen "Beta" would have enabled us to elucidate the mode of origin, or at least some definite part of the early history of the nodular vestiges, since these are obviously in process of involution in the older stage. The two stages, however, prove to be too remote from one another to yield positive and conclusive evidence as to the mode of origin of the nodules. A priori one might have expected to find the vestigial teeth represented in the younger specimen by precocious enamel-organs. But although the large permanent teeth "y" have not yet come into existence as distinct enamel-organs, and both "w" and "x" are in the condition of well-developed enamel-organs, there are no definite enamel-organs to represent the early phase of the nodular vestiges of the later stage.

Nevertheless we find that there are present, at intervals along the labial aspect of the dental lamina, a succession of peculiar structural differentiations which there is small room for doubting to be the beginnings of the nodular structures. These structural differentiations are of the nature of a series of invasions or deep indentations of the neck of the dental lamina, on its labial aspect, near the level of its continuity with the deep surface of the mouth-epithelium. They occur

only in those regions of the lamina which constitute the enamel-organs of the future teeth. They therefore appear to be appended to the enamel-organs in question. The later relationship of the actual nodules to the main cusps of the teeth is evidence that the early relation of these precursors of the nodules to the enamel-organs is not a chance one.

In fig. 1 there is reproduced a photomicrograph of a section through the enamel-organ of the upper tooth "x." Here there is seen a typical example of the structural arrangement of one of these early "nodular" differentiations. Imbedded in the neck of the dental lamina, at its junction with the enamel-organ, is a small group of cells occupying an otherwise clear central area. This is surrounded by a zone of enamel-epithelial cells, which show signs of differentiation into layers, and the entire mass appears as an appendage of the main enamel-organ. The group of central cells is mesodermal, and is in the next section seen to be continuous with the cellular tissue of the surrounding tooth-sac.

Another example of the structures in question, less striking but essentially identical, taken from the opposite side of the jaw, is represented in fig. 2. Here the continuity of the mesodermal core of the rudimentary nodule with the tooth-sac tissue is more obvious, but even here it is nearly surrounded by enamel-cells. It is plain that were the included pulp-cells seen in fig. 1 to assume an odontoblastic function there would result just such a shell of dentine as we figure in fig. 11 from our older stage, whilst the surrounding enamel-cells would account for the outer concentric layers of the fully-developed nodules.

Since no differentiation of the future cusps has occurred at the stage under consideration, no reference to the later condition in this respect is relevant. And, since the enamel-organ of the future tooth " $\frac{\text{y}}{\text{y}}$ " has not yet come into existence as such, posteriorly, the rudimentary stage of the interesting nodules connected with the upper tooth "y" is likewise in abeyance.

In connection with the tooth $\frac{“x”}{“x”}$, in the older specimen “Beta” we have seen that two nodules are present, “dx¹” and “dx²,” and that there was a possibility that the anterior of the three nodules in relation with the enamel-organ of the lower tooth “y” is really related to “x.” There is not the slightest doubt that there are multiple rudimentary nodules in connection with the large enamel-organ of $\frac{“x”}{“x”}$, in the younger specimen. But whether these are two or three in number it is difficult to say. In the upper jaw we believe that there are three, but the most posterior is at the hinder extremity of the enamel-organ of “x,” behind which we have the thick dental lamina from which “y” subsequently arises, so that we are unable to entirely exclude the possibility that the last rudimentary nodule may really belong to “y.” We incline to the contrary opinion. There is abundant evidence of the entire disappearance of some of the nodular rudiments originally present in the fact that in the younger specimen there appears to be two such differentiations of typical character present in relation with the enamel-organ of the tooth $\frac{“w”}{“w”}$, whilst this tooth in the latter stage is, as noted by Poulton, destitute of any vestigial nodule.

(6) GENERAL DISCUSSION OF TOOTH-HOMOLOGY IN ORNITHORHYNCHUS.

The facts set forth in the foregoing account seem to establish the existence in Ornithorhynchus of teeth belonging to at least two dentitional series. In the series to which the large multicuspidate teeth of the adolescent animal belong we believe that we are justified in reckoning five members. In the case of the upper jaw we lack positive evidence of the actual formation of the most posterior member of the series, and in the lower jaw, again, we believe that we are justified in supposing that the most anterior member has undergone

suppression. Of the five teeth which we take to be the full complement of members of the quasi-permanent series of which we have evidence, we hold the first and second $\frac{\text{“v”}}{\text{(v)}}$ and $\frac{\text{“w”}}{\text{“w”}}$ to correspond to premolars. This judgment is based largely upon the relative size and simplicity of the tooth $\frac{\text{“w”}}{\text{“w”}}$, which is the best developed of these (?) premolars, in both the earlier and later stages at our disposal. The proportions may be judged of by the schemes in text-figs. 1 and 2, and the form by a comparison of fig. 3 with figs. 1 and 2.

If the tooth $\frac{\text{“w”}}{\text{“w”}}$ be premolar, then the molar formula may be expressed, in accordance with our views and nomenclature, as $\frac{\text{“x - y - (z)”}}{\text{“x - y - z”}}$. There can be no hesitation in identifying these teeth as molar.

But if we are also right in our recognition of the “nodular” series, including the obvious denticle $\frac{\text{“dy}^2\text{”}}{\text{“dy}^2\text{”}}$ as vestigial teeth in series with the undoubted vestigial teeth $\frac{\text{“dv”}}{\text{“dv”}}$, then we have before us in Ornithorhynchus a demonstration of the presence of a whole series of precursors of the molar teeth. And it is not the least remarkable aspect of this demonstration that it exhibits these vestigial deciduous predecessors of the large molar teeth in the form of a much more numerous series of simple tooth-rudiments, each on the whole corresponding with one of the cusps of their multicuspitate molariform successors. The admission that there are exceptions to this general correspondence of deciduous nodule to permanent cusp, as seen in text-fig. 2, can hardly succeed in invalidating the idea of a deep-seated and definite correspondence which is otherwise suggested by the facts. It is not very difficult to imagine some explanation of the duplicity of the nodules $\frac{\text{“dy}^1 - \text{dy}^2\text{”}}{\text{“dy}^1 - \text{dy}^2\text{”}}$ on the lines of a theory of the

suppression or other modification of cusps. It is to be remembered that it is only the series of primary cusps which appear to have nodular correspondents.

It must also here be recalled that the examination of the younger specimen "Delta" has shown us that the early differentiation of nodules is not confined to the region to be occupied by molar cusps. Not only is there a vestigial quasi-nodular predecessor, "dv," to the enamel-organ "v," but there are one or perhaps two rudimentary labial nodular structures in relation to the neck of the enamel-organ "w," which disappear before the stage of specimen "Beta" is reached.

It cannot be denied that these facts tend towards the establishment of a doctrine which may be regarded as coming under the category of concrescence theories. In some sense or other it would appear that the molariform successors of the deciduous vestigial series represent compound structures corresponding to two or three of their simple (probably homodont) predecessors. It is not necessary to suppose that any ontogenetic fusion occurs. The mode of development of the successional molars in our younger stage is decisive against the occurrence of any fusion process. But the relation of the two series in the molar region cannot but be regarded as suggestive of some sort of phylogenetic substitution of a small number of compound teeth for a large number of simple teeth—a process which must be reckoned as covering the fundamental idea of concrescence.

It might well be accepted that a given segment of the dental lamina, whose constitutive material gave origin in one phylogenetic phase to a discrete series of simple teeth, might, in another and later one, lose its discrete or particulate character. On such a supposition the same morphological material would in its later history be concerned in the production of a larger composite structure within whose limits the individual elements of the pre-existing series might be only imperfectly preserved or represented.

An interpretation, practically identical with ours, of the concentric epithelial nodules and of their relations to, and significance for, the molar dentition in *Ornithorhynchus*, has been repeatedly advanced by Marett Tims (7). Thus on p. 135 of his memoir on "The Evolution of the teeth in the Mammalia" he says: "In the concentric epithelial bodies of *Cavia*, *Canis*, *Gymnura*, and *Ornithorhynchus* we have, I believe, the last traces of a vanishing dentition which must have preceded the cheek-teeth on account of their labial position. These bodies remain quite distinct from the teeth themselves and show no tendency to become fused." Again, in reference to the more general question of molar fusion he states (*ibid.*) that "an antero-posterior fusion of the teeth of the same dentition appears to me now to be the only solution of the difficulty in accounting for the duplex condition of the true molars of the greater number of mammals and of the complex cheek-teeth of the rodents and fossil multi-tuberculata. The repetition, so to say, of the development of the anterior and posterior halves of the rodent molars seems to me to render this highly probable, though I have not yet seen any actual fusion of enamel-germs. It may quite well be that this early stage may have become slurred over in the recapitulatory history, until it is entirely lost at the present day. Possibly the same may be true of the ungulates and proboscideans."

So far as *Ornithorhynchus* is concerned, this writer had only the data supplied by Poulton on which to base his interpretation, and his judgment must have been determined mainly by his experience of the occurrence of similar concentric epithelial bodies in the other forms specified. It is, therefore, noteworthy that with our wider opportunities of experience of the condition in the monotreme form we have been led to conclusions which do not differ in any important respect from those arrived at by Marett Tims. That view, then, which for him could not in the nature of the case be more than a surmise, as regards *Ornithorhynchus*, has through our observations received more or less definite confirmation.

In this connection we may note that we cannot now admit the validity of the further surmise of the same writer that the concentric epithelial cell-nests formerly described by us as occurring in *Perameles* are of the same order or significance as those we describe and figure in *Ornithorhynchus*. In our previous paper (8, p. 518) we have ourselves expressed the opinion, subsequently endorsed by Tims, as to their resemblance to the nodules of *Ornithorhynchus*. This, however, was prior to our study of the latter. We are now perfectly confident that the cell-nests we encountered in *Perameles* belong to a totally different category of structural differentiation. These latter are purely epithelial degeneration products formed in, or in connection with, the mouth epithelium overlying teeth which are about to undergo eruption; they are quite irregular in their occurrence and in their arrangement; they make their appearance, for the first time, in comparatively late stages of tooth-development—i. e. shortly before eruption, and they contain at no time any trace of structural elements save the concentrically arranged epithelial cells themselves, having no elements referable to an origin from the mesoderm as is the case with the genuine “nodular” structures in *Ornithorhynchus*. They are, in fact, entirely similar to the epithelial “pearls” which are met with in various situations, as, for example, in the neighbourhood of the median raphé of the palate; whereas we have shown that the nodules in *Ornithorhynchus* when fully developed show obvious traces of the typical structure of an enamel-organ.

A further conclusion from the view of tooth-development and tooth-homology in *Ornithorhynchus* set forth by us is that no support is to be derived from it for any theory which would seek to establish a serial distinction between the true molar teeth and those which appear, *prima facie*, to belong to the same series in front of them. It is quite true that the suppression of the antemolar teeth has proceeded so far that it would be rash to base any theory of serial homologies between molars and antemolars on the condition obtaining in *Ornithorhynchus*. All that we here affirm is that, so far

as they go, the facts rather point in the direction of serial continuity between the teeth of the molar series and their putative serial companions in front, meagrely represented in our older specimen "Beta" by the teeth $\frac{v-w}{(v)-w}$.

Finally, in several of the figures illustrative of the condition in the older specimen (figs. 8, 9, 10) it will be observed that a tolerably substantial "residual dental lamina" remains preserved along the lingual side of the large molar enamel-organs. We have not so far had an opportunity of observing the subsequent fate of this "residual lamina," but there is no reason for believing that it differs in any way as regards its destiny from the residual lamina so often found in a similar situation beside the developing molars of other mammals. Its presence here, therefore, need not be regarded as expressive of any special significance beyond that of representing the formal potentiality of "post-permanent" molar successors, the possibility of the occurrence of which cannot be excluded from the ordinary mammalian scheme of tooth-genesis.

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EXPLANATION OF PLATES 10—12,

Illustrating Professors Wilson and Hill’s paper, “Observations on Tooth-Development in *Ornithorhynchus*.”

All the figures are from photomicrographs of transverse sections. The symbol \div indicates the labial side.

FIG. 1.—Delta. Section of the enamel organ and dermal papilla of tooth “x” of the upper jaw, right side, with nodule rudiment on the labial side of neck of enamel organ. $\times 78$.

FIG. 2.—Delta. Tooth “x” of left side, upper jaw, also with a labially-situated nodule rudiment. In the lower jaw the dental lamina is swollen club-like to form the rudiment of the lower tooth “y.” $\times 78$.

FIG. 3.—Delta. Tooth “w” of lower jaw, right side. Note the narrow pointed character of the dermal papilla as seen in cross-section and compare with that of “x” as seen in figs. 1 and 2. On the lingual side the residual dental lamina is commencing to free itself from the enamel organ. $\times 78$.

FIG. 4.—Delta. Section through the dental lamina of the upper jaw, left side, about mid-way between its anterior extremity and the enamel organ “w,” showing the rudimentary toothlet “dv” indenting the oral epithelium to the labial side of the attachment of the lamina. $\times 175$.

FIG. 5.—Delta. Corresponding section through the vestigial toothlet “dv” on the right side of the upper jaw. $\times 175$.

FIG. 6.—Beta. Section through the upper tooth “x” passing through its main antero-internal cusp and the nodule “dx¹” related thereto. $\times 78$.

FIG. 7.—Beta. Corresponding section showing the tooth “x” and the nodule “dx¹” of the opposite side of the head. $\times 78$.

FIG. 8.—Beta. Section through the antero-internal cusp, upper tooth-let “y,” and its related nodule “dy¹.” $\times 78$.

FIG. 9.—Beta. Section shortly behind the preceding, showing the toothlet “dy²” underlying the posterior region of the antero-internal cusp of upper “y,” and well to the labial side of the residual dental lamina. The toothlet lies directly behind and in series with the nodule “dy¹” (cf. text-figure 2). $\times 78$.

FIG. 10.—Beta. Section through the postero-internal cusp of upper tooth "y," showing the nodule "dy³" apparently imbedded in the stellate tissue of the enamel organ to the labial side of the massive residual dental lamina. $\times 78$.

FIG. 11.—Beta. High-power view of the toothlet "dy²" showing its central connective-tissue core or pulp enclosed by a distinct ring of dentine, a distinct layer of columnar cells representing the enamel epithelium and a peripheral capsule of concentrically arranged connective tissue. $\times 345$.

FIG. 12.—Beta. Section through the nodule "dx²" to illustrate nodular structure. Note the central cellular core enclosed by concentrically arranged flattened cells forming a compact zone. $\times 216$.

FIG. 13.—Beta. Section through an epithelial strand to demonstrate its continuity with the enamel epithelium covering the apex of the cusp. $\times 345$.

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CONTENTS OF No. 202.—New Series.

MEMOIRS:

	PAGE
The Origin and Nature of the Green Cells of <i>Convolvula roseoffensis</i> . By FREDERICK KEEBLE, M.A., Sc.D., University College, Reading, and F. W. GAMBLE, D.Sc., Manchester University. (With Plates 13 and 14)	167
On the Development of the Plumes in Buds of <i>Cephalodiscus</i> . By W. G. RIDWOOD, D.Sc., Lecturer on Biology at St. Mary's Medical School, University of London. (With 11 Text-figures)	221
On the Structure of <i>Ænigma ænigmatica</i> , Chemnitz; a Contribution to our Knowledge of the Anomiacea. By GILBERT C. BOURNE, M.A., D.Sc., F.L.S., Fellow of Merton College; Linacre Professor of Comparative Anatomy in the University of Oxford. (With Plates 15—17, and 2 Text-figures)	253
On the Chromatin Masses of <i>Piroplasma bigeminum</i> (<i>Babesia bovis</i>), the Parasite of Texas Cattle-Fever. By H. B. FANTHAM, B.Sc.Lond., A.R.C.S., University College, London, and St. Mary's Hospital Medical School. (With Plate 18, and 44 Text-figures)	297
The Skin, Hair, and Reproductive Organs of <i>Notoryctes</i> . Contributions to our Knowledge of the Anatomy of <i>Notoryctes typhlops</i> , Stirling.—Parts IV and V. By GEORGINA SWEET, D.Sc., Melbourne University. (With Plates 19 and 20, and a Text-figure)	325
<i>Parorchis acanthus</i> , the Type of a new Genus of Trematodes. By WILLIAM NICOLL, M.A., B.Sc., Gatty Marine Laboratory, St. Andrews. (With Plate 21)	345

The Origin and Nature of the Green Cells of *Convoluta roscoffensis*.

By

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and

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With Plates 13 and 14.

CONTENTS.

	PAGE
Section I. Introduction	167
„ II. Proof of the Origin of the Green Cells by Infection	172
„ III. The Isolation of the Infecting Organism and the Synthesis of the Green <i>Convoluta</i>	179
„ IV. The Life-history of the Infecting Organism	182
„ V. The Normal Course of Infection	191
„ VI. The Significance and the Consequences of the Association of Animal and Green Cell	199
„ VII. General Summary	208
Tables I—VI	210
Literature	216
Explanation of Plates	217

SECTION I. INTRODUCTION.

XANTHELLÆ and Chlorellæ are widely distributed among the members of the basal groups of the animal kingdom. In

some animals they are present constantly, in others they occur sporadically.

Green, yellow, or brown cells have been described in representatives of every division of free-living Protozoa, in certain sponges, in most anthozoan Cœlenterates, in a few Hydrozoa and Scyphozoa, and in acœlous and rhabdocœlous Turbellaria. Their occurrence in the higher groups is rare—e. g. *Zoobothrium* (Polyzoa), *Elysia* (Mollusca), and *Echinocardium* (Echinoderms).

The association is obligate in *Convoluta paradoxa*, *C. roscoffensis*, and in *Hydra viridis*; it is facultative in the Protozoa, Anthozoa, and rhabdocœl Turbellaria.

In the former cases every individual of a species possesses green or yellow or brown cells; in the latter cases only certain individuals contain them.

Facultative association may exhibit itself in another manner: in one part of its range all the individuals of a species may exhibit the association, in another part the coloured cells may be absent from all the individuals. Thus *Noctiluca* is colourless in the North Atlantic and green in the Indian Ocean. British *Alcyonium* have no zoochlorellæ, whereas the closely allied *A. ceylonicum* possesses them (Pratt, 1905). It seems probable, indeed, that the maximum development of these associations occurs in the warmer seas. According as the association is facultative or obligate, it gives rise to a less or greater modification in the behaviour and in the structure of the animal.

Convoluta roscoffensis exhibits in a striking degree such modifications (Gamble and Keeble, 1903). It lives gregariously at mid-tide level on the beach and exposes itself to the bright light of mid-day. Its colourless young are as strongly phototropic as the green adults. With the advent and multiplication of the green cells it ceases to ingest solid food. Structural changes described in the body of this paper also follow consequent upon the association of animal and green cell.

Similar phenomena are exhibited by other animals. Thus

Cassiopeia, a medusa of warm seas, has adopted, as a consequence of the association with itself of green cells, a posture the reverse of that maintained by its allies. On certain reefs it may be seen fixed by its aboral surface, exposing its oral arms, and so the coloured cells contained therein to the light.

Various reef corals, richly provided with zoochlorellæ, show marked decrease in the size of their tentacles; in this respect, and also in the number and degree of development of their digestive filaments, they present a sharp contrast with their more freely feeding allies (Pratt, 1905). Facts such as these lead naturally to the hypothesis that the association of animal and green cell results in a supply of nutriment to the former from the latter.

Admitting, as it is reasonable to admit, this trophic hypothesis, it still remains to inquire—as we do in the case of *Convoluta roscoffensis* in the following sections—what food-substances are transferred from green cell to animal and how this transference is effected.

It is important to know how this hypothesis stands with respect to such cases of voracious feeders as anemones, *Hydra*, *Convoluta paradoxa*, and many green rhabdocæls. Here it is clear that the animal is not subdued to the working of the “plant” within it.

It may also be asked how this at present vague hypothesis meets the cases of facultative association. One of the two main purposes of this paper is to put forward a supplementary hypothesis (Section VI, page 205), which we think throws new light on the significance of chlorellæ and xanthellæ in animals. The other purpose of this paper is to provide absolute proof of the source of origin of the green cells in *Convoluta roscoffensis*.

The view generally held, that the green or brown corpuscles of animals are of algal nature, is based rather on probability than on certain evidence.

The usual arguments in favour of this view are as follows:

The corpuscles in question have been shown in certain cases to be capable of photosynthesis. In the light, gases

containing a high percentage (15-50) of oxygen may be evolved from the animals. Starch may be present in the corpuscles. They have been shown to contain chlorophyll, patent or masked by other pigment. Structurally the chlorellæ resemble certain algal cells. A wall of cellulosic or pectose substance, absent in some cases, may be present. The corpuscles may contain a nucleus, a pyrenoid, and occasionally an eye-spot. This circumstantial evidence is strong, but cannot be said to amount to proof.

Evidence of the origin of the green or brown cells to be final must be of a like nature to that demanded by pathologists in the case of a micro-organism suspected of pathogenic properties; the organism must be isolated in pure culture and the infection-test applied.

So in the cases of animals infected with green cells; these cells must be isolated, and, by introduction into the body of an animal previously free from them, be shown to give rise to the normal green animal. In other words, the final proof of the algal nature of the green cells can be provided only by the synthesis of the green organism from its algal and animal components. This synthesis has not as yet been effected in an indisputable manner in any single case. The green or brown cells of Turbellaria, Cœlenterates, or sponges have not as yet been isolated and cultivated.

Beijerinck (1890) and Entz (1881-1882), it is true, made cultures of green hydra, but owing to the proneness of the cultures to infection from within and from without both the authors and their critics have regarded the results with suspicion, especially in view of the fact that attempts at synthesis were unsuccessful.

Among green protozoa two or three cases of alleged isolation of the corpuscles have been recorded. By macerating the bodies of Stentor, Paramecium, and Frontonia, Famintzin (1889-1891), Dantec (1892), and Dangeard (1900) have obtained colonies of algæ. The results are, however, somewhat discrepant, for whereas the first two authors regard the alga of Paramecium as a true chlorella, the third considers it

to be a chlamydomonas. Schewiakoff (1891) successfully fed colourless *Frontonia* on macerated green specimens, and states briefly that multiplication of the green cells within the host occurred. Dantec obtained a like result with *Paramecium*.

Brandt (1883), after depriving sea anemones of their yellow cells by long confinement in the dark, caused the reappearance of these cells by placing the animal in fresh sea-water in the light. But from experiments made by ourselves, we venture to express some doubt as to whether the apparently colourless animal had lost absolutely all its yellow cells.

Haberlandt (1891), whose admirable work on *Convoluta roscoffensis* is referred to in the text of this paper, was unsuccessful in his attempts to cultivate the green cells of this animal. Pending more exact information it seems to us that Lankester (1882 and 1890) has done valuable service by his championship of the opposed view, that of the intrinsic nature of the corpuscles under discussion. For his view compels those who hold the "algal" theory to investigate each case separately and to vindicate their view by the synthesis of the green animal.

When this has been done in such cases as those in which cell-wall and nucleus are present in the corpuscles, there will remain others—e. g. *Hydra* and *Spongilla*, whose green cells are devoid of definite nuclei, and after these, such puzzling instances as *Vorticella campanula* (Engelmann, 1883), with its diffuse chlorophyll, as well as *Pelomyxa viridis* (Bourne, 1891), the green corpuscles of which seem difficult of explanation except on Lankester's hypothesis.

Influenced by the forementioned considerations, we have investigated the origin of the green cells of *Convoluta roscoffensis*. The results of this investigation are set forth in Sections II—V.

The experimental work recorded in this paper has been carried on in the laboratory at Trégastel, Côtes-du-Nord,

France; in the Zoological Department, Victoria University, Manchester, and in the Botanical Laboratory, University College, Reading.

We acknowledge with gratitude the assistance we have derived from a grant made by Section D of the British Association for the purposes of this investigation.

SECTION II. PROOF OF THE ORIGIN OF THE GREEN CELLS BY INFECTION.

The dark spinach-green colour of *Convoluta roscoffensis* is due, as is well known from the descriptions of von Graff (1905-1906), Geddes (1879, 1879 A, 1882), Haberlandt (1891), and ourselves (1903), to dense layers of green cells. These green cells are distributed with great uniformity in the body, and extend from just below the epidermis into the deeper tissues. Only in the anterior end of the body, in front of the otocyst and rudimentary eyes, are the green cells so few in numbers as to reveal the whitish colour of the animal.

The general appearance of an adult *Convoluta*, when examined microscopically, is not unlike that of the mesophyll of a green leaf. In surface view the green cells are flat, somewhat variously shaped bodies, now of rounded outline, now drawn out at one end into long tail-like extensions which appear to connect cell with cell.

The individual cells, described by Haberlandt (1891) in his important contribution to our knowledge of the green cells of *Convoluta*, are naked protoplasts, the larger part of each of which consists of a more or less cup-shaped chloroplast containing a large polygonal or irregular pyrenoid. The small remaining part of the protoplast is colourless, and lies either in the hollow cup-like invagination of the chloroplast or, when the shape of this latter is irregular, excentrically.

Though often free, or almost free, from starch, the green cells may, under certain conditions, contain considerable quantities both of pyrenoid starch—that is, starch distributed

in the layer surrounding the pyrenoid (the starch sheath)—and also of minute rods or granules scattered irregularly throughout the substance of the chloroplast.

Two views as to the nature of the green cells have been expressed—one, that they are algæ which have been ingested, and which have developed within the animal body, the other, that they are integral parts of the animal tissue.

The observations of Georgevitsch (1899) that the just hatched young are colourless lend support to the former of these views, but do not suffice to establish it. For, since the animals raised by Georgevitsch—hatched in filtered water—only survived for three days, it is conceivable that antecedents of green cells were present in a colourless form, but that, owing to the short period during which the animals lived, these colourless antecedents failed to give rise to the green cells.

Our own observations (Section V) that the earliest recognisable stages of the future green cells may be colourless justify this reservation, lending no more support to the infection-hypothesis than to the suggestion of Haberlandt that the green cells may be derived from colourless plastids, the persisting remnants of once independent algæ now transmitted, as are the plastids of green plants, by the egg-cell.

In order to determine finally the origin of the green cells of *Convoluta*, it is necessary, in the first place, to maintain newly hatched animals under such conditions that infection—if infection there be—cannot occur, and, in the second place, if the result of the foregoing is the production of colourless *Convolutas*, to expose the animals to infection and to determine whether this exposure brings about the development of green cells.

As we state in the Introduction to this paper, this rigorous proof of the intrusive nature of the green or yellow cells of animals has not hitherto been obtained in any single case, we therefore describe in detail experiments which fulfil these conditions, and which have led to a decisive result in the instance of *Convoluta roscoffensis*.

In our previous work (1903), where strong, but not decisive, evidence in favour of the infection-hypothesis was given, we showed that it was possible to maintain young *Convoluta* alive and colourless for several weeks. The freshly laid egg-capsules containing the groups of fertilised eggs were—in these experiments—removed from the neighbourhood of adult animals and placed in sea-water which had been filtered by means of a Pasteur-Chamberland filter. The animals hatched out in the course of two or three days as colourless larvæ and remained colourless for two or even three weeks (Table II, Columns A and D). Samples of these colourless animals taken at any time during this period and placed in fresh, unfiltered sea-water became green in three or four days.

So far the result of this experiment pointed most definitely to the environment as the source of the green cells; but this conclusion was rendered less certain by the subsequent behaviour of the animals reared in filtered sea-water. Certain among these, sometimes few, sometimes many, ultimately became green, and on microscopic examination were found to be possessed of normal green cells (Table II, Columns B and C). The sporadic and tardy appearance of green individuals among the *Convoluta* hatched in filtered sea-water could be accounted for best on the supposition that small numbers of the infecting organism had been introduced with the egg-capsules into the filtered water, and that the three or four weeks which elapsed before infection took place were required for the multiplication of this organism. Microscopic examination of the egg-capsules showed the high probability of this supposition, for they were found to be infested, even a day or so after they had been laid, and before the young had escaped from them, with numberless green and colourless cells of various kinds.

To eliminate this source of error it was necessary therefore to obtain capsules as clean as possible, and to take the precaution of isolating the young from this source of infection. This we succeeded in doing by the following somewhat laborious process.

Batches of *Convoluta* were collected in watch-glasses from the shore, care being taken to exclude particles of sand and other foreign matter. The animals so obtained were washed a number of times in filtered sea-water, and were then allowed to descend into a large glass dish containing filtered sea-water. This dish was illuminated unilaterally, and as soon as *Convoluta* had taken up its markedly positive phototropic position the dish was tilted, the water drained away, and the bottom of the dish cleaned. A fresh lot of filtered sea-water was added, and the dish so turned that the animals were impelled to crawl across to the opposite side. As soon as they had taken up their new light-position the tilting and cleaning were repeated, filtered sea-water was again added, and the vessel turned once more. In some experiments these processes were repeated as many as eight times. When the *Convolutas* in the dish began to lay, the egg-capsules were picked out daily and placed in glass vessels containing filtered sea-water. As a result of these precautions the capsules were much freer from those organisms which, when no precautions are taken, habitually infest them; and larger numbers of *Convolutas* hatched out under these conditions remained colourless. Nevertheless, even with these precautionary measures some green animals ultimately appeared among a great majority of colourless *Convolutas*. Hence an additional safeguard from possibility of infection had to be employed—namely, to separate the young as nearly as possible at the moment of hatching from the egg-capsules. This, though a tedious, was not a difficult, operation owing to the fact that when on the point of hatching, three or four days after the capsules have been laid, it suffices, in order to release the larvæ, to take up the capsule in a fine pipette and to eject it with some slight force into the water. Numbers of animals were obtained in this manner, free or almost free from capsule-remnants. Absolute freedom it is almost impossible to obtain, owing to the extreme tenuity and transparency of the capsules at the time of hatching.

As Table III shows, we have, by adopting this procedure, succeeded in obtaining batches of *Convoluta* which remained absolutely colourless and uninfected.

The several columns of this table give the results obtained in the cases of:

- (1) Animals left in association with their capsule-remnants.
- (2) Animals removed from their capsule-remnants.
- (3) Animals which, having been so removed, were subsequently submitted to the risk of infection by placing them in fresh unfiltered sea-water.
- (4) Animals hatched without any precautions in ordinary sea-water.

The animals hatched in unfiltered sea-water became uniformly green after two or three days. Among those left with capsule-remnants infection occurred less rapidly and more sparingly. Thus, as Columns 3, 4, 5 of Table III show, eleven individuals out of fifty-nine became infected during the eight days which followed after general infection had declared itself among the animals reared in unfiltered sea-water. After seventeen days, infection had become general in all these cases (Columns 3, 4, 5).

The contrast between this result and that set forth in Columns 1 and 2 of the same table is emphatic and conclusive. In those cases (Columns 1, 2, Table III) in which the animals had been separated at the time of hatching from their capsule-remnants the total numbers of animals showing infection were, in the one experiment (Column 1), five out of forty-four animals examined, and in the other none out of forty-seven examined. We conclude, therefore, that the green cells of *Convoluta* are of intrusive origin, or, to use the terms employed already, that they arise as the result of an infection from the water of the sea. From a single infecting cell, or at most from two or three, are produced the vast numbers of the green cells of the adult *Convoluta*: infection taking place normally during the first three days after hatching.

Experiments made in the course of this inquiry enable us to answer two other questions—viz. May the green cells of an

infected *Convoluta* escape from the body, live freely again, and again infect a young animal? and what is the origin of the cells which in the preceding experiments developed on the capsule and subsequently infected the larval *Convolutas*? are they derived from the interior of the body of the egg-laying parent or from the environment?

With respect to the first question, we have failed, as Haberlandt failed before us, to cultivate in nutrient media green cells liberated from the body of the adult. But more convincing than these negative results are those set forth in Table I (Column A) and Table III (column headed "Filtered sea-water with adults"). In these places are recorded the results of experiments in rearing young animals in association with large numbers of adults which had been washed many times in filtered water. Under these circumstances the larvæ, though surrounded by great numbers of adults, many of which, having ruptured in the course of egg-laying, had discharged large numbers of green cells, failed for twenty days to show sign of infection. It thus appears certain that the ordinary green cells, as they exist in the body of one animal, are incapable on their discharge from that animal of infecting another. Indeed, under no conditions known to us do the green cells of a *Convoluta* ever escape alive from the body. Each *Convoluta* leaves the body of the mother as a colourless, uninfected animal; as such it hatches out from its capsule, and the cells which then infect it are derived from the environment, neither they nor their direct ancestors ever having been before within the body of a *Convoluta*. We return to this matter again in Section V, where we give an account of the histological changes which the green cells undergo in the course of their development in the body of *Convoluta*, and show that these changes account for the at first sight extraordinary facts just described.

There remains to be considered the second question—as to the origin of the green cells which make their appearance on the capsules and which served in various of the foregoing experiments as sources of infection to larval *Convolutas*.

These cells must have been derived from one or other or both of two sources—viz. from the body of *Convolutas* disintegrated during egg-laying or from the environment. In the former case they must be special cells which, unlike the great majority of the green cells of the adult body, have undergone no degenerative changes, and so retain the capacity for development.

The evidence points to the environment as the place of origin of the green cells of the capsules. In the first place, such cells are by no means uniformly present on the capsules, and in the second place they are present less often on capsules laid by animals which have been freed in some measure by repeated washings from their associated flora and fauna. The effect of this repeated washing is not to render the surfaces of the animals free from extraneous organisms, but to reduce their numbers and to confine them to such as attach themselves most tenaciously to the slime which covers the surface of *Convoluta*. Chief among these most intimate associates are the infecting alga, certain other minute unicellular algæ, and various diatoms. Repeated washings, then, reduce the number of competitors for place on the capsules, and though some of the infecting organisms may themselves be swept away those which remain find their tenancy when they succeed in reaching a capsule less disputed than is the case under more natural conditions.

In the third place, as we show (Section IV), the infecting alga does not depend on chance for its association either with the surface of the animal or with the egg-capsule. It is attracted chemotactically thereto, and hence, though the numbers of infecting algæ in the water should be but few, they will inevitably distribute themselves upon some of the capsules.

Summary of Section II.

(1) *Convoluta roscoffensis* commences life as a colourless (non-green) animal.

(2) At this stage it has no germ of infection within its body.

(3) Infection occurs normally within three days of "birth."

(4) Water taken from the shore—not necessarily in the neighbourhood of *Convoluta*-patches—contains the infecting organism in such numbers as to induce wholesale infection in large batches of larvæ.

(5) The infecting alga habitually settles down and develops on the egg-capsules, which therefore serve as sources of infection (see also Keeble and Gamble, 1905).

(6) Infection does not take place directly or indirectly from the body of the parent.

(7) The green cells of an adult *Convoluta* are incapable of life apart from the body of the animal.

(8) Consequently the association must be regarded, not as a symbiosis, but as a case of parasitism, the host being the green cells and the parasite *Convoluta* (see also Section VI).

SECTION III. THE ISOLATION OF THE INFECTING ORGANISM AND THE SYNTHESIS OF THE GREEN CONVOLUTA.

It is natural that, in seeking to isolate the infecting organism of *Convoluta*, effort should be directed first toward the cultivation of green cells obtained from the body of the green animal. Haberlandt (1891) was the first to attempt this. His efforts were unsuccessful. We have attempted it again and again, but have failed. Miss Harriette Chick, who has had much experience in such work, was good enough to make in the laboratory at Trégastel in 1905 a large number of culture experiments, using a great variety of nutrient substances in liquid and solid media. The experiments gave no positive result. These failures make it in a very high degree probable that the task is impossible, and so lend some support to the conclusion arrived at in the preceding section that the green cells of *Convoluta*, once developed within the body of that animal, are no longer capable of separate existence.

It became necessary, therefore, if the search for the

infecting organism was to have a successful termination, to begin at the other end, namely, to seek for the organism before its entrance into the body of *Convoluta*.

Our observation that infection may take place, not only from fresh sea-water, but also from the remnants of egg-capsules laid in filtered sea-water suggested a mode whereby this search might be prosecuted with some hope of success: the mode being the isolation and observation of egg-capsules from which the larvæ had escaped. The isolation was necessary because, if left with the young animals, the capsules disappeared, either being torn to fine shreds by the frequent entrances and exits of the larvæ or, perhaps, being devoured by these larvæ. Accordingly, numbers of egg-capsules obtained from well-washed animals were put into filtered sea-water, and as soon as the young had emerged from them the transparent, gelatinous remains of the capsules were removed to another vessel of filtered sea-water. There they were kept under daily observation. After seventeen days (Table III, columns 3 and 5) several green spherical bodies of about the size of the egg-capsules made their appearance. Microscopic examination showed that these spherical bodies were composed each of a pure culture of vast numbers of a unicellular green organism. During the examination the slight pressure of the cover-glass sufficed to burst the delicate membrane of the green spherule and a swarm of active, flagellated cells emerged, leaving behind the recognisable remains of an egg-capsule (figs. 1, 2, 3, 4, Pl. 13). These flagellated cells, a detailed description of which is given in Section IV, presented so many features in common with the green cells of *Convoluta roscoffensis* as to leave but little doubt that they represented a free stage of these cells: the cup-shaped chromatophore containing a polygonal pyrenoid, the colourless part of the protoplast occupying, as is sometimes the case in the green cells of the animal, the narrow cavity of the cup, the red, lateral-lying eye spot, also to be met with in the green cells of recently infected young *Convolutas*, all pointed to this conclusion.

It only remained, therefore, to apply the inoculation test—that is, to submit colourless, uninfected animals reared in filtered water to the chance of infection by these flagellated cells. A batch of such animals was divided accordingly into three groups. One, serving as a control, was maintained in filtered water; another, also a control, was placed in unfiltered sea-water in order that its capacity for infection might be tested; the third was put into filtered sea-water to which numbers of the flagellated organism had been added. The result proved conclusively that these flagellated cells are a stage in the life-history of the infecting organism. Group 1 in filtered sea-water remained uninfected; Group 2 in unfiltered sea-water showed the susceptibility of the animals to infection: they became green in the course of three days; Group 3, exposed to infection by the flagellated cells, were observed to ingest these cells, to tolerate their active division, and to become in consequence normal green *Convolutas*. Subsequently, when we had perfected our procedure so as to be able to obtain fairly constant supplies of the flagellated cells, we repeated the experiment frequently, and in all cases with the same result. Thus *Convoluta* has been synthesised from its elements the colourless animal and the green, flagellated cell.

Summary of Section III.

(1) We have isolated and cultivated outside the body of *Convoluta* its infecting organism.

(2) It is not from the green cells of the body of *Convoluta* that the infecting organism may be isolated, but it may be obtained readily from the remnants of the egg-capsules.

(3) The infecting organism which occurs sporadically on the egg-capsules is derived, not from the green cells of the body of the parent, but from free cells frequenting the surface of the body at the time of egg-laying.

(4) If to filtered sea-water containing colourless *Convoluta* the infecting alga is added the synthesis of the green animal results.

SECTION IV. THE LIFE-HISTORY OF THE INFECTING ORGANISM.

The green spherules from which the swarms of flagellated cells issued as described in the preceding section served as a starting-point for the cultivation of the green alga which, as just shown, is the source of the green cells of *Convoluta*. The securing of material for this purpose was rendered comparatively easy owing to the well-marked positive phototropism of the alga in its motile stage. Issued from the egg-capsule, the flagellated cells swarm toward the more brightly illuminated side of the vessel in which they are contained; there they settle down sooner or later, either singly or in pairs, along and just above the water-line. Thus the position of the algæ is marked by a visible green patch. This patch consists of numbers of flagellated cells, and also of many which, having withdrawn their flagella, have surrounded themselves with a well marked and often stratified wall. A sample from such a patch was transferred by means of a platinum loop to a vessel containing filtered sea-water, to which a little potassium nitrate had been added, and in which had been placed a number of empty egg-capsules. After some days a green streak along the water-line made its appearance on the brighter side of the vessel. The vessel was taken from Trégastel to England (Reading) in September, 1905, placed in the light in a cool incubator, and kept under observation. The green scum gradually disappeared, and it was feared that the organisms had died. Toward the end of May, 1906, the vessel was placed on a bench in the laboratory in a good light, and within a fortnight a green scum re-appeared on the illuminated side of the vessel. Microscopic examination showed the identity of the organisms constituting this scum, with those added to the water the previous year. Beside the green layer on the side of the vessel loose masses of pale green mucilage, floated up to the surface by reason of included gas-bubbles, made their appearance. Imbedded in these mucilaginous masses were numbers of quiescent green cells, lying singly, in pairs, or in groups.

Subcultures in various media were made from this material. Some of these were taken back again to Trégastel in the summer of 1906, and proved sufficient for the infection of colourless young *Convolutas*. The cultures have also served to demonstrate that the alga which infects *Convoluta roscoffensis* has a very varied life-history. In the first place, the active flagellated cells are dimorphic. The macrocytes, $16\ \mu$ in length (figs. 4, Pl. 13 and 12 A, Pl. 14), are nearly double the size of the microcytes (4 : 2.5) (figs. 3, Pl. 13 and 12 B, Pl. 14). Except in point of size the large and small cells are similar in their histological details.

Both the large and small cells have four equal flagella arising from the anterior colourless part of the protoplast. The flagella in both bear the same relation to the length of the body (2 : 1). In both the chloroplast is cup-shaped, the pyrenoid single, the eye-spot lateral and situated in the anterior half of the cell. In both the wall is extremely delicate and gives no cellulose reaction—e. g. with sulphuric acid and iodine or with Schultze's solution or calcium chloride-iodine; but with zinc chloride-iodine it gives a faint rose-colour (chitin reaction).

The nucleus—a description of which is given in Section V, where a comparison between the free and imprisoned cells is drawn—lies, in both large and small cells, in the colourless part of the protoplast which fills the hollow of the cup-shaped chloroplast. It suffices to say here that the nucleus is suspended in a layer of the protoplast from which run strands, two downwards, serving as slings for the pyrenoid, and others outward through the chloroplast at regular intervals to meet the thin, colourless layer of protoplasm which forms a pellicle around the exterior of the chloroplast.

Large and small cells are alike equally phototropic, and both settle down after a period of activity, withdrawing their flagella and surrounding themselves with a more or less thick mucilaginous wall. The wall may form with great rapidity, so that encysting macrocytes are often to be met with whose flagella may be seen in undulating movement within the enclosing wall.

The resting-cells (Pl. 14, figs. 11 and 16) vary considerably in form and in behaviour. Thus, single flagellated cells may come to rest, withdraw their flagella and, without forming a thick wall, undergo longitudinal division into two or four cells contained within the wall of the mother-cell. These daughter-cells, at first without a distinct wall, organise flagella, form a delicate cell-wall, and escape from the deliquescent mother-wall as active flagellated cells. Again, in the case of the macrocytes, the active cell comes to rest, surrounds itself with an extremely thick stratified wall, takes on a spherical shape, and becomes green throughout as though the whole cell were filled with small, polygonal, green granules. This appearance is due to the colourless protoplasm, which in the active stage is in large measure confined to the cup-like hollow of the chloroplast, now radiating out in all directions through the chloroplast to the outer wall and so demarcating the green chloroplast into polygonal areas. In these rounded resting-cells the pyrenoid is at first recognisable, but later breaks up into a number of pieces, and finally into many granules. The eye-spot may take the form of a circular plate or ring lying near the periphery of the cell, or it may disappear altogether.

These rounded thick-walled cells (Pl. 14, fig. 11), after a period of rest, may organise four daughter-cells of oval shape and having all the characters of the active cells except that no pyrenoid is visible at first and no flagella are as yet developed. A third form of resting-cell resembles that just described, except for the fact that the green colour disappears. This colourless resting-cell appears, in surface view, to be divided up into small, regular, polygonal areas, and in this cell pyrenoid and eye-spot may be indistinguishable.

Yet, again, paired resting-cells are met with. These consist of two thick-walled cells, whose broad apposed surfaces are flattened as by mutual pressure (Pl. 14, fig. 16 A). Such paired cells are either green or colourless. Finally, yet another form of resting stage occurs. In this the resting-cells are paired, but one of the two cells is smaller than the

other; in short, a series of stages are met with—pairs of equal sized cells, pairs in which one cell is of normal and the other of reduced size, half normal, less than half, and even to a mere speck (Pl. 14, figs. 16 A, B, C).

This phenomenon we refer to again when dealing, at the end of the section, with the systematic position of the infecting organism. The flagellated cells which settle down, withdraw their flagella and surround themselves with a thick mucilaginous wall do not necessarily pass through a period of rest, nor do they necessarily divide up subsequently to two or four green or colourless cells as described previously; but the life-history is liable to be short-circuited in the following manner: a green cell may encyst itself temporarily and then cast off the cyst and escape once again as an active cell. The organism under consideration exists also in a colonial form (Palmella condition). The cells in the colony form plates imbedded in masses of mucilage produced by the coalescence of the outer layers of the walls of the individual cells. The cells in the colonial state are rounded, uniformly green, and possessed of a pyrenoid less refractive than that of the active cell (figs. 14 and 15, Pl. 14). The cells constituting the colony undergo rapid division, being rather budded off from the parent-cell than produced by equal division of that cell. Here and there among the mass mature cells occur. In these an eye-spot is visible, and the protoplast is differentiated into green chloroplast and colourless neck as in the active cells. The mature cell may be seen moving inside its wall and every now and then escaping from the wall as an active flagellated macrocyte. Hence, scattered among the green cells of the colony are empty cysts consisting of the walls left behind by escaped active cells (figs. 14 c and 15, Pl. 14). The green spherules from which we first obtained the infecting alga consist of such colonial stages which have developed within, and come to fill completely, the egg-capsule of *Convoluta*. Under other conditions the colonial form is made up of cells which show the reticular type of structure already described as occurring in resting-

cells. It is probable, therefore, that the constituents of a colony may, under certain conditions, all pass into the resting stage.

Another curious colonial form also occurs. Oval green cells showing the characters of the active cells, differentiated chloroplast, eye-spot, and pyrenoid, are attached to one another by sleeve-like branching columns of stratified mucilage (fig. 8, Pl. 13).

One of the most striking points in the life-history of this alga is the occurrence in approximately equal numbers of green and colourless resting-cells. These cells may be—as already stated—isolated or in pairs. The single green cells undergo, after a period of rest, division into four, green, oval cells which resemble active cells except that their walls are extremely thin and that flagella are lacking. The four daughter-cells are extruded together from the envelope of the mother-cell and undergo further division or become at once active cells.

Similarly the green paired cells divide to eight oval cells. The colourless cells behave in a precisely similar way, giving rise to four or eight daughter-cells which are extruded through a circular opening in the wall of the mother-cell. The colourless daughter-cells consist each of a highly vacuolated foam-like protoplast, including a large refractive mass which appears to correspond with pyrenoid and chloroplast of the green cell. These cells may either undergo further division or assume gradually and without further division a green colour, becoming first faintly yellow, then quite yellow, and finally green.

The simultaneous formation by the alga of green and colourless cells is certainly a curious phenomenon.

It is well known that the zygotes of various algae lose their chlorophyll, but we know of no case where resting-cells are alternatively colourless or green. The division of the green cell into a green group of daughter-cells and the division of the colourless cell into a corresponding group of colourless cells appears to indicate that a true dimorphism exists.

Among the Euglenæ and also among Diatoms it is known that green (or brown) cells may lose their pigments, and, assuming a colourless state, exchange a holophytic for a saprophytic existence.

From the fact that when infection of *Convoluta* occurs normally from sea-water the earliest recognisable stage of the infecting organism in the body of the animal is a colourless or faintly yellow cell it would appear that the colourless phase of the alga described above represents a perfectly normal stage of its life-history.

The suggestion may be hazarded that the formation of colourless cells capable of a saprophytic mode of life is an adjustment which widens enormously the range of distribution of the alga. The green, active cells swarm toward the light, and so have their distribution determined by the light factor of their environment. The colourless forms, living saprophytically, increase by division even in darkness. Thus the alga may be enabled to live, not only in the surface waters, but also below the surface of the sand wherever organic débris provides material for its support. Such a divided habit would undoubtedly be of the utmost service to the alga, for, attached to the mucilaginous film which surrounds the body of *Convoluta* or to the gelatinous egg-capsule, it undergoes immersion deep in the sand at every tide. As each tide arrives at the patch of sand covered by the vast colonies of *Convoluta* these latter descend, as we have described in an earlier paper, out of reach of the disturbance caused by the moving water. The eggs of *Convoluta*, moreover are laid, not on, but beneath the surface of the sand, so that an organism which adjusted its habits so as to become an associate of *Convoluta* would be compelled to pass many hours of the day, and not infrequently, when attached to the egg-capsules, many days, in darkness. This it could support only if it were capable of a saprophytic habit.

That the infecting alga is capable of such a habit cannot be doubted, for, apart from the colourless stage being regarded as evidence, the active green cells themselves give corroborated

tive testimony, since, in cultures containing disintegrating *Convolutas*, they take up positions among the breaking-down green cells of the animal and increase greatly in numbers. Again, the rapid increase of the alga which follows after infection is strong evidence of saprophytic powers of nutrition. Finally, we have proved by comparative cultures that the infecting alga increases by vegetative division quicker when supplied with organic nitrogen than when the nitrogen is in the form of nitrates. The details of these experiments are given in Table IV, which summarises the results of a series of cultures of the alga in sterile sea-water, to which nitrogen in different combinations was added. The most rapid increase took place in the culture containing uric acid, next in that containing urea, the increase in sea-water containing potassium nitrate being less than in either of these culture-fluids. The infecting organism, then, has even in its green-celled stage marked saprophytic tendencies—tendencies which appear to find full expression in the colourless stage and which offer the key to the origin of the remarkable association of green cell and *Convoluta*.

Two questions remain—(1) Is there any special adaptation in the habits of the infecting organism which brings it into close association with *Convoluta*, or is its prevalence in the surface slime of the animal and in the empty egg-capsules a matter of chance? (2) To what systematic position is this organism to be assigned?

To the first question the following experiments provide a decisive answer. If to a bulk of filtered sea-water containing egg-capsules which have been laid under the cleanest possible conditions a number of the flagellated cells are transferred by means of a platinum loop, then within a few hours one or more of these cells will be found to have settled down on each capsule. Yet more striking results are obtained if to a hanging-drop containing an egg-capsule with its group of developing eggs a number of the flagellated cells are added. In such a preparation the motile green cells are seen to approach the capsule, to

swarm about it, to press in close ranks into the soft gelatinous wall and so to imbed themselves in the envelope (figs. 6, 7; Pl. 13).

We conclude that the egg-capsule exercises a chemotactic influence on the active cells, and that the constant presence of one or more green cells of the infecting alga on egg-capsules contained in water to which a platinum loopful of the active cells has been added is thus accounted for. The behaviour of the cells which settle on the capsule proves that they find this a favourable medium for growth. Within a few hours each cell, having withdrawn its flagella, increases considerably in size, and whilst retaining its green colour takes on a granular appearance; the eye-spot and pyrenoid become fainter and the cell undergoes division. This division, like that of the temporary resting-cells already described, is in most cases longitudinal, but occasionally instances occur in which the division is transverse.

The mode of division is possibly determined by the state of symmetry of the cell; as long as the colourless protoplasmic plug occupies the cavity of the cup-shaped chromatophore the division will be longitudinal, but when the protoplasm becomes distributed radially and uniformly transverse division may occur, and this in turn may give place to the budding of the spherical units of the palmella stage.

The daughter-cells produced by the division of the green cells settled on the egg-capsule undergo a continuous series of divisions, and so give rise to a loose colony, which colony constitutes the green spherules, the discovery of which formed the starting-point for this description.

The second question, that of the systematic position of the infecting organism, remains to be considered. The assemblage of characters presented by this alga—its firm wall, its single cup-shaped chromatophore containing in its hollow the colourless protoplasm and nucleus, its pyrenoid and lateral eye-spot far removed from the bases of the flagella, its power of starch-formation—indicate that it must be assigned to a position in that primitive group of green algæ the Chlamydo-

monadeæ; and the possession by the cells of four flagella would indicate the genus *Carteria*.

Nevertheless we would wish to assign it only provisionally to this genus for the following reasons:

First, the infecting alga never has contractile vacuoles, two of which are described in the known species of *Carteria*.

Second, though like some species of *Carteria*—e. g. *C. multifilis* (Fresen)—it possesses cells of two sizes (West, 1904); yet, whereas in *C. multifilis* the small cells are gametes and the large, vegetative cells, neither the large nor the small cells of our organism appear to be obligate gametes. Numerous experiments which we have made of bringing together microcytes from different cultures, microcytes and macrocytes, and macrocytes from different cultures have given but meagre and extremely rare evidence for any sexual fusion. Leaving aside as unexplained the phenomenon already described in which one of a pair of resting-cells gradually decreases in size and finally disappears, we have only once obtained evidence of what may be a gametic union. Pl. 14, fig. 11 D, shows the case in point. Here two motile cells have come together, their walls have united, and the cells appear in process of fusion.

If this be a true case of sexual fusion, it presents features of great interest. For, as Blackman (1900) has pointed out, the *Chlamydomonadeæ* exhibit a remarkable series of modes of gametic fusion. In some forms the walls are thrown off whilst the gametes are approaching one another, in others at the moment of meeting. In *Chlamydomonas multifilis*, a four-ciliate form, partial fusion takes place first, so that the walls are left fused at one spot. Blackman adds that the series might be completed at its lower end by a form which fused without losing its walls. The figure just referred to appears to indicate that this primitive form of sexual union is exhibited by the infecting alga of *Convoluta*.

A third character which seems to separate it from *Carteria* is the peculiar branching habit which it sometimes presents. In this condition (Pl. 13, fig. 8) inverted green cells are

borne at the ends of branching gelatinous stratified stalks. This habit occurs, according to Oltmanns (1904), in other genera of the Chlamydomonadeæ, viz. Chlorangium (Stein), and Physocytium (Borzi). The nearest approach, however, to this habit of the infecting alga is exhibited by species of the genus Prasinocladus of the family Chlorodendraceæ, which stands, according to Oltmanns, between the Chlamydomonadeæ and the Polyblepharideæ.

We conclude that the infecting organism is a true alga and a primitive member of a primitive group, the Chlamydomonadeæ; and that whilst presenting many features characteristic of the genus *Carteria*, its possession of certain other characters, facultative gametes, branching as well as plate-like colonial form and its lack of contractile vacuoles, makes its assignment to that genus doubtful.

Summary of Section IV.

The infecting organism of *Convoluta* is an alga belonging to the Chlamydomonadeæ.

In its free stage it bears four equal flagella and possesses the general characters of members of this family.

The active cells are of two sizes, but neither large nor small cells appear to be obligate gametes.

The organism is capable of a saprophytic as well as of a holophytic existence; in the former state it may be colourless.

The active cells are attracted chemotactically to egg-capsules of *Convoluta*. They settle down and undergo active vegetative division in the capsules, and are finally liberated as a swarm of four-flagellated active cells.

SECTION V. THE NORMAL COURSE OF INFECTION.

We have demonstrated in Section III that the active flagellated cells of the infecting alga may be ingested by *Convoluta* and, dividing in the body of the animal, give rise to the green-celled "tissue" characteristic of the adult.

When, however, infection takes place from ordinary sea-water, when, for example, young *Convolutas* raised under sterile conditions are placed in a few cubic centimetres of sea-water brought fresh from the shore, it is not the green flagellated cell which constitutes the first stage of infection. The first sign of the presence of the infecting alga under these normal conditions is afforded by a larger or smaller colourless body lying in the central vacuole of the animal. The large body consists of a pair of closely apposed resting-cells, such as have been described already (Section IV). The small body is a single resting-cell of the infecting alga. Soon after ingestion the mucilaginous wall which surrounds the resting-cell swells considerably and the contents divide, in the case of the smaller cell into four, in that of the larger cell into as many as eight, colourless daughter-cells. These colourless cells are 15–16 μ in length—that is, of about the same dimensions as those of the macrocytes. They escape or are discharged from the central vacuole, and take up fairly definite stations in the body of *Convoluta*, two right and left of and a little behind the otcyst, and two on either side about the middle of the body. These cells, which are to form the starting-points from which the green tissue of the adult animal will be developed, lie each, like the mother-cell which gave rise to them, in a clear vacuole. The colourless cell at this stage has very granular contents, and a pyrenoid which is large and somewhat oily-looking. Even in this stage the subsequent differentiation of the protoplast into chloroplast and colourless protoplasm may be indicated by a plug or core of clearer protoplasm lying in the hollow of a more granular chloroplast. The cell now develops rapidly, an eye-spot makes its appearance, and a yellow tinge becomes visible in the leucoplast; at first extremely faint, the yellow becomes more marked, and is succeeded by a green colour which pervades the whole chloroplast. The cell is bounded by no well-marked wall, the limiting layer does not give a cellulose reaction, and is so delicate that the shape of the cell changes with the movements of the animal.

The infecting organism, though without flagella, now resembles in other respects the active flagellated free stage. It is, in fact, as both its histological features and its development show, a daughter-cell produced by the division of a colourless resting-cell. Such resting-cells on resuming activity undergo division into four (the paired cells into as many as eight) daughter-cells, which, as described in Section IV, are discharged together or severally through a circular opening in the thick mucilaginous wall of the cyst. Subsequently these cells surround themselves, in the free state, each with a thin wall. In the body of *Convoluta* the formation of a definite wall is suppressed.

The small colourless or faintly yellow cell, which is often the first recognisable sign of infection, is a daughter-cell which has been produced outside the body of the animal by the division of such a resting-cell as that just described.

Convoluta is then infected normally either by a resting-cell or by non-motile daughter-cells produced by the division of a resting-cell, though, as already shown, the organism in its flagellated phase may be taken up and give rise to normal infection.

We have now traced the infecting organism to its place in the body of *Convoluta*. At certain, fairly constant, stations of the body, several naked green cells lie, each in a clear vacuole. At about this stage, or after their further division, the green cells lose their regular oval outline, and the colourless part of the protoplast becomes more or less excentrically placed with respect to the chloroplast. It is easy to cause free active cells to undergo similar changes. All that is necessary to effect this is to transfer them from sea-water to a fluid—e. g. diluted sea-water—of lower osmotic pressure.

Hence we infer that the peculiar and variable shapes of the green cells of adult *Convolutas* are due to the osmotic pressure of the vacuolar fluid in which they lie being lower than that of sea-water. Incidentally, this observation serves to clear up another point. Haberlandt makes the suggestion that the colourless, excentrically-lying part of the green cell is in

reality an animal cell or part of an animal cell which has associated itself with the green cell, and that the nucleus lying in this colourless part belongs to this animal cell. From the preceding it follows that this suggestion cannot be maintained; for the colourless part of the green cell is nothing other than the plug or core of colourless protoplasm which in the free, active cell occupies the cavity of the cup-shaped chromatophore, and which in a fluid of osmotic pressure lower than that of sea-water becomes displaced excentrically. The question of nucleus we reserve (see p. 196). We turn now to consider the further development of the green cells which, as the result of infection, are planted about in the body.

The cells increase, sometimes very considerably in size, and undergo division. The process of this division in cells which have not yet become distorted is as follows: The colourless "neck" of protoplasm elongates, extending toward the base of the cell, where come to be placed pyrenoid and eye-spot. A vertical fold appears in the pyrenoid which later cleaves into two. The eye-spot degenerates during the division of the cell, taking on the form of a broken hoop of dull red colour. The eye-spot does not reappear in the daughter-cells which arise by longitudinal division. In the case of the larger originally colourless cells the colourless protoplasm occupies the middle of the cell. When about to divide a pyrenoid makes its appearance toward either end of the cell, and what appears to be a transverse division occurs.

The daughter-cells in either case separate from one another and, lying each in a clear space, undergo further divisions. These subsequent divisions are, like those which take place in the palmella state, more of the nature of budding than of equal division; hence are produced rows of cells of gradually decreasing size, which run from the periphery into the deeper tissues of the body (figs. 9 and 10, Pl. 13). The resemblance between the green tissue of loosely associated cells which occupies the body of *Convoluta* and the palmella stage of the alga is noteworthy.

The appearance presented by an abnormal *Convoluta*—

unique among the many thousands examined—lends support to the view which we put forward that the “*Convoluta* stage” of the alga is to be regarded as consisting of an hypertrophied, senescent palmella.

The animal in question presented an appearance under the low power of the microscope very like that of a fern-prothallus. Its body was lobed posteriorly, so as to present a conventionally heart-shaped form, and its green cells, so closely packed as to obscure all other elements of the body, were rounded and uniformly green, quite like those of the typical palmella-stage, which stage at the time of these observations was unknown to us.

To return to our consideration of the green cells of the normal animal. The rapid division of the green cells in recently infected *Convoluta* is accompanied by significant changes in the nucleus, changes which since they render intelligible the loss of power of independent existence on the part of the green cells of the adult animal we now describe in some detail.

The nucleus of the flagellated cell (fig. 12, Pl. 14) lies in the colourless part of the protoplast, about the middle of its depth, equidistant from the bases of the flagella and the pyrenoid. Its general appearance is that of a spherical uniformly staining body, from the periphery of which radiating branches run, two upward towards the points of insertion of the cilia and two downward toward the pyrenoid. These branches, though mainly cytoplasmic, show by their staining reactions with nuclear stains (e.g. Benda’s iron hæmatoxylin) that they also contain nuclear material.

In other flagellated cells the nucleus presents the appearance described by Dangeard (1899) as occurring occasionally in the *Chlamydomonadeæ*, of a clear spherical area, in the centre of which lies a deeply staining “nucleolus.” Again, in other specimens the nuclear substance consists of three fairly large granules lying either side by side or else pyramid-wise; the granules may be spherical or elongated, of equal or unequal size. Occasionally specimens show two vertical rows,

each of three granules. So far we have met with no indications of mitotic division except this separation of the nucleus into rows of granules.

In the division stages of the non-motile cells, groups of rods and granules may be made out and appear to represent phases of nuclear division.

The cells of the colonial stage (Fig. 15, pl. 14) present two types of nucleus. The resting nucleus, lying slung in cytoplasm in the centre of the cell, consists of a homogeneous, deeply-staining central body, surrounded by a clear area, the limits of which cannot be sharply distinguished from those of the cytoplasmic envelope containing it. The dividing nucleus consists of a diffuse group of fine granules, occupying a circular or oval area of the cytoplasm. The granules sometimes form two groups at opposed ends of a cell.

Thus the nuclei of the flagellated stage and of the actively developing palmella stage are, though small, distinct and readily recognisable. So, too, is the nucleus of the green or colourless cell, as it lies in the body of a larval *Convoluta* immediately after ingestion. After the first division in the body the nucleus of each daughter-cell has the appearance of that just described as characteristic of the actively dividing cells of the colonial stage—namely of an oval area occupied by diffuse granules (Fig. 13, pl. 14). As division of the infecting cells proceeds the presence of nuclear granules in the resultant cells is made out with increasing difficulty, till finally, in the adult animal, whose body is densely packed with green cells, it is often difficult to pick out any in which remnants of nuclear granules remain. The great majority of the green cells of *Convoluta* are not complete cells, but cells which show all stages of diminishing nuclear substance (Figs. 9 and 10, pl. 13). Thus the conclusion to which our physiological investigations led us that the body of *Convoluta* is the grave of the green cell receives histological confirmation. If this degeneration of the nucleus begins almost immediately after the ingestion of the infecting cells some light is perhaps thrown on the fact to which attention has frequently been

drawn—that the green cells of *Convoluta* are without cell-walls, for it is well known that the nucleus of the plant-cell presides over cell-wall formation. An enucleated fragment of a plant-cell, though capable of performing certain vital processes—e. g. photosynthesis—is unable to form a new wall.

We may therefore regard the suppression of cell-wall as the first result of a progressive nuclear degeneration. Enough, however, of the nucleus is left to preside over cell-division, which proceeds rapidly after infection; as this division goes on less and less nuclear material remains, till finally partial cells, incapable of independent life and probably also incapable of further division, alone are left.

It is noteworthy that we have come across no evidence of mitosis in the dividing green cells of the animal.

A significant phenomenon is revealed by a reference to Figs. 9 and 10, pl. 13, and to the description thereof. Here, as at (*Nu., gc.*), are isolated green cells, in each of which a nucleus is recognisable; in other parts of the section are ingrowing rows of cells budded off from the outermost cell, and of these rows only the outermost cells contain a distinct nucleus; others contain only deep-staining granules; and others, again, no nuclear substance whatever (Fig. 9, pl. 13).

A parallel suggests itself between the green cells of *Convoluta* and the red blood-cells of the higher vertebrates. As the red discs are enucleate partial cells budded off from the nucleated red cells, so may the green cells be regarded as enucleate partial cells budded off from the outermost row of nucleated green cells, and as the red discs are of limited life and specialised (respiratory) function, so are the green cells of limited life and of specialised (photosynthetic) function.

The green cell devoid of a nucleus of its own would not, however, appear to be shut off from all nuclear influences. For the enucleate green cell may be connected by a finely drawn process with another green cell still possessed of nuclear substance (Pl. 13, fig. 9). Moreover, such green cells as are without nuclear material are accompanied each by a large attendant nucleus of animal origin; this close

association of "attendant nucleus" and green cell is shown in Pl. 13, fig. 9, *Nu. Mes.* Further investigation of this phenomenon is required; but such observations as we have made point to the belief that these attendant nuclei are those of wandering cells which lie in wait, as it were, for enucleate green cells and at a subsequent stage bring about their destruction by digesting them.

The foregoing interpretation of the series of facts described offers some support in favour of the hardy suggestion, due originally to Schimper,¹ that the higher green plants are an association of two organisms—one a colourless organism, the other originally a green alga but now represented by the chloroplasts and by them alone.

For an adult *Convoluta* is a complex of two organisms—one the colourless animal, the other the chloroplast remainders of the original green alga; but in this case, unlike that imagined for the green plant, the synthesis is not a permanent one. It endures but for the lifetime of the animal and has to be recommenced in every larval *Convoluta*.

Summary of Section V.

(1) The colourless phase of the infecting alga normally supplies the cells which develop in the body of *Convoluta* into the green-celled tissue, which tissue is comparable with the palmella-stage of the alga.

(2) But infection may also result from the ingestion of the green active or green temporarily resting-cell.

(3) Ingestion is followed by the active division of these cells which develop transient eye-spots, etc., but which do not form a cell-wall.

(4) The distorted shapes of the green cells in the animal are due to osmotic conditions.

(5) The nucleus of the green cell dividing in the animal undergoes progressive degeneration and finally disappears, leaving the photosynthetic machinery intact.

¹ 'Bot. Zeit.,' 1883, p. 112, footnote.

(6) The green partial cells which result are incapable of independent existence.

SECTION VI. THE SIGNIFICANCE AND THE CONSEQUENCES OF THE ASSOCIATION OF ANIMAL AND GREEN CELL.

The relation between *Convoluta* and its green cells has sometimes been regarded as an example of parasitism, the parasite being the green cell and the host the animal. That this is an erroneous view we have already demonstrated.

More usually it is assumed that both partners in the association receive advantage therefrom, and consequently the relation is considered to be symbiotic.

It is obviously important, if such a term as "symbiosis" is to retain any value whatever, that a detailed examination of each case which appears to fall into this category should be made before it is relegated thereto. This is the purpose of the present section. In it we show that the relation between *Convoluta* and its green cells is both intricate and variable in the sense that the nature of the relation changes as the association becomes more intimate.

We show that it is only by taking the widest view of the case that it can be brought within the category of symbiotic phenomena.

The inquiry as to what is the significance and what are the consequences of the association of green cells and *Convoluta* resolves itself in large measure, but not entirely, into an examination of the modes of nutrition of infecting alga and of animal. The mode of nutrition of the infecting alga in its free state must be first described.

The fact that we have succeeded in obtaining luxuriant cultures of the alga in artificial sea-water to which traces of potassium nitrate were added proves that it is capable of a typical plant-like (holophytic) mode of nutrition. The alga under these conditions photosynthesises carbohydrates—storing the surplus as starch—and utilises the nitrogen of the nitrates in the formation of its proteids.

But, as we have already shown in Section IV, the alga in its free state grows more actively when enabled to obtain its nitrogen from organic compounds (uric acid and urea) than when "inorganic nitrogen" (nitrate) is supplied.

Next, as to the mode of nutrition of the alga in its "Convoluta stage."

Geddes (1879-1881) was the first to demonstrate that *Convoluta* gives off oxygen when exposed to light. He also proved the presence of starch in the green cells, stating that this substance is present always in small quantities. Our own experiments complete the proof that the green cells of *Convoluta* photosynthesise carbohydrates. Thus, we have shown that the green pigment of these cells is true chlorophyll (op. cit., p. 377), and that starch, present in the green cells, disappears in darkness and reappears when the animals are brought into the light. The photosynthetic activity of the green cells having been demonstrated, the questions remain, Does the animal receive a share of the elaborated carbohydrate? and, if so, How is the transference of this substance from green cell to animal tissue effected?

Geddes has stated that *Convoluta* survives only a few days' exposure to darkness, the implication being that when photosynthesis is arrested death from starvation ensues. But according to our experiments (op. cit. p. 375), if care is taken with respect to the water supply *Convoluta* may live in darkness for several weeks.

Again, Geddes' statement that starch occurs in *Convoluta* only in small quantities might be interpreted as meaning that the product of photosynthesis is passed on to the animal as soon as it is formed, and that only a small residue is accumulated in the green cell. The observation, however, is incorrect, for the green cells of *Convoluta* at certain times contain so much starch as to give to the body when stained with iodine a deep blue or blue-black colour.

We have endeavoured to ascertain to what conditions the variable amount of starch in the green cells is due.

For this, samples of animals were collected twice daily

during a lunar month from a certain patch of *Convoluta*. The samples were taken just after the tide had left the patch—i. e. immediately after the sojourn of the animals in darkness below the surface, and again after their spell of isolation, just as the tide was about to cover them.

The animals were fixed on glass slides, decolorised, stained with potassium iodide-iodine, and the amount of starch estimated by the resulting coloration.

The records obtained (Table V) during August, 1905, exhibit a bi-monthly periodicity in the amount of starch present in the animal. The amount is large during the spring tides and falls off during the slack tides. The result is perhaps susceptible of a simple explanation. For during the spring tides low water, and consequently exposure of *Convoluta* to light, occurs about the middle of the day (and night), whereas during the neap tides the exposure to light takes place during the early morning and late afternoon. Consequently, though the number of hours of daylight to which *Convoluta* is exposed may be actually greater during the slack tides than during the spring tides, the animals are exposed to a higher light intensity (of the mid-day) during the spring tides than during the neaps. Hence it seems reasonable to suppose that photosynthesis will be more active during the spring tides, and that this greater activity will be recorded by the larger deposit of starch. It is worth noting—though aside from the question under consideration—that the periodicity of egg-laying by *Convoluta* receives some explanation from the foregoing; for the egg-laying periods, occurring soon after the spring tides, follow closely on periods of abundant nutrition. Returning to the question, Does the animal receive a share of the carbohydrate elaborated by the green cells? we have just seen that on this hypothesis periodicity of egg-laying receives a simple explanation. But conclusive evidence is derived from observation of the rates of growth of recently infected *Convoluta*. The rapid increase in the numbers of green cells in the body—from one or two to thousands—is accompanied, not by any diminution of the

animal's tissues, but by the marked growth of the animal. Whilst uninfected *Convoluta*, as described presently, remain diminutive, those which become infected resume growth and increase rapidly in size.

It appears probable on several grounds that the animal cells are unable to lay hold of the starch contained in the green cells. Thus, though young *Convolutas* fed on starch-grains ingest them readily, they digest them not at all. Again, when adult *Convolutas* are kept in darkness the starch of the green cells disappears with extreme slowness—even after eight days some starch may still be present. This slowness of disappearance would seem to indicate that it takes place according to the requirements of the green cell, and not according to those of the animal. It is therefore likely that the animal cells obtain the product of photosynthetic activity directly as sugar. Just as from the green cells of a plant exposed to light there is a constant osmotic streaming of photosynthesised sugar to the colourless cells, so from the green cells of *Convoluta* sugar passes to the colourless animal cells. In general, the demand in adult *Convolutas* for food material is so great that but little starch accumulates in the green cells. It is only during the mid-day exposure to high light-intensity at the spring tides that photosynthetic activity considerably exceeds that of translocation. At all other periods during the adult life of the animal the photosynthesised carbohydrate passes away as fast as it is formed from the green cell to the animal. The sugar thus obtained is stored ultimately in the animal tissues or in the eggs in the form of fat.

We have next to consider the system, green cell and animal, with respect to nitrogen metabolism. To do this requires a review of the facts known with respect to the nutrition of the constituents of the system.

Von Graff (1905-1906) was the first to draw attention to the absence of any vestiges of solid food from the body of *Convoluta*. He concluded that this animal does not take up solid food. Geddes' observations, which, however, as we

have shown, require considerable modification, led him to conclude that the animal relies on the green cells for its food supply.

Georgevitsch (1899), finding that colourless larvæ die, if uninfected, within two or three days, seemed thereby to establish the existence of a yet closer dependence of animal on green cell.

We have shown in a former paper that von Graff's statement is too absolute, and that of Georgevitsch inaccurate.

We may summarise our own observations as to the ingestion of food by *Convoluta*.

A well-marked mouth—already described by von Graff in adult animals—is present in just hatched animals. This mouth, capable of a wide gape, is situated on the under surface, about the middle of the body. When open the mouth is connected by a short, ciliated, ectodermal invagination with an axial mass of highly vacuolated tissue which constitutes a rudimentary gut.

By means of this mouth a young *Convoluta* takes up almost any objects which it can encompass; diatoms and unicellular green algæ form the staple diet in the open, while in the laboratory starch-grains, litmus, lamp-black, and sand-grains may be swallowed.

If colourless uninfected *Convolutas* are cultivated in water devoid of the infecting alga but rich in other organisms, such as diatoms, they continue to feed actively for some time—for a week or more. But after this period they become increasingly inert and ultimately cease altogether to take up food.

In this state they await, as it were, the infecting alga. If the latter is supplied they ingest it, and, as it develops, the animals resume their activity. If, on the other hand, the infecting organism is still withheld, the lethargic condition becomes more pronounced, and undergoing gradual diminution in size, *Convoluta* finally dies though lying in the midst of food of a kind which, at an earlier stage, it took up and digested readily enough, and on which young infected *Convolutas* are still feeding. It is to this remarkable behaviour

that we attribute the failure of all our attempts to raise a colourless race of *Convolutas*.

The infected young animals continue during the period of development of their green cells to ingest solid food. But when this period is complete and the reproductive organs are mature all ingestion of solid food ceases.

The only food materials now available are such substances synthesised by the green cells as may pass in solution from green cell to animal cell.

This mode of nutrition of the animal is of short duration. It is soon succeeded, or rather overlapped, by another which consists in the digestion by the animal cells of whole groups of green cells. During the adult life of *Convoluta* large or small aggregates of green cells in all stages of disintegration are to be met with, lying in vacuoles in the axial tissue which constitutes the gut of the animal.

From this stage onward the ultimate significance of the relation between animal and green cells stands more and more clearly revealed. The animal is now and henceforth parasitic on its green cells. As a result of this habit old animals are not infrequently to be met with in laboratory cultures whose bodies are half green and half colourless.

If in order to summarise these conclusions we employ the terms used by plant physiologists in describing the principal modes of nutrition, and if we consider for this purpose a green *Convoluta* as an organism, then we may distinguish the following styles of nutrition:

(1) In the pre-infection stage the animal feeds heterotrophically—i. e. typically animal-wise.

(2) Young infected stage; the green animal is nourished mixotrophically, viz. by (solid) ingested food, and by photosynthesised food-substances.

(3) Mature green *Convoluta*; the mode of nutrition is holophytic—typically plant-like. The green cells are the photosynthetic agents, the animal's colourless cells receiving, just as do the colourless cells of plants, the products of photosynthetic activity.

(4) Old green *Convoluta*; the only term that can be applied is autotrophic; the green animal behaves to its green cells just as any animal or plant may behave to any reserves of food-material; it digests them.

If we consider the same facts from the point of view of the animal *Convoluta*; then stages two, three, and four represent the progressive parasitism of the animal on its contained green cells.

We have next to consider the sources of the supplies of nitrogen compounds to the green cells and to the animal.

The facts already described, proving that the infecting alga thrives better on organic than on inorganic nitrogen compounds suggest that the habit of this alga of settling down on the egg-capsules of *Convoluta* originated in obedience to these nitrogen-requirements.

The following considerations and observations lend support to this view. Whereas most Turbellarians have a well-developed excretory system of flame-cells, *Convoluta roscoffensis* has none. Nor do we find in this species those granular accumulations, often appearing as localised refractive bands or patches, which are constant features in allied species and which von Graff regards as being of an excretory nature. The fruitlessness of our prolonged search for flame-cells justifies us in holding that none exist in *Convoluta roscoffensis*. Moreover, green *Convolutas* show no signs of any excretory substances. The green cells we know are capable of utilising such excretory nitrogen compounds as uric acid and urea. It seems, therefore, probable in a high degree that the green cells of *Convoluta* are its excretory system.

The following observations lend a certain measure of support to this hypothesis. If larval *Convolutas* are protected from infection and kept without food, their large stores of reserves gradually disappear, and vacuoles charged with long acicular crystalline bodies make their appearance. The numbers of these vacuoles and of their contained crystals increase till they form one of the most striking features of the

body. We have not yet succeeded in determining the chemical nature of these crystals; they disappear when the "vacuole" containing them is destroyed, as it is by every fixative or reagent we have used. Nevertheless it cannot be doubted that these crystals are products of metabolism, and it seems likely that they are of the nature of excretory nitrogenous substances. Now, these acicular crystals do not occur in the infected green animal.

Admitting the argument here set forth, the conclusion follows that the green cells, on gaining entrance to the body, take over the business of disposing of the waste products of nitrogenous metabolism. We have learned already that the life of the animal depends on the occurrence of infection, that failing infection the animal ceases to feed and dies, possibly as the result of auto-intoxication by the accumulated waste products. Thus the closeness of the relation between animal and green cells is such that they together constitute, in the green *Convoluta roscoffensis*, one organism.

The conclusions concerning the rôle of the green cells in utilising the nitrogenous waste of the animal as material for proteid synthesis appear to us to throw light on the last phase of the nutrition of the animal. That phase is characterised by the digestion of the green cells. In the early stages of its development *Convoluta* offers to the green cells ample supplies of waste nitrogen compounds, the products of its proteid metabolism the materials for which were derived from the reserves in the egg and from the products of digestion of solid food. On these excretory nitrogen compounds, and on the product of its own photosynthetic activity, the green cell flourishes, and in turn furnishes soluble carbohydrates to the animal. But when ingestion of solid food ceases there follows a shortage of nitrogenous waste substance. The green cell, adjusted to utilise organic nitrogen, now has only available for proteid synthesis the soluble nitrogen compounds of the sea-water. A dearth of nitrogen compounds available for anabolic processes subvenes. This dearth falls earliest and acutest on the animal. Urged by its

nitrogen hunger, it turns upon the green cells and raids such stores as they contain by digesting them. These stores are but limited, and when exhausted, death, both of the animal and of its green cells, inevitably follows.

The election by the infecting alga of the egg-capsules and the surface of the animal as a normal habitat may also be regarded as a symptom of nitrogen hunger; so, too, is the ultimate digestion of the green cells by the animal.

Taking the broadest view of the whole relationship, including therein, not only the green cells inhabiting the body of *Convoluta*, but also the free green cells living on soluble organic waste contained in the egg-capsules, we may classify it as a symbiotic relationship, for it is now only by reason of its infection by the green cells that *Convoluta roscoffensis*, the species, persists, and though the green cells which enter the animal never escape alive, this is, as it were, but the price which the species has to pay for its lodging.

But if we confine our attention to a green *Convoluta*, not looking beyond the association of green cells and animal, then the relation constitutes a case of parasitism, the host being the green cells and the parasite the free-living animal.

The extraordinary restriction of the range of the species *C. roscoffensis* we may regard as the result of this peculiar and economically unsound attempt on its part to solve the "nitrogen question."

Summary of Section VI.

(1) *Convoluta* exhibits four phases of nutrition, passing from the typically animal to the completely parasitic.

(2) The infecting alga shows specialisation in the direction of saprophytism.

(3) This habit enables the green cells to utilise the products of the animal's nitrogenous metabolism, and so to develop rapidly within the body, where they serve as an excretory system to the animal.

(4) The habit of the infecting alga, in its free state, of fre-

quencing the egg-capsules and also the surface slime of the body of *Convoluta* is to be ascribed to its nitrogen requirements; this habit developed originally with no reference to *Convoluta* (being shared with various *Chlamydomonadae*; e.g. *Carteria subcordiformis*, Wille n. sp. (1903)), was nevertheless an essential preliminary to the association of animal and green cell.

(5) The association entails ultimately the death of the green cell and of *Convoluta*; but whereas the former dies without issue the latter first produces one or more batches of eggs.

(6) The consequences of the association are: To the green cell hypertrophy, nuclear degeneration, premature senescence, and death. To the animal: suppression of excretory system, cessation of feeding, resignation of power of existence apart from the green cells—i. e. obligate parasitism; adaptations facilitating the photosynthetic activities of the green cell—e. g. marked positive phototropism identical with that displayed by the infecting alga in its free state.

SECTION VII. GENERAL SUMMARY.

Convoluta roscoffensis hatches out from its egg-capsule as a colourless animal whose body contains neither green cells nor antecedents of green cells.

Infection takes place neither directly nor indirectly from the body of the parent, but from the sea-water or from the egg-capsules to which the infecting organism is chemotactically attracted and on which it habitually settles down and develops.

Experiment shows that the green cells of adult *Convoluta* are incapable of life apart from the body of the animal; histological examination, proving that the development of the green cell within the body is accompanied by degeneration of its nucleus, supplies the explanation.

The infecting organism has been isolated, and, by the addi-

tion of it to colourless *Convoluta*, the green animal has been synthesised.

In its free stage the infecting organism shows the essential characters of the *Chlamydomonadeæ*; its four equal flagella point to its inclusion in the genus *Carteria*; certain peculiarities suggest that the assignment of the infecting organism to this genus should be only provisional. The infecting organism is capable of a saprophytic as well as of a holophytic mode of life and occurs in both colourless and green forms. Its saprophytic habit leads to its association with the egg-capsules and constitutes a first physiological step toward its association with the body of *Convoluta*.

The green cells serve as an excretory system to the animal.

The relation between green cell and animal changes with their development, passing from a symbiotic relation to one in which the animal is parasitic on the algal cells.

The association leads to marked changes of habit on the part of the animal—e. g. to its ceasing from the ingestion of food—and is best interpreted as an economically unsound attempt on the part of both green cell and animal to solve the “nitrogen problem.”

TABLE I.—Infection Experiments.

Convoluta roscoffensis, Trégascl, 1903. Cultures in sterile and in ordinary sea-water.

Method.—Some thousands of mature *Convolutas* placed in each of three large glass vessels (A, B, C), eggs laid, and larvæ hatched and left with adults during time of experiment. Sea-water sterilised by filtering through Pasteur-Chamberland filter.

	A	B	C
Time of records, August 29.	Convoluta washed 8 times with sterilised sea-water and then put in sterilised sea-water.	Convoluta washed 8 times with sterilised sea-water and then put in fresh (unfiltered) sea-water.	Convoluta not washed and put in sterilised sea-water.
Aug. 31	Capsules laid	Capsules laid	Capsules laid.
Sept. 3	Larvæ hatched (colourless)	Larvæ hatched (colourless)	Larvæ hatched (colourless).
Samples, 12 from each of A, B, C, Sept. 7, examined microscopically	Larvæ colourless	10 colourless; 2 with colourless vacuoles in gut	All colourless.
Ditto, Sept. 9	" "	5 colourless; 7 with colourless or yellow cells in vacuoles	6 colourless; 6 with colourless cells in vacuoles.
Ditto, Sept. 11	Larvæ colourless; in 6, 5, or fewer, colourless vacuoles scattered over the body	1 containing a typical green cell; 11 others with colourless or yellow cells in vacuoles	1 containing a (doubtful) green cell; 2 with colourless inclusions in vacuoles; 9 colourless and no inclusions.
Ditto, Sept. 13	Larvæ colourless; vacuoles persistent and empty	All infected; in some 6 or more green cells	All colourless; vacuoles empty.
Ditto, Sept. 20	Larvæ colourless; vacuoles persistent; some full of long spicules; = no infection	10 larvæ, many green cells; 2 larvæ, no infection; = infection	All colourless; = no general infection.

TABLE II.—Infection Experiments.

Convoluta roscoffensis, Trégastel, 1904. Cultures in sterile and ordinary sea-water. Procedure as in Table I, but eggs picked out as laid (August 9th) and washed in filtered sea-water, then put in a series of vessels containing :

Aug. 13 hatched.	Filtered sea-water.					Fresh unfiltered sea-water.
	A.	B.	C.	D.	E.	
Aug. 28. {	10 uninfected 0 infected	9 uninfected 1 infected	9 uninfected 1 infected	10 uninfected 0 infected	2 uninfected 8 infected	
Sept. 16. {	None remain	3 uninfected 1 infected	1 uninfected 9 infected	2 uninfected 1 infected	—	

TABLE III.—Infection Experiments.

Convoluta roscoffensis, Trécastel, 1905. Cultures of larvæ in sterile and in fresh sea-water, with and without capsule-remnants, and with and without adults.
 Method; July 19th.—Large numbers washed 3 times in filtered sea-water. Egg-capsules transferred when laid to filtered sea-water in covered watch-glasses—3 eggs per watch-glass. When hatched the young placed under the conditions indicated:

The fractions represent the ratios: $\frac{\text{infected}}{\text{uninfected}}$.

	Filtered sea-water without capsules.	Filtered sea-water without capsules.	Filtered sea-water with capsules.	Filtered sea-water with capsule remnants.	Filtered sea-water with capsules.	Filtered sea-water with adults.	Fresh sea-water without adults.	Fresh sea-water with capsules.	Fresh sea-water with adults.
July 30	$\frac{2}{8}$	$\frac{0}{10}$	$\frac{0}{10}$	—	$\frac{0}{10}$	0	—	0	infection in many.
Aug. 2 and 3	$\frac{0}{10}$	$\frac{0}{10}$	$\frac{4}{6}$	$\frac{3}{2}$	$\frac{1}{10}$	0	$\frac{10}{0}$	$\frac{5}{5}$	—
" 4-6	$\frac{0}{10}$	$\frac{0}{10}$	—	$\frac{3}{1}$	$\frac{0}{10}$	—	$\frac{20}{0}$	—	—
" 8-11	$\frac{0}{10}$	$\frac{0}{10}$	Visibly green	—	$\frac{3}{34}$	0	young form a visibly green cloud	—	$\frac{5}{5}$
" 11-15	$\frac{3}{6}$	$\frac{0}{7}$	and flagellate-cell colonies formed	$\frac{1}{0}$	$\frac{10}{0}$	$\frac{35}{0}$	—	general infection	—
Totals	$\frac{5}{44}$	$\frac{0}{47}$		—	—	$\frac{0}{75}$	—	—	—

TABLE IV.—Comparison of the Effects of various Compounds of Nitrogen when added to Cultures of the Infecting Organism of *Convoluta roscoffensis* in Filtered Sea-water.

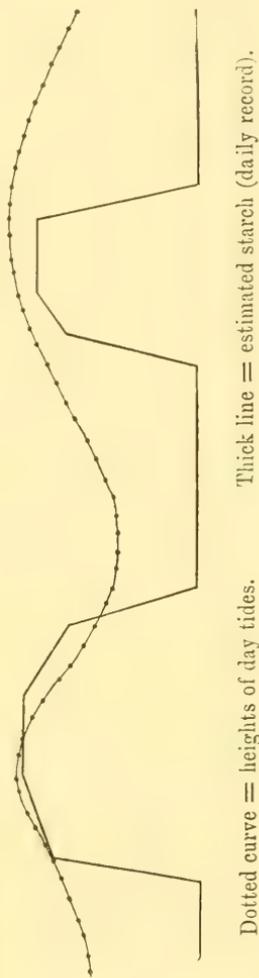
Trégaste!, August 31st, 1906.—Samples of active flagellated stage planted out by platinum loop in test-tubes containing sea-water + the following:

	A	B	C	D	E
	Asparagine.	Urea.	Uric acid.	Potassium nitrate.	Ammonium chloride.
Sept. 8	No growth	A little growth	Considerable growth	No growth	No growth.
Sept. 20.	„	Fair growth	Much growth, the alga forming a visible green scum	A little growth	„

TABLE V.—Daily Estimate of Starch-content of Green Cells of *C. roscoffensis* during Twenty-eight days. Trégastel, 1905.

Date.	Tide.	Recorded day-period during which <i>Convoluta</i> zone was uncovered.	Starch.
Aug. 18 . . .	82 decimetres	11—3.30	Much.
„ 19 . . .	80 „	11.20—4.15	„
„ 20 . . .	78 „	11.30—4.15	„
„ 21 . . .	75 „	11.45—4.50	„
„ 22 . . .	72 „	12.30—6	„
„ 23 . . .	68 „	1—6.15	„
„ 24 . . .	67 „	1.50—6.40	Little.
„ 25 . . .	68 „	3.30—7	„
„ 26 . . .	70 „	daylight—9.30	}
		4.30—dusk	
„ 27 . . .	74 „	7—11.30	} Fair amount.
		5—11.30	
„ 28 . . .	79 „	6.50—12 50	Much.
„ 29 . . .	83 „	7.50—1.30	„
„ 30 . . .	85 „	8.30—2.30	„
„ 31 . . .	90 „	9—3.15	„
Sept. 1 . . .	91 „	9.45—4	„
„ 2 . . .	90 „	10.15—4.15	„
„ 3 . . .	88 „	11—4.40	„
„ 4 . . .	84 „	12—6	Less.
„ 5 . . .	79 „	1—6.40	„
„ 6 . . .	73 „	—7	}
		2—7.30	
„ 7 . . .	71 „	—8.15	}
		3—8.30	
„ 8 . . .	71 „	—9	} More but still little.
		4—9.30	
„ 9 . . .	73 „	—10	}
		5.30—11.20	
„ 10 . . .	76 „	6.50—12 noon	} Little.
		6.30—12 night	
„ 11 . . .	79 „	7—1.10	Much.
„ 12 . . .	82 „	8—1.50	„
„ 13 . . .	84 „	8.30—2	„
„ 14 . . .	84 „	10.30—3	—

TABLE VI.—Diagrammatic Reproduction of Table V, showing Coincidence of Periodicity of Starch-formation in the Green Cells of *Convoluta roscoffensis* with Tidal Periodicity.



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EXPLANATION OF PLATES 13 & 14,

Illustrating the paper by Dr. Keeble and Dr. Gamble on
“The Green Cells of *Convoluta roscoffensis*.”

REFERENCE LETTERS.

Bact. Bacteria on the caps. (egg-capsule). *B. M.* Basement membrane. *C.* Empty cyst left by an escaped flagellated cell from the palmella state. *CHL.* Chloroplast. *Cil.* Cilia. *C. Pl.* Cytoplasmic plug forming the “neck” of the green cell. *Ep.* Epidermal layer. *G. c.* Green cells. *Mes. C.* Mesenchyme (probably phagocytic) cells of *Convoluta*. *Mu.* Mucilage of wall of green cell. *Nu. G. C.* Nucleus of green cell. *Nu. Mes.* Nucleus of mesenchyme cell associated with the green cell. *Ov.* Eggs of *Convoluta* in the common capsule. *Pyr.* Pyrenoid. *St.* Stigma (eye-spot).

PLATE 13.

FIG. 1.—Egg-capsule of *Convoluta* filled with a dense mass of flagellated green cells forming a pure culture of the infecting organism. $\times 35$.

FIG. 2.—The same capsule under slight pressure. The flagellated cells are swarming actively and escaping through the ruptured wall.

FIG. 3.—The smaller green cells (microcytes) of the infecting organism in the active phase, showing the delicate wall, four flagella, chloroplast, stigma and clear "neck" of cytoplasm in which the nucleus is lodged. (For histology see also Fig. 12 B, Pl. 14.)

FIG. 4.—The larger green cells (macrocytes). (No contractile vacuoles in either form of green cell.) Cf. Fig. 12 A.

FIG. 5.—A portion of the egg-capsule; the green cells have come to rest and lie in pairs.

FIG. 6.—The infection of an egg-capsule by the green cells chemotactically attracted thereto (see pp. 188, 189 of text).

FIG. 7.—Infected capsule, more highly magnified. Green cells (and bacteria) seen on the outer edge of the capsule. Other green cells have made their way through the capsule to the egg (*ov.*).

FIG. 8.—Peculiar colonial form of the green-celled infecting organism (for regular palmella stage see Figs. 14 and 15, Pl. 14). Non-flagellated green cells in inverted position borne at the ends of branching columns of stratified mucilage.

FIG. 9.—Dorsal portion of transverse section through the body of an adult *Convoluta roscoffensis* showing the structure, arrangement, and relations of the green cells. The majority of the green cells have no nuclei, some mere granular traces. The green cells are arranged in rows running inward from the periphery; the outermost green cell of a row has a nucleus (red). Indications of continuity between green cells are seen and the close relation between them and the mesenchyme cells of *Convoluta* is shown. $\times 750$.

FIG. 10.—Another more highly magnified section through the body of *Convoluta* (fixed with Fleming, stained with safranin). Four green cells suspended by slender processes from the basement membrane and separated by mesenchymatous cells. The green cell on the left hand has a nucleus (*Nu. gc.*), the others contain only granules which stain deeply with safranin; similar deep-staining granules are also plentiful in the subjacent mesenchyma (Cam. luc., oc. 12, obj. $\frac{1}{2}$).

PLATE 14.

FIG. 11.—Life-history of the green-celled infecting organism in the free state. A, Encysted colourless macrocytes, resting and dividing into colourless daughter-cells. B, Microcyte dividing. C, Encysted green macrocytes, resting and dividing into green daughter-cells. D, Apparent fusion of two macrocytes (see text, p. 190). E, Apparent fusion of two unequal colourless cells.

FIG. 12.—A, A typical free macrocyte. B, A typical free microcyte. The nucleus is homogeneous, and in A sends processes towards the bases of the flagella and also towards the pyrenoid. The reticulate surface-cytoplasm is shown, the more internal chloroplast being drawn in optical section.

FIG. 13.—Green cells from the body of just-infected larval *Convolutas*. The large nucleus of the recently ingested green cell contrasts markedly with the degenerate nucleus of the daughter green cell budded off later from such a mother-cell. (Cam. luc., oc. 12, obj. $\frac{1}{12}$ Zeiss.)

FIGS. 14 and 15.—The infecting organism in the palmella (colonial) stage. A, B show green cells of the palmella organising active cells. C, The empty cyst left after the escape of a flagellated cell. Fig. 15 illustrates the origin of new cells of the palmella by budding.

FIG. 16.—A, B, C, the gradual disappearance of one of a pair of apposed resting-cells (see text, p. 184). D, E, Colourless and green resting-cells. F, G, Formation of colourless daughter-cells within a colourless mother-cell. H, F, Formation and discharge of green daughter-cells.

On the Development of the Plumes in Buds of Cephalodiscus.

By

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With 11 Text-figures.

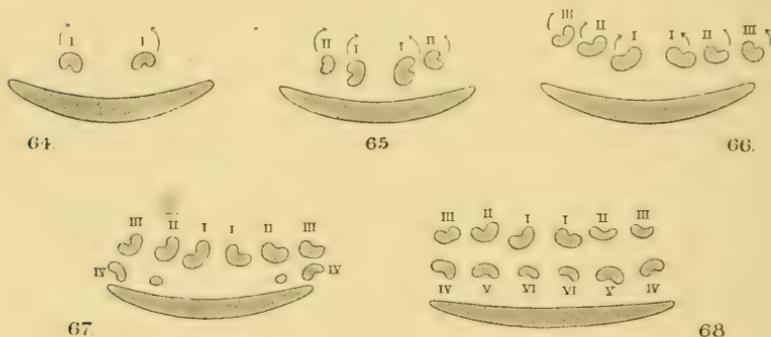
	PAGE
Introduction	221
Methods	223
Development of the Plumes in Buds of <i>Cephalodiscus</i>	
<i>hodgsoni</i>	224
Development of the Plumes in Buds of <i>Cephalodiscus</i>	
<i>dodecalophus</i>	230
Development of the Plumes in Buds of <i>Cephalodiscus</i>	
<i>nigrescens</i>	235
Development of the Plumes in Buds of <i>Cephalodiscus</i>	
<i>gilchristi</i>	242
General Remarks on the Collar and its Appendages	245
Summary	251
Explanation of the Abbreviations employed in Text-figures 2, 3, 4, 6, 7, and 9	252

INTRODUCTION.

During the progress of an investigation on the anatomy of three recently discovered species of *Cephalodiscus* (*C. nigrescens*, *C. hodgsoni*, and *C. gilchristi*), kindly entrusted to me for description by Prof. E. Ray Lankester, F.R.S., Director of the Natural History Museum, London, I noticed that in the development of the buds there is no torsion of the first and second plume-axes such as is described by Masterman as occurring in buds of *C. dodeca-*

lophus,¹ and that the last plumes do not develop between the earlier plumes and the buccal shield, as recorded by the same writer, but on the side of those plumes remote from the buccal shield. His figures 64—68 are reproduced as text-fig. 1.

I was unable to settle the point satisfactorily in time for the introduction of my results into the two papers describing the structure of the polypides and tubaria of the new species,² but since the completion of those papers I have made an exhaustive study of the growth of the plumes in buds of



TEXT-FIGURE 1.—Diagrammatic transverse sections of plumes and buccal shield of *Cephalodiscus dodecalophus* at five stages of development. Copied from A. T. Masterman's paper in the 'Trans. Roy. Soc. Edin.,' vol. xxxix, part 3, 1898, plate 4, figs. 64—68. The original numbering has been retained.

Cephalodiscus hodgsoni, *C. nigrescens*, and *C. gilchristi*, and having made from the abundant material at my disposal a well-graded series of buds of each species, I was enabled to confirm my earlier conclusions.

I finally prepared a series of buds of the "Challenger" species, *Cephalodiscus dodecalophus*, with the result that I am satisfied that the buds of this species agree with

¹ 'Trans. Roy. Soc. Edin.,' vol. xxxix, part 3, 1898, p. 521.

² "'Discovery" Expedition Reports,' vol. ii, 1907, and 'Marine Investigations in South Africa,' vol. iv, 1906.

those of the other three species. The torsion described by Masterman does not occur, but the grooves of the first and second pairs of plumes, developed in such a manner that they face ventrally towards the buccal shield, continue to face the shield. The last two pairs of plumes are not developed between the first two pairs and the shield, but on the dorsal side of them, i.e. on the side remote from the shield. The grooves of these last plumes (fifth and sixth pairs) are not directed towards the shield, but away from it.

Although the four series of plumes of buds were prepared for the purpose of deciding the points raised by Masterman, they serve to show a number of other interesting features, such as (1) the difference of plume development in the buds of the four species; (2) the relation which the last-formed pair of plumes bear to the edge of the post-oral lamella; and (3) the conversion, in the later stages of growth, of the line of the bases of the collar-outgrowths (plumes and post-oral lamella) from a circle to a double crescent, or two incomplete ellipses. I have selected from each of the four series a number of preparations to illustrate these points; they are reproduced in text-figures 2, 3, 4, 6, 7, and 9, and are described under headings of the respective species.

METHODS.

In the case of the youngest stages the whole bud was mounted in diluted glycerine, and gold size was run round the edge of the cover-glass to keep it firmly in position, and to prevent the glycerine from accumulating dust. Most of the buds were dissected, the shield being first removed by tearing through its stalk by the aid of fine mounted needles, and then the collar region, with plumes and post-oral lamella (e.g. text-fig. 2, 1), was removed by carefully manipulating the needles between these parts and the "body" of the bud. The three parts, shield, collar region, and "body" with its stalk, were then mounted on the same slide in dilute glycerine.

The relation which the collar region, as dissected out, bears to the whole bud will be made clear by reference to text-fig. 5, A, a diagrammatic side view of a bud with three pairs of plumes, and text-fig. 10, a diagram of a longitudinal section of the adult polypide; in the latter figure the limit of the collar region is marked by two lines of heavy dashes.

The results here recorded and illustrated are based upon 135 preparations of buds made and mounted as above explained. In some cases (e.g. text-fig. 6, F) the three parts—"body," collar appendages, and shield—were drawn separately on tracing paper, and the perfect bud was reconstructed by a superposing of these transparent sheets.

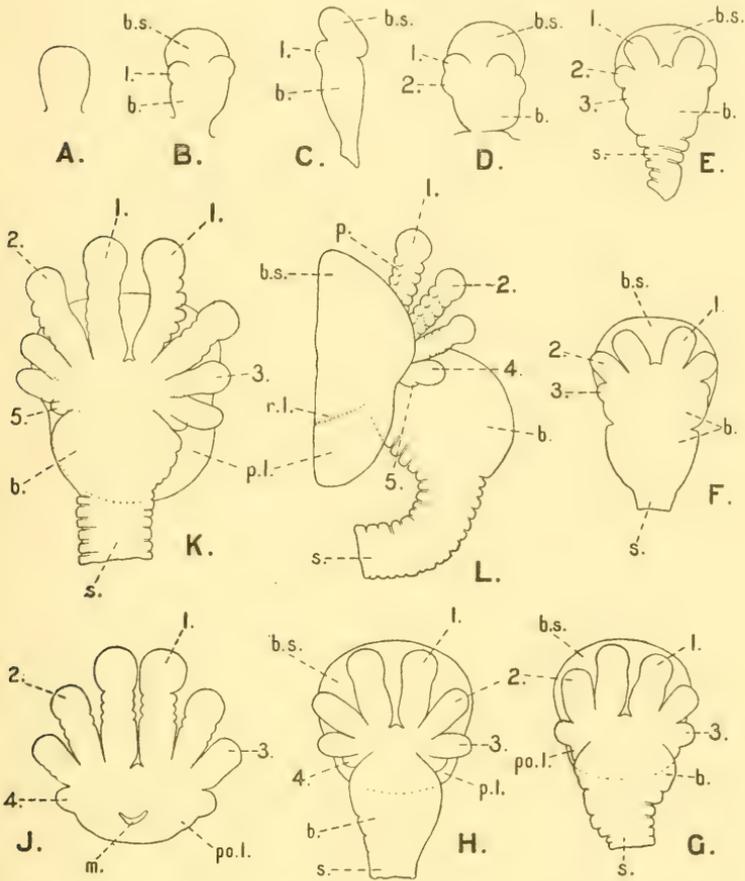
No staining fluids were used. The dissections were made under a Greenough binocular erecting microscope magnifying 20 and 40 diameters; the drawings were made by the aid of a compound microscope with Zeiss apochromatic 8 mm. objective and No. 6 compensating ocular with eye-piece micrometer. The figures in text-figs. 2, 3, 4, 6, 7, and 9 are all reproduced to the same scale of enlargement, viz. 62 diameters.

DEVELOPMENT OF THE PLUMES IN BUDS OF CEPHALODISCUS HODGSONI.

In the earliest phase of development the bud is ovoid or pyriform (text-fig. 2, A) without signs of buccal shield or plumes. The shield and the first pair of plumes make their appearance simultaneously (B and C). The plumes are at first small mounds, later hemispherical (D), and still later longer than broad (E, F, etc.). The second pair of plumes appear on the external side of the first pair (D, 2), and in contact with them. The two plumes of the first pair are not in contact with one another at their bases. The third pair of plumes appear lateral to the second pair (E, 3), and in contact with them.

The bud increases considerably in size before the fourth

plumes appear; the buccal shield assumes its definitive shape by becoming flattened, and by the differentiation of the pos-



TEXT-FIGURE 2.—*Cephalodiscus hodgsoni*. Figures illustrating the development of the plumes of the buds. C and L are side views, the others are dorsal views. J represents the collar and its appendages (plumes and post-oral lamella) dissected from a bud a little older than H. The first pair of plumes are just appearing in B and C, the second in D, the third in E, the fourth in H, and the fifth in K and L. The series is continued in text-fig. 3. All figures enlarged 62 diameters. For explanation of the lettering see end of paper.

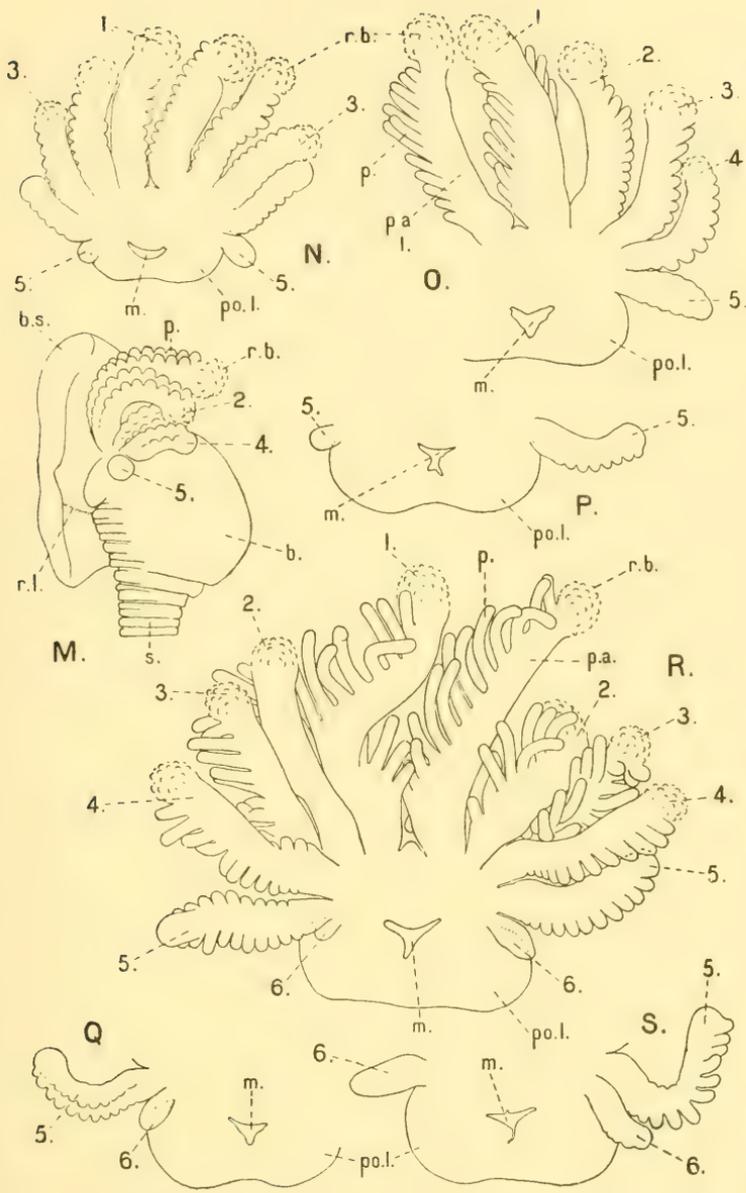
terior lobe. In G and H the dotted line marks the position of the free edge of the posterior lobe. The fourth plumes

appear when the bud has reached the stage of development represented in π . The structure which in g is marked *po.l.* might at first glance be taken for the developing fourth plume; it is the lateral part of the post-oral lamella.

Figure j represents the collar region and its appendages (the plumes and post-oral lamella) of a bud a little older than that shown in figure π ; the buccal shield has been dissected off from the one side, and the "body" and stalk from the other. The position of the mouth is indicated in the figure, but this region is always more or less damaged in making such a dissection as that described. The torn stalk of the buccal shield in this preparation—as also in the similar preparations shown in text-fig. 3, n , o , and r , and in other of the text-figures—occupies a position intermediate between the mouth and the bases of the first pair of plumes. In figure j the fourth plumes are hemispherical projections at the bases of the third plumes, touching these on the one side, and in continuity with the post-oral lamella on the other. Traces of pinnules are appearing on the first and second plume-axes, and the terminal end-bulbs of these same plumes are swelling out.

In κ the fourth plumes are now longer than broad, and the fifth pair are making their appearance. l is a side view of a bud of about the same stage as κ , as may be determined by comparing the development of the fourth and fifth plumes in the two buds. Pinnules are now appearing on the third plumes, and it will be noticed, in the case of the first, second, and third plumes, that the pinnules are not laterally placed on the plume-axes, but ventro-laterally, and the groove between the two rows of pinnules of each of the plumes in question opens ventrally, or ventro-laterally, towards the dorsal or stalked side of the shield.

The figures in text-fig. 3 continue the series shown in text-fig. 2. m is a little more advanced than l of text-fig. 2; the fifth plume is now hemispherical in shape; pinnules are appearing on the fourth plumes, and the end-bulbs are becoming differentiated. The end-bulbs of the first, second,



TEXT-FIGURE 3.—*Cephalodiscus hodgsoni*. Figures illustrating the development of the plumes of the buds; series continued from text-fig. 2. M shows the left side of a bud in which the fifth pair of plumes are swollen knobs; the other figures are dorsal views of the collar and appendages (plumes and post-oral lamella) of older buds, represented in whole (N and R) or in part only (O, P, Q and S). The fifth pair of plumes have the form of clavate knobs in N, and the sixth in R. All figures enlarged 62 diameters. For explanation of the lettering see end of paper.

and third plumes—the last concealed in the figure by the second plume—are showing refractive beads (*r.b.*), and their surface is no longer smooth, but warty; the pinnules are larger and more numerous than in *L*. The figure *M*, like *I*, shows how the ciliated groove between the two rows of pinnules of each of the plumes 1, 2, and 3 faces towards the dorsal surface of the buccal shield.

Figure *N* is a dissection made in the same manner as that shown in figure *J*, by the removal of the buccal shield on the one side of the collar and the "body" of the bud on the other. The preparation is viewed from the dorsal surface, so that the grooves of the plumes are facing away from the observer. The fifth plumes are seen to be continuous with the bases of the fourth plumes, and with the post-oral lamella; they are unequally developed on the right and left sides of the body.

In *O* the pinnules on the first plumes are now finger-like processes, those on the fourth are in the same stage of development as the pinnules of the first plumes were in the stage represented in figure *M*. There are twelve or thirteen pairs of pinnules on the plumes of the first pair, and about nine on the fourth plumes, but the numbers later become increased by the development of new pinnules at the basal end of each series. The fifth plumes are larger than in the last stage, and show an indication of pinnules, but as yet they have no end-bulbs. There is no sign of a sixth plume between the fifth plume and the post-oral lamella. *O*, like *N*, is a dorsal view, and the ciliated grooves of the plume-axes are not seen; the grooves of the first and second plumes are facing ventrally, those of the third ventro-laterally, and those of the fourth postero-laterally.

Figure *P* shows an unequal development of the right and left plumes of the fifth pair. On the right side the plume has about eight pairs of pinnules and a moderately differentiated end-bulb, whereas on the left side the length of the plume is only a little greater than the width. The groove on the right-hand plume is facing posteriorly.

Figure q shows the developing sixth plume, wedged in between the base of the fifth and the antero-lateral edge of the post-oral lamella;¹ it is already pear-shaped, and with a narrowing base. The preparation here drawn is presenting its dorsal surface to the observer, and the fifth plume is showing its groove facing postero-dorsally.

In figure r the fifth plumes are larger and with better developed pinnules than in q, but the sixth plumes are not more advanced; indeed, on the left side the sixth is less developed than it is in q. It is worthy of special remark that, while in the case of the first and second plumes the development of the right and left plumes of each pair is regular and symmetrical, in the fifth and sixth plumes the one is very frequently more advanced than the other of its pair. The grooves of the fifth plumes should be facing posteriorly or postero-dorsally; by the pressure of the cover-glass upon the preparation the plumes have twisted round somewhat. For the same reason the first plumes have their grooves facing to the left, while the third plume on the right side is twisted right round so as to present its grooved surface to the observer. The pinnules on the first plumes are now fairly long, and the pinnules of the third and fourth plumes are finger-like, and resemble those of the first plumes in the stage shown in figure o.

s differs from r in that the sixth plumes are more advanced, the right hand one showing signs of pinnules. The fifth plume shows its groove facing posteriorly. The bud from which the preparation shown in figure s was made was a very late bud, so large, and with such great development of the visceral mass, bringing the base of the stalk from a posterior to a ventral position, that, if it was not still in connection with a full-grown polypide by means of its stalk, one might easily mistake it for an adult polypide.

¹ The close relation obtaining between the base of the last-formed plume and the edge of the post-oral lamella was, I believe, not known until it was pointed out by Harmer two years ago ('Pterobranchia of the "Siboga" Expedition,' 1905, p. 36).

Possibly in some cases the sixth plumes are permanently arrested in their development on one side, or on both sides, or they are not developed at all, for some polypides of *Cephalodiscus hodgsoni* of full size and with mature gonads have five pairs of plumes only. In the cases of those polypides which are found with eleven plumes the explanation may just as likely be that the sixth plume has failed to develop on one side as that one of the plumes has been lost by injury.

As regards the development of the pinnules on a plume-axis, about five pairs appear simultaneously, and at the time of their origin they occupy nearly the whole, or, at all events, more than half of the length of the plume-axis behind the end-bulb. The later pinnules are developed a pair at a time at the basal end of the series.

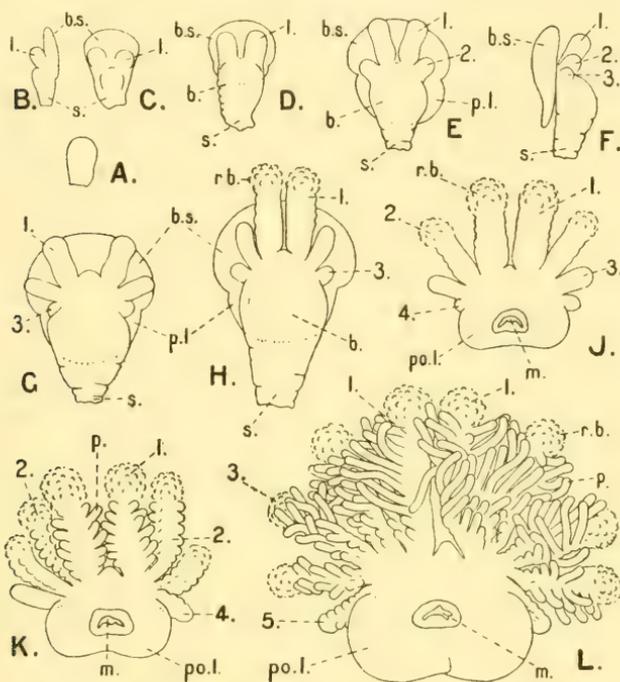
DEVELOPMENT OF THE PLUMES IN BUDS OF *CEPHALODISCUS DODECALOPHUS*.

The bud of *Cephalodiscus dodecalophus* in its earliest phase (text-fig. 4, A) resembles that of *C. hodgsoni* except in being somewhat smaller. The first pair of plumes and the buccal shield begin to differentiate simultaneously (B and C). By the time the second plumes appear (E) the first pair are relatively longer than in *C. hodgsoni*. The third plumes develop before the second pair have grown very large (F and G).

In buds with three pairs of plumes present the size of the first pair varies considerably, as may be seen by reference to H and G. Although H is a larger bud than G, it is not much more advanced in its development; the second and third plumes are only slightly more developed than in G, and there is no sign of the fourth plumes. Yet the first plumes are very large in H, and have refractive beads in the terminal end-bulbs, whereas the corresponding plumes in G are

no larger in proportion than in *C. hodgsoni* (cf. text-fig. 2, G).

In J the fourth plumes are appearing, and the gradation from the first plumes to the fourth is fairly uniform; refractive beads are now visible on the second plumes. On



TEXT-FIGURE 4.—*Cephalodiscus dodecalophus*. Figures illustrating the development of the plumes of the buds. J, K and L are ventral views of the collar and appendages (plumes and post-oral lamella); B and F are side views of complete buds; the remaining figures are dorsal views of complete buds. The first pair of plumes are just appearing in B and C, the second are fairly large in E, the third are appearing in F and G, and the fourth in J; in K the fourth plumes are moderately well developed, but there are no fifth plumes; in L the fifth plumes are fairly well developed. All figures enlarged 62 diameters. For explanation of the lettering see end of paper.

comparing J of text-fig. 4 with J of text-fig. 2 it will be seen that in *C. dodecalophus* the lateral lobes of the post-

oral lamella are more prominent as free flaps than in *C. hodgsoni*.

Pinnules first appear in the stage represented in J, i. e. in the stage when the fourth plumes are appearing, or possibly a little earlier (H). They are at first confined to the first pair of plumes, and they appear as five or six pairs of small eminences arising simultaneously; additional pairs develop in succession at the basal end of the series. In K there are seven pairs, and in L eleven or twelve pairs of pinnules on the plumes of the first pair. The pinnules on the second, third, and subsequent plumes arise later than those on the first, as might be expected. About four pairs of pinnules are just appearing on the second plumes in J.

Masterman,¹ in figuring a bud about as advanced as G and H of text-fig. 4 of this paper, shows four pairs of long slender pinnules on the first plumes. There is nothing comparable with this in my preparations. My observations also fail to accord with those of Dr. Masterman in the matter of the development of the first few pinnules on the plumes. In figs. 69—74 of his Plate 4 he gives figures illustrating the growth of a plume—he does not say which. In fig. 71 there are no pinnules, in fig. 72 one pair, in fig. 73 the first pair have elongated considerably, and there is a new pair on their proximal side, in fig. 74 there are four pairs of pinnules shown. My own observations go to show that the first five or six pairs of pinnules arise simultaneously on the first plumes; on the second and third plumes the first four or five arise simultaneously, and on the fourth pair (text-fig. 4, K, 4) the first three or four. At a stage when any one plume has but four pairs of pinnules I have not seen the pinnules nearly so long and slender as those shown in Masterman's fig. 74.

In L of text-fig. 4 the fifth plumes have attained considerable size and exhibit about four pairs of pinnules. The pinnules on the first and second plumes are now long, except the youngest of the series near the base of the plume-axis. End-bulbs with refractive beads (*r.b.*) are present on the first four

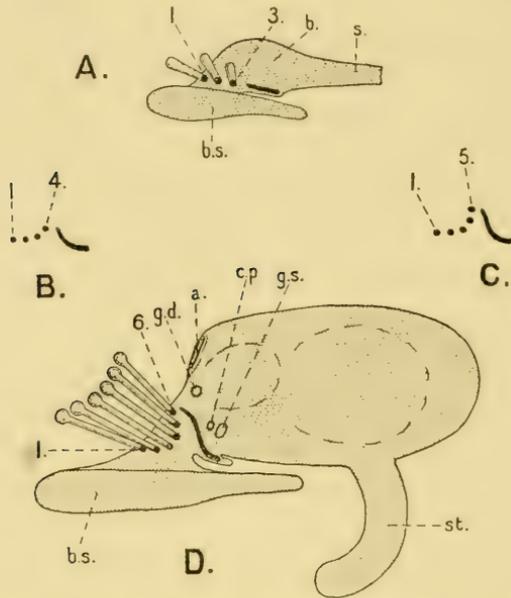
¹ 'Trans. Roy. Soc. Edin.,' vol. xxxix, 1898, pl. 2, fig. 24.

pairs of plume-axes. Both κ and L are ventral views, and the groove between the two rows of pinnules of each plume-axis is directed towards the observer.

It will be noticed that in J , κ , and L the right and left plumes of the first pair are separated by a small interval, in which is situated a slight mound of the basal part of the stalk of the buccal shield, obliquely torn in the making of such preparations as those here figured; but the several plumes of the same side of the body are in close contact with one another at their bases, and the last developed plume is wedged in, with a certain amount of over-lapping, between the last plume but one and the antero-lateral edge of the post-oral lamella.

Except in distorted specimens the last developed plume lies slightly dorsal to the edge of the post-oral lamella, and lies slightly ventral to the last plume but one (κ). It must be noted, however, that in stages such as that shown in L , and in later stages, the line of plume bases and base of the post-oral lamella does not lie in a plane, and a certain amount of distortion or crumpling is bound to result from any attempt at mounting the dissection between two pieces of glass. Note the wrinkle in the middle of the posterior edge of the post-oral lamella in L . In such a stage as is shown in J the line of plume-bases and attached edge of the post-oral lamella is nearly in one plane, a plane slightly oblique to the long axis of the bud. The fourth, fifth, and sixth plumes, however, are not developed in this plane, but more dorsally, and this is how it comes about that in the adult polypide the bases of the six plumes are set in a circle or ellipse in one oblique plane of the body, while the attached edge of the post-oral lamella lies in another oblique plane, which intersects the first plane at the points of contact of the sixth plumes with the edge of the post-oral lamella. This will be made clear, I think, by reference to text-fig. 5, and its accompanying legend. See also text-fig. 8, a diagram of *Cephalodiscus nigrescens*.

Masterman has stated¹ that the first three pairs of plumes arise with their grooved faces directed towards the buccal



TEXT-FIGURE 5.—Diagrams showing that the later developed plumes (4, 5 and 6) have their bases set more dorsally than the earlier. The heavy dots mark the several plume-bases, the heavy line the line of attachment of the post-oral lamella. In A, a side view of a bud with three pairs of plumes, the plume-bases and the post-oral lamella are in one plane, a plane nearly parallel with the buccal shield. In B the base of the fourth plume is more dorsally placed than the others, and the line of the post-oral lamella rises at its anterior end. Similarly with the fifth plume and the post-oral lamella in figure C. In D, a diagram of an adult polypide, the base of the sixth plume is not only dorsal to that of the fifth, but is anterior also; the right and left plumes of the sixth pair are thus fairly close together; the line of attachment of the post-oral lamella is produced forward so that the end of the line is, as before, close to the base of the last-formed plume. In D the pinnules are not shown, and the plume-axes are represented very diagrammatically.

a. Anus. c.p. Collar pore. g.d. Gonad duct. g.s. Gill slit. st. Stolon. Other lettering as before.

shield, but later rotate upon their axes so that the grooves face away from the shield; further, that the last three pairs

¹ 'Trans. Roy. Soc. Edin.,' vol. xxxix, 1898, p. 521.

of plumes arise between the first three pairs and the shield and have their grooves directed towards the shield (see text-fig. 1 of this paper). I am in a position to confirm Harmer's opinion¹ that this statement is without foundation, and that Masterman's earlier enumeration of the plumes² is the correct one. In *Cephalodiscus dodecalophus*, as in *C. hodgsoni*, the grooves of the first and second pairs of plumes, developed in such a position that they face the buccal shield, continue to face the shield, and the last two pairs of plumes are not developed between the first two pairs and the shield, but on the side of the first two plumes remote from the shield, i. e. to their dorsal side; and the grooves of the last-formed plumes are not directed towards the shield, but away from it. The grooves of the third and fourth plumes face somewhat laterally.

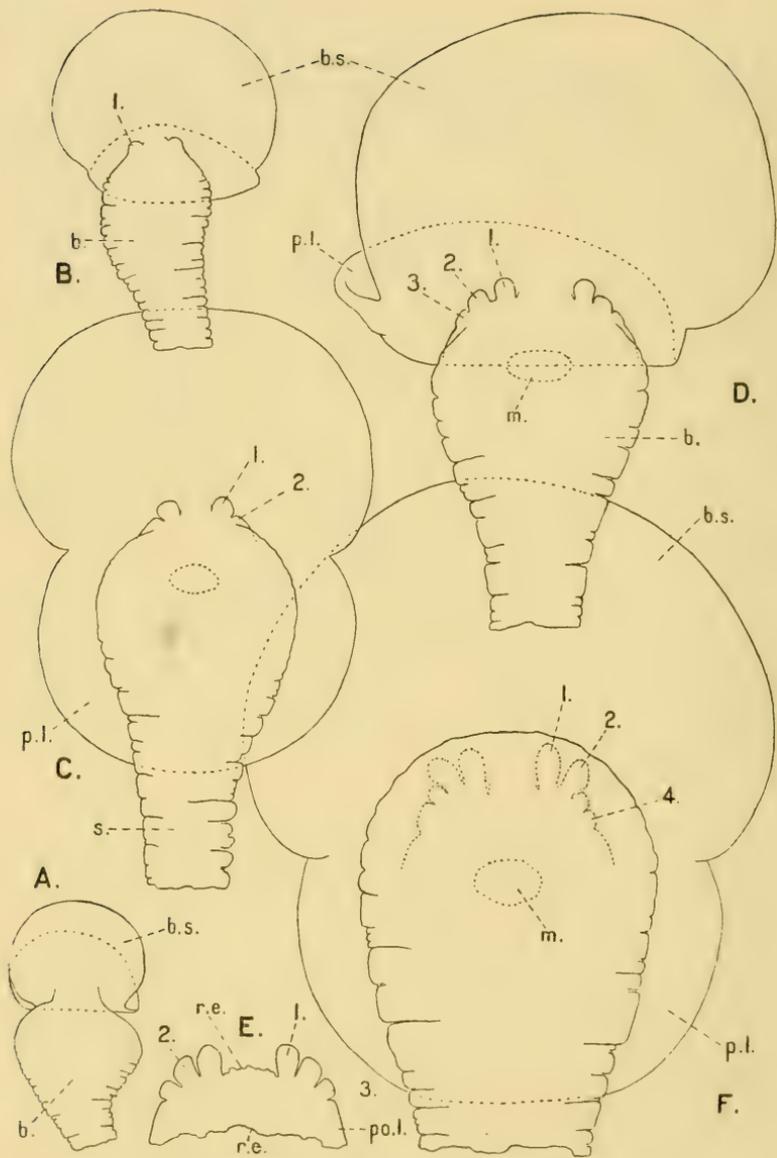
The six pairs of plume-bases are set on an ellipse, and the grooves of the twelve plumes face away from the foci of the ellipse. The six grooves of the right side lead down into the space between the shield and the post-oral lamella on the right side of the mouth; similarly the six grooves of the left side lead into the left side of the mouth (see text-fig. 8, *C. nigrescens*).

DEVELOPMENT OF THE PLUMES IN BUDS OF CEPHALODISCUS NIGRESCENS.

While in buds of *Cephalodiscus hodgsoni* and *Cephalodiscus dodecalophus* the first pair of plumes begin to appear at about the same time as the buccal shield, in those of *Cephalodiscus nigrescens* the buccal shield is well differentiated, and with a well-defined posterior lobe (text-fig. 6, A) before there is any sign of plume development. When the first pair of plumes make their appearance (B, 1) they are small as compared with the bud as a whole, and when the second plumes begin to develop (C, 2) the buccal

¹ 'Pterobranchia of the "Siboga" Expedition,' 1905, pp. 36, 37.

² 'Quart. Journ. Mic. Sci.,' vol. 40, 1897, p. 346, pl. 26, fig. 36.



TEXT-FIGURE 6.—*Cephalodiscus nigrescens*. Figures illustrating the development of the plumes of the buds. A, B, C, D and F are dorsal views of complete buds; E shows the plumes dissected from a bud a little earlier than F. The first pair of plumes are just appearing in B, the second in C, the third in D, and the fourth in F. The series is continued in text-fig. 7. All figures enlarged 62 diameters. For explanation of the lettering see end of paper.

shield is of remarkably large size. Compare text-fig. 6, c, with text-fig. 2, D; and text-fig. 4, E.

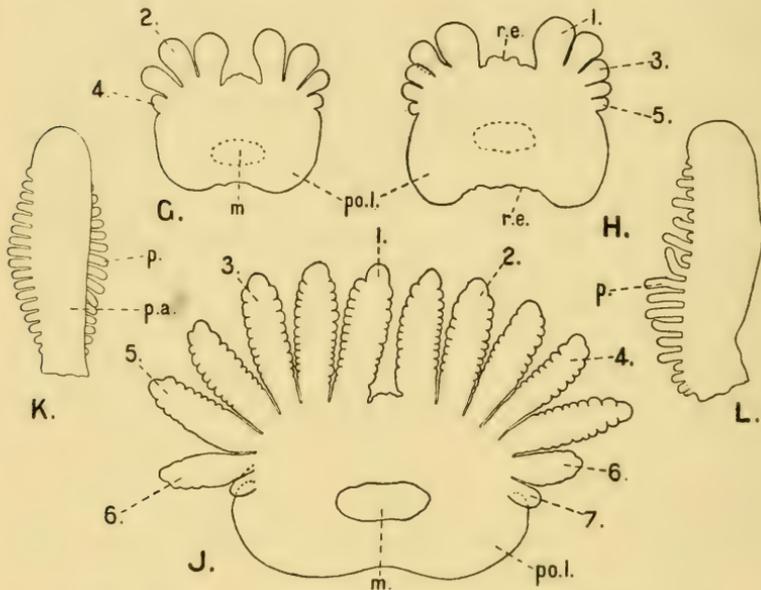
The third plumes appear to the outer side of the second, and are in contact with them at their bases (D, 3). The two plumes of the first pair are widely separated from one another (D, 1). When in buds of *Cephalodiscus hodgsoni* and *Cephalodiscus dodecalophus* the third plumes make their appearance (text-fig. 2, E, 3, and text-fig. 4, G, 3) they are directed strictly laterally, whereas in *Cephalodiscus nigrescens* (text-fig. 6, D and E, 3) they point more anteriorly than laterally. This doubtless is connected with the relatively late origin of the plumes, and the relatively small size of the plumes during the early stages of their development.

A, B, and D illustrate a peculiarity of many buds and adults of *Cephalodiscus nigrescens*, the bending forward of the posterior lobe of the buccal shield. The occurrence of the posterior lobe in this position may be due to an exceptional contraction of the muscles of the shield brought about by the irritating properties of the formalin solution in which the animals were killed, but whether this be so or not, it indicates considerable mobility of the organ in question in normal conditions of existence.

Text-fig. 6, F, shows the dorsal view of a bud in which the fourth pair of plumes are making their appearance. The "body" of the bud here figured was placed so far forward as compared with the stalk of the shield that the plumes, instead of being set on the anterior edge of the "body" as in D, lie in a deep groove between the "body" of the bud and the dorsal wall of the buccal shield. The plumes are drawn in a dotted line to signify that they would be seen in the positions they occupy in the figure if the "body" were transparent; as a matter of fact the "body" is so black that the employment of the usual clarifying reagents fails to make it transparent. The bud in question was drawn as a whole, with no plumes visible; then the "body" was dissected off and the characters and positions of the plumes

noted. This relation of the "body" and shield whereby the plumes are not visible except by dissection is a common one; it is only exceptionally that the "body" is so much drawn back as to expose the developing plumes as completely as in text-fig. 6, D, and in fig. 66 of Plate 7 of the "Discovery" Expedition Reports,' vol. ii, 1907.

The post-oral lamella is not well defined until after the fourth plumes have developed. In text-fig. 7, G, the two



TEXT-FIGURE 7.—*Cephalodiscus nigrescens*. Figures illustrating the development of the plumes of the buds; series continued from text-fig. 6. G, H and J represent the collar and appendages (plumes and post-oral lamella) as seen in dorsal view; K and L are single plumes. The fifth pair of plumes are appearing in H, and the seventh in J. All figures enlarged 62 diameters. For explanation of the lettering see end of paper.

lateral flaps are complete, but the free edge connecting the two flaps behind the mouth is not yet entire, and so in making such a dissection as is shown in G and H this part is left with a ragged edge (H, *r.e.*). The plumes of the first three pairs are elongating and are swelling out so that the base of each

appears comparatively narrow (G and H). The fourth plume in G is set between the base of the third plume and the anterior part of the free edge of the post-oral lamella, and so when the fifth plume appears (H, 5) it would seem as though that plume must have arisen from the edge of the post-oral lamella itself. Similarly also with the sixth and seventh plumes.

I have not met with a bud showing the early development of the sixth plumes, so that there is a greater interval than I could have wished between H, with fifth plumes appearing, and J, with seventh plumes appearing.

Up to the time when the fifth plumes are beginning to develop, the size of the plumes diminishes from the first to the fifth (H), but when the seventh is making its appearance (J) the plumes first formed have failed to maintain their initial superiority in size, and the first five pairs of plumes are nearly of equal size.

Pinnules arise rather late. In text-fig. 7, J, the seventh plumes have already appeared, and yet the pinnules of the first plumes are not more than hemispherical projections. On the plumes of the first pair the first ten or twelve pairs of pinnules arise simultaneously, the later ones are added at the basal end of the series. In *Cephalodiscus hodgsoni* (text-fig. 3, o) and *Cephalodiscus dodecalophus* (text-fig. 4, L) the pinnules on the first plumes are already finger-like projections at the time when pinnules first make their appearance on the fifth plumes, and before the axes of the last plumes (the sixth) have shown signs of development. In *Cephalodiscus nigrescens*, however (text-fig. 7, J), pinnules begin to appear simultaneously on the first five pairs of plumes, and are very little advanced at a time when the last plumes (the seventh in this species) have already come into existence. The bud from which fig. J was drawn is of the same size and general appearance as that represented in fig. 67 of Plate 7 of the 'Antarctic "Discovery" Expedition Report on *Cephalodiscus*,' 1907.

In text-fig. 7, J, the first five plumes have elongated con-

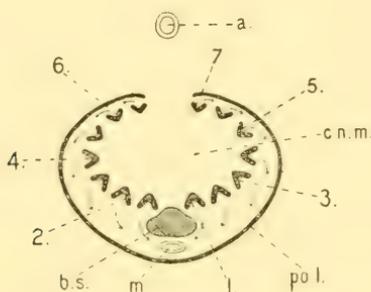
siderably since the stage shown in H, and the axes are less bluntly tipped. The interval between the right and left plumes of the first pair is not relatively, but absolutely less than in earlier stages—all these figures are drawn to the same scale of magnification. The exigencies of pictorial delineation demand that the structures under consideration be drawn flat; it is well to bear in mind, therefore, that, while the structures represented near the median line of the figure are on the antero-ventral surface of the "body" of the bud, just above the buccal shield, the lateral parts (plumes 6 and 7, and lateral parts of the post-oral lamella) are set high up the sides of the "body." The figure if cut out and bent back would give approximately the correct relation of the parts.

In the adult the right and left seventh plumes are as close together as are the right and left first plumes. The line of plume-bases in the adult, when viewed from the front, is an ellipse, incomplete on the upper side; and the line of the attached edge of the post-oral lamella is another ellipse similarly incomplete. The two ellipses join where they are incomplete (see text-fig. 8). The parts seen are, of course, at very different distances from the observer; the base of the fifth plume is in the middle distance, the base of the first plume is nearest to the observer, and the mid-ventral part of the post-oral lamella farthest away. The stalk of the buccal shield and the mouth lie in the middle distance between the two ellipses, and the anus dorsal to both (compare text-fig. 8 and text-fig. 5, D).

The seventh plumes of the adult, and in some polypides the sixth plumes also, do not arise directly from the "body" of the polypide, but are processes of a paired lophophoral upgrowth, which is connected with the "body" at the base of the fifth plume and the more ventral parts of the collar-outgrowths (see "Cephalodiscus," "Discovery" Expedition Reports, 1907, p. 31, last paragraph).

Late buds of *Cephalodiscus nigrescens* are extremely rare in the material at my disposal. Possibly after the stage

of development represented in figure J has been reached the buds migrate. Whatever be the explanation the fact remains that from an abundant supply of buds of various stages I have only discovered one which shows pinnules in a further stage of development than that drawn in figure J. The bud is figured in the 'Report on Cephalodiscus' ("Discovery" Expedition, Pl. 7, fig. 68). It has fourteen plumes, of approximately the same size and development, standing forward in a bunch, parallel with one another; the stalk of the bud is no longer terminal, but projects from the ventral surface of the "body." Two of the plumes of this bud are shown in text-fig. 7, K and L; K is a nearly dorsal view, L a



TEXT-FIGURE 8.—Diagrammatic representation of the view obtained on looking backward at an adult polypide of *Cephalodiscus nigrescens* after cutting short the plumes and post-oral lamella, and removing the buccal shield. The cut bases are represented in black. *pol.* The edge left after clipping away the post-oral lamella. 1, 2, 3, 4, 5, 6 and 7. The stumps of the several plume-axes, showing the grooves facing away from the middle of the ellipse upon which they are set. *b.s.* Stalk of the buccal shield. *c.n.m.* Position of central nerve mass. *m.* Mouth. *a.* Anus. The arrows indicate the direction of the food currents from the grooves of the plumes into the mouth. This figure is in the main similar to Harmer's figures of *C. levinseni* ('Pterobranchia of the "Siboga" Expedition,' 1905, pl. 12, figs. 158—160), but it is treated more diagrammatically.

nearly lateral view. The plume-axis is very massive and pigmented, and has a smooth hemispherical extremity; some of the other plumes of this bud, however, have the ends less rounded, more like those of J. There are about seventeen or

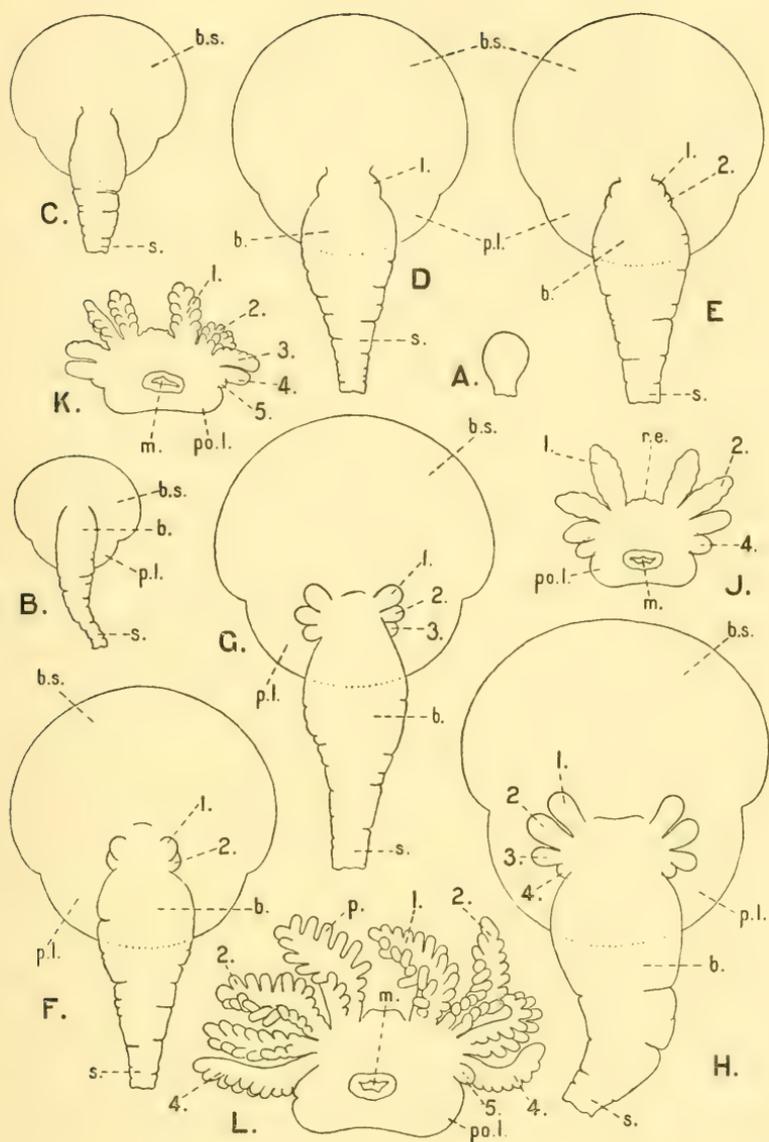
twenty pairs of pinnules, and the middle ones of the series are the longest.

DEVELOPMENT OF THE PLUMES IN BUDS OF CEPHALODISCUS
GILCHRISTI.

Cephalodiscus gilchristi resembles *Cephalodiscus nigrescens*, and differs from *Cephalodiscus dodecalophus* and *Cephalodiscus hodgsoni*, in the tardy development of the plumes in comparison with the rapid growth of the buccal shield. The bud, at first pear-shaped (text-fig. 9, A), soon becomes differentiated into a buccal shield and a "body," with stalk (B). Even at this early stage of development the posterior lobe of the buccal shield is well defined (B, *p.l.*). A pair of mounds appear on the right and left sides of the "body," near its junction with the middle of the shield (C and D), and these develop into the first pair of plumes. It is to be noted that these mounds are strictly lateral structures, and do not project forward as do the first plumes of the other three species under consideration during the early stages of growth.

The second pair of plumes arise postero-ventrally to the bases of the first (E and F, 2), and in a dorsal view such as that shown are partially concealed by the first pair. When the third plumes begin to develop (G, 3), the first pair project antero-laterally instead of strictly laterally, possibly owing to the pressure put upon them by the second and third plumes, which arise posteriorly to their bases. Each of the six plumes now present is wider at the middle than at its base.

When the fourth plumes make their appearance (H, 4) the length of the first and second plumes is more than twice their width at the base; and before there is any sign of the fifth plumes pinnules begin to differentiate upon the first two pairs of plumes (J, 1 and 2). The first plumes are now directed more anteriorly than laterally, and the post-oral lamella is clearly distinguishable (J, *po.l.*).



TEXT-FIGURE 9.—*Cephalodiscus gilchristi*. Figures illustrating the development of the plumes of the buds. J is a dorsal view, and K and L ventral views of the collar and appendages (plumes and post-oral lamella); the other figures are dorsal views of complete buds. The first pair of plumes are just appearing in D, the second in E, the third in G, the fourth in H, and the fifth in K. All figures enlarged 62 diameters. For explanation of the lettering see end of paper.

At a time when the fifth plumes are just appearing (κ , 5) the first plumes point well forward and not laterally, the fourth plumes are twice as long as broad, and pinnules are making their appearance on the third plumes. It would seem that the fifth plumes grow very slowly, for in the specimen figured in text-fig. 9, I, the fifth plume on the one side is not much longer than broad, and on the other side it is even less developed, and yet the pinnules on the first pair of plumes are digitate processes, twice as long as broad, and pinnules have already made their appearance on the fourth plumes. The first four pairs of plume-axes are now almost of the same length.

In all of the buds of *Cephalodiscus gilchristi*, except the very young ones, the surface of the "body" is closely studded over with refractive beads similar to those which occur on the end-bulbs of the plumes of *Cephalodiscus dodecalophus* and *Cephalodiscus hodgsoni*. In the present species the refractive beads occurring on the plumes are negligible; the beads occur mostly on the general surface of the body, and, in the adult particularly, on the upper surface of the anterior margin of the buccal shield (see description of *Cephalodiscus gilchristi*, 'Marine Investigations in South Africa,' vol. 4, 1906, p. 184). The refractive beads are not shown in text-fig. 9.

Buds in which the fifth plumes are more advanced than in I, and buds in which the sixth plumes are developing I have been unable to find. This is not without significance when taken in conjunction with the fact that in the material of *Cephalodiscus nigrescens* I found only one late bud showing the plumes more advanced than is represented in text-fig. 7, J, and with the fact that in *Cephalodiscus hodgsoni* the later stages in plume development, showing the development of the sixth plumes, are not rare. The species *gilchristi* and *nigrescens* have tubaria in which the polypides are isolated, and live in separate tubular cavities, which open individually on the surface, and do not communicate the one with the other. The species *hodgsoni*,

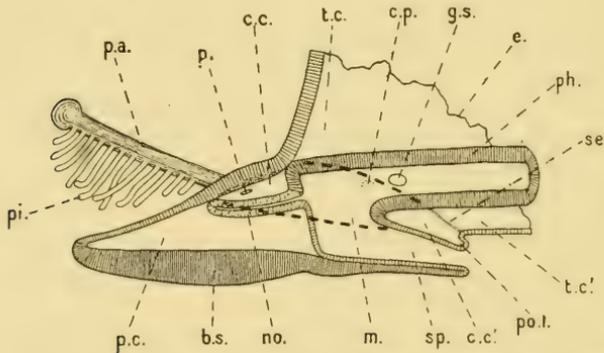
like dodecalophus, has a tubarium in which the polypides live in a common cavity, which opens by several ostia to the exterior. In my report on *Cephalodiscus* in the '“Discovery” Expedition Reports' (pp. 23, 24) I ventured to suggest that, in the species of the former kind (i. e. species of the sub-genus *Idiothecia*) the late buds severed their connection with the parental stolon, and wandered over the surface of the colony in order to settle down in some convenient situation, usually the apex of a branch, and to secrete tubes of their own. The fact now recorded, namely, the inability to discover in the material of *Cephalodiscus gilchristi* any individuals intermediate in growth between buds with small fifth plumes and full-grown polypides with well-developed sixth plumes, supports that suggestion, for the late buds, while developing their fifth and sixth plumes, would be migratory forms on the surface of the colony, and would be brushed off while the specimen was in the trawl. In the species of the sub-genus *Demiothecia* (e. g. *Cephalodiscus hodgsoni* and *C. dodecalophus*) the late buds would complete their development within the common cavity of the tubarium, and while being drawn up in the trawl, and while undergoing fixation in preservative fluids, would not be more likely to be lost than the younger buds and the adults of the colony.

GENERAL REMARKS ON THE COLLAR AND ITS APPENDAGES.

Harmer in his recent 'Monograph on the Pterobranchia of the "Siboga" Expedition' (p. 30) lays stress on the relations of the plumes (or "arms") to the post-oral lamella (or "operculum") in *Cephalodiscus*. He points out that "in *Balanoglossus* the anterior margin of the collar forms a projecting fold encircling the base of the proboscis-stalk. The ventral half of this fold may be regarded as constituting a lower lip, while the dorsal part is connected, in the middle line, with the anterior neuropore. In *Cephalodiscus* the

neuropore is not represented, and the collar forms no projection in the median dorsal line above the base of the proboscis. Except for this interval the whole of the anterior margin of the collar forms a strongly-developed fold, split up dorsally to form the arms, and ventrally constituting the operculum.”

My observations on the development of the plumes and post-oral lamella of buds of *Cephalodiscus* entirely bear out this contention. While, however, Harmer finds it necessary to insist that the post-oral lamella is a derivative of the anterior edge of the collar, and not of its posterior edge as



TEXT-FIGURE 10.—Diagram of the collar and adjacent parts of *Cephalodiscus* as seen in a longitudinal section of the polypide. *b.s.* Thick ventral wall of the buccal shield. *c.c.* Anterior part of collar cavity. *c.c'.* Posterior part of same. *c.p.* Position of right collar pore, in distance. *e.* Torn edge of body-wall of right side. *g.s.* Internal opening of gill slit. *m.* Mouth. *no.* Notochord. *p.* Opening of the cavity of the plume-axis into the collar cavity. *p.a.* Axis of first plume of right side. *p.c.* Proboscis cavity. *ph.* Dorsal wall of pharynx. *pi.* Pinnules. *po.l.* Median part of post-oral lamella. *se.* Wall or septum between collar cavity and trunk cavity. *sp.* Space between the posterior flap of the buccal shield and the post-oral lamella, leading into the mouth (*m.*). *t.c.* Dorsal part of trunk coelom. *t.c'.* Ventral part of same.

he formerly supposed,¹ the point does not appear to me to be of special consequence. The post-oral lamella is really a pair of ventro-lateral flaps united together behind the mouth,

¹ “Challenger” Reports, vol. xx, part 62, 1887, p. 43.

the connection being but a mere hollow ridge. The ridge is certainly connected with the hinder edge of the collar (text-fig. 10, *po.l.*), and the middle part of each lateral flap with the middle of the length of the collar, i.e. not with the front edge nor the hind edge; in the region of the collar canal, which is set on the postero-dorsal edge of the collar, about midway between the dorsal and ventral surfaces, the base of the flap is near the front edge. But the relations of the plumes to the post-oral lamella can be clearly understood in spite of this; and it is a very significant circumstance, in connection with the view that the post-oral lamella and the plumes belong to one and the same system, that in the development of the buds of *Cephalodiscus* each new plume arises between the last-formed plume and the end of the post-oral lamella; one might almost say that each new plume is differentiated from that part of the post-oral lamella immediately in contact with the last-formed plume. The post-oral lamella may thus be regarded as composed of postero-ventral plumes which fail to differentiate as separate plumes.

In the adult the food-grooves of the axes of the first and second pairs of plumes face ventrally,¹ i.e. towards the dorsal wall of the front part of the shield; the grooves of the third and fourth pairs face laterally, and those of the fifth and sixth pairs dorsally or dorso-laterally. Tracing these grooves basally one finds that those of the right-hand plumes bend round and converge to the right side of the mouth, and those of the plumes of the left side of the body to the left side of the mouth, the food current being in all probability

¹ It is to be noted that Harmer orientates the polypide of *Cephalodiscus* in such a way that the antero-posterior axis of the body is a line passing from the middle of the buccal shield through the central nerve mass, and ending on the rectal side of the body a little below the anus, so that the visceral mass is regarded as a ventral downgrowth ('Pterobranchia of the "Siboga" Expedition,' 1905, p. 23). In the present paper the ordinary orientation is adopted, namely, plume-apices anterior, stolon and face of shield ventral, intestine dorsal, rounded end of visceral mass posterior.

guided into the mouth and prevented from escaping by the free edge of the post-oral lamella being at the time in close contact all round with the posterior flap of the buccal shield. The lateral lobe of the post-oral lamella thus keeps the food current distinct from the exhalent current through the gill slit and from the water passing in or out of the collar canal, both the gill slit and the collar pore being situated posterior to the lateral lobe of the post-oral lamella.

If *Phoronis* is justly to be regarded as a member of the Hemichordata, and the tentacles are collar-structures comparable with the plume-axes of *Cephalodiscus*, one must admit, with Harmer,¹ that what is in *Cephalodiscus* known as the post-oral lamella is in *Phoronis* produced into a row of tentacles instead of a paired flap. On this admission there is no great difficulty in effecting a comparison between the plume-systems of the two.

The line of the bases of the tentacles of *Phoronis* runs in the double spiral well shown in Benham's figure of the end view of the animal with the tentacles cut short.² This figure, if inverted, is closely comparable with text-fig. 8 of this paper. Below the mouth is a row of tentacles, corresponding with the post-oral lamella of *Cephalodiscus*, continued on each side to the centre of the spiral, which point is equivalent to the uppermost limit of the post-oral lamella in text-fig. 8. Here it becomes continuous with the tentacles of the supra-oral series, as in *Cephalodiscus* the extremities of the base of the post-oral lamella are continuous with the terminal members of the series of plumes. The notch in the supra-oral series of tentacles, marked *x* in Benham's figure, is the equivalent of the space between the right and left plumes of the first pair in *Cephalodiscus*.

Not only has this correspondence between plumes and post-oral lamella of *Cephalodiscus* with the two series of tentacles of *Phoronis* been indicated by Harmer, but that

¹ 'Pterobranchia of the "Siboga" Expedition,' 1905, pp. 116, 117.

² 'Quart. Journ. Mic. Sci.,' vol. 30, 1889, pl. 10, fig. 7.

author also directs attention¹ to a still more remarkable similarity between the processes of the lophophore and the lower lip of the Sipunculoid *Phymosoma*, described by Shipley,² and the plume-axes and post-oral lamella of *Cephalodiscus*. The ends of the lower lip of *Phymosoma* are continuous with the ends of the series of finger-like processes of the lophophore, and Shipley's figure 32 on Plate 4 of his paper bears a close similarity to my text-figure 8. It may be not without significance that, when viewed from the front, the nephridiopores of *Phoronis* and of *Phymosoma* occupy the same positions with regard to the anus and other parts as do the gonadial apertures of *Cephalodiscus*.

While the position of the epistome of *Phoronis* does not militate against the view here favoured of an affinity between *Phoronis* and *Cephalodiscus*, it is to be noted that, according to the observations of de Selys Longchamps,³ the pre-oral lobe of the larva *Actinotrocha*, which is homologous with the proboscis of *Balanoglossus* and the buccal shield of *Cephalodiscus*, disappears during the metamorphosis, and does not persist as the epistome of the adult *Phoronis*.

Although the occurrence of the five divisions of the coelom in *Actinotrocha*, corresponding with the proboscis cavity, two collar cavities and two trunk cavities, claimed by Masterman,⁴ has been contested by Goodrich,⁵ Ikeda,⁶ and de Selys Longchamps,⁷ these last three writers agree that there is a division of the coelom into a pre-septal and a post-septal part, the septum in question following the course of the tentacles of the larva. It is still possible, therefore, that

¹ L. c., p. 119.

² 'Quart. Journ. Mic. Sci.,' vol. 31, 1890, figs. 1 and 2.

³ "Développement Postembryonnaire et Affinités des *Phoronis*," 'Mém. Classe Sci. Acad. Roy. Belgique,' i, 1904, p. 73.

⁴ 'Proc. Roy. Soc. Edinb.,' vol. xxi, 1896, pp. 62, 63, and 130; also 'Quart. Journ. Mic. Sci.,' vol. 43, 1900, p. 395.

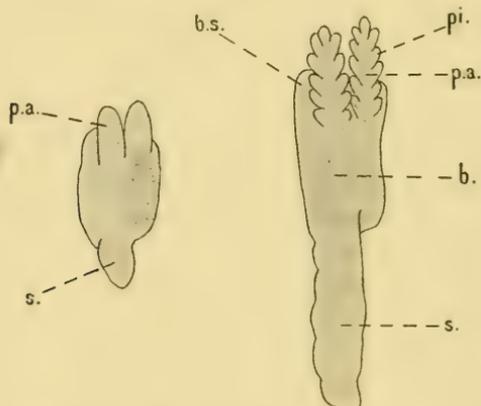
⁵ 'Quart. Journ. Mic. Sci.,' vol. 47, 1904, p. 111.

⁶ 'Journ. Coll. Sci. Imp. Univ. Tokyo,' xiii, part 4, 1901, pp. 540 et seq.

⁷ Loc. cit., pp. 15, 16.

the pre-septal cavity may represent the pair of collar cavities of *Balanoglossus* and *Cephalodiscus*.¹ The obliquity of the cœlomic septum of the *Actinotrocha* is exactly similar to the obliquity of the boundary between the collar cavities and trunk cavities of *Cephalodiscus* (see the upper line of dashes in text-fig. 10), the dorsal part being much more anterior than the ventral.

A comparison of such published figures of buds of *Rhabdopleura* as show developing plumes, with the buds of *Cephalodiscus* described in the earlier part of this paper, confirms the general impression that the two plumes of the



TEXT-FIGURE 11.—Two buds of *Rhabdopleura normani*, dorsal aspect; copied from Lankester, 'Quart. Journ. Mic. Sci.,' vol. 24, 1884, pl. 39, fig. 3, buds 5 and 7. *b.* Body of the bud. *b.s.* Buccal shield. *p.a.* Plume-axis. *pi.* Developing pinnules. *s.* Stalk.

adult *Rhabdopleura* are equivalent to the first pair of plumes of *Cephalodiscus*. The plumes of *Rhabdopleura* arise as a pair of digit-like processes (see text-fig. 11), close together, and dorsal to the shield; later on the pinnules develop in pairs, and in the adult the pinnules are directed ventrally towards the shield, exactly as are the pinnules of

¹ See Fowler, 'Festschr. 70ten Geburtstage R. Leuckarts,' 1892, p. 297, and Harmer, 'Pterobranchia of the "Siboga" Expedition,' 1905, p. 116.

the plumes of the first pair in *Cephalodiscus*. The second and later plumes of *Cephalodiscus* are not represented in *Rhabdopleura*. Besides the figures of buds of *Rhabdopleura* published by Lankester and reproduced here as text-fig. 11, those by Allman¹ and Schepotieff² may be consulted with advantage.

SUMMARY.

1. The torsion of the axes of the first and second plumes of the buds of *Cephalodiscus* described by Masterman does not take place. The grooved faces of the axes of these plumes are in the first instance directed towards the dorsal face of the buccal shield, and they maintain this relation through life.

2. The last two pairs of plumes do not arise between the first two pairs of plumes and the buccal shield, as described by Masterman, but they arise on the dorsal side of those plumes, the side remote from the shield. Their grooves are directed, not towards the shield, but away from it.

3. The evidence derived from a study of the buds of *Cephalodiscus* bears out the contention of Harmer that the series of plumes and post-oral lamella are continuous. The plume-axes develop in pairs successively, the median pair first, then a pair lateral to these, and so on. When the post-oral lamella appears, usually at the time when the third or fourth pair of plume-axes are beginning to develop, its edge is in contact with the last-developed pair of plume-axes. The fifth plume-axis appears between the fourth plume-axis and the end of the margin of the post-oral lamella, the sixth between the fifth and the end of the post-oral lamella, and, in the case of *Cephalodiscus nigrescens*, in which there are seven pairs of plumes in the adult, the seventh arises between the sixth and the end of the margin of the post-oral lamella.

¹ 'Quart. Journ. Mic. Sci.,' vol. 9, 1869, pl. 8, figs. 7 and 8.

² 'Zool. Anzeiger,' vol. xxviii, 1905, p. 802, fig. 6.

4. The line of plume-bases in the adult, when viewed from the front, is an ellipse, incomplete on the upper side (text-fig. 8); and the line of the attached edge of the post-oral lamella is another ellipse, similarly incomplete. The two ellipses join where they are incomplete.

5. Separate accounts are given in this paper of the plume-development in buds of *Cephalodiscus hodgsoni*, *C. dodecalophus*, *C. nigrescens*, and *C. gilchristi*.

6. The plumes develop relatively later in buds of *C. nigrescens* and *C. gilchristi* than those of *C. hodgsoni* and *C. dodecalophus*.

EXPLANATION OF THE ABBREVIATIONS EMPLOYED IN TEXT-FIGURES
2, 3, 4, 6, 7, and 9.

1, 2, 3, 4, 5, 6, 7. The first, second—seventh pairs of plumes of the buds.
b. The "body" of the bud. *b.s.* Front lobe or main portion of the buccal shield. *m.* Mouth, or position of mouth so far as can be ascertained in the dissected preparations, this region being much disturbed during the dissection.
p. Pinnules. *p.a.* Plume-axis. *p.l.* Posterior lobe of buccal shield. *po.l.* Post-oral lamella. *r.b.* Refractive beads in the swollen ends of the plume-axes in *C. hodgsoni* and *C. dodecalophus*. *r.e.* Ragged edge caused by dissection of the part figured from the rest of the bud. *r.l.* Red line of the buccal shield. *s.* Stalk of the bud.

On the Structure of *Ænigma ænigmatica*,
Chemnitz; a Contribution to our
Knowledge of the Anomiacea.

By

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With Plates 15—17, and 2 Text-figures.

IN a recent paper in the 'Arbeiten aus dem zoologischen Institut der Universität Wien,' Sassi (16) has given a full account of the anatomy of *Anomia ephippium*, and has elucidated several hitherto obscure points relating to the kidneys and the reno-pericardial orifices in that species. Before Sassi's paper had come into my hands I had made some observations on the allied genus *Ænigma*, a tropical member of the Anomiacea, the principal outcome of which has been to confirm Sassi's results. At the same time I have found certain differences between the genera *Anomia* and *Ænigma* which are of sufficient importance to make it worth while publishing a short account of the latter genus.

I am indebted to Mr. R. Shelford, of Emmanuel College, Cambridge, and of the Hope Department of Zoology at Oxford, for the specimens of *Ænigma ænigmatica*, Chemnitz, which form the subject of this memoir. They were found living under conditions described by previous authors, on the roots and branches of *Nipa* (a palm of the family *Phytelephantinæ*) at Sarawak, and Mr. Shelford tells

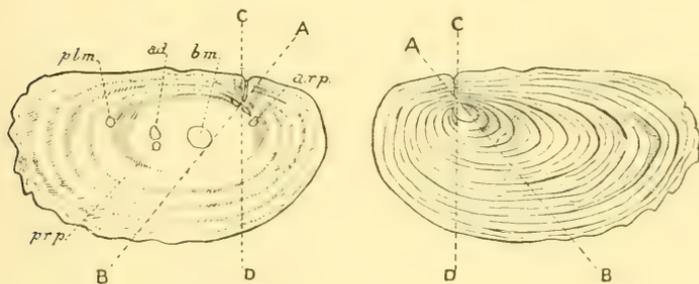
me that the specimens he collected were uncovered not only at low tides, but also at high neap tides, so that they remained exposed for days together to the rays of a tropical sun, but nevertheless always remained moist and fresh. The power of resisting desiccation for so long a time is unusual among lamellibranchiate molluscs, and indicates some structural adaptations enabling the animal to survive in such a habitat. I shall show in the course of this paper that the thickened mantle lobes and the presence of certain irregular passages and channels connected with the mantle cavity may be regarded as modifications for the retention of a sufficient supply of moisture, but in the essential features of its anatomy *Ænigma* is very similar to *Anomia ephippium*.

Ænigma, like other Anomiacea, is firmly fixed by a stout byssus passing through a large sinus or circular perforation in the right valve of the shell. The specimens given me by Mr. Shelford have been carefully preserved for histological purposes, and the right valve has in every case been wholly or partially removed. I am therefore unable to give an exact account of it, but from the fragments remaining in several specimens, I can confirm the descriptions given in conchological works, namely, that the upper extremities of the sinus through which the byssus passes curve round above the byssus and meet one another above the ligament, but do not fuse together as in adult specimens of *Anomia ephippium*.

The left valve varies very much in size and shape, and its form seems to be moulded to some extent upon the substratum to which the animal is attached. It is generally more or less elongate-oval in shape, of a dark purple colour, nacreous and iridescent internally. It is very thin and more or less translucent, especially when wet, this latter character being apparently of some importance to the economy of the animal, as will appear later on.

There are certain points of difference between the left valve of *Ænigma* and that of *Anomia* which require some little explanation. In the latter genus the left valve is more

or less symmetrical; the umbo is close to the dorsal margin and is more or less median in position and the ligament is immediately within and below the umbo. In *Ænigma*, as is clearly shown in the text-figure 1, the left valve is asymmetrical; the umbo is prominent and obliquely curved, so that it points toward the anterior and upper margin, and it is situated at a distance of some 3—5 millim. from that margin, but is connected with it by a narrow slit or fissure. Internally this fissure extends as far as the ligament, and is more or less at right angles to it. The ligament, which is 3—4 millim. long in large-sized specimens, is situated obliquely



TEXT-FIG. 1.—The left valve of *Ænigma ænigmatica*; internal view (left-hand figure) and external view (right-hand figure). A, B. The true dorso-ventral axis of the shell. C, D. The apparent dorso-ventral axis. *ad.* Impression of the adductor muscle of the valves. *a.r.p.* Impression of the anterior retractor pedis muscle. *b.m.* Impression of the byssus muscle. *pl.m.* Impression of the branchiopallial muscle. *p.r.p.* Impression of the posterior retractor pedis muscle.

above the umbonal fossa, and a line, A B, drawn at right angles to it indicates the original dorso-ventral axis of the shell. It is obvious that an inequality of growth, clearly indicated by the growth lines on the outer surface of the valve, has produced a secondary symmetry, and that the apparent dorso-ventral axis has been rotated through an angle of 40° in a postero-anterior direction. Sassi has shown that in *Anomia ehippium* the animal has undergone a similar rotation to an extent of 90° , that is to say through an angle twice as great

as that in *Ænigma*, but there is also this difference between the two genera; that whereas in *Anomia* the rotation has affected the position of the animal with respect to its shell, the latter retaining its symmetry, in *Ænigma* the inequality of growth has affected the shell as well as the contained animal, and both are asymmetrical to the same degree, as may be seen by a comparison of pl. 15, fig. 1, with the text-figure. Notwithstanding the change of symmetry, it will be convenient to describe the upper and nearly straight margin of the shell as dorsal, the lower convex margin as ventral, and the two ends as anterior and posterior respectively.

The impressions of the posterior adductor muscle, the anterior and posterior extractor muscles of the foot (here serving as retractors of the byssus) and of the large byssus muscle are shown in the text-figure and do not require special description. But it should be noticed that there is a fifth muscular impression, *pl.m.*, which has been overlooked in previous descriptions of this genus, but Woodward (17) describes and figures a similar muscle under the name of the branchio-pallial muscle in *Placuna placenta*. This impression marks the point of attachment of a specialised band of pallial muscles running forward and downward in the left mantle lobe.

GENERAL ANATOMY.

Fig. 1, pl. 15, is a representation of the animal lying in the left valve of the shell. The right mantle lobe has been largely cut away, and the left mantle lobe is obviously contracted by the action of preservative fluids; otherwise the animal is shown in its natural position. As has been explained above, *Ænigma* has not undergone so great a degree of rotation as *Anomia*, and retains its original symmetry with regard to the shell. We find accordingly that pallial cavity or sinus lying dorsad of the (posterior) adductor muscle, and containing the posterior recurved free ends of the gills, is

smaller than in the latter genus, and the dorsal sutural union of the mantle edges is considerably longer, extending from the posterior edge of the ligament to the ventricle between the points marked *x, x* in fig. 1.

Before proceeding to a description of the different organs it is necessary to point out that, in addition to the postero-anterior rotation described above, the anterior half of the body of *Ænigma* (and of all other *Anomiacea*) has been twisted round from left to right in connection with the peculiar development of the byssus and its retractor muscles. The nature and effect of this torsion has already been described by de Lacaze Duthiers (8) and Sassi (16); but a repetition is not out of place, if only to save the reader the trouble of reference to their papers. In a normal *Lamelli-branch* the byssus cavity and groove are situated on the posterior margin of the foot, and the retractor muscles of the byssus are paired and symmetrical, passing dorsally to their insertions on the right and left valves on either side of the hinge. In the *Anomiacea* the byssus, instead of passing out between the valves, passes through the well-known hole or sinus in the right valve, and drags the posterior margin of the foot over to the right. The retractor muscle of the byssus, instead of being paired and attached near the hinge line of both valves, is single and is attached near the centre of the left valve. As a consequence of these displacements, the whole of the anterior half of the body is twisted over to the right in such a way that the paired organs of the right side come to lie above the byssus muscle, and those of the left side below it; the visceral nerve commissures and the kidneys being specially affected. When this torsion of the foot and the lower part of the visceral mass is kept in mind, much that is puzzling in the anatomy of the adult is made clear.

The Mantle.—The edges of the mantle are free for nearly the whole of their extent, and are only united dorsad of the visceral mass between the points marked *x, x* in fig. 1. The left mantle lobe is entire, and covers in the whole left side of

the animal, its continuity being broken only by the surfaces of attachment of the adductor muscle, the retractor muscles of the foot and byssus, and the special slip of pallial muscle referred to above. The edges of both mantle lobes are thickened and bear small pallial tentacles covered by a columnar epithelium. There are also a few much larger tentacles on the posterior lower margin of the right mantle lobe. There are no marginal pallial eyes or pigment spots, but at the bottom of the groove formed in the thickened pallial margin a track of columnar cells, supplied by twigs of the circumpallial nerve, can be distinguished. In the region of the visceral mass, that is to say in its upper half, the left pallial lobe is very thin; but in its lower and posterior half, where it covers the large recurved gills, it is greatly thickened by the abundant development of lacunar tissue. The slip of pallial muscle, attached to the left valve of the shell, is shown at *pl.m.* in fig. 2. It coincides in position with the attachment of the axis of the left gill to the mantle lobe, and must function as a retractor of the gill. The left gonad extends backward to the level of the anus in the left pallial lobe, and in the same lobe there is a large anterior extension of the left gonad, forming a conspicuous sausage-shaped swelling in front of the mouth (figs. 1 and 2, *go.a.*).

The most remarkable feature in the left pallial lobe is the presence of a number of deeply pigmented spots, arranged in an irregular oval at a considerable distance from the pallial margin. These eye-spots, as I must call them, vary in number in different individuals. In the specimen shown in fig. 2 there are twenty-three. They are, however, very constant in position: the most anterior eye-spot is always situated just behind the surface of attachment of the anterior retractor pedis muscle, and the most posterior close to the attachment of the pallial muscle. Each eye-spot consists of a ring of black pigment surrounding a central opaque white area. The histology of these organs will be described in the latter part of this paper; but I may say here that their minute structure leaves little doubt that they are sensory in function and

adapted for visual functions. As they are situated at a greater or less distance from the edge of the mantle, they must always be covered by the shell, and the existence of visual organs in such a position is somewhat extraordinary. But, as I have pointed out, the left valve of the shell is thin and translucent enough to allow a considerable amount of light to pass through. As *Ænigma* spends a large part of its existence uncovered by the sea, with its valves tightly closed to prevent evaporation, it is probable that these eyes are efficient in informing the animal of the duration of daylight, or, at any rate, of the incidence of direct sunlight. It is probable enough that after sunset the valves of the shell are slightly opened to admit of the aeration of the water contained in the pallial chamber, and are kept tightly closed to prevent evaporation during the heat of the tropical day.

The right mantle lobe is very irregular in shape, and presents a large anterior sinus corresponding with the sinus of the right valve of the shell, and serving for the passage of the byssus. In the anterior part of the body the right mantle lobe is attached by a very narrow band of tissue to the visceral mass, the line of attachment running above and nearly parallel to the upper edge of the byssus cavity. In this part of the body, indeed, the viscera are thrust over to the left side of the body and the visceral mass is adherent to the left pallial lobe. But in the hinder part of the body the rectum and cæcum of the crystalline style, passing respectively above and below the adductor muscle, cross over from the left to the right side, and are here adherent to the right mantle lobe and embedded in the mass of the right gonad and right kidney (compare figs. 11 and 13). The lower and posterior part of the right mantle lobe is exceedingly thick, and its inner surface is pitted and folded in a very irregular manner. As the animal lies with its right valve lowermost these folds and pits must serve for the retention of water during the long periods in which it is uncovered by the tides.

The mouth, as is shown in fig. 1, lies asymmetrically on the

right side. It is concealed in a labial groove formed by two deep folds of the integument, which are nothing else than the greatly enlarged and modified labial palps. The extent and relations of these labial folds in *Anomia* were first described by de Lacaze Duthiers (8) and more fully by Sassi (16); they have very much the same relations in *Ænigma* as in that genus. The external labial fold passes round in front of the mouth forming a hood and the internal fold passes behind the mouth, the two enclosing between them a groove of varying depth lined by a high columnar ciliated epithelium, which contains numerous gland-cells in the region of the mouth. On the left side of the body the two folds, as they pass backward from the mouth, become very deep and prominent, and enclose between them a deep groove or gutter whose walls are thrown into numerous vertical folds covered by a ciliated epithelium. At a short distance behind the mouth the folds hang far down in the mantle cavity on the left side of the foot and are suspended from the visceral mass above by a thin sheet of tissue. Towards the posterior end of the foot the groove becomes shallower, and its walls are less folded, and eventually the external labial fold unites with both the direct and the reflected lamella of the left external demibranch, and the internal labial fold with the direct lamella of the left internal demibranch. Thus the left labial groove becomes continuous with the inter-branchial chamber of the left side (see text-figure 2, A and B). On the right side the two labial folds run back above the byssus cavity, parallel with and below the line of attachment of the right mantle lobe to the visceral mass. Dorsad of the byssus the labial folds are inconspicuous, the labial groove contained between them is shallow, and the epithelium lining is ciliated and glandular, but not ridged. Towards the posterior end of the byssus the groove turns downward and backward, and the labial folds enclosing it increase rapidly in vertical depth. At the same time the walls of the now very deep groove are thrown into numerous vertical ridges, and the epithelium of the ridges is richly

ciliated but not glandular. The outer right labial fold, continuing to increase in vertical depth, eventually passes into the upturned reflected lamella of the right external demibranch, and the internal right labial fold becomes continuous with the thin membrane by which the direct lamella of the right internal demibranch is attached to the body-wall below the byssus muscle. The right labial groove thus becomes continuous with the inter-branchial chamber of the right side, but it is continued backwards as a cul-de-sac for some distance beyond the point of union with the gill, and in sections the right branchia appears to be suspended from the lower wall of this cul-de-sac, as is shown in text-figure 2, E.

The foot (fig. 1, *f.*) is reduced to a flat muscular projection at the anterior angle of the byssus cavity. In most of the spirit-preserved specimens it is contracted to a small, lanceolate, muscular mass, but in the individual figured it is unusually long and ribbon-shaped. The extremity of the foot is always pointed, and bears a single small tentacle, similar in all respects to the marginal tentacles of the mantle. There is no infundibuliform cavity at the end of the foot as in *Anomia ephippium*, but the right (morphologically the ventral) surface is grooved and covered with numerous transverse, ciliated ridges. The mass of the foot is highly muscular and contains numerous mucous glands, and it is evident that this organ is very extensile. It seems probable that it can be protruded some distance beyond the shell, and that it is auxiliary to nutrition, minute particles being swept by ciliary action along the groove on its right surface, and thence to the right labial groove.

The principal muscles connected with the foot have not undergone as much modification in *Ænigma* as in *Anomia* owing to the lesser degree of rotation in the former genus. The two tapering muscular bands running respectively forwards and backwards from the great retractor muscle of the byssus to their surfaces of attachment on the left valve, are clearly the homologues of the anterior and posterior retractors of the foot of other Lamellibranchia (fig. 2, *a.r.p.* and *p.r.p.*).

But here they have shared in the torsion of the other organs of the anterior part of the body, have become twisted round to the right, have lost their primitive connection with the right valve, and are inserted on the left valve only, serving rather as accessory retractors of the byssus than as retractors of the foot. It will be observed that both the anterior and posterior retractors of the foot arise from double origins above and below (morphologically right and left) of the retractor muscle of the byssus, but that each is formed into a single strand shortly before its attachment to the shell.

The retractor muscle of the byssus is a large, coarsely-fasciculated muscle, nearly circular in section, and passes transversely from its attachment to the left valve to the byssus cavity. It probably represents the right and left retractors of the byssus of symmetrical Lamellibranchia, but betrays no sign of its primitive paired origin. De Lacaze Duthiers (8), however, considers that the right retractor byssi muscle is aborted in *Anomia*, but it should be observed (fig. 10) that the muscle is equally well developed above, that is on the morphological right, and below, that is on the morphological left of the byssus cavity.

The byssus cavity is large and shallow, more or less oval or lozenge shaped, and bordered above and below by muscular lips, which are really the posterior continuations of the right and left margins of the foot. The bottom of the cavity is lined by a number of close-set, parallel folds or lamellæ running fore and aft in the direction of the long axis of the shell. These lamellæ form the byssus gland, the histology of which will be described in the latter part of this paper. But I may state here that the byssus of *Ænigma* is not calcified like that of *Anomia*, but consists of a number of parallel plates of the byssus substance, secreted by the epithelial cells covering the ridges of the byssus gland. These lamellæ are fused externally into a plate which is directly and firmly fixed to the substratum to which the animal is attached.

The Gills.—The natural position of the gills, as seen

from the right side is clearly shown in fig. 1. They are relatively even larger than in *Anomia*, and, as in that genus, their posterior ends are recurved and form the posterior boundary of the dorsal or supra-branchial chamber into which the anus opens, and in which the ventricle of the heart lies. In their microscopic as well as their macroscopic structure the gills of *Ænigma* are singularly like those of *Anomia ephippium* as described by Ridewood (15), to whose paper the reader is referred for details. Thus the reflected filaments of the right and left internal demibranchs are fused together and the whole series of filaments are in organic continuity along the line of union, and are traversed by a blood-vessel. The upper ends of the reflected filaments of the right and left external demibranchs are secondarily reflected downwards to form the so-called velar fold or flap, and at the angle of reflection the whole series of filaments are united in organic continuity, and are traversed from end to end by a blood-vessel (text-figure 2, *b.v.*). The lower ends of the velar filaments are, however, independent, and have an arrangement of cilia which appears to have been overlooked by previous observers. As is shown in fig. 6 these velar filaments are sub-triangular in section, and the chitinous lining of their cavities is thickest on the internal or morphologically ventral side, this being the reverse of what obtains in the direct and reflected limbs of the filaments. The outer (morphologically dorsal) side of the velar filament is broad and flat, and is covered by a cubical epithelium bearing a number of short stiff cilia. The opposite face of the filament is narrowed and covered by longer columnar epithelial cells bearing long fine cilia, continuous with the frontal cilia of the reflected or ascending limb of the filament. There can be little doubt that the short stiff cilia borne on the pallial faces of the velar filaments have the function of ciliated discs, and give a sufficient amount of friction against the inner surface of the mantle to prevent the whole of the reflected lamella from slipping down. One might expect to find a corresponding ciliated ridge, or row of ciliated discs, on the mantle, but I

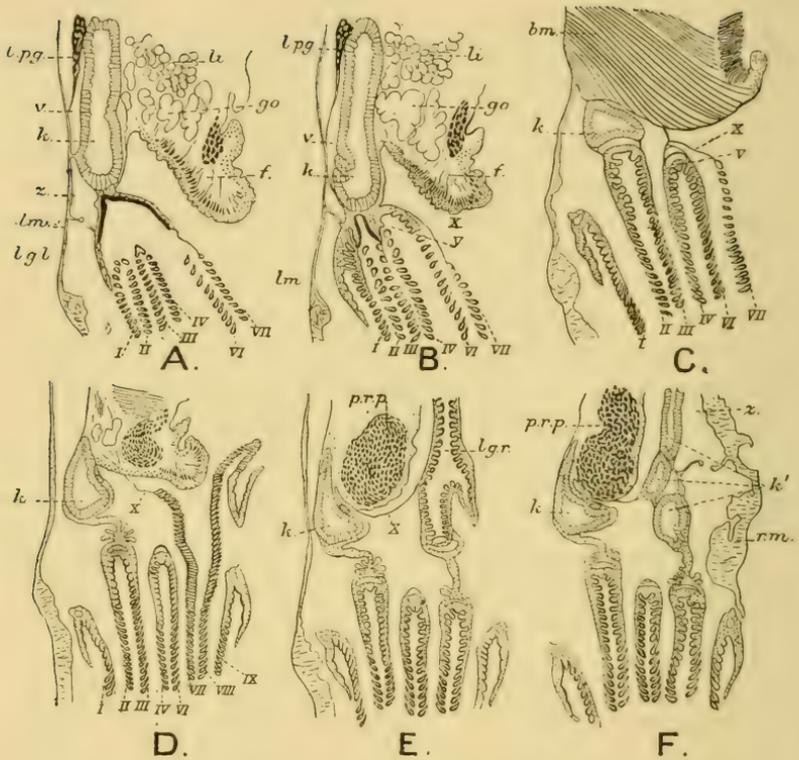
can find no trace of them, and am certain that they do not exist. It should be noted that the velar flap on each side abuts on the thickened and corrugated lower moiety of the mantle lobe, so that ciliated discs on that side are unnecessary.

As in *Anomia ephippium*, there are no ciliated discs on either the direct (descending) or reflected (ascending) limbs of the filaments, but there is a well-developed single row of discs at the angle of reflection, the details of which are shown in fig. 5. There is, as in *A. ephippium*, a very low inter-filamentar septum close to the angle, but beyond this no other interlamellar junctions whatever. A transverse section of two filaments, showing the frontal and lateral cilia and other details, is given in fig. 7. The attachments of the gills to the body-wall and mantle are affected to a considerable extent by the asymmetry of the anterior half of the body and by the fact, mentioned above, that the visceral mass is adherent to the left mantle lobe in the anterior part of the body but to the right mantle lobe in the posterior part. As these relations have not been sufficiently fully described in *Anomia*, I will enter into them somewhat closely in *Ænigma*. The posterior recurved ends of the gills lie free in the mantle cavity and are not attached to the mantle. In the more anterior part of their courses the right and left branchia are differently affected by the asymmetry due to the twisting of the byssus and foot over to the right. The relations of the left branchia are on the whole those of a normal symmetrical Lamellibranch. At a point nearly vertically below the anus its axial fold becomes attached by a deep suspensory fold to the left mantle lobe. In this fold run the muscular fibres which have already been described as a specialization of the pallial musculature forming a retractor muscle of the branchia (figs. 2 and 13). Passing forward the axial attachment of the left branchia becomes shifted more and more towards the middle line, largely in consequence of the interposition of the fibres of the posterior retractor pedis muscle between it and the mantle (fig. 12).

In front of the adductor muscle the axis of the left branchia is suspended in the mantle cavity by a deep suspensory fold attached to the lower surface of the visceral mass just below the lower limb of the left kidney, and has lost all connection with the left mantle lobe. These relations are continued forward as far as the posterior edge of the foot, where the direct and reflected lamellæ of the outer demibranch become continuous with the external left labial fold and the direct lamella of the inner demibranch passes into the internal left labial fold as described above (p. 260).

The fold formed by the united upper ends of the reflected filaments of the right and left inner demibranchs acquires no attachment till it reaches the anterior end of the great extractor byssi muscle. Here it is pushed over to the left and unites with the body wall close alongside of the attachment of the direct lamella of the left inner demibranch, and its blood-vessel passes into the blood sinus lying below the recurrent limb of the left kidney, this sinus discharging its blood into the left auricle (text-figure 2, B and C, v).

As may be seen in fig. 1, the anterior end of the right outer demibranch curves round dorsally behind the byssus cavity. As explained above (p. 261) its reflected lamella becomes continuous with the right external labial fold, and its direct lamella with the right internal labial fold behind the byssus (see text-figure 2, D and E). The anterior filaments of both lamellæ of the right outer demibranch are very short, but those of the direct lamella of the right inner demibranch are very long and, as may be seen in fig. 1, *d.b*², extend below and to the left of the byssus cavity as far forward as the foot. As is shown in text-figure 2, A to E, and in fig. 10, these elongated anterior filaments of the right inner demibranch are connected with the ventral surface of the body, below the byssus muscle, by a thin membrane, *x*, which stretches across to the left side and eventually, as in A, becomes attached to the left inner labial fold. Thus, as a consequence of the displacement of organs several times referred to, the inner demibranch of the right side is carried



TEXT-FIG. 2.—Transverse sections through *Ænigma ænigmatica* to show the attachments of the gills to the body wall. A. Part of a section through the anterior edge of the foot. B. A section taken a short distance behind A. C. A section through the middle of the byssus muscle. D. A section through the posterior edge of the byssus muscle. E. A section taken through the heart. F. The tenth section of the series behind E. *b.m.* Byssus muscle. *f.* Foot. *go.* Gonad. *k.* Left kidney. *k'*. Right kidney. *li.* Liver. *l.g.l.* Left labial groove. *l.g.r.* Right labial groove. *l.m.* Left mantle lobe. *l.pg.* Left pericardial gland. *r.m.* Right mantle lobe. *v.* Anterior venous branch of the left auricle; the pericardial gland forms a thickening of its outer wall. *x.* Membranous attachment of the right inner demibranch to the lower, morphologically the left, side of the foot and byssus muscle. *z.* Spaces serving as reservoirs for water. I. Reflected lamella and—II. Direct lamella of the left outer demibranch. III. Direct lamella and—IV. Reflected lamella of the left inner demibranch. V. Sutural union of the reflected lamella of the right and left inner demibranchs. VI. Reflected lamella and—VII. Direct lamella of the right inner demibranch. VIII. Direct lamella and—IX. Reflected lamella of the right outer demibranch.

across the body and attached to what is morphologically the left side of the foot.

THE ALIMENTARY TRACT.

The mouth leads into a narrow, and, for a Lamellibranch, relatively long, œsophagus, lined by a ciliated glandular epithelium, whose characters will be described in the latter part of this paper. The œsophagus passes into a capacious stomach occupying the greater part of the visceral mass dorsad of the foot. The roof of the stomach is thin, and lined by a few ciliated but non-glandular columnar epithelium, which extends down for some distance on the right wall, especially in the anterior half of the stomach. The right wall and floor, and in the posterior half of the stomach, the left wall are, on the contrary, lined by an epithelium consisting of very long attenuated ciliated cells, intermixed with which are numerous elongated claviform gland-cells filled with yellow granules. The floor of the stomach is also thrown into longitudinal folds, and is covered by a thick cuticular layer, the "flèche tricuspide" of Poli, which is apparently secreted by the yellow gland-cells. This question will be discussed more fully in the latter part of this paper. The stomach is embedded in the liver, which opens into it by several large ducts. One of these ducts is dorsal, and communicates with the superior lobe of the liver; the remainder are posterior and ventral, and some of them run far back in the posterior mass of the liver before breaking up into branches (figs. 9 and 10, *li. d.*). Posteriorly the stomach presents a dorsal cæcum, into which one of the largest of the posterior liver ducts opens. Ventrally it narrows in diameter, and gives off the intestine and sac of the crystalline style.

The intestine opens into the stomach by an aperture common to itself and the sac of the crystalline style. The entrance to the intestine is guarded by a number of promi-

ment longitudinal ridges covered by ciliated epithelium. The intestine itself is a narrow tube running back for some distance close to and on the right of the sac of the crystalline style. In this part of its course its lumen is narrowed by the projection of four prominent ciliated ridges into its interior. Just anterior to the heart the intestine enlarges somewhat suddenly in diameter, the four internal longitudinal ridges disappear, and it turns sharply upwards, makes a single complete turn, bends up at a sharp angle towards the ventricle, and then runs a straight course below the ventricle to the anus. In the last section of its course there is a distinct typhlosole in the large intestine. The rectum is very short and funnel-shaped. Its epithelium differs entirely from that of the large intestine, consisting of clear, attenuated, ciliated cells with deeply-staining nuclei, indicating that it is a proctodæum. The histology of the alimentary tract will be described further on.

As in *Anomia*, the sac of the crystalline style is excessively long. At first nearly median in position (fig. 10), it passes over to the right and runs back, closely attached to the right mantle lobe, below the adductor muscle. In the posterior part of its course it lies parallel to and above the attachment of the right branchia, and finally it curves forward and ends blindly (figs. 10—13, *cry.*).

THE CIRCULATORY SYSTEM.

The ventricle of the heart, as in all *Anomiacea*, lies free in the dorsal bay of the mantle cavity, and is not enclosed in a pericardial sac. Situated dorsally to the intestine, it sits, so to speak, astride of the latter organ, the auricles passing down on either side of it like a rider's legs. The walls of the ventricle are very thick and muscular, as also are the walls of the auricles, but the latter not to so great a degree as in *Anomia*. The aorta arises from the antero-ventral angle of the ventricle (its aperture is guarded by a valve),

and, running towards the right dorsad of the rectum, it at once divides into three branches. The median branch penetrates the liver mass, and soon breaks up into branches and disappears. The left-hand branch constitutes the anterior aorta. This vessel runs forward to the right of and above the intestine, passes through the coil of the intestine to the left upper side of the visceral mass, and runs forward over the stomach to the œsophagus, where it divides into numerous branches. The right-hand branch of the aorta runs down on the right of the intestinal coil towards the cæcum of the crystalline style, and then bends back to take a posterior course, supplying the gills and right mantle lobe with blood.

The course of the veins is similar to that described in *Anomia ephippium* by Sassi. The left auricle divides below the level of the intestine into two branches. Of these the posterior runs down to the outside of the large upper posterior lobe of the left kidney, and, turning back, may be traced as far as the visceral ganglia, where it receives several vessels from the gills and left mantle lobe. The anterior branch courses forward in close connection with the upper limb of the left kidney, and its outer wall is thickened by an abundant glandular tissue, which will be described in connection with the excretory organs. The right auricle dilates to form a large thin-walled sac lying to the right of and partially above the intestine. The walls of this sac are locally thickened by the same glandular tissue that accompanies the anterior branch of the left auricle, which, from its relations to the reno-pericardial funnels, must be regarded as the representative of the pericardial gland. The venous cavity may be traced back along the right side of the upper lobe of the right kidney, and in the posterior part of its course it receives numerous accessions from irregular sinuses and vessels bringing back blood from the right mantle lobe, the right branchia and the sac of the crystalline style.

The right and left auricles communicate with one another by a sinus passing ventrad of the intestine, immediately below the hinder end of the ventricle. A similar vessel

occurs in *Anomia ephippium*, and was interpreted by Pelseener (13) as a relic of the pericardium, a mistake which anybody might be excused for falling into, seeing how peculiar are the relations of the kidneys, venous channels, and remnants of the pericardium in that genus.

THE EXCRETORY ORGANS.

Sassi (16) is the first author who has given a correct and intelligible account of the kidneys in *Anomia ephippium*. Fig. 4, which is a reconstruction from one of my series of sections, shows that these organs are extremely similar in *Ænigma*. The chief difference between the two genera consists in the fact that, whereas in *Anomia* the left kidney forms a complete loop behind the byssus muscle, in *Ænigma* the upper and lower limbs are free from one another posteriorly. In the right kidney the upper limb extends much further back along the intestine in *Ænigma* than it does in *Anomia*. In all other respects I can fully confirm Sassi's observations. In *Ænigma*, as in *Anomia*, the right and left kidney sacs communicate by a wide opening situated below the auricle (fig. 4, *ap.*). The left renal sac runs forward, dorsad of the byssus muscle, as far as the foot, and then turns sharply back, running just above the attachment of the axis of the left branchia as far as the visceral ganglion, where it turns upward and opens into the suprbranchial chamber somewhat in front of the right renal aperture. The left reno-pericardial canal is, as in *Anomia*, situated far forward near the anterior bend of the renal sac (*l. rp.* in fig. 4), and the left gonaduct opens into the renal sac by a distinct ciliated funnel situated close above and nearly opposite to the left reno-pericardial canal. The right renal sac is roughly crescentic in shape, the concavity of the crescent embracing the adductor muscle. The right reno-pericardial funnel (*r. rp.* in figs. 4 and 11) is situated immediately below the right auricle, and the right gonaduct opens

into the right wall of the kidney sac nearly opposite to, but a little further back than, the reno-pericardial funnel. So far the only difference between the structure and position of these organs in *Ænigma*, and those in *Anomia*, as described by Sassi, consists in the fact that whereas that author says there are no ciliated funnels to the gonaducts in the latter genus, there are very distinct gonaducal funnels, with a well differentiated ciliated columnar epithelium (fig. 15) in *Ænigma*.

In respect of the relations of the reno-pericardial funnels to the remnants of the pericardium, there is, however, a great difference between the two genera. Sassi has shown that in *Anomia ephippium* the reno-pericardial canals lead into a system of short and slightly branched tubules ending in glandular diverticula, and these he identifies, no doubt correctly, as the remnants of the pericardium. I have already shown that *Ænigma* is in several respects a less specialised form than *Anomia*, and in this particular matter of the reno-pericardial ducts and their connections it has clearly undergone less modification than the latter genus. Fig. 14 is a drawing of a section through the left reno-pericardial canal of *Ænigma*. The canal is lined by a columnar epithelium, each of whose constituent cells bears a long flagellum. The upper end of the canal is lined by a flatter non-ciliated epithelium, and passes into a mass of reticulate glandular tissue containing many inter-cellular spaces. Though it is not particularly well shown in the section figured, the communication between these spaces and the reno-pericardial canal can be easily traced in the series of sections. Some few sections further back the canal appears to open, by an aperture so distinct that it might almost be described as a ciliated funnel, into a central lumen in the mass of glandular tissue, and this as it is traced backwards breaks up into a number of irregular branches or channels communicating with the spaces in question. From its relation to the reno-pericardial canal there can be no doubt that this lumen together with the irregular channels

opening into it represents the pericardial cavity, nor can there be any doubt that the branching canals are homologous with the structures described by Sassi in *Anomia ephippium*. If the lumen represents the pericardial cavity, the glandular tissue clearly is the homologue of the pericardial gland, and the chief point of difference between *Ænigma* and *Anomia* is that in the former the pericardial gland is well developed, and retains not only its function as an excretory organ, but also its connection with the heart. As is shown in figs. 4 and 14, and in text-figure 2 A and B, *l. pg.*, the left pericardial gland is of considerable vertical extent in the immediate neighbourhood of the left reno-pericardial canal. Posteriorly it narrows to a thin band of glandular tissue, which may be traced backwards, lying to the outside of and above the upper limb of the left renal sac, and in close connection with the anterior of the two veins into which the left auricle divides, as far as the muscular wall of the left auricle itself. As it approaches the heart the pericardial gland spreads out over the walls of the auricle and becomes intimately fused with them, extending, as I shall show further on, into the thickened walls of the ventricle.

On the right side the right reno-pericardial canal (figs. 4 and 11, *r. rp.*) leads into the lumen of a similar but larger mass of glandular tissue, occupying the right wall and floor of the dilatation of the right auricle described on p. 269. This dilatation of the blood-vessel has no apparent analogue in *Anomia*. The glandular sac forming part of the thickness of its walls is of course the right pericardial gland, and its histological structure as well as its relations to the upper posterior lobe of the right renal sac and to the right auricle are analogous to those of the left pericardial gland. The extent of the two glands and their relations to the kidneys and heart are indicated by the spaces enclosed between the black lines in fig. 4.

The structure and distribution of the pericardial glands of the Lamellibranchia has been worked out in great detail by Grobben (6). Without entering into the details of his careful

and voluminous research on this subject, I may say briefly that he has shown that in a large number of Lamellibranchs belonging to different families the epithelial walls of the pericardium are glandular and have an excretory function. In many species, and among the Filibranchia in the Arcidæ, Mytilidæ, and Pectinidæ, the glandular tissue is localised on the auricles and in some other forms, e.g. *Venus verrucosa* (see Grobben, loc. cit., fig. 15), it extends to the ventricle of the heart. The characteristic histological elements of these glands are oval or somewhat irregular cells with a spherical nucleus, alveolar protoplasm containing a few granules, and in the latter a distinct brown concretion. Though the shape, size, and appearance of these cells vary in the various species examined by Grobben, they are present in the pericardial glands of all, and are very distinct in character from the concentric concretions found in the kidney.¹ The structure of the pericardial gland in *Ænigma* is shown in fig. 14. The tissues have doubtless undergone contraction, and are otherwise altered by the action of spirit, but it is clear enough that the bulk of the gland in the vicinity of the reno-pericardial canals is formed by a mass of branching cells, whose processes unite to form a reticulum. Or the structure might be otherwise described as a mass of vacuolated protoplasm containing numerous oval nuclei, smaller, and with a denser chromatic network than the nuclei of the kidney cells. This tissue may be traced along the upper margin of the kidney from the reno-pericardial funnel to the roots of the auricles on either side of the body, and on arriving at the thickened muscular walls of the auricles below the intestine it seems to thin out and disappear. In the spaces or vacuoles of this tissue are olive-brown concretions, which vary in size and appearance with

¹ The concretions in the kidney are commonly said to consist of uric acid; but Letellier (9) states that no uric acid is excreted by any Lamellibranchiate, and that the urinary concretions consist of calcium carbonate and acid phosphates of lime and magnesium. According to this author the Lamellibranch kidney excretes urea, the Gastropod kidney uric acid.

the metabolic condition of the animal. In some of my series of sections the concretions are small and are contained in oval cells, as shown in fig. 16, *a*. These cells are almost identical with the pericardial cells of *Venus verrucosa* and *Cardium edule* figured by Grobben (*loc. cit.*, figs. 53 and 54). In another of my series the concretions are much larger, and are either surrounded by a thin cell-envelope with the nucleus lying to one side, as in fig. 16, *b*, or the cell structure is no longer distinguishable. Similar conditions are figured by Grobben for divers Lamellibranchs.

The presence of these highly characteristic cells and concretions not only enables us to identify the above-described tracts of tissue as pericardial glands, but also to trace the latter beyond their apparent limits. The pericardial glands appear to thin out on the auricular walls, and to stop short of the heart. But an examination of the walls of the heart with high powers of the microscope reveals the fact that they are penetrated by the glandular tissue. The two auricles, embracing between them the intestine, lie, like the ventricle, in the pallial cavity, and have relatively thick muscular walls, covered externally by a columnar epithelium continuous with the external epithelium of the body.

The muscle-fibres, both of the auricles and ventricle, cross one another in all directions, forming a sort of sponge-work with numerous spaces. A high power of the microscope shows that the tissue of the pericardial glands runs through these spaces in the muscular walls of the auricles, and extends into the ventricle. The characteristic cells containing olive-brown concretions can be distinguished even with a low power in the inter-muscular spaces of both auricles and ventricle (fig. 19). Furthermore, a careful examination of the columnar epithelium of the heart shows that the inner ends of its component cells do not rest on a basement membrane, but are prolonged internally into fine processes which run in between the muscular fibres. In other words, the external epithelium of the auricles and ventricle is fused to and partly immersed in the subjacent muscular tissue,

much as the epidermal cells of many *Platyhelms* are immersed in the subdermal muscular and connective tissue.

With these facts before us we have a ready explanation of the hitherto unsolved problem of the fate of the pericardium in the *Anomiacea*.

In a typical *Filibranch* with a well-developed pericardium we should find the following layers in transverse section:—
 1. The external epithelium. 2. The outer epithelial wall of the pericardial cavity. 3. The inner glandular wall of the pericardial cavity adhering to the auricles or ventricle. 4. The muscular wall of the auricles or ventricle. It is clear that in *Ænigma* the pericardial cavity has disappeared in the vicinity of the heart, and that the external epithelium, the outer and inner walls of the pericardium, and the muscular wall of the heart itself have become more or less intimately fused together; in particular the glandular tissue of the inner pericardial wall has become incorporated with the muscular wall of the heart. None the less all these forms of tissue—epidermic, glandular, and cardiac muscular—can be recognised by careful microscopical examination. It is obvious that the disappearance of the pericardial cavity and the fusion of its walls with those of the heart is in some way correlated with the torsion of the body produced by the excessive development of the byssus muscle, and the attachment of the latter to the centre of the left valve of the shell. The result of this is, that the left kidney has been dragged forward to pass round the byssus muscle and the left reno-pericardial canal has been shifted forward far from its typical position, involving a great anterior extension of the left pericardial cavity. The corresponding structures on the right side have not suffered so much displacement, but the strain to which the whole system of organs must have been subjected during the passage from the larval to the adult condition is sufficient to account for the obliteration of the pericardial cavity, except in the immediate proximity of the reno-pericardial canals. Sassi has suggested that the whole of the venous vessel from the ventricle to the left reno-

pericardial aperture is the representative of the left auricle, and if we regard all that vessel as auricle which is covered with pericardial glandular tissue, his suggestion is correct. But as the muscular coat of the afferent vessels of the heart does not extend as far in *Ænigmia* as in *Anomia*, and as the left afferent vessel divides close below the intestine into an anterior and a posterior vessel, I have preferred to restrict the term auricle to those muscular vessels which embrace the intestine.

The gonads.—The sexes are separate. The ovaries and testes occupy the same position in the two sexes, so a description of one will apply equally well to the other. The left gonad is much the larger of the two, and extends for a long distance in front of and behind the gonopore. Its anterior moiety has the same relations as in *Anomia*, that is to say, it runs forward below the stomach and liver, and just above and to the right of the left labial groove to the mouth, where it passes to the right of the attachment of the anterior retractor pedis muscle, and passing into the mantle forms the large præoral mass which is so conspicuous a feature when the animal is opened. The posterior moiety runs back as a fine canal, bearing a few slightly branched diverticula, below the cæcum of the crystalline style and above the byssus muscle; its course being here on the right rather than on the left side of the body. Behind the byssus muscle it passes over to the left side of the body, and divides into two branches running respectively along the upper and lower edges of the posterior retractor pedis muscle. Behind the attachment of this muscle the gonad passes into the left mantle lobe, and there forms a large follicular mass extending back for some distance beyond the level of the anus, as shown in figs. 2, 12, 13. The left gonad does not extend into the mantle in *Anomia*, and Pelseueer (14) has stated that it does not do so in any of the *Anomiacea*, a somewhat rash generalisation which must now be corrected. The right gonad in the anterior part of its course lies dorsad of the stomach and liver on the upper side of the visceral mass,

where it forms a large lobe above and to the right of the intestinal loop. From this lobe two main branches pass backward. The one passes to the dorsal side of the cæcum of the crystalline style, and accompanies the cæcum for the whole of its course in the right mantle lobe, and forming a large mass above its extremity, as shown in fig. 13. The other branch, into which the gonaduct opens, maintains a more dorsal position, and, passing above the adductor muscle, forms a considerable follicular mass to the right of and above the posterior part of the intestine.

The gonaducts, as has been described above, open by distinct ciliated funnels into the renal sacs close to the renopericardial apertures of the sides of the body to which they belong. A gonaducal funnel of a male is shown in fig. 15. There is no difference in the histological characters of the funnel in the two sexes. The gonaducts, that is to say those sections of the ovarian or testicular tubules that are lined by a low cubical instead of a germinal epithelium, are extremely short. The histology of the ovaries and testes does not call for special description, but in one of my series of sections the ovary was penetrated throughout by a number of green filaments, whose nature I could not satisfactorily determine. They have the appearance of filamentous algæ, and may possibly be symbiotic or parasitic within the mollusc. But they are not of constant occurrence, for I could find no trace of them in two other females examined.

The Nervous System.—As may be seen in fig. 3, the nervous system is of the usual lamellibranchiate type, and the modifications it has undergone are attributable only to the torsion which has affected these in common with all the other organs of the anterior moiety of the body. Thus the right cerebro-pleural ganglion lies above and somewhat behind the left. The cerebro-pedal connectives are relatively short, and the right pedal lies above the left pedal ganglion. The right visceral connective passes above, and the left visceral connective below, the byssus muscle and the right visceral ganglion is somewhat above and in advance of the

left. But beyond this distortion which it shares with the other organs of this region, the nervous system presents few features requiring special description. The otoliths lie a short distance behind the pedal ganglia, and are connected with the latter by short nerves. The two stout nerves passing from the pedal ganglia, that from the right ganglion distributed to the upper and that from the left ganglion distributed to the lower surface of the byssus muscle are worthy of mention, because de Lacaze Duthiers (8) describes a large nerve passing from the visceral ganglia to the byssus muscle in *Anomia*. No such nerve is present in *Ænigma*, the byssus muscle being wholly innervated from the pedal ganglia. The branchial and pallial nerves issuing from the right and left visceral ganglia should be noted. The branchial nerve of either side runs straight down into the suspensory fold of the gill axis, and there enters a distinct branchial ganglion (fig. 3, *br.g.*), from which fine nerves run forwards and backwards in the gill axis. The pallial nerve issues separately from the branchial nerve on the right side, but the two have a common origin from the left visceral ganglion. The branches of the two visceropallial nerves diverge in the mantle lobes and unite in each mantle lobe with a distinct circumpallial nerve, which forms a complete ring in the thickened margin of the mantle, and forms a connection anteriorly with nerves issuing from the cerebro-pleural ganglia.

Histology.—The specimens of *Ænigma* collected by Mr. Shelford were so well preserved that I have been able to work out the histology of some of the organs in some detail, but I do not propose here to do more than give an account of some of the more striking features that came under my notice. A detailed account of the histology of various members of the Lamellibranchia, including the *Anomiacea*, is indeed a desideratum, but for such a task fresh specimens are necessary, and where they are not available it is inexpedient to attempt more than a description of such characters as can be accurately studied in sections.

Pallial Organs.—The lobes of the mantle were so much contracted in spirit that I have been unable to make out the details of the histology of the marginal tentacles to my satisfaction. The tentacles are covered by a high columnar epithelium, and receive an abundant nerve supply from the circumpallial nerve. There are some specially large tentacles on the lower posterior edge of the right pallial lobe (fig. 13, *t.*), and near the bases of some of these there are ganglionic enlargements on the circumpallial nerve. The thickened edges of the mantle present the three reduplications commonly occurring in the Lamellibranchia, and in the bottom of the groove formed by the two outermost reduplications there is, in the hinder part of both mantle lobes, a tract of very definite columnar cells supplied with twigs from the circumpallial nerve. These cells are probably sensory in function.

As may be seen in the sections, figs. 9—13, the lower moieties of the mantle lobes overlapping the gills are very much thickened, and their inner surfaces are thrown into a number of folds, which are specially prominent in the right lobe. These foldings are no doubt exaggerated by contraction in spirit, but there can be little doubt that they exist in the fresh state, and serve to retain water when the animal is uncovered for long periods by the tide. The thickened lobes of the mantle are nearly entirely composed of a lacunar tissue, traversed by very few branching muscle-fibres. The greater part of the musculature of the mantle lies immediately below the external layer of epithelium, and all within this is a spongy lacunar tissue, which must serve as a reservoir for the retention of fluid.

In this connection it may be observed that the asymmetrical arrangement of the organs, and in particular the narrow attachments of the mantle lobes to the visceral mass except in the regions of the attachments of the muscles to the valves of the shell, induces a certain laxity and independence of the organs, which can be better understood by an examination of figures 11—13 than by any description. As a consequence there are numerous irregular spaces communicating with the

suprabranchial or the pallial cavities, and some of them, such as those marked *z* in figs. 11 and 13, end in veritable culs-de-sac. The entrances to these culs-de-sac is guarded by two ridges, whose thickened borders are covered by a columnar epithelium furnished with stiff cilia. A transverse section of one of these ridges is shown in fig. 8. There can be little doubt that when the thickened borders of these ridges are in apposition the cilia interlock, and thus close the entrance to the cul-de-sac, which then serves as a reservoir for water. The largest of these reservoirs are situated between the hinder part of the right labial groove and the visceral mass (fig. 11, *z.*), between the right kidney and the right mantle lobe, below the level of the heart (text-figure 2, F, *z.*), and between the hinder part of the intestine and the right mantle lobe (fig. 13, *z.*). It is evident, then, that *Ænigma* is well provided against the danger of desiccation when exposed for days together to the rays of the sun.

The most remarkable of the pallial organs are the pigment-spots or eyes of the left pallial lobe, whose position has been described on p. 258. The structure of these organs is altogether peculiar in that two epidermic layers, namely, those of the outer and inner face of the mantle, share in their formation. As is shown in fig. 20, each eye comprises a cornea, a lens, a vitreous body, and a layer of deeply-pigmented cells forming a rudimentary retina. The cornea is formed by a local modification of the external epithelium of the mantle, which, elsewhere formed of columnar cells with an admixture of glandular cells, here becomes flatter; its component cells are transparent and vacuolated, and the cell outlines are scarcely distinguishable, but the nuclei are distinct and oval, with scattered chromatin granules. The lens (fig. 21) is a biconvex lenticular mass lying below the cornea, but separated from it by a thin sheet of tissue continuous with the vitreous body. The lens, in spirit specimens, is of an opaque white colour, and no definite structure can be discerned in it. It is a vacuolated mass of

finely granular substance containing a few nuclei, and as the latter are identical with the nuclei of the cornea, it is probable that the lens is formed by a proliferation of the epithelial cells. The lens is embedded in a vitreous body consisting of a mass of finely granular, vacuolated protoplasm containing numerous nuclei which are smaller and stain more deeply than the nuclei of the lens or cornea. This vitreous body, in which no cell outlines are distinguishable, is evidently a local modification and concentration of the sub-epidermal tissue. The retinal layer, as is clearly shown in fig. 20, is formed by the epithelium lining the inner face of the mantle. Some of the eyes are borne on very thin parts of the mantle, and in these, as the vitreous body occupies the whole thickness of the mantle, the retina simply consists of a modification of the cells lining the inner face of the mantle. They become very large and columnar (fig. 21), and are thickly loaded with black pigment granules, but there are no chitinous rods or rhabdomes, such as are commonly found in retinal cells. On the other hand they bear a close resemblance to the retinal cells of the visual organs of the siphons of *Mya arenaria*, *Solen vagina*, and *Dreissensia polymorpha*, figured by Sharp (17). In the case of those eyes situated on thickened portions of the mantle, the internal epithelium is deeply invaginated, as is shown in fig. 20, and the extremity of the invagination spreads out below the vitreous body to form a two-layered optic cup which in section bears some resemblance to the optic cup of a developing vertebrate eye. The cavity of the optic cup is in free communication with the pallial cavity by the "optic stalk," if we may give this name to the stalk of invagination. In most cases the whole of the invaginated cells are pigmented, those of the stalk as well as those of the cup, and both layers of the cup are always pigmented, but it is only the cells of the anterior layer of the cup, that is to say, those in contact with the vitreous body, that are enlarged and columnar. The hinder wall of the cup and the walls of the stalk are composed of low cubical or flat epithelial cells and the transition between

these and the large columnar pigmented cells at the margin of the cup is well shown in fig. 21. The preservation of my specimens was not good enough to allow of my working out such delicate details as the nerve supply of these organs. In every case a nerve derived from the circumpallial nerve could be detected in close proximity to an eye, and in fig. 20 two such nerves are seen in section, one on each side of the optic stalk. But I have been unable to trace nerve-fibres running into those columnar pigmented cells which, because of their characters and position I have called retinal. Further investigations are necessary before the exact nature and function of these "eyes" can be determined with certainty. But their structure points to their being photoscopic, or possibly thermoscopic. I have already alluded to their somewhat paradoxical position and suggested that they may serve to warn the animal to keep the valves of the shell closed during the day-time.

The Byssus and Byssus-gland.—In looking through the literature of the subject one notes with surprise the controversy about the nature of the byssus in *Anomia ephippium*. The calcareous plate or "ossicle" was a stumbling-block to many of the older authors, who regarded it as a third valve of the shell, and, though de Lacaze Duthiers gave cogent reasons for regarding the ossicle as a calcified byssus, and Moore (12) described the fixation of the young forms and the modification of the left valve produced by the asymmetrical attachment of the byssus, neither of these authors gave any account of the microscopical structure of the byssus gland, and as recently as 1878 von Jhering emphatically denied any homology between the "Faltenorgan" of *Anomia* and the byssus-cavity of other *Lamelli-branchia*.

The question was finally decided by Barrois (1), who showed that the macroscopic and microscopic structure of the "Faltenorgan" is in all essential particulars identical with the byssus cavity of *Arca tetragona*. But Barrois contented himself with two very diagrammatic woodcuts of

the byssus cavity of the two genera, and I have been unable to find any accurate drawing or description of the minute structure of the byssus of the Anomiacea. The "ossicle" being absent in *Ænigma*, the byssus and byssus-gland of this genus is a more suitable object for microscopical study than the corresponding organ in *Anomia*, and its resemblance to the byssus gland of *Arca tetragona* as described by Boutan (4) is obvious. In the latter genus the foot is very small, and has a groove on its hinder or posterior surface; the byssus cavity is very large, and the byssus is a stout oval structure consisting of a number of lamellæ which, as Boutan says, overlie one another like the coats of an onion. The byssogenous gland consists of some twenty to twenty-five parallel epithelial folds or laminae, which traverse the interior of the byssus cavity, and the chitinoid lamellæ of the byssus are formed from, and their inner ends are contained between, these laminae. Following Boutan, I will describe the internal divisions of the byssus itself as "lamellæ" and the epithelial folds of the byssogenous gland as "laminae."

The extent of the byssus-cavity in *Ænigma* and its relation to the massive byssus-muscle are shown in the small scale drawing fig. 10, and a transverse section through both byssus and byssus-gland as seen under a higher magnification in fig. 17. The left-hand part of the latter figure shows that the lamellæ of the byssogenous gland are folds of the epithelium lining the byssus cavity, each fold having in its centre a core of connective tissue. The figure, though drawn under a magnification of 600, is on too small a scale to show histological details clearly, but it may be seen that the edge of each lamina is covered by large columnar cells filled with granules. To the right of the figure the byssus itself is seen in situ. It consists of a number of chitinoid lamellæ lying between the byssogenous epithelial laminae. The dark line down the centre of each lamella shows its double origin from the walls of adjacent laminae. The outer ends of the lamellæ are united to form a plate, and it is evident that this plate increases in thickness by the addition of material secreted by

the large glandular cells on the edges of the laminae. This plate is firmly attached to the substratum on which the animal rests. There is no trace of calcification in it, and in several of my specimens the bark of the root to which it was fixed remains adherent, showing that there is no question of the existence of an "ossicle," which has been torn off when the animal was detached. A comparison of this drawing with Boutan's figures (loc. cit., pl. 14, figs. 18, 21, and 22) leaves no doubt as to the identity, in all essential particulars, of the byssus and byssus-gland of *Ænigma* with the corresponding structures in *Arca tetragona*. The laminae are much more numerous and the byssus cavity is relatively wider and shallower in the former genus, that is all.

Fig 18 is a very highly-magnified drawing of the outer end of a single byssogenous lamina lying between two lamellæ of the byssus of *Ænigma*. The sides of the laminae are clothed by a clear, generally-cubical, ciliated epithelium. I have no doubt that this is a ciliated epithelium, and that it corresponds with the ciliated epithelium lining the byssus cavity of other Lamellibranchia, as, for instance, in *Cyprina islandica* (Carrière, 5), *Dreissensia polymorpha* (Horst, 7), and *Jousseaumia* (Bourne, 3). Boutan, however, is of a very different opinion. He says, of similar cells in *Arca*, "Au-dessus de l'épithelium, en contact avec le produit sécrété, on aperçoit une striation très nette qu'on serait tenté de prendre, au premier abord, pour des cils vibratiles; en réalité, ce ne sont que des petits bâtonnets de matière sécrétée, absolument immobiles." Immobile they may possibly be, as I suspect that their function is to afford sufficient surface friction to prevent the byssus lamellæ from slipping out of place, but that they are true cilia is shown by their insertion on a striated border of each epithelial cell, by their correspondence with the cilia undoubtedly borne by similar cells in other Lamellibranchia, and by the fact that they are present where the secretory activity is in abeyance, but absent where it is still in progress, which is the reverse

of what would be the case if they were "little rods of secreted material."

Between the epithelial walls of the lamina is a plexus of connective-tissue cells, among which there are elongate pyriform or spindle-shaped masses of granules in which no nuclei can be distinguished. Deeper down in the laminae a few glandular cells loaded with granules are scattered through the connective-tissue core, but there is no compact mass of byssogenous cells, such as is usually to be found in other Lamellibranchia. The elongated strings and globules of granules must be identified with the streams of granules which I have described in *Jousscaunia* (3) as travelling by intercellular paths from the byssus gland to the byssus cavity. I am of the opinion that the byssogenous cells break up, and that the secretion travels between the irregular spaces of the connective tissue, and that there are not definite ducts as described by Horst (7) in *Dreissensia*. Boutan figures irregular branching ducts in *Arca tetragona* (4, pl. 13, fig. 12), but he does not enter into histological details, and his figure might equally well be interpreted according to Horst's views or my own. Perhaps the most remarkable feature in *Ænigma* is the cap of granular columnar cells on the edge of each byssogenous lamina. These cells are clearly continuous with the ciliated epithelium of the sides, but they are not ciliated, and are filled with byssogen granules. It may be inferred that they have taken up these granules from the intercellular channels of the connective tissue, and that they secrete them again at their free surfaces, thus adding to the thickness of the byssus plate. The concentric lines in the latter (fig. 18) clearly indicate that there has been a continuous addition of fresh matter from the large granular cells capping the edge of the lamina. There is no possibility of confusing the byssogenous with the mucous cells in *Ænigma*; the latter are, indeed, numerous in the foot and in the lips of the byssus cavity, but they never penetrate into the laminae, and are easily distinguished by their oval or polygonal shape small nuclei and clear contents.

I will conclude with a few remarks on the histology of the labial grooves and alimentary tract.

The right and left labial grooves pass without any distinct line of demarcation into the mouth. The right groove as stated on p. 260 is shallow and smooth for a large part of its course posterior to the mouth; the left groove, on the contrary, soon becomes deep, and is thrown into numerous vertical ridges. In both grooves the vertical ridges are covered by a very high, ciliated, columnar epithelium, in which no gland cells can be distinguished. But the smooth portions of both grooves are lined by a characteristic epithelium shown in fig. 23. The ciliated columnar cells are very distinct, and have a doubly refractive border. Between them are two kinds of gland cells, elongated granular, and ovoid clear cells. The former are elongated and occupy the spaces between the ciliated cells, their free ends reaching to the surface. They are filled with fine yellow granules, and their nuclei are to be found in the inner third of their length. As these nuclei are identical with those of the ciliated cells it is probable that the granular gland cells are modifications of ciliated cells. The ovoid clear cells are very large, with clear contents staining pink in picro-indigo carmine; their nuclei stain uniformly dark red in borax carmine. From their staining properties these ovoid cells appear to be mucous cells, and they are similar in size and appearance to the mucous cells of the foot, but, unlike the latter, are not rendered polygonal by mutual pressure.

The epithelium of the labial grooves passes into the œsophagus, but the finely-granular cells soon disappear, and their place is taken by large coarsely-granular gland cells. The mucous cells at the same time disappear. The œsophagus is surrounded by a very distinct layer of subepithelial muscular fibres. The epithelial lining of the œsophagus passes gradually into that of the stomach. In this cavity the glandular cells, as has been already described on p. 267, are restricted to the side walls and floor; the roof is thin and lined by moderately long ciliated cells only. It is noticeable

that the thick, cuticle-like lining of the stomach, the "flèche tricuspidé" of Poli, corresponds exactly in extent to the area in which the yellow glandular cells occur, and is not present in the roof and upper portion of the left wall where these cells are absent. A series of transverse sections shows that the thick glandular tract of the stomachal epithelium is continued posteriorly into the cæcum of the crystalline style, while the ciliated non-glandular tract of the roof and left side passes into the small intestine, and at the entrance into the latter is thrown into a number of stout, ciliated ridges which form a straining apparatus, and are continued into the four prominent ridges projecting into the lumen of the intestine. The transition from the glandular epithelium of the floor of the stomach into the characteristic epithelium of the cæcum of the crystalline style is a gradual one; the ciliated cells of the stomach gradually become shorter and stouter, the yellow gland cells gradually become scarcer, until shortly after its origin from the stomach the cæcum is lined exclusively by the epithelium shown in fig. 22. I have shown (3) that in *Jousseaumia* there is a similar localised tract of glandular cells in the stomach passing through a similar transitional epithelium into the cæcum of the crystalline style, and I have suggested that the last-named structure is secreted by the gland cells in question.

Our knowledge of the structure and functions of the "flèche tricuspidé" and the crystalline style is due to the researches of Barrois (2), List (10) and Mitra (11). Thanks to the last author, we know that the style consists of a proteid material belonging to the globulin class, and that it contains an active amylolytic ferment. He supposes, without giving any very cogent reasons for his conclusions, that the substance of the style is secreted by the liver, and is stored up as a flexible solid in the cæcum, or in some forms, in a special compartment of the stomach or intestine. Barrois has carefully investigated the structure of the "flèche tricuspidé" of the stomach and the crystalline style in *Donax trunculus*, and has entered much more fully into the histology of the

tissues in contact with these structures than has Mitra.¹ He describes the "flêche tricuspidè" of *Donax* as forming a complete lining of the cavity of the stomach, and as being more or less adherent to the anterior end of the crystalline style. He does not recognise any gland cells in the epithelium of the stomach, but suggests that the "flêche tricuspidè" is formed from a granular mass detached from the ends of the epithelial cells, and gives some not very satisfactory figures in support of his statement. He further suggests that the function of the "flêche tricuspidè" is to protect the walls of the stomach from injury. In his description of the cæcum of the crystalline style of *Donax*, Barrois calls attention (and as far as I can determine he is the only author who has done so) to the existence of a groove lined by a modified epithelium, running the whole length of the right side of the cæcum of the crystalline style, from its origin from the stomach to its extremity. There is a similar groove along the right side of the cæcum of *Ænigma*, showing the same histological characters as those described and figured by Barrois in *Donax*. I have given a careful drawing of this groove and the adjacent parts of the wall of the cæcum in fig. 22. As regards the characters of the epithelial cells, it corresponds so exactly with Barrois' description that I need give no further account of it, except to call attention to the extremely long cilia borne by the short cells lining the bottom of the groove, and the short and fine cilia borne by the tract of modified columnar cells on the upper side of the groove. These are not described by Barrois, who says on the contrary, "toute la surface épithéliale est tapissée de cette épaisse et forte couche de cils vibratiles d'ont j'ai parlé à maintes re-

¹ Mitra appears to have been very imperfectly acquainted with the researches of his predecessors on this subject, and his quotations of literature are mostly derived from text-books. Had he read Barrois' memoir, he would have found that as early as 1686 v. Heide suggested that the crystalline style was subservient to digestion: "aliquando cogitavi hunc stylum suppeditari alimini fermentum." Barrois gives a very interesting account of the various views that have been held on the origin and function of the crystalline style.

prises, et sur d'excellentes préparations au carmin aluné, j'ai pu la suivre aussi bien au niveau de l'épithélium modifié que sur la reste de la section." I think Barrois must have been mistaken on this point, but however that may be, the modified cilia are very conspicuous in *Ænigma*. Their arrangement leaves no doubt that the groove has a function analogous to that of the endostyle of an Ascidian, and that its cilia sweep a current of liquid or viscous matter (I could find no solid particles in it) along the length of the cæcum.

From a consideration of these histological details, I suggest that the material of the crystalline style is secreted by the yellow glandular cells of the epithelium of the stomach, and that the so-called cuticular lining of the stomach, or "flèche tricuspide" of Poli, is nothing more than the coagulated viscous secretion of these glands. This viscous secretion, I suggest, is swept into the cæcum by the action of the cilia of the ciliated groove, and is there moulded and solidified into the substance of the crystalline style which, as Mitra has proved, is a globulin containing an amyolytic ferment. During the process of digestion, the style as a whole is moved forward into the cavity of the stomach by the action of the stiff brush-like cilia of the normal cæcal epithelium, and is gradually dissolved, liberating the amyolytic ferment. It is true that several authors, including Barrois, have asserted that the anterior end of the crystalline style is continuous with the so-called "flèche tricuspide," but this confirms rather than contradicts my suggestion. These same authors assert, and I agree with them, that the substance forming this cuticular coat or "flèche tricuspide" is identical with the crystalline style. When one examines this substance carefully, one finds that it forms a lining to the wall of the stomach, thin in some places, thicker in others, and where it is thicker its inner surface (that is the surface farthest from the epithelium) passes insensibly into a mass of granular coagulum, in which the anterior end of the style appears in some cases to be imbedded. But there is no real continuity between the two. The anterior end of the style is in all cases much reduced in

diameter, and is evidently undergoing dissolution. It cannot at one and the same time be losing material and receiving additions from the substance which has been called the cuticle or the "flèche tricuspidé." My interpretation is that the crystalline style is added to at its hinder end, and that the material for its renewal is carried down the cæcum by the ciliated groove. List (10) has given an interesting account of the formation of the crystalline style in specimens of *Mytilus* fed with Indian ink, which is not inconsistent with my suggestion. He does not, however, appear to have subjected the style to the same careful chemical analysis as Mitra.

The numerous follicles of the liver are formed of two kinds of cells. Large, coarsely-granular, clear cells with distinct nuclei, and wedged in among the outer ends of these a smaller number of deeply-staining, finely-granular cells resembling the demilune cells of mixed salivary glands of mammals.

The intestine, in the first part of its course where it is of narrow calibre, with four ridges projecting into its lumen, is lined by an epithelium consisting of attenuated ciliated cells with deeply-staining, densely-crowded nuclei, among which are a few goblet cells, smaller, and with more finely-granular contents those occurring in the stomach. In the loop of the intestine the ridges die out, the goblet cells disappear, and the attenuated ciliated epithelium alone remains. In the straight part of the intestine this is replaced by a columnar-ciliated epithelium with a clear, somewhat granular cytoplasm and pale oval nuclei containing a sparse chromatic reticulum (fig. 24). There are no gland cells in this region, but the cytoplasm of the epithelial cell stains bluish-green in picro-indigo-carminé, and is very distinct from the section of the intestine preceding it and from the proctodæum. It is usual to call this part of the intestine the "rectum." But it is so sharply marked off from the terminal portion of the alimentary tract that I prefer to describe it as the large intestine, and to restrict the name rectum to the short, somewhat enlarged section of the gut which opens to the exterior by the anus

This rectum, which is clearly proctodæal in origin, is lined by an epithelium composed of clear and very attenuated ciliated cells with deeply-staining nuclei.

The whole of the large intestine, and a section of the small intestine preceding it, is infested by sporozoan parasites, whose characters are shown in fig. 24. They are not sufficiently well preserved to admit of careful description, but they are evidently the trophozoites of a Coccidian, some of which are forming cysts containing sporoblasts. They have some likeness to the genus *Klossia*, but as I have not been able to discover the spores, or to trace the various stages of the life-history of this parasite, it will be better to record its existence without conferring upon it a new and probably a misleading name.

SUMMARY OF RESULTS.

1. *Ænigma*, though modified in the same direction as *Anomia*, has undergone a less degree of torsion, and has retained more of the typical features of a normal Lamellibranch.

2. There is, on the left side, a specialised pallial muscle, attached to the left valve, and acting as a retractor of the left gill.

3. A ring of eye-spots, of peculiar structure, is found on the left mantle lobe, at a considerable distance from the edge of the mantle.

4. Adaptations for resisting desiccation during long exposure to the sun and air are found in the thickening and corrugation of the lower moieties of the mantle lobes and in the existence of cæcal extensions of the pallial cavity, which can be closed by the apposition of the ciliated edges of ridges developed on the mantle and body-wall.

5. The structure of the byssus gland is of the same type as that of *Arca tetragona* and *Anomia ephippium*. There is no calcified ossicle.

6. The inner demibranch of the right gill is attached to

what is morphologically the left side of the foot. The minute structure of the gills is curiously similar to that of *Anomia ephippium*. The velar filaments of the external demibranchs bear special cilia.

7. The intestine is coiled.

8. The kidneys and the openings of the reno-pericardial ducts and gonoducts into the kidneys are similar to those of *Anomia ephippium*. The gonopores have ciliated funnels.

9. There are extensive remnants of the pericardial gland. The wall of the pericardial cavity is shown to be incorporated with the walls of the ventricle and auricles.

10. An internal ciliated groove runs along the whole length of the cæcum of the crystalline style.

11. The left gonad extends far back into the left lobe of the mantle.

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EXPLANATION OF PLATES 15—17,

Illustrating Prof. G. C. Bourne’s paper on “The Structure of *Ænigma ænigmatica*, Chemnitz: a Contribution to our Knowledge of the Anomiacea.”

LETTERING IN ALL THE FIGURES.

ad. Adductor muscle. *an.* Anus. *ap.* Aperture between the right and left renal sacs. *a.r.p.* Anterior retractor pedis muscle. *br.g.* Branchial ganglia. *by.c.* Byssus cavity. *by.l.* Lamella of byssus. *by.lm.* Laminæ of byssogenous gland. *by.pl.* Byssus plate. *c.cm.* Cerebral commissure. *c.d.* Ciliated discs. *c.fl.* Latero-frontal cilia of gill filaments. *c.fr.* Frontal cilia. *c.s.* Chitinous skeleton of gill filaments. *c.v.* Ventral cilia on velar filaments. *cn.* Cornea. *cry.* Cæcum of crystalline style. *db¹.* Right external demibranch. *db².* Right internal demibranch. *db³.* Left internal demibranch. *db⁴.* Left external demibranch. *ep.ext.* External epithelium. *es.* Pallial eye spots. *f.* Foot. *gl.c.* Gland cells. *go.a.* Anterior lobe of left gonad. *go.l.* Posterior lobe of left gonad. *go.r.* Right gonad. *int.* Small intestine. *int¹.* Large intestine. *k.cn.* Concretions in cavity of kidney. *l.cp.* Left cerebropleural ganglion. *lep.* Left excretory pore. *lfr.* Right labial folds or palps. *l.g.d.* Left gonoducal opening into kidney. *la.l.* Left labial groove. *lg.r.* Right labial groove. *li.* Liver. *li.d.* Hepatic ducts. *lk.* Left kidney. *ln.* Lens of eye. *l.p.g.* Left pericardial gland. *l.r.p.* Left reno-pericardial

aperture. *m.* Mouth. *m.f.* Muscle fibres. *ml.* Left mantle lobe. *ml'*. Right mantle lobe. *o.* Oesophagus. *p.c.c.* Pericardial cells with endoplastic concretions. *pd.g.* Pedal ganglia. *pl.m.* Branchio-pallial muscle. *pr.p.* Posterior retractor pedis muscle. *r.* Rectum. *r.ep.* Right cerebro-pleural ganglion. *r.ep.* Right excretory pore. *r.k.* Right kidney. *r.p.g.* Right pericardial gland. *r.r.p.* Right reno-pericardial aperture. *rt.* Retina. *st.* Stomach. *v.* Ventricle of heart. *v.cm.l.* Left visceral connective. *v.cm.r.* Right visceral connective. *vel.* Velar fold of gill filaments. *v.j.* Visceral ganglia. *vi.* Vitreous body. *v.m.* Visceral mass. *x.* The dorsal pallial suture. *z.* Cavities between various parts of the body, ending blindly and serving for storage of water. I. Reflected lamella of left external demibranch. II. Direct lamella of left external demibranch. III. Direct lamella of left internal demibranch. IV. Reflected lamella of left internal demibranch. V. Union between the reflected lamella of the right and left internal demibranchs. VI. Reflected lamella of right internal demibranch. VII. Direct lamella of right internal demibranch. VIII. Direct lamella of right external demibranch. IX. Reflected lamella of right external demibranch.

PLATE 15.

FIG. 1.—View of the animal lying in the left valve of the shell, after removal of the right mantle lobe. \times about $2\frac{1}{2}$.

FIG. 2.—The animal removed from its shell and viewed from the left side, showing the ring of pallial eye-spots. The principal organs of the left side are seen through the transparent left mantle lobe.

FIG. 3.—A reconstruction from a series of sections to show the course of the alimentary canal and the nervous system.

FIG. 4.—A reconstruction from a series of sections to show the renal organs and the extent and relations of the pericardial glands. The right kidney is represented in a darker, the left kidney in a lighter tone. The spaces enclosed by the black lines represent the pericardial glands.

FIG. 5.—A horizontal section through the angle of the direct and reflected lamellæ of one of the demibranchs, showing the single row of ciliated discs, *c.d.* Highly magnified.

FIG. 6.—A transverse section through three velar filaments, showing the stiff cilia, *c.v.*, on the ventral faces of the filaments. Zeiss' $\frac{1}{12}$ hom. imm. comp. oc. 4.

FIG. 7.—A transverse section through two adjacent gill filaments. Zeiss' $\frac{1}{12}$ hom. imm., comp. oc. 4.

FIG. 8.—A transverse section through a pallial fold guarding the entrance to one of the water reservoirs, showing the ciliated ridge, *c.r.*, on the edge of the fold. *z.* A branch of the pallial nerve.

PLATE 16.

FIG. 9.—A transverse section through the body at the level of the foot *f.br.* The “*flèche tricuspidè*” of Poli.

FIG. 10.—A transverse section passing through the posterior half of the byssus muscle.

FIG. 11.—A transverse section passing through the ventricle of the heart.

FIG. 12.—A transverse section passing through the middle of the adductor muscle.

FIG. 13.—A transverse section passing about 2 millim. in front of the anus.

FIG. 14.—A section through the right reno-pericardial canal and the adjacent portion of the pericardial gland. Highly magnified.

FIG. 15.—A section through the ciliated funnel of the opening of the left gonoduct of a male into the kidney. The columnar ciliated cells of the funnel are conspicuous. *go.* A follicle of the testis. *sp.d.* Sperm duct.

FIG. 16.—*a.* A group of pericardial cells containing small concretions. *b.* A group of larger concretions from the pericardial gland of another specimen of *Ænigma*.

PLATE 17.

FIG. 17.—A transverse section through the byssus and byssogenous laminae. $\times 600$.

FIG. 18.—A transverse section through the distal end of a byssogenous lamina and two adjacent lamellæ of the byssus. *cil.* Ciliated epithelial cells at the sides of the lamina. *grn.* A mass of byssogen granules in the connective tissue core of the laminae. Zeiss' $\frac{1}{12}$ hom. imm., comp. oc. 4.

FIG. 19.—Portion of a section through the wall of the ventricle of the heart, showing *ep. ext.*, the external epithelium; *m.f.*, the branchial cardiac muscle fibres; *pc.c.*, the characteristic pericardial gland cells with endoplastic concretions.

FIG. 20.—A section through a pallial eye, showing the deep invagination of the epithelium lining, the inner face of the mantle, and the modification of the deeper invaginated cells to form a retinal layer.

FIG. 21.—A portion of another section through a pallial eye. Zeiss' $\frac{1}{12}$ hom. imm., comp. oc. 4.

FIG. 22.—A portion of a section through the cæcum of the crystalline style, showing the ciliated groove, *gt.*, and the tract of modified epithelium on the dorsal side of it. The section is reversed in the drawing, so that the ventral side is uppermost.

FIG. 23.—A section through the right labial groove, close to the mouth.

FIG. 24.—A section through the epithelium of the large intestine. *spor.* Sporozoan parasites.

On the Chromatin Masses of *Piroplasma bigeminum* (*Babesia bovis*), the Parasite of Texas Cattle-Fever.

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With Plate 18, and 44 Text-figures.

CONTENTS.

	PAGE
I. Introduction	297
II. Methods	299
III. Previous observations on the Chromatin of <i>Piroplasma bigeminum</i> and <i>P. canis</i>	300
IV. The Chromatin Masses of <i>P. bigeminum</i> in (α) double pyriform, (β) double ovoid, (γ) single pyriform, (δ) rounded, (ϵ) groups of more than two, (ζ) dividing, and (η) other intra-corpuseular forms; also in (θ) free parasites	302
V. Note on the Cytoplasm of <i>P. bigeminum</i>	317
VI. The Probable Significance of Nuclear Dimorphism in <i>Piroplasma</i>	318
VII. Summary of Results	320
VIII. References to Literature	323
IX. Explanation of Plate	324

I. INTRODUCTION.

Some time ago, while looking at a preparation of *Piroplasma bigeminum* lent to me for examination, I was at once somewhat surprised to find that many of these very small parasites distinctly exhibited what has been called "nuclear dimorphism," for quite typical and non-dividing

forms showed the presence of more than one "nucleus" or chromatin mass. This feature of "nuclear dimorphism" is of great interest at present in elucidating the probable close affinities of the Hæmosporidia and Hæmoflagellates.

As is well known, the micro-organism *Piroplasma bigeminum* (*Babesia bovis*) is the pathogenic agent of Texas Fever (Red-water) in cattle. The preparation examined and now described was a blood-smear, made immediately after the death of the Bovine, labelled "scraping from heart-muscle," and came from Australia. There have been serious ravages among cattle in Queensland, due to Red-water. A few other preparations of the parasite were also examined.

In this particular blood-film from heart-muscle about 90 per cent. of the red corpuscles were infected, one corpuscle usually containing two pyriform parasites, especially characteristic of this, the type-species of *Piroplasma*, and which led to the specific name *bigeminum* (Smith and Kilborne).

All members of the genus *Piroplasma* are small, usually occurring in pairs inside various mammalian erythrocytes, so that the diameter of the host-cells only averages 7μ , and the parasite is considerably smaller, about 3μ by 1.5μ . The minute character of the parasite has been a source of great difficulty in the present research. Great care has been necessary with the illumination, and high magnifications have been used.

For the photo-micrographs, from which the figures in Plate 18 were drawn, I am greatly indebted to Dr. N. H. Alcock, Lecturer on Physiology at St. Mary's Hospital Medical School, assisted by Mr. G. R. Lynch. To these gentlemen I would tender my best thanks for the trouble they have taken in photographing this exceedingly difficult object. Since it was found that the photo-micrographs, which clearly exhibited the "nuclear dimorphism" in question, were too delicate to sustain clear reproduction in the number of copies required for circulation, the photographs have been drawn (twice the size of the original) by the lithographic artist.

II. METHODS.

The blood-smear examined had been fixed and stained by the Romanowsky method. The resulting film was a thin, even, and good one, and was covered and mounted in Canada-balsam. The stain of the host-cells (red blood-corpuscles) was somewhat faint, which was afterwards found to be a distinct advantage, but the parasites were still perfectly well stained, and exhibited the red or purplish-red colour in the chromatin and blue in the cytoplasm characteristic of the Romanowsky coloration.

In order to eliminate, as far as possible, sources of error incidental to stained preparations—errors more especially applicable, however, to the shape and structure of the cytoplasm of the parasite—the slide was examined under various kinds of illumination. These, in brief, were—first, critical illumination, using as a source of light the sharp edge of a paraffin flame; second, monochromatic light (green or yellowish-green was best); and thirdly, less critical illumination from a Welsbach burner or an electric lamp. The last mentioned should, on the whole, be rejected as somewhat untrustworthy and uncomfortable in a research of this kind, where so much depends on the correct determination of the colour of the chromatin masses. The monochromatic light is comfortable and useful for structural detail, but a source of white light, such as is obtained from a paraffin flame, is necessary for the determination of colour, especially of the looser chromatin. In all cases, however, the chromatin could be distinguished, either absolutely or relatively, from the cytoplasm of the parasite, but with very bright (white) sources of light, the relative sizes of the chromatin masses to each other were sometimes misleading, and there was, in such cases, a lack of finer detail. Wrong impressions were also apt to be obtained with the very powerful sources of light, concerning the size and condition of the vacuoles mentioned hereafter. Daylight was also used when possible (winter).

The objectives employed were Zeiss' 2 mm. and 3 mm. apochromatics, aperture 1.40, and compensating oculars 8, 12, and 18, the various combinations of these all giving, in the main, precisely similar results, with little or no increase in detail, for a maximum amount of detail is obtained with compensating ocular 8 and 3 mm. apochromatic homogeneous immersion objective.

Lastly, it seems quite certain that relatively pale-stained preparations are much to be preferred to more deeply stained ones. Nuttall and Graham-Smith (8, p. 588) lay stress on the necessity of differentiation with methylated spirit after coloration with comparatively dilute solutions of Giemsa's stain. It seems to me probable that, in the past, the frequent deep-blue staining, together with the more or less blue coloration of the enclosing blood-corpuscle, usually obtained by the various modifications of the Romanowsky method, using strong solutions, after one quarter to half an hour's staining, has perhaps obscured the finer chromatic details and masked the looser chromatin in such small endoglobular forms as Piroplasmata, and has also hidden the finer structural details of the cytoplasm, vacuolated or otherwise.

III. PREVIOUS OBSERVATIONS ON THE CHROMATIN OF PIROPLASMA BIGEMINUM AND P. CANIS.

The parasite of Texas fever was discovered by Smith and Kilborne, and carefully described by them in 1893 (10), though they saw the parasite in 1889 and apparently "noticed [it] in the spleen of a case as early as 1886" (10, p. 213). Their classical monograph is perhaps, nowadays, hardly consulted as much as it still deserves to be, though the memoir is not always easy of access, unless one procures a copy direct from Washington. The chief stain used by the American investigators was the alkaline methylene blue of Löffler, and the presence of a nucleus in the parasite is not specifically mentioned by them. However, there can be little doubt that the nucleus, as described later by Laveran and Nicolle (3),

was really seen by Smith and Kilborne, for on page 215 of their monograph they remark, regarding fresh specimens, that: "The smaller forms [of the parasite] are as a rule homogeneous; the larger forms are very frequently observed to be provided, in the rounded end of the pyriform body, with a very minute spherical body probably not more than 0.1 to 0.2 μ in diameter, which contrasts dark with the body itself In the largest pyriform bodies there was seen in the centre of the enlarged end a somewhat larger round or oval body which seemed to take the place of the smaller body or else be associated with it. This second body was from 0.5 to 1 μ in diameter. It changed its appearance with the focus One or both of these bodies were observed in some of those forms undergoing amœboid changes." Judging by the latter part of this quotation Smith and Kilborne even may have seen "nuclear dimorphism" in parasites occurring "in fresh blood of the acute disease during life."

Laveran and Nicolle, apparently, were the first definitely to describe and figure¹ the nucleus of *P. bigeminum* in 1899 (3), from stained specimens treated with Borrel blue and eosin, according to Laveran's modification of the Romanowsky method, after fixation by heat and corrosive sublimate. In twin intra-corpuseular forms they found a spherical or oval karyosome at the blunt end of the parasite, measuring 0.7 μ to 0.9 μ in diameter, with a clear zone surrounding the karyosome, which clear area they regarded as the peripheral part of the nucleus. Laveran and Nicolle also mention the frequent presence of numerous, easily stained granules at the pointed extremity of the parasite. These granules, they state, might easily be mistaken for a second karyosome in deeply stained specimens, but, on decolorising a little, are seen to be really only "agglomerations of granules." Personally, I consider that at the pointed end of the parasite loose chromatin is not infrequently seen (see p. 303).

¹ These figures are reproduced in Minchin's 'Sporozoa' (7), p. 269, fig. 80.

It should also be noted that Ziemann (12), in 1898, very briefly mentioned the occurrence of chromatin in *P. bigeminum* without figuring it.

Schaudinn (1904), in a brief note at the end of his remarkable memoir on *Trypanosoma noctuæ* and *Spirochaeta ziemanni* (9, p. 438), points out the presence of nuclear dimorphism in *P. canis* and *P. bigeminum*. Lühe (4, 5), in 1906, confirmed the observation as regards *P. canis*, and further considered the question. Schaudinn and Lühe described a large mass of chromatin, the "principal" nucleus, and a smaller, dense, punctiform mass of chromatin, which Schaudinn suggested was homologous with the blepharoplast of a *Trypanosome*, to which Lühe agrees.

Lastly, Nuttall and Graham-Smith (8), in October, 1906, also saw these two chromatin masses in *P. canis*, and, in addition, a third mass of loose chromatin, described by them for the first time.

My own observations on the chromatin of *P. bigeminum*, as seen more especially in blood from the heart-muscle, may now be set forth.

IV. THE CHROMATIN MASSES OF *P. BIGEMINUM*.

The observations to be described were always made on those parts of the preparation which appeared well fixed and properly stained, especially in so far as the parasite was concerned.

It may be stated at the outset that to give a general account of the distribution of the chromatin, which might be taken as the average or type, is difficult, as considerable variation was noticed. This variation has been already notified by Nuttall and Graham-Smith (8) in the case of the chromatin of *P. canis*, a species larger than *P. bigeminum*. It would seem best, then, to illustrate, by accurate diagrams, as many different forms as possible, at the same time noting their relative frequency of occurrence.

(a) The Chromatin in Pairs of Pyriform Intra-cytopuscular Parasites.

These were the most numerous forms observed, and often in each parasite two distinct masses of chromatin were seen, situated on the sides of the body of the micro-organism at about the middle of its length (text-figs. 1, 2, 3). For convenience, this position may be termed "the equatorio-lateral"—a somewhat cumbersome term, perhaps, and not very correct geometrically—but the position of the chromatin masses in ovoid forms has also to be considered, and in such cases the term will be rather useful. Of these lateral chromatin masses one is nearly always larger than the other (text-figs. 1, 2, 3, etc.), though it cannot always be stated that the smaller chromatin mass is merely "punctiform," as it often appears relatively larger than the idea of size conveyed to my mind by such a term, and may be surrounded by a somewhat deeply-staining area of cytoplasm. Sometimes the lateral chromatin masses are very nearly equal in size (text-fig. 13). Besides these lateral chromatin masses there is not infrequently a third rather loose mass of chromatin, more mesh-like or woolly in character, often situated at the pointed end or apex of the parasite in the midst of rather deeply-staining granular cytoplasm (text-fig. 2). Laveran and Nicolle (3) mentioned the occurrence of numerous granules, easily stained, at the pointed end of the parasite, as we have already briefly noted. They did not consider that these granules formed a "second karyosome"—that is, that they were composed of chromatin, as at first sight appeared, because after slightly decolourising the preparation—which, they state, appeared too deeply stained at first—it could be seen that this apical mass consisted only of granules ("une agglomération de granulations"). It seems to me likely that, in decolourising the preparation a little, the characteristic colour-reaction of the chromatin became very faint or was lost, for among these granules of cytoplasm, situated at or near the pointed end of the parasite, I feel

sure that there is also chromatin present, sometimes perhaps only loosely packed—at any rate in many cases (Pl. 18, figs. 1, 2, and 4 show position of this). Further, Koch depicts chromatin as occurring at the apex of *P. bigeminum* from Africa (1, taf. 1, figs. 1 to 4), and Nuttall and Graham-Smith figure masses of apical chromatin in *P. canis* (8, Diagram 1, figs. 3, 4, 14, 20; Diagram 3, figs. 4, 6, 8, 9, 13, 16, 19).

In a *Trypanosoma*, carefully stained by the Romanowsky method, as by Giemsa's solution, the nucleus appears bright red and the blepharoplast violet-red in colour. In *Piroplasma*, if the two lateral chromatin masses, more or less unequal in size, be strictly homologous with the nucleus and blepharoplast¹ of a *Trypanosome*, one would expect similar differences in staining reaction. In the specimens which have come under my observation it is difficult occasionally to distinguish in depth and colour of staining between the larger and smaller chromatin masses of this minute parasite, as sometimes the one, sometimes the other, may appear slightly more deeply stained, and a difference in tint, as regards violet, is exceedingly difficult to determine in this case. However some specimens certainly showed the blepharoplast more violet in tint after staining. Furthermore, *Piroplasma* is generally intra-corpuseular, and relatively not often free in mammalian blood, and is much smaller than any *Trypanosome*, while the exact method of staining and mounting the preparation, together with its age, are of the utmost importance in determining the finer staining reactions of chromatin.

As regards the structure of the chromatin masses, it is very difficult to make out any details in such small objects. The principal nucleus and blepharoplast appear compact, and separate granules of chromatin cannot be made out in them with any degree of certainty, though, as a rule, the nucleus

² The term "blepharoplast," as relating to *Piroplasma*, is used in this memoir for convenience, and without prejudice to its literal meaning as a nuclear body related to a flagellum. In the present state of our knowledge it is impossible to state definitely whether a flagellate stage occurs in the life-history of *Piroplasma* or not (vide Sect. VI).

is rather less dense than the blepharoplast. The loose chromatin varies somewhat in texture in different forms. It often consists of relatively coarse, chromatic granules, closely associated and touching each other, yet loosely packed compared with the principal chromatin masses, and staining less deeply than these; and this structure may be well seen under monochromatic light, after one has carefully determined the loose chromatin area under white light.

The foregoing description applies to the distribution and structure of the chromatin masses as seen in the most commonly-occurring parasites (about one-fifth of the whole).

Another fairly common form of distribution of the chromatin in pyriform parasites may be termed the "polar" distribution (text-figs. 5, 6, 7, and 8). In these forms there is a large nucleus near the blunt end of the parasite, and a small mass of chromatin—the so-called blepharoplast—at some distance from it, sometimes near the apical end (text-figs. 5, 6, 7). The position of the nucleus in these parasites corresponds with that often noted in the past before the blepharoplast was observed. Occasionally, however, the relative position of these two nuclear masses may be reversed (text-figs. 11 part,¹ 14 part), and the larger chromatin mass then appears near the pointed end.

The nucleus and so-called blepharoplast occasionally occur close together (text-figs. 10, 9 part, 18 part).

Forms in which the chromatin masses appear much alike, both as regard size and density, are sometimes found (text-figs. 13, 15).

One of the parasites represented in text-fig. 18 has much chromatin of varying density, along one side (cf. Koch [1, taf. 1, figs. 1, 2, 4]).

Other types, such as are drawn in text-figs. 9, 12, and 16, are rare, especially the latter, where the parasites possessed only a paucity of chromatin.

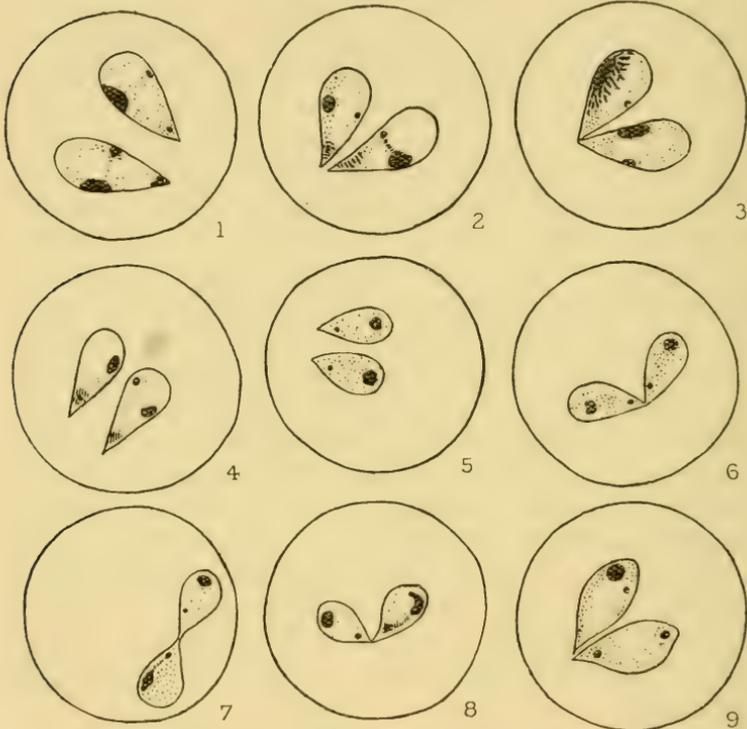
Smaller, and presumably younger, intra-corpuseular pairs

¹ "Part" here refers to the fact that only one member of the pair of parasites shows the particular feature.

of parasites usually exhibited two nuclei already differentiated; it was somewhat rare to find only one "nucleus" in small forms, and then the single chromatin mass was polar, as figured by Laveran and Nicolle (3).

Having noted the principal types of chromatin distribution

TEXT-FIGS. 1—9.



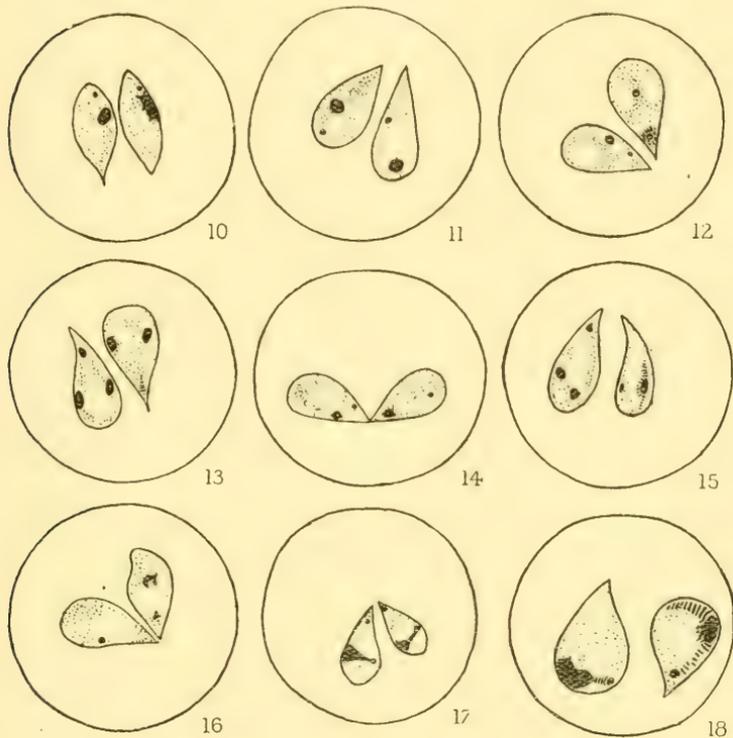
In these and following text-figures the dense chromatin masses of a parasite are represented black; the loose chromatin is shaded. The cytoplasm is diagrammatically indicated by small dots, more closely aggregated and deeply marked where the cytoplasm was more deeply stained. Very faintly-stained areas and vacuoles are left clear. Intra-corpuseular parasites are figured inside more or less circular areas, representing the outlines of infected red blood-corpuscles.

in intra-corpuseular pyriform pairs, a few words are necessary regarding the inter-relation of the three chromatin masses already mentioned. There is sometimes a bridge of some-

what loose chromatin connecting the nucleus and blepharoplast (Pl. 18, fig. 1; and text-fig. 2 part).

In many cases a marked cytoplasmic band stretches across the parasite equatorially between these two "nuclei" (text-fig. 1 part).

TEXT-FIGS. 10-18.



The loose chromatin sometimes surrounds one or other of these denser chromatin masses (text-fig. 3 part, 12 part, 18 part, see also text-fig. 34), or forms a rod in relation with one of these masses (text-fig. 15 part).

Attention may be drawn to the small forms shown in text-fig. 17, where the blepharoplast occurs at the apical end, and the principal chromatin mass stretches across the parasite near the blunt "polar" end. Loose chromatin cannot be

definitely distinguished as a separate mass in these small and young forms.

Usually the shape of the principal chromatin mass is ovoid, especially when occurring laterally. At other times, but less commonly, it is round, as in the case of "polar" distribution. Occasionally it is seen to be irregular (text-fig. 10 part). The so-called blepharoplast is usually round, occasionally ovoid. Rarely, the principal chromatin mass is in the form of a curved rod (text-figs. 8 part, 16).

These different forms and appearances, more especially the varying distribution of the chromatin masses, whether "equatorio-lateral" or "polar," may be explained, at any rate in part, by regarding the parasite as viewed from different aspects, as Christophers has pointed out in connection with the Leishman-Donovan body. It may also be that, as the parasite grows older, the blepharoplast becomes further removed from the nucleus. The intra-corpuseular pyriform parasites are almost certainly flattened to a greater or less extent, as Nuttall and Graham-Smith (8, p. 639) have shown in the case of the larger form, *P. canis*, from observations on living blood.

These observers also mention the occurrence of an achromatic halo around the nucleus or blepharoplast in some cases (8, p. 592). I have very rarely seen this in *P. bigeminum*, and then somewhat imperfectly (cf. text-fig. 9, part).

On the whole, in the case of pairs of parasites, each member of the pair is usually very like its fellow in size, shape, and distribution of chromatin. Variations, however, occur (e. g., text-figs. 9, 15), which are comparatively few in proportion to the large number of parasites examined. Reference has already been made, incidentally, to these variations, whenever it has been necessary to use the word "part" in referring to a figure, thereby indicating variation among the individuals of a pair.

Minute isolated masses or dots of chromatin, other than the three masses already mentioned, were rarely, if ever, seen with certainty in these minute parasites. Further, no really

definite cases of the entire absence of chromatin from a parasite were encountered.

Regarding the relative positions of the nucleus and blepharoplast, it may be noted that although the latter is fairly often seen in close proximity to the former, and even connected with it by loose chromatin, yet I have never seen the blepharoplast actually on the nucleus, or directly budded off from it, as stated by Lühe in *P. canis*. Also, the relative sizes of these two chief chromatin masses can only be determined by careful differentiation and sharp focussing from the granular cytoplasm, sometimes surrounding one or both of them.

(β) The Chromatin in Pairs of Ovoid Intra-corporal Parasites.

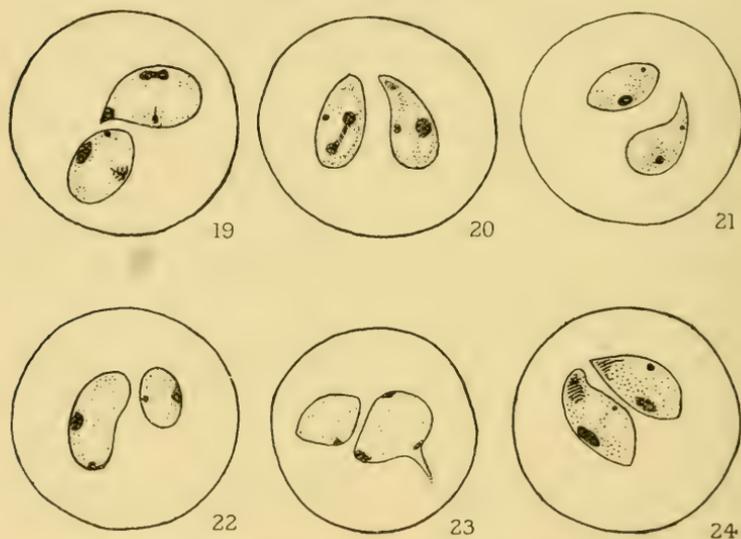
Pairs of strictly ovoid parasites, in which each member of the pair is truly ovoid, are rare. Under this heading it will be convenient to consider cases of pairs of parasites where one member is strictly ovoid in shape while the other is more or less so, yet still shows something of a pyriform contour and may be rather fusiform (text-fig. 24), for the gradation in shape between the two forms is progressive. Such pairs of parasites merit separate consideration as a group after the large number of more strictly pyriform types already discussed.

In these cases we have the same broad distribution of chromatin as before. The "equatorio-lateral" arrangement of the nucleus and so-called blepharoplast preponderates. This distribution of the chromatin, together with the ovoid contour of the micro-organism, recalls, superficially, the appearance of the Leishman-Donovan body.

A few interesting forms showing the connecting bridge of chromatin between the nucleus and blepharoplast were seen (text-fig. 20 part). Three masses of chromatin in each parasite of a pair are shown in text-fig. 19, which masses appeared stained about the same colour and intensity, while the mass which might probably be termed the blepharoplast was in

each case triangular in surface view, with the apex tapering towards the centre of the parasite and surrounded by loose chromatin in one case. The principal nucleus of the more pyriform parasite (text-fig. 19) was rather dumb-bell-shaped, suggesting division, but the general facies of this parasite did not lend support to this view. In text-fig. 24, where each parasite is seen to be somewhat pointed at the ends, there were well-marked masses of loose chromatin at one end. In

TEXT-FIGS. 19-24.



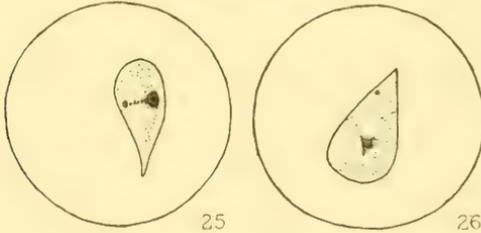
text-fig. 23 two pale-staining parasites are drawn, with peripheral, flattened chromatin. One of them showed a pointed process of cytoplasm, rarely seen in stained preparations apart from spleen-blood. In text-fig. 22 a rather unusual difference in size between the individuals is shown, the larger one being bean-shaped. Both exhibited nuclear dimorphism.

(γ) The Chromatin of Single Pyriform Intra-Corpuscular Parasites.

These forms were not numerous, and no new disposition of the chromatin masses was found. Text-fig. 25 represents a

typical parasite, possessing nucleus and blepharoplast, with, in this case, a rather well-marked bridge of looser chromatin joining them (c.f. preceding text-figs. 2, 20). The presence of such a connecting bridge or strand of chromatin in forms of

TEXT-FIGS. 25 AND 26.



P. canis is mentioned and figured by Nuttall and Graham-Smith (8, p. 594, fig. 12).

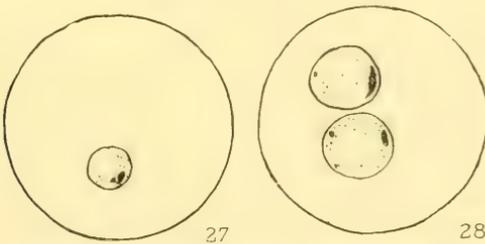
In text-fig. 26 a large pyriform parasite is represented with less well-marked masses of chromatin, the larger of which is irregular in shape.

The loose mass of chromatin was not always to be found in these single intra-corpuseular forms.

(δ) The Chromatin of Rounded Intra-Corpuseular Parasites.

These are not very common, and the parasites may occur either singly or in pairs—usually only one in a corpuscle.

TEXT-FIGS. 27 AND 28.



They generally exhibit a clear, very faintly stained, or quite unstained, area in the centre, and the chromatin is, in some

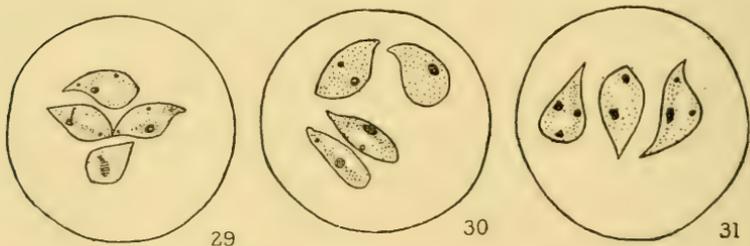
cases, not easily identified. However, chromatin is always present, and is situated strictly peripherally, the chromatin masses being flattened against the circumference of the parasite. One, two, or three (text-fig. 28) masses of chromatin were seen in various specimens. In text-fig. 27 there are really two masses of chromatin, as a blepharoplast occurs close to the nucleus.

These parasites are truly rounded forms, not merely pyriform ones seen blunt-end-on, as could be determined by focussing, and from the fact already mentioned, that the central area was clear; also the corpuscle-hosts are only thin, bi-concave discs. Some of these parasites are ring-like.

(ε) On the Chromatin of Endoglobular Parasites occurring in Groups of More than Two in a Corpuscle.

Cases of three or four parasites, more especially four, occurring together within a corpuscle are fairly common. These may result from multiple infection or from two binary

TEXT-FIGS. 29—31.



divisions after a single infection. All the members of such a group usually exhibit the same or similar distribution of the chromatin elements. In text-figs. 29—31 there are slight differences in the chromatin masses among the members of a group, and it is for this reason more especially that they are figured.

Of the group represented in text-fig. 29, one of the parasites only possessed a mass of loose chromatin. In text-fig. 30

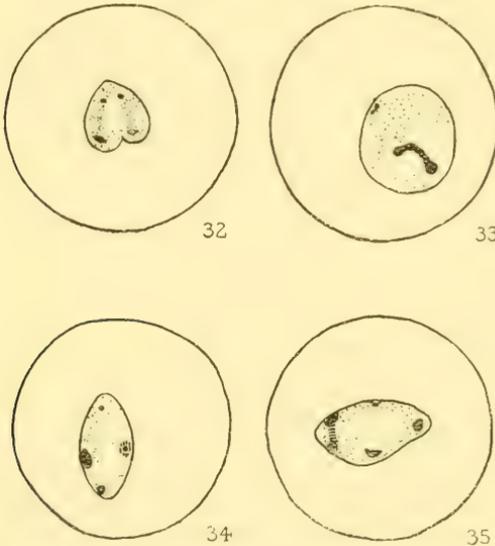
two easily distinguished pairs are shown, which would appear almost certainly to have resulted from a multiple (binary) infection. In text-fig. 31 a group of three parasites is represented, all differing to some extent. Such groups are rare.

The general distribution of the chromatin in groups of parasites conformed to that already given.

(ζ) The Chromatin of Intra-corpuseular Parasites suggesting Division.

Parasites in process of division were not common. In text-fig. 32 is represented a typical, heart-shaped form in process of longitudinal division. Such forms have been

TEXT-FIGS. 32—35.



figured previously, but without showing the blepharoplast in the partially separated forms. In this dividing parasite two nuclei, "polar" in position, and two blepharoplasts, "apical" in position, were clearly seen.

In text-fig. 33 nuclear division is depicted in a large, ovoid form. The principal nucleus is here drawn out into a somewhat curved rod, with slightly enlarged ends, the outline of

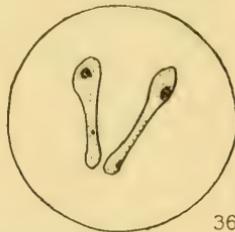
the rod in its middle portion being not quite sharp, but a little irregular. Nothing in the nature of chromosomes could be definitely distinguished, nor reduction division with certainty. The blepharoplast (text-fig. 33) had divided into two dots, lying side by side and still touching, at the periphery of the parasite, away from the dividing nucleus. A vacuole was present near the latter.

Text-figs. 34 and 35 represent ovoid forms, suggesting parasites in process of division. Each contains several chromatin masses of varying size, and more or less peripheral in position. In text-fig. 34 the relative sizes of the four chromatin masses suggest the presence of two nuclei and two blepharoplasts.

(n) Other Intra-corpuseular Forms.

In text-fig. 36 is shown a rare form, consisting of two intra-corpuseular parasites, closely approximated at one end, which

TEXT-FIG. 36.



may be termed rod-shaped, but are really more like a dumbbell or spoon in outline. Bacillary or rod-like forms of *P. bigeminum* are known, but from immune cattle (Theiler). The parasites drawn in text-fig. 36 may possibly be pyriform ones seen edge-on. Two masses of chromatin, one larger than the other, were seen in each parasite situated near the ends.

Irregular forms are very rare. Nuttall and Graham-Smith (8) describe intra-corpuseular parasites showing processes in stained preparations and also in the living condition, and remark (more especially concerning free parasites) that cytoplasmic processes seen in living parasites are often not found

in stained preparations, owing to retraction at death or to the preparation of the film or smear and the difficulty of staining such fine processes. I have only seen one good case of a stained parasite with a protoplasmic process (text-fig. 23), already mentioned.

(θ) The Chromatin of Free Parasites.

Extra-corpuseular or free parasites are not very numerous, and are found chiefly, as might be expected, at the edges of the smear. Regarding epi-corpuseular forms, emphasised by Lühe (4, 5) in the case of *P. canis*, I have only seen one or two doubtful cases, not sufficiently definite to merit detailed description, and my observations have been strictly confined to stained specimens.

The free parasites are nearly always more or less pyriform in shape (text-figs. 37 to 42), occasionally rounded (text-figs. 43 and 44), and are usually slightly larger in size than the corresponding intra-corpuseular forms.

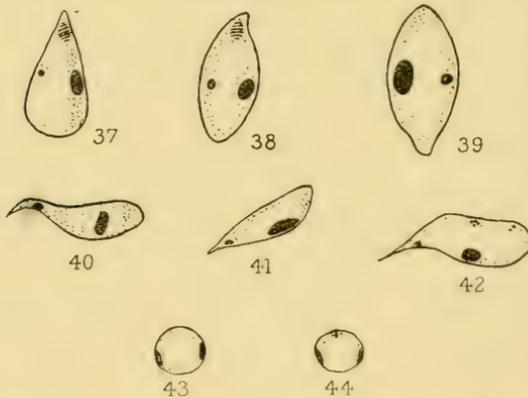
The distribution of chromatin in free forms on the whole closely corresponds with that found in pyriform intra-corpuseular parasites. Common cases are shown in text-figs. 37 and 39, where nucleus and blepharoplast are distinguished, and in some cases an apical mass of looser chromatin (text-figs. 37 and 38) is seen. In text-figs. 38 and 39 the strictly pyriform contour is modified, approaching the fusiform.

Occasionally the pyriform outline is seen to be modified in the direction of its long axis. We then have interesting forms like those shown in text-figs. 40 to 42. These are long and thin, and sometimes exhibit a prolongation of the pointed extremity into a short, pointed cytoplasmic process ("flagellum"¹ of some authors). Such processes, however, hardly suggest great motility. These forms always possessed a nucleus and blepharoplast, the latter at the pointed end. Rarely, smaller chromatoid granules could, with difficulty, be distinguished (text-fig. 42). These forms bear at least

¹ Unfortunately this term ("flagellum") is used, in the Protozoa, for both a cytoplasmic process and a chromatic process rather indiscriminately.

a superficial resemblance to *Crithidia* (Léger) and some species of *Herpetomonas* (vide Woodcock [11, p. 268, fig. 38]). Similar forms have been observed in different species of *Piroplasma*, often without "nuclear dimorphism" having been noticed therein. Such free forms of *Piroplasma bigeminum* are certainly very interesting, more especially to those parasitologists who attach great weight to the observations of Schaudinn ([9], p. 438) on blood-films prepared by Weber at night from a cow kept in the dark, and dying of

TEXT-FIGS. 37-44.



piroplasmiasis, wherein a small Trypanosome was found accompanying *P. bigeminum*. Trypanosome-like (trypaniform) bodies were also found in old films of Kossel and Weber made from the contents of the digestive tract of ticks, which had fed on cows suffering from piroplasmiasis. However, the inference that *Piroplasma* possesses a trypaniform stage is not in the least supported by the researches of Koch on *P. bigeminum* and *P. parvum* (1) from the gut of ticks, nor by the work of Kleine (2) on culture forms of *P. canis*. Kleine also carefully searched for flagellate stages in the blood of infected dogs, without success.

Regarding the chromatin of free rounded forms (text-figs. 43, 44) it closely resembles in distribution that of similarly shaped intra-cytoplasmic forms, and needs no further comment.

V. NOTE ON THE CYTOPLASM OF *P. BIGEMINUM*.

As judged by the distribution of the stain, the cytoplasm is more especially peripheral, and often a clear area is seen in the centre. Such a clear area may be regarded as a vacuole, though one must beware of such appearances, sometimes deceptive, when observations are confined to stained preparations. However, vacuoles have been described in Piroplasmata by various observers while examining the parasites in the living condition. The vacuolated condition, as determined from stained specimens, is well shown in text-figs. 1, 2, 3, 9, 12, 21, 26, 27, and 32. A vacuole with a sharp and definite edge is shown in text-fig. 33. It does not always follow that the clear space inside a parasite, as diagrammatically represented in the text-figures, is a vacuole, for when one examines the preparation under monochromatic light a slight shadow may sometimes appear therein, suggesting a continuous cytoplasm within such parasites, which may be more clear and hyaline in the centre, or less permeable to stain than that at the periphery. The vacuoles, of which more than one may occur in a parasite, usually lie between the chromatin masses, and appear only in the larger parasites. Two vacuoles may occur in a parasite separated by a marked cytoplasmic strand (text-figs. 3 part, 21 part, 22 part).

The cytoplasm shows a granular structure, more especially at the apical end of some forms (text-figs. 3, 12 part). The very finely-granular character of the protoplasm is difficult to represent in drawings. The very small dots in the text-figures, which figures are semi-diagrammatic, only show the limit of the obviously stained area, yet that is almost the limit of certainty in such small and delicate (endocorpuscular) objects as Piroplasmata.

The central clear area seen in most rounded parasites would appear to be a vacuole.

I was not able to obtain any further evidence as to the character of the cytoplasm from an extended examination of the stained free forms.

It may be stated at this point that the dimensions of the larger intra-corpuseular parasites examined was broadly about $3\ \mu$ by $1.5\ \mu$, and agreed well with the measurements given by former observers, though variations occur. The nucleus (largest dense chromatin mass) was from $0.3\ \mu$ to nearly $1\ \mu$ in long diameter in some forms, and exhibited variations in size in different specimens. The so-called blepharoplast was sometimes only about half that in diameter.

VI. THE PROBABLE SIGNIFICANCE OF NUCLEAR DIMORPHISM IN PIROPLASMA.

As far as evidence goes at present—that is, considering the observations of Schaudinn (9), Lühe (4, 5), and Nuttall and Graham-Smith (8), as well as those herein set forth, it would appear that there is some justification for the terms “nucleus” and “blepharoplast,” as applied to the larger and smaller dense chromatin masses respectively in Piroplasmata, at any rate, as a “working hypothesis” (Schaudinn), and so suggesting affinities of this rather aberrant Hæmosporidian genus with the Hæmoflagellates.

The loose, reticulate chromatin, it seems to me, might similarly be tentatively compared with the chromatoid granules often recorded in Trypanosomes, though the loose chromatin of Piroplasma appears to be relatively of greater bulk.

Mention has already been made of Schaudinn’s observation on Weber’s blood films taken at night from a diseased cow, and Kossel and Weber’s films from the gut of ticks. It is much to be regretted that the trypanosome-like organisms occurring in these preparations were not subjected to a lengthy and detailed examination and carefully figured. Even then the question of a double infection would not have been disposed of satisfactorily.

Also, it has been mentioned that Koch’s (1) and Kleine’s (2) researches do not at all support, so far as known at present, the hypothesis of a trypaniform phase in the life-history of Piroplasma. However, in Koch’s large pyriform parasites

from the gut of ticks, with protoplasmic radiations at the blunt ("polar") end, there is a larger nucleus at this end and a smaller blepharoplast-like body nearer the other ("apical") end. Three chromatin masses are seen in the zygote-like forms, with radiations at opposite poles. It is remarkable that Kleine (2) obtained similar results by cultural methods in the case of *P. canis*.

The superficial resemblance of Piroplasmata, exhibiting well-marked nuclear dimorphism, to the Leishman-Donovan body is striking. At one time it occurred to me that, perhaps, the loose chromatin of Piroplasma, in some forms of its distribution, might be compared with the "tail"¹ of chromatin in the Leishman-Donovan body, though this "tail," when present, seems to be always attached to the blepharoplast of the Leishman-Donovan body, and stains rather darkly, and the resemblance to loose chromatin is none too well marked. Laveran and Mesnil would seem to be perhaps justified in placing this body in the genus Piroplasma (as *P. donovani*), considering superficial appearances only, but the difference of habitat must be borne in mind, and the Leishman-Donovan bodies are rarely, if ever, intra-corpuseular. The chromatin masses of the Leishman-Donovan bodies, too, are well defined. Flagellate stages most probably do occur in the life-cycle of these bodies (c f. Rogers, Christophers, Leishman, and others on "cultures.').

I will venture, however, to make a suggestion on the "nuclear dimorphism" of Piroplasma from a rather different point of view, namely, its relation and interpretation in terms of the distribution of chromatin in the Protozoa generally (vide Mesnil [6]). From this standpoint it is probable that the nucleus (large chromatic body) of Piro-

¹ This "tail," which is stated to occur in some of the larger forms of the Leishman-Donovan parasite, is perhaps, on the other hand, only the developing flagellum of the flagellate stages first found by Rogers in cultures, though one hardly gathers this from the published accounts of the formation of the flagellum. This chromatin "tail" merits further consideration by workers with the necessary material, for a flagellum does not occur in parasites in the human spleen.

plasma consists of vegetative chromatin, and is, indeed, a trophic nucleus, while the blepharoplast, according to the upholders of the flagellate affinities of *Piroplasma*, would be a kinetic nucleus—that is, a specialised and separate portion of vegetative chromatin. But the so-called blepharoplast, usually denser and more deeply staining, may really consist of generative chromatin (cf. the micro-nucleus of *Paramœcium*). The loose chromatin would be a reticulum of extra-nuclear chromatin or chromidia. Whether this loose chromatin consists of tropho-chromidia (chromidia properly so-called) related to the cell-metabolism of *Piroplasma*, or whether the loose chromatin consists of idio-chromidia and represents a reserve of generative chromatin, is difficult to say at present. The significance of the so-called blepharoplast at the moment is most problematic, and precludes more definite statements.

Nuttall and Graham-Smith (8, pp. 641-643) consider the various hypotheses which have been advanced at different times regarding the development of *Piroplasma*. They reject them all, after careful studies on the living parasites.

It would appear, then, premature and useless to discuss further the possible life-cycle and affinities of *Piroplasma*; there have been, recently, it seems to me, too many “phylogenies” of such problematic organisms, often founded on slender evidence, and the complete life-history of *Piroplasma*, and even *Trypanosoma*, has yet to be ascertained.

Lastly, as definite and further pronouncements¹ on the developmental stages of *Piroplasmata* in ticks may be expected shortly, it is better to wait for these, when we may learn whether a flagellate stage really does occur in the life-cycle of *Piroplasma*, and what is the true significance of Koch's forms, so difficult to interpret alone.

VII. SUMMARY OF RESULTS.

(1) All the specimens (stained) of *Piroplasma bigeminum*, which were examined, showed the presence of a

¹ See Addendum.

chromatin mass or masses. Usually there was more than one chromatin mass in each parasite (see pl. 18, figs. 1 to 7).

(2) In the pyriform and ovoid parasites there are usually present (α) a rather large and dense chromatin mass—the nucleus; (β) a second, somewhat smaller, usually denser mass of chromatin—the blepharoplast, which is sometimes only punctiform; and many parasites possess in addition (γ) a rather looser mass of chromatin, of a woolly or mesh-like structure (chromidial reticulum).

(3) Variations occur in the relative positions of these chromatin masses, and less frequently in their relative sizes. These variations may be due in part to the parasite being viewed from different aspects.

(4) The loose chromatin mass is often relatively well-marked in *P. bigeminum*.

(5) Free parasites were sometimes seen in which the pyriform contour was slightly modified, and the apical end prolonged into a short cytoplasmic process. Such forms possessed a nucleus and blepharoplast, and somewhat resembled *Hæmoflagellates* (text-figs. 40 to 42).

(6) In round forms nuclear dimorphism also probably occurs, for there is usually more than one chromatin mass present (text-figs. 27, 28, 43, and 44). Amœboid forms were not available for examination.

(7) The cytoplasm of *P. bigeminum* appears to be vacuolated in character. Unfortunately, the observations were, of necessity, confined to stained preparations.

(8) The possible significance of these results is briefly discussed in the preceding section. More definite pronouncements are premature, pending further knowledge of the developmental stages of *Piroplasma* in the tick.

December 31st, 1906.

ADDENDUM.

Since writing the foregoing, two papers have appeared relating to *Piroplasma* (*Babesia*) *canis*, one by Chris-

tophers (13) on the developmental forms of the parasite in the dog-tick (*Rhipicephalus sanguineus*) of Madras, and the other by Kinoshita (14) on the forms of the parasite in the dog's blood and in sodium-citrate cultures.

The paper by Christophers, though only a preliminary account, is most important. Most of the forms mentioned by Koch in the cases of *P. bigeminum* and *P. parvum* in the gut of adult ticks were seen by Christophers, except the markedly radiate forms. Stages in the development of *P. canis* were traced in the tick-egg, in the nymph, and in the developing adult of the dog-tick. The life history appears to be simple. Loose chromatin was seen in several stages, but no mention of any flagellate form occurs, nor is such figured. Also the presence of a blepharoplast is not mentioned. This absence of a flagellate stage in the developmental cycle in the tick is most important, and Christophers' fuller memoir will be awaited with the keenest interest.

Kinoshita's paper is interesting, though to me it is a little difficult to summarise. The author gives excellent figures of nuclear dimorphism in the comparatively large form, *P. canis*, the largest species of *Piroplasma*, and sufficiently large to provide finer morphological detail often invisible in the smaller *P. bigeminum*. He describes schizogony and gametogony, and considers the blepharoplast to consist of "animal" chromatin. He figures two forms, each with a chromatic appendage arising from a large blepharoplast, which he considers to be microgametes. Kinoshita himself, apparently, does not think that a flagellate stage normally occurs in *Piroplasma*, though in an editorial footnote, p. 306, additional and independent evidence for the presence of a flagellate stage is set forth.

It is most probable, indeed certain, that a flagellate stage does occur in the life-cycle of the Leishman-Donovan body, and may be expected in the alimentary tract of a blood-sucking Arthropod, namely, the bed-bug, as suggested by Rogers, and now being worked out by Patton (vide 'Ind. Med. Gaz.,' 1906, p. 302). On the other hand, a flagellate stage appears to be

absent in the case of *Piroplasma*. The pathogenic agents of kala-azar and hæmoglobinuria would not appear, then, to belong to the same genus. However, the evidence at present available is conflicting, and further discussion is premature.

February 14th, 1907.

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IX. EXPLANATION OF PLATE 18.

Illustrating Mr. H. B. Fantham's paper, “On the Chromatin Masses of *Piroplasma bigeminum* (*Babesia bovis*), the Parasite of Texas Cattle-Fever.”

Figs. 1-7.—Lithograph drawings made from photo-micrographs of *Piroplasma bigeminum* (*Babesia bovis*) from heart-smear, showing chromatin masses and their distribution. These photo-micrographs were taken under monochromatic (yellowish-green) light, using a Thorpe's grating. Zeiss's 3 mm. apochromatic homog. immers. objective was used, with compensating oculars 8 or 12.

The lithograph drawings are twice the size of the photographs.

These minute intra-corpusecular parasites were very difficult to focus for photography in order to bring out detail successfully, and so the relative sizes of the nuclear masses do not appear always precisely correct.

The red blood-corpusecles were in all cases only very faintly stained.

FIG. 1.—Shows bridge of chromatin between nucleus and blepharoplast in parasite to left. Note also pair of parasites on right.

FIG. 2.—Pair of large pear-shaped intra-corpusecular parasites, showing nucleus and blepharoplast in each, and loose chromatin at apex of one. Magnification greater than in other figures.

FIG. 3.—Blood-corpusecles showing rather better than in other figures, one pair of parasites distinct.

FIG. 4.—One parasite shows three chromatin masses, and chromatin bridge.

FIG. 5.—One parasite, on right, shows chromatin bridge faintly.

FIG. 6.—Several pairs of parasites, on left, show nuclear dimorphism somewhat faintly.

FIG. 7.—Parasites at top-left-hand part of field show chromatin masses.

**The Skin, Hair, and Reproductive Organs
of Notoryctes.**

Contributions to our Knowledge of the Anatomy of *Notoryctes typhlops*, Stirling—Parts IV and V.

By
Georgina Sweet, D.Sc.,
Melbourne University.

With Plates 19 and 20, and a Text-figure.

INTRODUCTION.

The following is a continuation of the work on *Notoryctes typhlops*, of which Part I, on the Nose and Jacobson's Organ; Part II, on the Blood-vessels; and Part III, on the Eye, have already been published [11, 12].

It has been thought well to make the record of the anatomy of *Notoryctes* more complete by incorporating here a fuller account of the skin and hairs, and also of the reproductive organs and associated parts, than has previously been given.

Professor Baldwin Spencer has therefore kindly handed to me the drawings and notes he had made in connection with these structures; for this, as also for access to his stock of animals, I wish to record my indebtedness and thanks.

As stated previously in another place no embryonic material is forthcoming, so that no facts concerning the development of these parts can be given.

Part IV.—The Skin, Hairs, and Certain Associated Structures.

The skin forming the surface of the body generally, consists of two or three layers of cells, covered by a thin corneous layer (Pl. 19, figs. 3, 4, 12, *e.*). Over the snout and tail the epidermis becomes most highly developed forming the strong horny coverings to those parts. The skin undergoes modification, also in the region of the special "ischiotergal patch," on the hinder part of the back, overlying the ischio-tergal slip of muscle previously referred to by Dr. Stirling [10, p. 159], Professor Baldwin Spencer [8, p. 46], and Professor Wilson [13, p. 6]. The diameter of this patch varies as measured in sections, from 9 mm. to 10 mm., 9.6 mm. being an average width. It can always be seen from the exterior by its darker golden-brown colour.

The varying thickness of the skin in different parts is seen in the following table :

Thickness of	Snout	Chin and Side of Head.	General Surface.	Centre of Ischio-tergal Patch.
Stratum Corneum	.13 mm.	.009 to .014 mm.	.040 mm.	.018 to .021 mm.
„ lucidum	.04 mm.	.01 to .021 mm. }	.014 mm.	.007 to .012 mm.
„ Malpighi	.071 mm.		.034 mm.	.050 to .063 mm.
Total thickness of Epidermis	.244 mm.	.019 to .035 mm.	.088 mm.	.075 to .096 mm.
Total thickness of Dermis	.3 mm.	.85 mm.	.52 mm.	1.54 to 2.45 mm.

The thinness of the dermis in the snout is due to the absence of hairs in that part, while in the chin and general surface of the body, and especially in the ischio-tergal patch, these structures are highly developed. Immediately below

the level of the hair-roots in this modified region, is a thick layer of fat cells. The greater thickness of the dermis here does not end abruptly, but gradually decreases from the centre of the area till it reaches the margin, where there is a small sudden decrease in thickness, marking the boundary of the patch [cf. 8, pp. 45, 46].

The difference in thickness of the corneous layer in the modified patch, and over the general body surface, is occasioned by the compactness of this layer in the former case, and its looseness in the latter.

Hairs.

As in *Ornithorhynchus* [cf. 7 and 9] and *Echidna* [cf. 9] the hairs are of two kinds, large and small, arranged in bundles (Pl. 19, figs. 1 and 3) containing usually one large and a greater number of small hairs [cf. 8, pp. 45, 46]. The hairs of each bundle have a common neck and opening (figs. 2-4, *c.f.o.*) to the surface, where the individual follicles are absent.

The bundles are furthermore arranged in groups of three (Pl. 19, fig. 1), or sometimes four or even five, in more or less straight lines, each bundle, however, having its own follicular opening not communicating with that of the other bundles in the group (see Pl. 19, fig. 2), so that in transverse sections near the surface, the group arrangement is often lost.

The arrangement at deeper levels is shown in Pl. 19, fig. 1, where we have a typical group of three bundles from the skin of the back. The number of small hairs in each bundle varies from nine to twenty, the most numerous examples showing from eleven to nineteen. Occasionally the large hair seems to be absent. As will be seen, there are in the group figured—

GROUP 1.	{	Bundle 1.	1 large hair and	15 small hairs.	
		,, 2.	1 ,,	,, 13	,,
		,, 3.	1 ,,	,, 12	,,

Other bundles contain—

GROUP 2.	{	Bundle 1.	1 large hair and	12 small hairs.
		„ 2.	1 „ „	13 „
		„ 3.	1 „ „	22 „
GROUP 3.	{	Bundle 1.	1 large hair and	21 small hairs.
		„ 2.	1 „ „	15 „
		„ 3.	1 „ „	19 „
GROUP 4.	{	Bundle 1.	0 large hair and	21 small hairs.
		„ 2.	1 „ „	26 „

Successional hairs are only occasionally seen.

Each bundle is separated from those around it by a deeply staining, more or less compacted fibrous tissue. In the ischiotergal patch, and on the chin and side of the head, the bundles are more closely packed together than on the shoulder, thigh, and body generally.

The large hair grows in various positions in the bundles, generally posteriorly, but by no means always so.

The muscles connected with the hairs are as usual unstriated, both in the ischiotergal patch and elsewhere.

Glands.

In the modified patch, around the cloacal opening and on the head, the sebaceous gland mass often almost entirely surrounds the bundle, lying both between the latter and its muscles, and between this bundle and its fellow. The glands in this modified area, and those around the cloacal opening are also very long, extending well down towards the hair-roots, and even below them (Pl 19, fig. 4), but on the side of the head and under the chin, though the glands often extend round the bundles, they are very much shorter than in the ischiotergal patch specially.

On the shoulder and thigh, as well as the general body surface the gland mass is very much smaller and shorter (see Pl. 19, fig. 4). The main duct of the gland is found most often just behind the large hair of a bundle, and should the

large hairs of the three bundles in a group vary in size, as often happens, the largest hair and gland duct is generally to be found in connection with the most central bundle.

The greatly marked glandular development of the ischiotergal region, and the peculiar structure of the ends of the hairs, as described below, no doubt account for the usually matted character of the hair-covering over this area.

Structure of the Hairs.

The ordinary hairs, both large and small, forming the fine silky golden covering of Notoryctes vary considerably in diameter below the epidermis as seen in the following table:

General Surface.		Ischiotergal Patch.	
Large hair.	Small hair.	Large hair.	Small hair.
·021 mm.	·005 mm.	·027 mm.	·005 mm.
·032 "	·007 "	·034 "	·006 "
·045 "	·009 "	·048 "	·007 "
·055 "	·009 "	·056 "	·009 "

The average size for the shaft enclosed in the skin is for the large hair ·040 mm., and for the small hair ·007 mm.

In depth below the surface of the epidermis we find variations according to the region; thus over the body generally a typical large hair may extend for ·49 mm. below the stratum corneum, while in the "ischiotergal" patch it is commonly 1·66 mm. in depth, though it may be more.

Such difference in length may easily be found only 9 or 10 mm. apart. The hair roots are also very long in the head region, but not so much so as in the modified patch.

In structure [cf. 8, pp. 45, 46] the large hairs are not unlike, though much smaller than, those of the Ornithorhynchus [cf. 7 and 9]. They have a rapidly tapering smooth tip (Pl. 19, figs. 5 and 6). This is succeeded by a

wide flattened part, which in a typical hair at a distance of 1 mm., from the tip is .09 mm. wide. From this point downwards we find a diminution in size till at its exit from the follicle it is round, and .03 mm. in diameter. The tip is solid, but in the wider shield and long smooth shaft, the medulla is much vacuolated; the air cavities become regularly arranged, so as to give the ladder-like appearance at about 2 mm. below the extreme point. There is no pigment in the older hairs, though in the successional large hairs there is pigment in the centre of the flattened shield.

In the ischiotergal patch, however, these large hairs are much pigmented right to the free end, causing the darker colour of this area. The tip, moreover, instead of being smooth and pointed as elsewhere, is deeply split (Pl. 19, figs. 9 and 10), giving rise to a somewhat brush-like appearance at the end of each hair. These are frequently .1 mm. in width just below the division.

The small hairs are imbricated all over (Pl. 19, fig. 7), and taper gradually from base to tip over the general surface, but in the ischiotergal region (Pl. 19, fig. 8) they are irregularly waved near the tip, somewhat resembling a badly made spear.

The roots of both large and small hairs are situated at about the same level in the dermis. In minute structure the hair sheaths, as well as the hairs, are normal.

The long straight hairs found around the cloacal opening in both male and female are in distinction to those of the rest of the body surface, not disposed in bundles, but are isolated. In diameter they average .047 mm. to .05 mm.

Special Cutaneous Structures.

When examining the epidermis over the region of the rudimentary eye, I found curious modified groups of epidermic cells with a more or less definite arrangement; and, on further investigation, they are met with over most of the head region,

over the modified ischiotergal patch, and in the pouch region. The epidermis over the head region, i. e. behind the snout, consists of at most three layers of cells, except at special points (Pl. 19, fig. 12). Here the epidermis is much thicker (Pl. 19, fig. 12, *s. o.*), having four or five layers of cells, causing the Malpighian layer to become depressed into the dermis, and the stratum corneum to become slightly raised. Sometimes the whole structure resembles a raised platform, often like a much-truncated cone in outline. In horizontal diameter the top of the modified area varies from $\cdot 11$ mm. to $\cdot 15$ mm.; while in vertical depth, excluding the corneous layer, it is $\cdot 04$ mm. to $\cdot 06$ mm. The whole area of the Malpighian layer involved may have a diameter of 2 mm. The slightly larger examples were found in the ischiotergal patch, though these structures are much more conspicuous in the region of the head and neck, since there, as previously stated, the whole normal Malpighian layer may be only $\cdot 01$ mm. thick. These structures are normally separated by a space of 2.5 mm.

The loose outer part of the corneous layer, which so readily becomes detached elsewhere, tends to adhere more firmly to the underlying part over these areas. The cells of the Malpighian layer are much elongated and thinner, and, in addition to sloping upwards and inwards, they are much crowded horizontally.

A tendency to become arranged around a central core is to be noted in some cases. Their nuclei are also much elongated and larger. In the modified "ischiotergal" patch the whole epidermis is, as previously stated, much thickened, but these cutaneous structures are equally, if not more commonly, developed there than in the skin of the head region. Over the shoulder and thigh regions, as over most of the body, these cutaneous organs do not appear to be present. The structure is often pierced by a group of hairs, or it may be flanked by such a group, while, on the other hand, they would seem often to exist without any relation to hair-groups.

Beneath the modified epidermic areas, there can generally be seen a group of more numerous connective tissue cells, not unlike those found in a developing hair-papilla. Sometimes there is a group of about a dozen oval cells staining less deeply than the cells above, and with two or three well-defined nucleoli, I have not been able to detect either nerve or blood supply to these structures, though in a few cases the oval cells appeared to give off processes towards the Malpighian layer, the cells of which immediately above are very pointed, and almost spindle shaped, penetrating the second layer of more cubical cells.

The general appearance of these structures is that of a taste bud with the stratum corneum continuous over it, and not unlike a developing cutaneous sense organ of Triton.

Of the sensory function of these structures I can therefore offer no conclusive evidence, but it is reasonable to suppose that in the absence of the sense of sight and *pari passu* with the apparently increased olfactory function, there might exist some special organ or organs of tactile sense in *Notoryctes*, as in some other forms. It is at least possible that such may be the function of these special cutaneous structures. They cannot be early stages of developing hairs from the fact that they are present in the fully-grown animal alongside well-developed bundles of hairs and often pierced by such bundles.

Further they are quite different in structure and size from those very early stages of a developing hair which at first sight they slightly resemble.

So that, although direct proof of such nervous character is wanting, one is led to consider it probable that they are some form of tactile sense-organ, which must be of special use to such a burrowing, sightless animal as *Notoryctes*.

Part V.—Reproductive Organs.

URINARY AND MALE REPRODUCTIVE ORGANS.

My observations confirm those of Dr. Stirling as to the position, appearance, structure and relations of the testes, epididymis, vasa deferentia, and their associated blood-vessels; also as to the absence of any external scrotum. The following additional points are worthy of note.

Around the base of the thick muscular-walled bladder (Pl. 20, figs. 13—16, *blr.*), and extending over the origin and anterior part of the urethra, is a well-marked glandular part evidently prostatic in character, and enclosed by a strongly-developed circular muscle coat. Within each of the large muscular bulbs attached to the root of the penis is a well-developed corpus cavernosum with thick fibrous walls. These corpora cavernosa leave the muscular masses and approach each other posteriorly in the mid-ventral line beneath the urethra, forming the “cylindrical body” described by Dr. Stirling [10, p. 182], though, owing to the absence of sections, he was not able to determine the presence of the corpora cavernosa. At the plane of coalescence of the median walls of the corpora cavernosa (Pl. 19, fig. 18, *c.c.*), the corpus spongiosum (Pl. 19, fig. 18, *c.s.*) appears ventral to them again, and with them, extends posteriorly into the penis. This organ lies in a preputial sac ventral to the rectum, and on its dorsal side some distance from the tip (which contains only a blood-vessel) the urethra opens by a long slit-like opening.

In this region the rectum loses its typical glandular character, the cloaca being lined by a very thick stratified epithelium (·095 mm. in thickness), which has a thick, corneous layer. When seen in section, it is very conspicuous when contrasted with the epidermis which consists of two or three layers only (·028 mm. thick) in this region. The lining epithelium of the cloaca is also very much folded, and rests upon a thick layer of submucous connective tissue. Extending

along the length of the cloaca, to near its external opening there are as in *Perameles* [4, p. 57] a number of much-coiled branching diffuse tubular glands lying amongst the muscle bundles of the cloacal wall, and in the connective-tissue surrounding it, especially dorsally and laterally. These gland tubes, which may be found also to a less extent around the rectal wall (Pl. 19, fig. 18, *g. t.*), have very thin walls, composed of cells, which are small and distinctly non-glandular in appearance, and yet the lumen always contains secretion.

Their ducts, in number about thirty, open along the whole length of the cloaca, dorsally, ventrally, and laterally, though most numerous dorsally.

On either side of the anterior half of the cloaca, or even more anteriorly still, lies an "anal gland" (Pl. 19, fig. 18, *a. gl.*) similar to those of some other Marsupials, a single duct from each [contrast Dr. Stirling, 10, Pl. 9, fig. 5] running backwards in the adipose tissue outside the muscle-layers of this part to enter the cloaca laterally, close to its hinder end [cf. *Perameles*, 4, p. 57]. These are readily distinguishable in sections from the diffuse gland ducts by their size and structure, while the other two pairs of duct-like structures described and figured by Dr. Stirling are seen to be simply fibrous bands anchoring the gland in position. This anal gland is most curious in structure, as indicated by Professor Hill in *Perameles*. It consists in *Notoryctes* of an almost spherical hollow ball, 2.4 mm. in external diameter, with a fibrous capsule, from which processes pass inwards, just comparable to the trabeculæ of a lymphatic gland, and forming a more or less complete network. Unlike a lymphatic gland, however, the centre of the ball is hollow (1.4 mm. in diameter), and the whole gland appears to be surrounded by striated muscle-fibres [cf. 1, pp. 157, 161, and contrast 4, p. 57]. Between the trabeculæ the alveoli are filled with long oval cells, those nearest the capsule containing protoplasm with deeply-staining nuclei. As they pass inwards towards the central cavity, however, these cells lose

their nuclei and become disintegrated, so that the ball contains a greater or less quantity of broken-down material, which is passed to the exterior by the duct and cloaca.

In the region of the cloacal opening are numerous solitary large hairs. Associated with the bundles of hairs are also very greatly developed sebaceous glands, which may extend below the hair-roots, but do not occur unless in connection with the hairs. They are only found around the hinder end of the cloaca, and always lie outside, and generally separated from, the muscular wall of the cloaca.

We have in *Notoryctes*, therefore, representatives of three of the types of glands, described by Professor Disselhorst and others as found in connection with the reproductive organs in Marsupials, viz. in *Cuscus orientalis*, *Sminthopsis crassicaudata*, *Macropus robustus*, etc. [cf. 1, pp. 154—163], viz.:

(1) The tubular glands lined by a single layer of cylindrical epithelial cells.

(2) The rectal or anal gland with a duct unbranched, as in *Sminthopsis*, and *Cuscus*, and *Antechinomys*, leading into the cloaca.

(3) Hair glands—large complicated sebaceous glands which do not here, however, lose their close relationship to the hair-follicles, as they do in *Sminthopsis*, etc., where they come into connection with the tubular and the rectal glands, and lie amongst the muscles of the cloacal wall [1, p. 161, fig. 159]. Nor is there here any special development of muscles around these enlarged sebaceous glands, as described by V. Den Broek for *Cuscus orientalis* [1, p. 161].

As to the morphology of the rectal or anal gland which V. Den Broek has considered to be a highly developed modified sebaceous gland [1, p. 163], this seems to me most probable from the somewhat similar general internal arrangement of some of the larger groups of sebaceous glands found around the cloacal opening of *Notoryctes*—though these have not in this form a striated muscular investment

such as surrounds the anal gland; and also from the fact that the secretion of both is necrobiotic involving the death of the cells [2, p. 29]. Of the fourth type of gland described by V. Den Broek [1, p. 163] there is no special representative in Notoryctes.

FEMALE REPRODUCTIVE ORGANS.

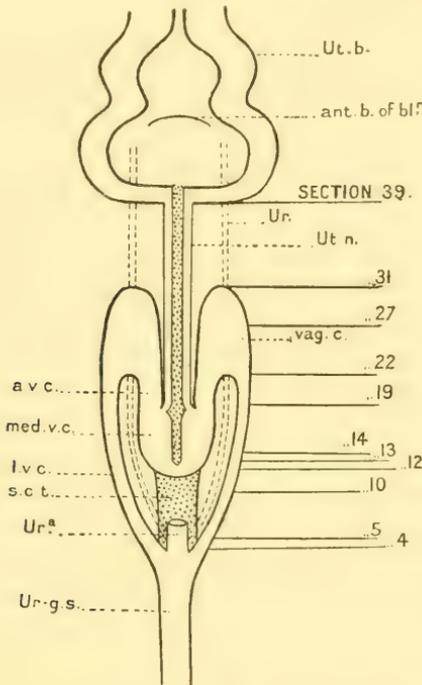
It is of especial interest to note the relations and comparative morphology of these organs in all Marsupials since Professor Hill's valuable work on those in Perameles.

Hence to the admittedly incomplete description and somewhat erroneous figure given by Dr. Stirling, and the brief comments of Professor Spencer, further details are here added, together with the necessary figures. The whole of these organs have been examined by means of several sets of serial sections, and in addition by dissection.

The various relations herein described may be better understood by reference to the accompanying text-figure, in which the parts are represented as seen from the ventral surface, and to scale as far as is possible for serial sections.

In Notoryctes, as in some other marsupials, there are in addition to the ovaries, oviducts, uteri, and vaginae proper, two lateral vaginal canals, with vaginal caeca, and a median vaginal apparatus. The bodies of the uteri (text-figure *ut. b.*) are much twisted, and their internal surface is folded and glandular. They lie ventrally to the rectum with their mesial surfaces almost touching in the middle line anteriorly, while posteriorly they are separated by the anterior end of the bladder (*ant. b. of blr.*). Their posterior ends approach each other almost transversely to the length of the animal, to enter the uterine necks (*ut. n.*), which are much smaller and lie close together ventrally to the rectum, and dorsally to the fundus of the bladder (see Pl. 20, fig. 13, *ut. n.*). The convoluted bodies of the uteri may in some extend for a short distance alongside the bladder, and ventral to the

necks of the uteri, the long axis being parallel to that of the animal. In some of these respects *Notoryctes* resembles *Myrmecobius fasciatus* [5, p. 520] rather than *Pera-meles obesula*. The uterine necks, the internal surfaces of which are much folded, pass backwards often in contact with each other, and embedded in connective tissue, to open into the vaginæ on a small papilla (see text-figure, and



TEXT-FIGURE.

Pl. 20, fig. 14, *ut. n.*) as in *Tarsipes* [5, p. 523] and *Acrobates* [5, p. 525].

Passing backwards from these openings are two large median vaginæ (text-figure, and Pl. 20, figs. 14, 15, *med. v. c.*), separated for some distance back by a septum which is absent, however, at the extreme posterior end, leaving a small opening, apparently a natural one, by which the two

median vaginal canals may communicate with one another. In the possession of this communication *Notoryctes* is quite unlike the young *Perameles* [4, p. 54], young *Dasyurus* [6, p. 371], *Myrmecobius* [5, p. 526], or the young *Trichosurus* [5, p. 528], etc., and more like *Petaurus* [5, p. 526] or *Acrobates* [5, p. 525].

It is, however, normal in the termination of the median vaginae "blindly in the connective tissue between the posterior ends of the lateral vaginal canals, and in comparatively close proximity to the urogenital sinus." The anterior limb of each vagina (text-figure, and Pl. 20, fig. 14, *a. v. c.*) passes forwards from the opening of the uterine neck. It is very short, but wider and thinner walled than either the neck of the uterus or the lateral vagina. It lies laterally to the uterine neck of its own side, and very soon opens into a fair-sized expansion (text-figure, and Pl. 20, fig. 13, *vag. c.*) corresponding to the much larger vaginal cæcum on either side in *Perameles*, its anterior border in *Notoryctes* being situated at about half the length of the uterine necks. In *Notoryctes*, moreover, in contradistinction to *Perameles*, the vaginal cæca being smaller, never lose their close relationship to the uterine necks. Each lateral vagina (text-figure, and Pl. 20, figs. 14—16, *l. v. c.*) runs back as a small, thick-walled, straight, or slightly-curved tube laterally to the anterior limb of the vagina and the median vaginal canal, and separated from the latter by the ureter (text-figure, and Pl. 20, figs. 14—16, *ur.*), which comes here to lie more ventrally than in its anterior part. Thus the whole of these tubes in this region, i. e. the median vaginal canals, the ureters, and the lateral vaginae, lie in the same horizontal line ventral to the rectum and dorsal to the bladder (Pl. 20, figs. 14 and 15).

As in other allied forms, there is here a urogenital strand which contains anteriorly the necks of the uteri, the anterior limbs of the vaginae, the vaginal cæca, and the lateral vaginae, and more posteriorly the median vaginal canals, the ureters, and the lateral vaginae, with the hinder half of the bladder,

and the whole length of the urethra. Indeed, so closely bound together are these parts that a true conception of their relations cannot be obtained by dissection. Notoryctes then, in this respect, also may be compared with such forms as *Perameles*, *Tarsipes*, and *Acrobates*, and contrasted with the free coiled lateral vaginæ of *Myrmecobius*.

Posteriorly to the median vaginals, and occupying the short space between it and the anterior extremity of the long, narrow, urogenital sinus, there lies a mass of dense deeply-staining connective tissue (text-figure, and Pl. 20, fig. 16, *s.c.t.*) exactly similar in appearance and position to that in *Perameles* [4, p. 54, 55], and other forms, through which in *Perameles* birth takes place. Careful examination of this part in *Notoryctes* has failed to show any indication of a split such as occurs in other forms, such as *Perameles*, *Tarsipes*, *Macropus*, *Trichosurus*, and *Dasyurus*, but it is open to question whether this means that the animals examined had not borne any young, as might possibly be indicated by the absence of spermatozoa and their accompanying secretion in the ducts, and by the general appearance of the parts, or, that parturition having taken place similarly to *Perameles*, the epithelium has been completely renewed, as in *Perameles* and *Dasyurus*, and even the split in the connective tissue has been quite closed, as in *Dasyurus* also.

Certainly, unless these animals were virgins, there is here no permanent pseudo-vaginal passage [cf. *Acrobates*], and even were they not so, the connection between the two median vaginal canals, shows no trace of any such forced opening as occurs at birth in older forms of *Perameles* and *Acrobates*, so that we may infer that a median vaginal connection is normally present, as in *Petaurus*.

At the same time, I should judge that birth takes place, as in *Perameles*, through a pseudo-vaginal cleft in the connective tissue, and not through the lateral vaginal canals.

We may notice particularly in concluding this part—

(1) The sharply marked distinction between the uterine and vaginal parts of the ducts.

(2) The large size of the median vaginal canals in comparison with those of other forms and with its own vaginal cæcum, and the intercommunication of the two median vaginal canals.

(3) The presence of the urogenital strand which encloses the anterior vaginal canals as well as the other ducts, as in *Perameles*, *Peragale*, etc., and that of the dense connective tissue anterior to the long urogenital sinus.

Notoryctes then, like *Perameles*, seems to be more primitive in type, as far as its female reproductive organs can show.

Pouch.

As previously described by Professor Spencer [8, p. 49] the pouch is present in both male and female forms, as in some other Marsupials. In the male it is but slightly, though unmistakably, developed, while in the female, it is naturally much larger. My observations confirm the statements hitherto published [8 and 10] as to the position and relations of the pouch and mammæ, etc. The general appearance is shown in Pl. 19, fig. 17, herewith.

The epidermis of this region is similar to that of the rest of the body, but has, here and there, some of the special "sense organs" described in Part IV. The dermis is dense and deeply staining around the pouch area, least so on the roof of the pouch cavity. The sphincter marsupii is present, and lies deep down in the very thick layer of adipose tissue in the walls of the pouch. This tissue also contains numerous bundles of non-striated fibres, mostly transverse, with others longitudinal to the body. The dermis has a very abundant blood-supply.

The groups of hairs are less closely packed in this area, especially on the roof of the pouch cavity, and usually consist of three bundles each. The hairs are chiefly of three average sizes—the largest yellow-brown in colour $\cdot 018$ mm. thick, the smaller ones, which are colourless and much imbricated, being $\cdot 006$ mm. to $\cdot 009$ in diameter.

The sebaceous glands are very well developed, especially around the circumference of the mammæ. Beneath the mammæ the dermis extends even more deeply than elsewhere, and contains numerous mucous ducts. The mammary ducts, three to four in number, open close together on the apex of each mamma, and have, in a marked degree, the characters of sudoriparous glands, as opposed to sebaceous glands; so that, as in Monotremes [2, p. 40] the mammary glands and sweat glands are apparently homologous in Notoryctes also.

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EXPLANATION OF PLATES 19 AND 20,

Illustrating Miss Georgina Sweet's paper on "The Skin, Hair, and Reproductive Organs of *Notoryctes*."

REFERENCE LETTERS.

a. Artery. *a.gl.* Anal gland. *a.t.* Adipose tissue. *a.v.c.* Anterior vaginal canal. *blr.* Bladder. *c.c.* Corpora cavernosa. *cf.o.* Common follicular opening. *c.s.* Corpus spongiosum. *cl.o.* Cloacal opening. *d.* Dermis. *e.* Epidermis. *gt.* Gland tubes. *l.h.* Large hair. *l.v.c.* Lateral vaginal canal. *m.* Muscles of hair. *med.v.c.* Median vaginal canal. *r.* Rectum. *r.s.* Root sheath of single hair. *s.c.t.* Dense connective tissue. *s.h.* Small hair. *s.g.* Sebaceous gland. *s.g.d.* Sebaceous gland duct. *s.o.* Sense organ. *ur.* Ureter. *ura.* Urethra. *ur.gs.* Urogenital sinus. *ut.b.* Body of the uterus. *ut.n.* Neck of the uterus. *v.* Vein. *vag.c.* Vaginal cæcum.

(All figures except Fig. 17 were drawn by the aid of the camera lucida. For Figures 1—11 and 17 I am indebted to Professor Spencer.)

PLATE 19, Figs. 1—12 and 17—18; PLATE 20, Figs. 13—16.

FIG. 1.—Horizontal section through the skin of the back, across a typical group of three bundles of hairs, showing large (*l.h.*) and small hairs (*s.h.*), sebaceous gland (*s.g.*), and duct of same (*s.g.d.*). Zeiss C, oc. 2.

FIG. 2.—Portion of stratum corneum, showing two bundles of hairs, each emerging from common follicle (*cf.o.*), and showing distinct root sheaths (*r.s.*) below. Zeiss C, oc. 2.

FIG. 3.—Longitudinal vertical section through two bundles of hairs in ischiotergal region, showing slightly thickened epidermis (*e.*), large (*l.h.*) and

small hairs (*s.h.*), with separate roots and sheaths below (*r.s.*); and common follicular opening (*c.f.o.*) above. Also gland (*s.g.*) and muscles (*m.*). Zeiss C, oc. 1.

FIG. 4.—Small bundle of hairs from the side of the ischiotergal patch, showing greatly lengthened sebaceous gland, and also diminishing length of base of hairs. Zeiss C, oc. 1.

FIG. 5.—Shows length and shape of pointed large hairs found over general surface of body, with root attached. Zeiss A*, oc. 4.

FIG. 6.—Same hair as in Fig. 5, under higher magnification, showing solid tip and broad shield. Zeiss C, oc. 2.

FIG. 7.—Small hair from general body surface. Zeiss C, oc. 2.

FIG. 8.—Small hair from ischiotergal patch, showing irregular surface. Zeiss C, oc. 2.

FIG. 9.—Large hair from ischiotergal region, showing outline and length. Zeiss A*, oc. 2.

FIGS. 10 and 11.—Two large hairs from ischiotergal patch, showing the brush-like, deeply cleft extremity in contrast to the pointed tip of Fig. 6. Zeiss C, oc. 2.

FIG. 12.—Section through epidermis of side of head, showing "sense organ" (*s.o.*), with bundle of hairs cut transversely. Zeiss C, oc. 4.

FIGS. 13—16.—Transverse sections from a complete series taken through the female urogenital ducts, their position being indicated by section lines in Text-figure (page 337). All drawn under Zeiss A*, oc. 4.

FIG. 13.—Section No. 27 through the bladder (*blr.*), vaginal cæca (*vag.c.*), necks of the uteri (*ut.n.*), ureters (*ur.*), and rectum (*r.*). (Contrast Hill, 4, fig. 3.)

FIG. 14.—Section No. 19 cut obliquely, showing on one side the opening of the uterine neck (*ut.n.*) into the anterior vaginal canal (*a.v.c.*) to form the median vaginal canal (*med.v.c.*), while on the other side the section is slightly anterior to this opening. (Cf. Hill, 4, figs. 5 and 6.)

FIG. 15.—Section No. 14 cut through septum between the two median vaginal canals (*med.v.c.*) just anterior to their fusion, showing ureters (*ur.*) in same horizontal plane as lateral (*l.v.c.*) and median (*med.v.c.*) vaginal canals. (Cf. Hill, 4, fig. 7.)

FIG. 16.—Section No. 10 taken through dense connective tissue (*s.c.t.*) connecting the walls of the median vaginal canals with the wall of the urogenital sinus, showing ureters (*ur.*) passing ventrally to open into the base of the bladder (*blr.*) close to the opening from it of the urethra. (Cf. Hill, 4, figs. 11, 12.)

FIG. 17.—View of pouch cut open ventrally, showing position of the two mammæ, and the relation of the pouch to the cloacal opening (*cl.o.*). $\times 2$.

FIG. 18.—Section through rectum of male, showing anal glands (*a.gl.*), urethra, corpora cavernosa, corpus spongiosum, and gland tubes in rectal wall. Zeiss A*, oc. 2.

**Parorchis acanthus, the Type of a new Genus
of Trematodes.**

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With Plate 21.

THE form with which I am about to deal is one possessing several distinctive features of importance, and for that reason I have thought it worthy of special consideration. The following account is by no means exhaustive, but it is sufficient to place the species beyond any doubt of recognition, and to establish its claim to be regarded as the type of the genus to which it belongs.

Parorchis acanthus, mihi.

1906. *Zeugorchis acanthus*, gen. and sp. n. Nicoll, Ann. and Mag. Nat. Hist. (7), xvii, p. 519—522, pl. xii, figs. 4, 5; xiii, figs. 6, 7.

1907. *Parorchis acanthus*, Nicoll. Ibid (7), xix, p. 128.

The original description of this form as *Zeugorchis acanthus* was made from two preserved specimens found in the bursa Fabricii of a Herring-gull (*Larus argentatus*). It is incomplete and erroneous in parts. I have since

obtained numerous examples from the same situation, and in the only common gull (*Larus canus*) which I have had an opportunity of examining an adult individual occurred in the rectum. The habitat is invariably the bursa Fabricii and the rectum, never the coeca or the intestine proper. The infection is not very numerous, there being seldom more than a dozen parasites in one host. In several instances, especially in young birds, examples of all sizes were found ranging from small immature forms measuring $\cdot 45$ mm. to fully developed adults over 4 mm. long. When removed from the host the parasite displays considerable vitality, several having been kept alive in distilled water over twenty-four hours, and doubtless if a proper temperature were maintained they would survive much longer.

The general outline of the body is roughly oval, narrower in front and rounded behind. In the extended state the breadth is comparatively uniform, but on contraction a characteristic shape is assumed, like that depicted in my original representation of the species. Three well-marked regions are then differentiated, a small head, a stout neck, and a broad flat posterior part. The head is always distinct, and possesses a raised ridge surrounding the oval sucker. The ridge forms a shoulder-like prominence on each side of the sucker, and its ends, which do not meet ventrally, are tucked up towards the mouth. On the edge of the ridge is a single row of regularly-arranged spines, about sixty in number, and having a fairly uniform length of $\cdot 037$ mm. The ant-acetabular region or neck is thick and muscular. Its ventral surface is flattened and beset with numerous strong spines; the dorsal surface is more convex and devoid of spines, except for one or two near the extreme edge of the body. On the ventral surface there is a sort of ridge between the genital papilla and the ventral sucker, which extends a short distance round the sucker on each side. The post-acetabular region is flatter and more delicate in structure. It is not nearly so muscular nor capable of so much contraction as the ant-acetabular region. Its ventral surface

has a few spines near the ventral sucker, but the dorsal surface has none.

The usual length of adult individuals is 3—5 mm. Sexual maturity is seldom attained below a length of 3 mm. The various measurements which will follow have reference to an individual of about 4 mm. length. The breadth of the head is fairly constant, .82—.87 mm. In the extended state the breadth of the rest of the body is 1.2—1.4 mm., but on contraction the posterior acetabular region may be as much as 3 mm. broad. The average breadth is about one third of the length of the body. In young examples the cephalic breadth is proportionately greater than in adults. The thickness of the body varies from .4 mm. in the neck to .8 mm. at the ventral sucker. The post-acetabular region has an average thickness of .5 mm.

The suckers are globular in shape and extremely muscular. The apertures are circular. The oral sucker may attain a diameter of .5 mm., while the ventral sucker is usually rather more than twice as great. In young specimens the proportion between the diameters of the two suckers more nearly approximates 2 : 3, the oral sucker being proportionately larger.

The cuticle is well developed and has an average thickness of .005—.007 mm., although in contracted parts it may be twice as thick. The spines, where they occur, are imbedded deeply in it. The anterior spines have a length of .019 mm. Passing backwards they increase in size, and may be found as long as .031 mm. with a base measurement of .012 mm.

The alimentary system is very well developed. There is a short thin-walled pre-pharynx, .11 mm. long (Plate 21, fig. 2, *p.ph.*). The pharynx is muscular, and measures .24 by .17 mm. On contraction of the animal the pharynx is thrust up inside the pre-pharynx, and the walls of the latter instead of contracting are bent down round the pharynx. The œsophagus is about three times as long as the pharynx. Its walls are crinkled so that numerous small dilatations are formed. In section its dorso-ventral diameter is much greater than its transverse diameter. The epithelium of its lumen is well

developed, usually in one layer, sometimes in two or three. The bifurcation takes place a short distance in front of the ventral sucker, almost on the same level as the genital aperture. The diverticula pass round on either side of the sucker; behind it they are directed in towards the middle line of the body, pursuing a more or less zigzag course. Further back they again bend out to pass round the outer border of the testes, and their terminal part, which is usually somewhat dilated, again approximates the middle line. Like the œsophagus the diverticula are wider dorso-ventrally than from side to side. The characteristic features of the alimentary system are thus its shape, the slightly sacculated condition of its walls, and the lateral flattening.

The excretory system is one of the most peculiar features of this Trematode, and differs very much from the types usually found. The vesical proper (Plate 21, fig. 1, *Ex.*) is not of large size, but is remarkable for its shape. The outline is very irregular, there being on each side about four short unsymmetrical branches, which may be bifurcated at their ends. It is situated at the posterior end of the body; the aperture is not terminal, but is a little forward on the dorsal surface. The excretory system comprises, in addition, two median and two lateral trunks, which communicate posteriorly with the vesicle. The median trunks are wide, compressed dorso-ventrally, and occupy a dorsal position. The lateral trunks are more irregular, and fill up the side-areas of the body. Their lumen is traversed by numerous septa, which divide it, as it were, into a system of anastomosing vessels. Viewed from externally the appearance produced is that of a mosaic of irregular patches, amongst which the excretory fluid circulates. The lumen of the median trunks is not so much divided. Behind the ventral sucker they begin to branch, and communications between the two trunks of one side, as well as between the two median trunks, are not infrequent. At the level of the ventral sucker the median trunks each send out a branch, which forms anastomosing connections almost completely surround-

ing the sucker. In sections the sucker has thus the appearance of being separated from the rest of the body by a cavity (Plate 21, fig. 2). In front of the ventral sucker the four trunks are no longer distinguishable, but are represented by a number of smaller vessels, which still retain their dorsal or lateral position. A few of these small vessels extend as far forward as the head.

The system which has just been described probably corresponds to the usual simple Y-shaped or V-shaped excretory vesicle. The remainder of the excretory apparatus does not differ from that commonly met with. On each side of the body there is a long narrow unbranched collecting tubule, having a ventral situation not far to the outer side of the intestinal diverticula. They are circular in section, and are lined with flagella throughout the greater part of their course. Their walls are distinctly marked, and differ in this respect from those of the excretory trunks, which have the appearance of being mere sinuses in the connective tissue. The fluid in the vesicle and trunks is limpid, and contains comparatively few granules. In the living animal it can be seen to be driven to and fro by the movements of the body.

I am not aware of any Distomid which possesses an excretory system exactly corresponding to this type. Cases in which the limbs of the excretory vesicle are put into communication with each other by means of anastomosing vessels are not uncommon, but they are not of the same nature as the present instance. A condition displaying more resemblance is found in *Mesometra* (*Monostomum*) *orbicularis* (Rud.),¹ in which there is on both dorsal and ventral surfaces a system of anastomosing canals, mapping out little polygonal masses of parenchyma. The form of the vesicle, however, in this species differs entirely from that of *Parorchis*.

The musculature is not essentially different from that of other forms already described. Beneath the cuticle there is a single layer of circular muscles. This is continued round

¹ Cf. 'Bronn's Thierreich,' IV, Vermes I i, p. 650, pl. xxxi, fig. 3.

the outside of the oral sucker, but is apparently absent from the ventral sucker. Within this is a much thicker layer of muscles having mainly a longitudinal direction. Numerous transverse fibres stretch across the body, especially in the anterior region.

The genital organs exhibit many features of peculiar interest. The testes are two comparatively large bodies measuring $\cdot55$ — $\cdot60$ mm. in diameter, situated not very far from the posterior end of the animal. They are placed side by side, almost, in some cases quite, touching each other, and practically on the same level. The obliquity, if any, is very slight, the left testis being possibly a little in advance of the right. They are compressed dorso-ventrally; the outline is roughly circular or polygonal, there being from six to eight distinct, though not very deep, lobes on each. The ovary lies a short distance in front of the testes, almost median or very slightly to the left of the middle line. Its outline is a regular oval, the long axis being transverse; its section is also oval, the long axis again being transverse. It is smaller than the testes, from which it is separated by part of the uterus, and measures $\cdot33$ by $\cdot25$ mm. The yolk glands are not of very great extent. They consist of a series of unequal-sized follicles on each side of the body, extending from the ventral sucker to the testes. Anteriorly they are situated close to the outer side of the intestinal diverticula. They retain this position throughout the greater part of their extent, but posteriorly they cross the diverticula ventrally, and a few follicles are to be found on the inner side near the testes. From this point the yolk-ducts run obliquely towards the middle line in a narrow strip of connective tissue lying between the testes and the median trunks of the excretory system. The shell gland is of large size, lying close to the ovary, dorsal and behind it. There is a small globular receptaculum seminis situated between the testes. Laurer's canal is present.

The vasa deferentia occupy a somewhat dorsal position in the body. They are not symmetrically situated, one being

nearer the middle line and more dorsal than the other. They unite in a fairly small, pear-shaped vesicula seminalis. This is a simple structure, not included in the cirrus pouch, and lying dorsal to the posterior half of the ventral sucker, behind which it extends a short distance. It is near the middle line of the body. The ductus ejaculatorius runs dorsal to the ventral sucker; its terminal third is surrounded by numerous prostatic glands. The penis, in its retracted state, is represented by a short dilated sinus, the wall of which is beset with numerous comparatively large spines. The penis and prostate are enclosed in a thin membrane forming the cirrus pouch. The genital aperture is situated a short distance in front of the ventral sucker on a conspicuous oval papilla in the middle line of the body.

The uterus has its beginning in the triangular area bounded by the ovary and testes. After executing several windings between these organs it proceeds to form convolutions running transversely from one side of the body to the other. These convolutions extend some distance to the outer side of the intestinal diverticula. They overlap the testes to a slight extent, but do not pass behind them. Anteriorly they are bounded by the ventral sucker. The vagina is well developed, situated to the left of the ductus ejaculatorius and dorsal to the ventral sucker. It opens on the left of the male duct at the genital aperture.

The ova are large and numerous, arranged in single file in the uterus. They are elliptical in shape, and the shell is of a light yellow colour. Near the ovary the ova are darker, and present the appearance of being in the process of segmentation. They measure $\cdot 081$ — $\cdot 095$ mm. by $\cdot 040$ — $\cdot 044$ mm. Development takes place rapidly, however, so that towards the middle of the uterus the almost fully formed larva can be observed within the egg-capsule. The latter has by this time increased in size to about $\cdot 106$ — $\cdot 113$ mm. by $\cdot 056$ — $\cdot 062$ mm. Shortly after the extrusion of the ova, which process I observed on one occasion,¹ the capsule is

¹ The previously-noted occurrence of ova in the ventral sucker was, I

burst open at the blunt pole and the larva is set free. This is a small actively-moving Miracidium (Plate 21, fig. 6), not differing much from the usual type. It measures $\cdot 18$ by $\cdot 05$ mm., and the body is differentiated into two distinct parts, a head and a posterior part. The surface is completely covered with long cilia. Near the centre of the head is situated a large, dark, usually five-lobed pigment-spot (fig. 6 *e.s.*).

I shall now proceed to define the genus of which this species is to be regarded as the type.

Genus *PARORCHIS*, mihi, 1907 (= *ZEUGORCHIS*, mihi, 1906). Body of moderate size and roughly oval outline, in the contracted state differentiated into three regions, head, neck, and hinder part, varying considerably in breadth. Anterior part of ventral surface beset with strong spines and oral sucker surrounded by an incomplete row of spines (or spines entirely absent?). Anterior part of body muscular, posterior part more delicate. Suckers well developed; ventral sucker much larger than oral sucker. Intestine with short pre-pharynx, powerful pharynx, œsophagus of considerable length with irregularly sinuate walls, and diverticula which pass round the outer side of the ventral sucker, then bend in towards the middle of the body, and, after bending out again, extend nearly to the hinder end; usually somewhat dilated at their termination. Excretory system consisting of a small median, irregularly-shaped vesicle posteriorly, into which open two median and two lateral excretory vessels; the latter are divided by septa into numerous lacunæ, and as they pass forward branch into numerous smaller vessels. Genital aperture median in front of ventral sucker. Cirrus-pouch includes only the penis and the pars prostatica. Penis beset with spines. Vesicula seminalis at some distance to the rear, small, oval, extending a short way behind the ventral sucker. Vagina well developed. Testes distinctly lobed; situated side by side, on approximately the same level near the hinder end of the body. Ovary transversely believe, purely accidental. I have not observed them in that situation again.

oval, pretty close in front of the testes, almost median. Shell gland well developed. Receptaculum seminis small, between the testes. Laurer's canal present. Yolk-glands not extensively developed, composed of unequal follicles; situated for the most part close to the outer side of the intestinal diverticula, and extending between the ventral sucker and the testes. Uterus also confined between the latter limits; convolutions fairly numerous, and having a transverse direction from side to side of the body. Eggs of large size, with light yellow shell, containing even at some distance from the genital aperture a fully developed Miracidium larva.

Habitat.—The terminal portion of the intestine of birds.

Type.—*Parorchis acanthus*, mihi.

In my previous description of the type I made mention of the remarkable similarity which it bears to *Distomum pittacium*, Braun, apart from the fact that the latter form entirely lacks spines. Further investigation of *Parorchis acanthus* has convinced me that the two species are very closely related, and, indeed, differ only in minor details. The outstanding feature of distinction is the absence of spines in *Distomum pittacium*. I am inclined to believe, however, that this difference does not actually exist, and that the spines have been removed as a result of the method of preservation, and their traces unnoticed by Braun. As far as I can gather, his description is based on a single specimen from the Vienna Museum collection, and he has had no opportunity of examining the living animal.

Immersion in a weak acid solution for even a comparatively short time causes the spines to disappear wholly or in great part, and something of this nature has possibly occurred to Braun's specimen. The circum-oral collar, of which one would have expected at least a trace to remain, is not represented in his figure, but, in the absence of spines, it might be apt to be passed over. As sufficient ground for venturing the foregoing supposition I would adduce the remarkable similarity in the internal structure of the two forms. It is

needless to recapitulate the features of resemblance; I shall rather point out the particulars in which they differ. Braun's specimen measured 3.5 mm., so that it is about the same size as an average example of *Parorchis acanthus*. In the former the oral sucker is much smaller than the ventral sucker, the diameters having a ratio of 1 : 3; in the latter the ratio is nearly 1 : 2. In *P. acanthus* the pharynx is slightly larger than that of *D. pittacium*, and in the latter the testes are much smaller. As a consequence the yolk ducts pass in front of the testes. The yolk glands do not quite reach the ventral sucker anteriorly, but the uterus has several convolutions on either side of the sucker as far forward as the middle of it, and, in addition, it extends very far back, passing even beyond the testes. The convolutions extend to the extreme edge of the body, and thus the whole organ is more extensive than that of *Parorchis acanthus*. Braun doubts the existence of a true cirrus-pouch, but marks it in his figure. Owing to what is, no doubt, an oversight, he represents an aperture in the centre of the cirrus pouch (*c.b.*), while on the right side of it the male duct appears to open separately. No mention is made of the excretory system.

From the foregoing considerations there can be little hesitation in including *Distomum pittacium*, Brn., in the genus *Parorchis*, which, therefore, comprises *P. acanthus* as type and *P. pittacius* (Brn.).

With regard to the systematic position of the genus I have previously made some remarks. It displays much affinity with the genus *Pygorchis*, Looss, a member of the sub-family *Philophthalminæ*. It might, though with some difficulty, be included under this sub-family, and is, in any case, very near it. It agrees in the size and muscular nature of the body, the relative sizes and development of the suckers, the well-developed alimentary system, the position of the genital aperture and genital glands, the situation and small extent of the yolk glands, and the condition of the uterus and ova. The differences consist in the spines, the cephalic ridge, the long œsophagus, the weakly-developed cirrus

pouch, and the symmetrical-lobed testes. Looss does not describe the form of the excretory system in the sub-family. The genus is thus brought into close relation with a sub-family, from which it differs externally, and affords a good illustration of the point which Looss¹ insists upon, namely, that external character is often a fallacious guide to the systematic position of a form.

EXPLANATION OF PLATE 21,

Illustrating Mr. William Nicoll's paper on "Parorchis acanthus, the Type of a new Genus of Trematodes."

The following letters apply to all the figures. *Bs.* Ventral sucker. *D.St.* Yolk glands. *Ex.* Excretory system. *J.* Intestinal diverticula. *K.St.* Ovary. *K.G.* Oviduct. *L.C.* Laurer's canal. *M.S.* Oral sucker. *Oe.* Œsophagus. *P.* Penis. *P.G.* Genital aperture. *Ph.* Pharynx. *P.Ph.* Pre-pharynx. *Pr.* Prostate glands. *T.* Testis. *Rs.* Receptaculum seminis. *S.D.* Shell gland. *Ut.* Uterus. *Vg.* Vagina. *V.S.* Vesicula seminalis.

FIG. 1.—Parorchis acanthus, extended, slightly compressed. Ventral aspect. *Pa.* Pre-acetabular ridge. *Ex*^l. Lateral excretory trunk.

FIG. 2.—Sagittal section through anterior part of body, a little to one side of the middle line. *Pa.* Pre-acetabular ridge.

FIG. 3.—Transverse section through genital aperture.

FIG. 4.—Sagittal section through ovary and shell gland; almost median.

FIG. 5.—Transverse section through receptaculum seminis and testes; somewhat oblique. *S.R.* Collecting tubule.

FIG. 6.—Miracidium larva. *e.s.* Pigment spot.

¹ 'Zool. Jahrb.,' Syst. xii, p. 596.

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CONTENTS OF No. 203.—New Series.

MEMOIRS:

	PAGE
The Chaetognatha, or Primitive Mollusca. With a Bibliography. By R. T. GÜNTHER, F.L.S., F.R.G.S., Fellow of Magdalen College, Oxford. (With 10 Text-figures)	357
The Structure, Development, and Bionomics of the House-fly, <i>Musca domestica</i> , Linn.—Part I. The Anatomy of the Fly. By C. GORDON HEWITT, M.Sc., Lecturer in Economic Zoology, University of Manchester. (With Plates 22—26)	395
<i>Trichomastix serpentis</i> , n.sp. By C. CLIFFORD DOBELL, B.A., Scholar of Trinity College, Cambridge. (With Plate 27, and 2 Text-figures)	449
Notes on Common Species of <i>Trochus</i> . By H. J. FLEURE and MURIEL M. GETTINGS, University College, Aberystwyth. (With Plate 28)	459
Note on the Formation of the Skeleton in the Madreporaria. By MARIA M. OGILVIE GORDON, D.Sc.(London), Ph.D.(Munich), F.L.S.	473
Studies in Spicule Formation. VII.—The Scleroblastic Development of the Plate-and-Anchor Spicules of <i>Synapta</i> , and of the Wheel Spicules of the <i>Auricularia</i> Larva. By W. WOODLAND, The Zoological Laboratory, King's College, London. (With Plates 29 and 30, and 6 Text-figures)	483

The Chætognatha, or Primitive Mollusca.

With a Bibliography.

By

R. T. Günther, F.L.S., F.R.G.S.,
Fellow of Magdalen College, Oxford.

With 10 Text-figures.

It is the purpose of the present paper to demonstrate that, so far as our present knowledge of the Chætognatha goes, more numerous and more cogent reasons can be given for allying them with the Mollusca than with any other group. We shall endeavour to show that no organ of importance has been described in Chætognath anatomy which is not closely paralleled by similar and, we believe, homologous organs among the Mollusca. Indeed, we believe we can go further and demonstrate that the divergences of structure between the Chætognatha and the Mollusca are slighter than those known to exist between different orders belonging to the latter phylum. The theory of the Molluscan and Chætognath affinity explains in a simple manner many facts in the anatomy and developmental history of both groups, while there are no well-established facts which are inconsistent with it.

HISTORICAL SKETCH.

It is not necessary to attempt here a complete review of the stages by which our knowledge of the Chætognatha has

been accumulated. Several of our predecessors have already published such historical résumés, and so it will suffice to refer the reader to the works of Hertwig, Langerhans, and Grassi for fuller details than we give here.

In his original description Martin Slabber (1775) expressed the opinion that the "arrow-shaped worms" should constitute a sub-division of the Linnean group of Vermes. Quoy and Gaimard (1827), after examining the large *Sagitta bipunctata* from Gibraltar, were uncertain as to whether it was Zoophyte or Mollusc. D'Orbigny (1834), however, decided in favour of the Mollusca, and associated the arrow-worms with the Heteropoda, an association in which he was followed by Milne-Edwards (1845), Troschel (1845), Siebold (1848), and Burmeister (1856). Milne-Edwards, indeed, went so far as to point out that the "prépuce" together with the head of *Sagitta* were the equivalents of the Molluscan head.

The anatomical work of Krohn (1844) marks a new epoch. Having been fortunate in obtaining specimens of large size at Messina, Krohn was able to investigate many hitherto undiscovered details of the internal organisation, and amongst others the nervous system, testes, and buccal apparatus. The result was an alliance of the Arrow-worms with the Annelida. Five years later Oersted (1849) suggested that *Sagitta* should rather be considered as a Nematode, an association favoured by Leuckart (1854) and by several writers of zoological text-books (e.g. Claus). The comparison rests principally upon a certain similarity of arrangement of the musculature.

Passing over a suggestion of relationship with the Tardigrada and lower Arthropods by Huxley (1852 and 1878), which has been reconsidered by Grassi (1883) on account of fancied resemblances between the cerebral ganglia, we come to the more serious proposal that a new subdivision of the worms should be constituted for the reception of the *Sagittæ*; and of the names proposed, that of Leuckart (1854)—*Chaetognatha*—has taken precedence of *Oesthelminthes* of

Gegenbaur (1856) and of Pterhelminthes of Harting (circ. 1865).

In 1857 a fantastic theory of Vertebrate affinity was put forward by Meissner. It has, however, failed to find further support than that accorded to it by Haeckel. Metschnikoff (1867) included the Chætognatha in his new group of Ambulacrata, together with the Echinodermata, Brachiopoda, and Enteropneusta. This view has been supported by Bütschli, who, while believing the Chætognatha to have some affinity to the Annelida, considered that the method of the development of the hypoblast is a sufficient justification for regarding them as being near akin to the Echinodermata and to the Tunicata; still more recently (1876) he has proclaimed the Brachiopoda to be their nearest relations.

A revival of the Molluscan theory is due to Langerhans (1878), who re-investigated the nervous system, and discovered the circumœsophageal nerve ring.

In more recent times, while the Annelidan theory has found some support, notably by Giard (1875) and Gourret (1884), it has also found a vehement opponent in Grassi, who maintains that among the Chætognatha there is not to be found any trace either of the metamerisation of the body, or of setæ, or of parapodia. On turning to recent zoological text-books we find that the majority of writers content themselves with expressing their uncertainty concerning the exact position of the group in the Zoological System, but, on the whole, there is a distinct feeling in favour of an association with the "Vermes."

INTRODUCTORY.

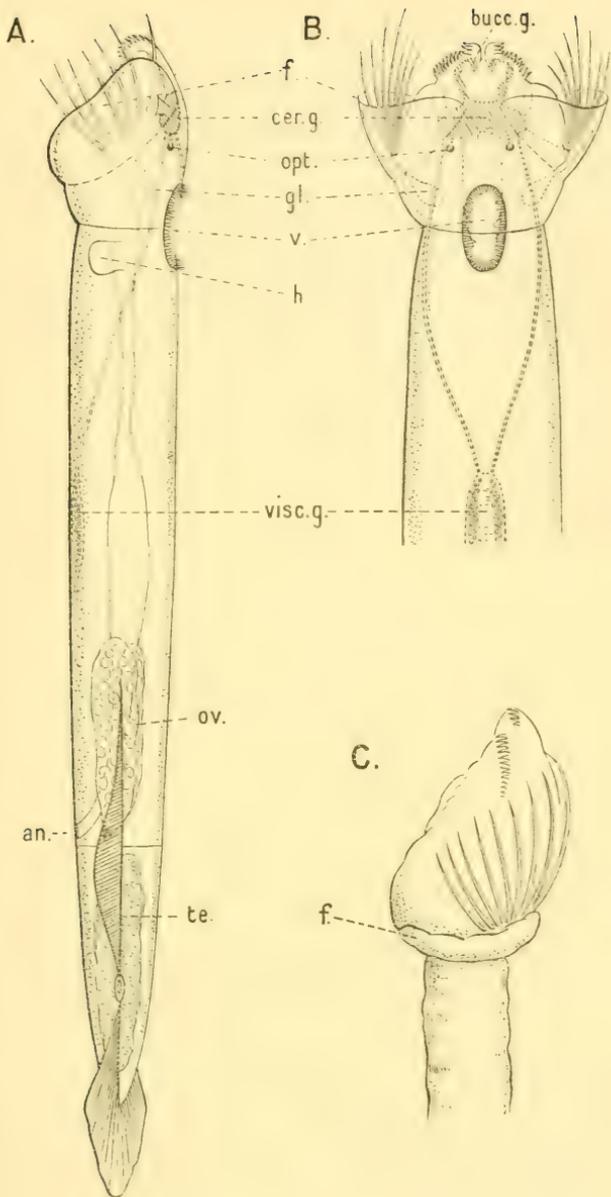
The theory that the Chætognatha are primitive Mollusca is on first thoughts open to the criticism that they do not possess features which have hitherto been generally accepted as essential to members of the Molluscan phylum, and that the obvious points of resemblance are the results of convergence rather than indications of phylogenetic affinity.

We shall endeavour to meet these objections, and to show that the resemblances are shared by all the chief organs in the Chætognath anatomy, namely, by the nervous, alimentary, genital, and other systems; that also in their development the Chætognath approaches very close to the primitive Molluscan type, and that in the case of those features in which the Chætognath organisation appears not to be of the Molluscan type, interesting parallels are to be found within the limits of the Molluscan phylum itself.

At the same time it is not proposed to merge the Chætognatha in any one of the existing Molluscan classes; they are rather to be regarded as modern and modified representatives of a free-swimming ancestor from which the Molluscan phylum has sprung. They represent, in fact, Archimollusca modified for an active swimming life in the open waters of the ocean, with affinities to all existing classes of the phylum. They have, to our mind, more primitive features than the "Archimollusc" of Lankester, which we shall term the creeping Archimollusc to distinguish it from our swimming type of archimollusc; and to emphasize this relationship we propose to consider the Chætognatha as belonging to the lowest class in the Molluscan phylum from which the others have sprung.

EXTERNAL ORGANISATION.

The external configuration of the Chætognath body has justly earned for them their common name of "Arrow-worms." Human ingenuity has not got further in striving after the form best adapted to fast swimming beneath the surface of the water. The lines of the Sagitta and the Whitehead torpedo are the same. Comparatively few of the Mollusca, which have taken to a swimming mode of life, have reached such perfection of outline; in all the difficulty of disencumbering themselves of unwieldy shells and feet and mantle flaps has had to be overcome, and even when these have all gone, as in the languid Phyllirhoe, the

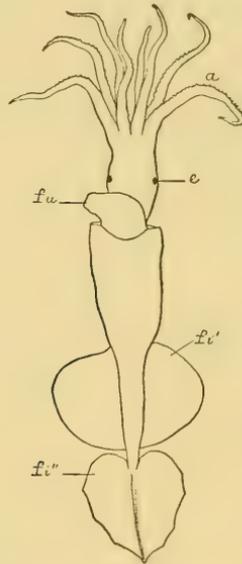


TEXT-FIG. 1.—General view of Chætognath anatomy. A. Side view. B. Dorsal view of anterior region. C. Side view of head of *Sagitta macrocephala*¹ showing the reduced hood (*f*).

¹ From a specimen captured by Mr. G. Murray on November 21st, 1898, in lat. 52°—18'·1 N., long. 85°—53'·9 W., in 1510 fathoms.

result is not satisfactory. The Pteropods are the butterflies of the sea rather than its dragon-flies; and the elaborate hydraulics of the Cephalopods effect locomotion by a totally different process, which we are not considering here.

The separate regions of body merge gently into one another, and all projections unnecessary for functions of swimming or feeding have been smoothed down. For this



TEXT-FIG. 2.—Grimalditeuthis (from Pelseneer, after Joubin).
l', *f''*, lateral and caudal fins.

reason the visceral sac is all contained within the cigar-shaped contour of the body-wall. In Mollusca the integument of the visceral sac is typically folded near its junction with the head and foot, but no such mantle-flap or pallium is distinguishable in Sagitta. The pallial ectoderm is, however, drawn out in the horizontal plane to form the lateral and caudal fins of many of those Dibranchiate Cephalopoda (text-fig. 2) in which the shell is most degenerate, and the resemblance is strengthened by the deposition of a chitinous or

conchyolinous substance within the fold either in the form of fin-rays or of a continuous lamina.

If we may be permitted to imagine the alterations of structure which a typical Mollusc would undergo if it were to become adapted for an active, free-swimming, predacious life; I think that, judging by living examples, we should find that they would be in the direction of the structure of the Chætognatha.

That foot, visceral hump, shell, and mantle cavity might disappear, is clearly shown by the structure of *Phyllirhoe*. But the Chætognatha differ from *Phyllirhoe* in their smaller size, and in the far greater activity of their swimming movements. To these two factors we attribute the relatively large development of certain organs, such as the longitudinal muscular system and the non-existence of others, e. g. separate vascular and respiratory systems respectively.

It may be premised at once that there is nothing in the Chætognath anatomy or development which we have felt able to homologise with the shell or shell-gland of the Mollusca. We cannot, however, consider that the absence of this pallial organ is very material to the main issue, for even among Mollusca of very moderate swimming powers the shell is usually the first structure to dwindle and disappear (*Heteropods*, *Phyllirhoe*, etc.), and might therefore be expected to be altogether absent in a Mollusc built upon such perfect lines for swimming as *Sagitta*. But it may be argued, if the ancestor of Chætognatha had a typical Molluscan shell, surely some trace of a shell-gland would be noticeable in the younger stages of their growth, it being so conspicuous a feature in Molluscan developments. For its absence two explanations may be given: firstly, by our hypothesis, the Chætognatha are presumed to have become modified for their pelagic life at a very early period, before the Molluscan phylum and the Molluscan shell had been in existence for a long time, in other words, the Chætognatha are a far older group than any of the shell-less Mollusca.

If the loss of all trace of the shell-gland may have been the

effect of time, its loss has no doubt also been accelerated by the marvellous rapidity with which Chætognath development takes place.

SEGMENTATION.

Although the body cavity appears to be divided into three paired cavities by septa, there is no justification for regarding the Chætognatha as exhibiting any real metameric segmentation at all. In the first place the two transverse septa cannot be regarded as homologous with one another, or with the septa which separate the metameres of Annelida, because in time, mode, and purpose of origin they are absolutely different. Embryological examination shows that the anterior septum is produced by the meeting and fusion of the somatic and splanchnic mesoblast very early in embryonic life, at a time when differentiation of tissues has not begun and the mesoblast is still continuous with the hypoblast; and that the posterior septum appears in close connection with the genital cells, and is not formed by the whole thickness of the mesoderm, but probably only by the cellular envelopes of the genital cells, and certainly by the splanchnic layer exclusively.

In the light of our new knowledge concerning these developmental phenomena, the theory of a close relationship of Chætognatha to an unsegmented group of animals of a high grade of organisation is in no way prejudiced by the division of the body into three sections which have the guise but not the reality of metameres.

INTEGUMENT.

The Chætognatha differ from the majority of Mollusca in the absence of a more or less extensive ciliated epithelial covering of the body both in the larval and adult stages. But though devoid of a general investment of cilia which might

impede their active movements in the water, most of them possess a circular or oval ring of ciliated cells on the dorsal side of the head and behind the eyes. This ciliated organ is innervated directly from the cerebral ganglion, and is believed to have an olfactory or gustatory function. It will be referred to again as the homologue of the velum or preoral circlet of cilia of the Trochophor larva of the Mollusca (text-fig. 1, *v*).

The external epithelium of the Chætognatha, however, agrees with that of the Mollusca in the presence of gland-cells, which are especially abundant upon the ventral surface of those littoral species, which live among algæ, such as *Spadella cephaloptera*; and similar cells, though less well developed, have been noticed in *Sagitta darwinii*, Grassi, and to less extent in *Spadella marioni* by Gourret.

The two large glands (text-fig. 1, *gl.*) on the upper side of the head have been described by Gourret in *Spadella marioni*. They are covered by the "hood," and may be variously regarded as mucous glands for the lubrication of the hood, as poison glands for the spines, or as extra-buccal glands. It is unlikely that their function is excretory, as Gourret suggests.

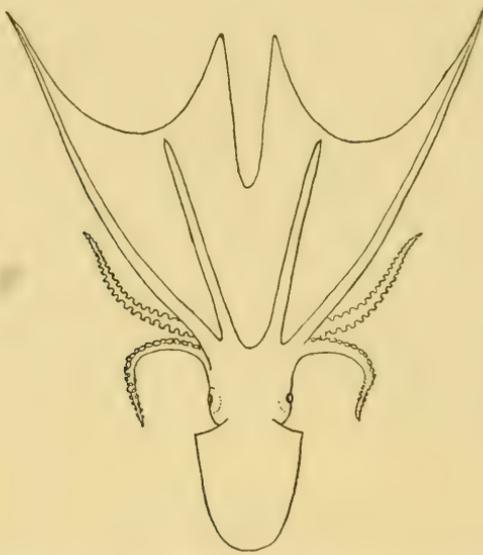
SUB-EPITHELIAL TISSUE.

In some species of Chætognatha, e. g. *Spadella draco*, the sub-epithelial tissue presents a peculiar vesicular condition which is particularly noticeable in the neck region anterior to the lateral fin. Similar cells occur in the sub-epithelial (mesodermal) connective tissue of many Mollusca.

FOOT.

There is no distinct organ in the Chætognatha which can be compared with the Gastropod or Lamellibranch foot, but it must be remembered that the Chætognatha are not creep-

ing animals, nor is there any reason to suppose that their recent ancestors (like those of the Heteropoda) were so, or, indeed that they have ever had a creeping ancestor at all. On the other hand the circumoral hood, "Kappe" or "prépuce," situated, as it is, between the head and visceral regions, occupies the same relative position as the "foot" of a Dibranchiate Cephalopod, or the lateral lobes of Nautilus, and to these structures we desire to compare it. The method



TEXT-FIG. 3.—Tremoctopus (from Pelseneer, after Joubin).
Note the interbrachial membrane.

of innervation would, no doubt, settle the question of the homology of these organs easily were the hood a more powerfully developed muscular organ than it is. According to our theory the Chaetognath "hood" is the Cephalopod circumoral foot in its most primitive condition, or else it represents that organ in a reduced condition as a mere reduplication of the ectoderm into which the body cavity extends but a short distance. It is therefore to be compared with the interbrachial membrane of many of the Cephalopoda (Histi-

teuthis, Tremoctopus [text-fig. 3], Leioglossa) rather than with the arms themselves. In the deep-sea form *Sagitta macrocephala* it is in a much reduced condition (text-fig. 1, c, f.). In development, its bilateral origin already indicated by the notch in the mid-ventral line is very clear, for it is formed from two separate ectodermal thickenings, one on either side of the mouth (Doncaster).

The two glands near its dorsal attachments may secrete mucus of the inner surface of the hood (text-fig. 1, A, B, gl.). According to the theory that hood and Cephalopod inter-brachial membrane are homologous organs, these hood-glands and the aquiferous pores of Tremoctopus and other Cephalopoda, cannot be regarded as homologous.

MUSCULATURE AND SKELETON.

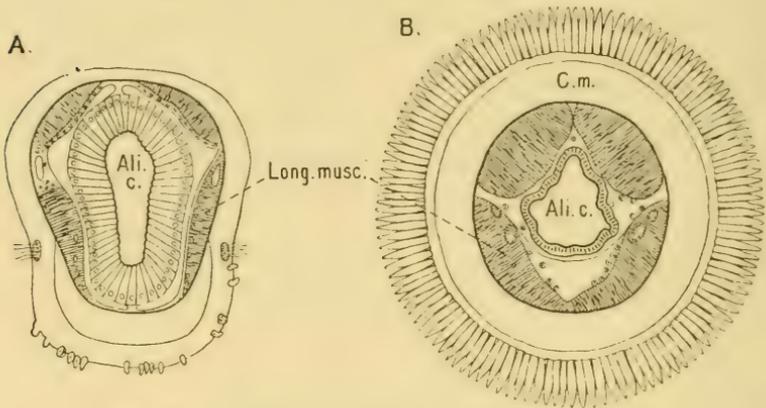
The musculature of the Chætognatha, which has so often been cited as their point of resemblance to the Nematoda, is as distinct as possible from our ordinary conception of Molluscan musculature, but among the varied members of the Molluscan phylum there are some which present features closely resembling the Chætognath condition.

Further, the Chætognath muscle fibres are striped, whereas Molluscan fibres are as a rule unstriped.¹ But were striped muscle entirely unknown in living Mollusca we should not feel inclined to attribute much importance to this difference, believing that muscle striation is merely an indication of efficiency from the point of view of rapidity of contraction. Molluscs are slow in their movements, the Chætognatha are quick, that is all. However, Spillmann has shown that well-striated muscle may occasionally appear among the Mollusca, for instance, in the hearts of *Turbo rugosus* and *Acmæa virginea*.

The arrangement of the longitudinal musculature in a

¹ Several text-books, e.g. those by Lang and Sedgwick, are in error in stating that true striated muscle does not occur in the Molluscan phylum.

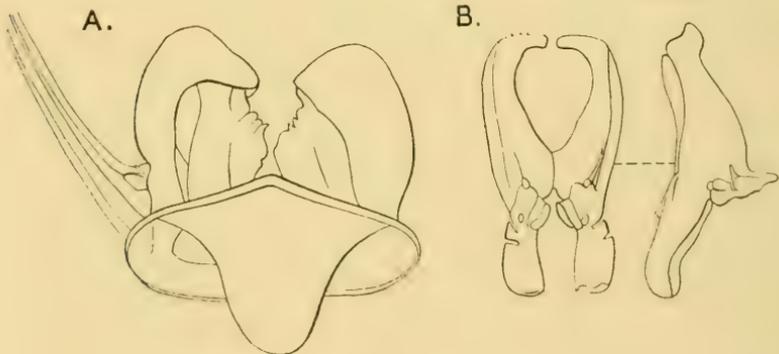
sheath surrounding the viscera is an adaptation for swimming similar to that in *Phyllirhoe*, whose ancestors, no doubt, possessed both foot and shell. The subdivision of the muscle



TEXT-FIG. 4.—Transverse sections showing musculature.

A. *Chaetognath* (after Hertwig). B. *Chaetoderma* (after von Graff).

bands into four quadrants finds an exact counterpart in *Chaetoderma* (von Graff, text-fig. 4). The homology of these



TEXT-FIG. 5.—Endocephalic skeletons.

A. *Spadella marioni* (after Gourret). B. *Nautilus* (after Lankester).

muscles with the four longitudinal retractor muscles of the head of the Cephalopoda will be noticed later.

The occurrence of three pairs of supporting plates in connection with the spines on the surface of the buccal mass is an important feature in Chætognath anatomy, but of far wider significance is the complicated chitinous (?) skeletal piece with its large lamella and two lateral wings serving for the attachment of the retractor, extensor, and other muscles in the head. This internal cephalic skeleton we believe to be the homologue of the cartilaginous cephalic skeleton of the Cephalopoda, and bears a superficial resemblance to that of *Nautilus* (text-fig. 5). Its presence in addition to the many other points of resemblance between the two groups should be something more than a mere case of analogy.

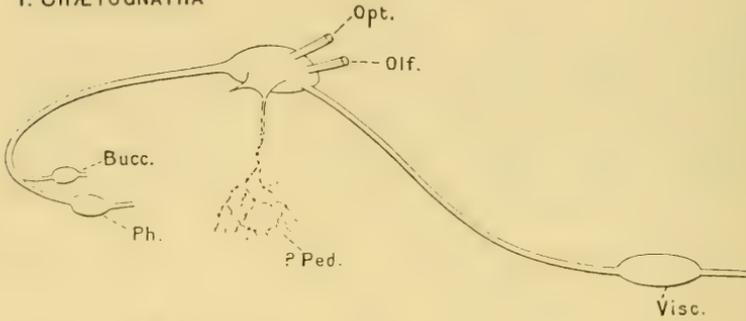
NERVOUS SYSTEM.

The remarkable resemblance of the Chætognath and Molluscan nervous systems was recognised as long ago as 1844 by Krohn, the discoverer of the nervous system in *Sagitta hexaptera*, and again by Langerhans, who discovered the buccal ganglia. The two most recent accounts by Hertwig and Gourret are not entirely in agreement, but both may be readily referred to the Molluscan plan. Indeed, the resemblance is so close that, in a description of the Chætognath nervous system, the Molluscan terminology may be appropriately employed.

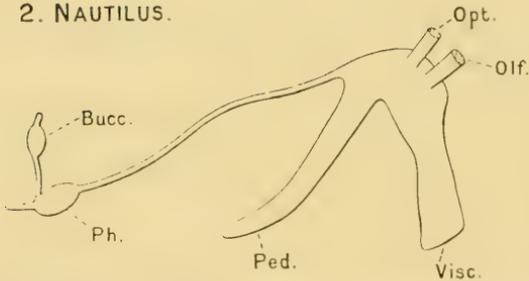
Certain characteristics which are primitive among the Mollusca are retained in the nervous system of the Chætognatha, but these co-exist with others which appear to be directly associated with the more active mode of life of the latter. In two features, namely, in the widely distributed nerve plexus which enwraps the body, and in the close adhesion of the central nervous system to the ectoderm, the Chætognatha are more primitive than the Mollusca; the ladder-like arrangement of the nerves that occurs in *Amphineura*, for instance, is not a Chætognath feature, although the nerves issuing from the ventral ganglion are

highly suggestive of it. The uniform sheathing of ganglionic cells, indicating lack of concentration, which gives peculiar interest to primitive nerve-cords, as in the Chitonidæ, is not to be found in Chætognatha, whose active movements demand a more perfect apparatus for their co-ordination, and in

I. CHÆTOGNATHA



2. NAUTILUS.



TEXT-FIG. 6.—Nervous systems. *Bucc.* Buccal ganglion; *Ph.* Pharyngeal ganglion; *Ped.* Pedal ganglion; *Visc.* Visceral or ventral ganglion of Chætognatha.

which a more centralised nervous system has accordingly been evolved.

The cerebral ganglion, consisting of a median group of nerve-cells and of two lateral ones joined by commissural fibres, gives off posteriorly a pair of rhinophoral and a pair of optic nerves to the olfactory organs and eyes respectively,

and the great cerebro-visceral connectives which lead back to the "ventral" or visceral ganglion. Lateral extensions of the cerebral ganglion give off two nerves, which are, at any rate, partly motor, to the cephalic or buccal muscles, and a pair of lateral nerves to the integument of the head.

To demonstrate the existence of ventral commissures below the œsophagus is not always an easy matter. Langerhans definitely affirmed the presence of a circumœsophageal ring completed by a subcutaneous commissural nerve just behind the mouth, a statement which Hertwig was not able to confirm. My own observations on Neapolitan material have led me to believe that there is a commissural plexus of ganglion cells beneath the skin, which, if more concentrated, might give rise to a commissural nerve strand such as Langerhans figures. Such a nerve-loop is to be compared with the stomatogastric loop of the Mollusca, the commissural nerves of which are often extremely attenuated, and, owing to their position on the buccal muscles, difficult to demonstrate; in *Nautilus*, for example, the completion of the buccal nerve-loop has only been recently proved by Graham Kerr. The co-existence of two pairs of ganglia, which may be termed buccal and pharyngeal in Chætognatha, in many Gastropoda (e. g. *Patella*), and in *Nautilus* is a significant feature.

The descriptions of Hertwig and of Gourret differ in regard to the mode of origin of certain nerves from the cerebral mass, but they may be reconciled if we suppose the cerebro-buccal connectives and motor nerves to the muscles of the mandibles, to run side by side for a short distance after leaving the cerebral ganglion in *Sagitta hexaptera* (Hertwig), but to be separate from the start in *Spadella marioni* (Gourret).

If it could be established that the buccal ganglia of the Chætognatha are derived from the stomodæal ectoderm the resemblance to the Mollusca would be complete.

Concerning the existence of the posterior or visceral loop there can be no doubt. The visceral ganglia appear early as

ectodermal thickenings of relatively enormous size. Though at first lateral in position, they soon become approximated, and give rise to the "ventral ganglion," which, however, never loses its primitive character of lying immediately beneath the ectoderm.

The pedal ganglia and their commissural loop do not exist in any conspicuous form, nor should we expect to find them in the absence of a well-developed and muscular foot. It might be possible to trace their homologues by making a more minute study of the innervation of the hood; but at present we merely suggest that cells corresponding to those of Molluscan pedal ganglia may be merged in the lateral expansions of the cerebral ganglion already mentioned. Von Jhering, on the other hand, believed that the "ventral ganglion" consisted of visceral and pedal ganglia united.

Pedal ganglia and nerves excepted, the *Chætognath* nervous system admits of a close comparison in point of detail with that of *Mollusca*, but the particular type to which it exhibits the closest affinity is that of *Nautilus*, as will be seen by reference to the diagrams (text-fig. 6).

A peculiar feature in *Nautilus* is the undivided nature of the cerebral ganglion, which is also found in *Chætognatha*, though unusual among *Mollusca*. The chief differences are the absence of the pedal loop, the more centralised arrangement of ganglionic cells on the visceral loop, the close adhesion of the nervous system to the ectoderm and the superficial nerve plexus.

SENSE ORGANS.

The tactile organs of *Chætognatha* consist of isolated neuro-epithelial cells terminating in tactile hairs, and of groups of tactile bristles which belong to a type of sense organ which is widely distributed among *Mollusca*.

The "olfactory" (?) ring of ciliated cells and the area within it (text-fig. 1, *c.*) is undoubtedly an organ of great importance.

It seems to be the homologue of the velum and the rhinophoral organs enclosed within it of the Mollusc, for the innervation from the cerebral ganglion by two nerves lying just within the optic nerves, is precisely similar. It is possible that the minute organs which Weiss has described in certain Oigopsid Cephalopoda are to be compared with them. And, in this connection, both the tentacles of *Spadella cephaloptera* and the olfactory spoon-shaped organs of *Chiroteuthis* should be made objects of further study.

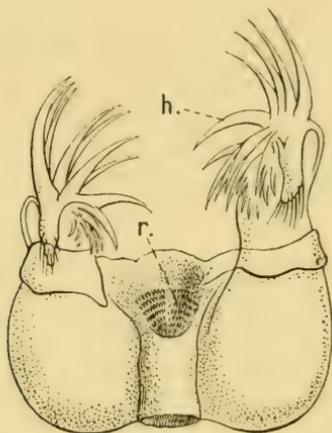
The eyes are undoubtedly of the Molluscan vesicular type. The retinal cells contain rods; the crystalline lens is secreted within an invagination of the ectoderm, which has become overgrown by a flattened epithelium. The fact that in the Molluscan eye the pigment forms a complete cup to the retina, but is mainly restricted to one side of the ocellus in *Sagitta*, is a minor point resulting from the peculiar method of the grouping of Chætognath eyes in threes.

As in the Amphineura, otocysts are unknown.

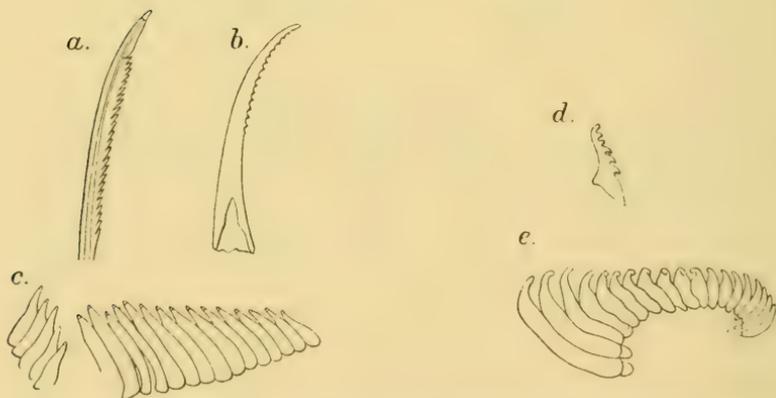
ALIMENTARY CANAL.

If our hypothesis of the origin of the Mollusca be correct, it follows that the straight and symmetrical alimentary canal of the Chætognatha is in a more primitive condition than that of any other Mollusc. Excepting for the absence of the radula, an organ better adapted to quiet browsing than to predatory Chætognath habits, the buccal armature is singularly like the armature which predatory Molluscs develop. The resemblance both in position and structure between the lateral groups of mandibles of a Gymnosomatous Pteropod, such as *Clio* (text-fig. 7, *h.*) and the "hooks" of *Sagitta*, is very close. No chitinous teeth are developed in the mid-ventral line, but the repeated series of spines in marginal and lateral groups above the entrance to the buccal cavity suggests a possibility of the successional development and replacement

of chitinous elements near the mouth. And if this were to happen in the mid-ventral line a radula would result. At



TEXT-FIG. 7.—Buccal armature of *Clio borealis*.
h. "Hooks." *r.* Radula.



TEXT-FIG. 8.—Buccal armature of Chaetognatha and Amphineura.
a. *Krohnia hamata* (after Fowler). *b.* *Sagitta serrato-dentata*.
c. *Sagitta bipunctata* (after Fowler). *d.* *Lepidomenia* (after Kowalewsky). *e.* *Proneomenia* (after Hubrecht).

the same time it must be remembered that the radula is absent in several Amphineura and in Lamellibranchia, in the

Leioglossal Cephalopoda, and that it even tends to disappear in certain groups of carnivorous Gastropoda.

A comparison between the forms of teeth characteristic of Chætognatha and of Amphineura is instructive (text-fig. 8).

Compare the saw-like hooks of *Krohnia hamata* and *S. serrato-dentata* (*a*, *b*) with a tooth of *Lepidomenia* (*d*), and the teeth of *S. bipunctata* (*c*) with those of *Proneomenia* (*e*).

No special salivary glands have been noticed, but the alimentary canal of *Sagitta cephaloptera* is furnished with two (liver?) diverticula (text-fig. 1, *A*, *h*).

GENITAL ORGANS.

The genital organs of the Chætognatha are situated in the hinder part of the visceral sac. The gonads are paired and hermaphrodite, but the male and female gonads develop in separate cavities, and pass to the exterior by separate paired ducts. The Mollusca too are typically hermaphrodite, and although there are some reasons for regarding the diœcious condition as primitive in the group, both ova and spermatozoa are derived from the epithelial lining of the same cavity; but in Mollusca belonging to very different groups there is a tendency for the male and female gonads to develop apart, either in separate male and female acini (*Pleurobranchidæ*, *Nudibranchs*, *Cardium oblongum*, etc.), or in separate regions of the same gland (*Cycladidæ*), or in different glands (*Anatinacea* and *Septibranchia*). The longitudinal partition between the paired gonadial cavities of the Chætognatha reminds us of the division between the paired gonads of the *Aplacophora*, and is typical, there is good reason to believe, of the primitive condition in Mollusca (text-fig. 10).

Thanks to the minute observations of Doncaster we are able to draw attention to an astonishing similarity between the behaviour of the gonads both of the Mollusca and of the Chætognatha, which we believe may throw a new light upon the morphology of the transverse septum of the latter group.

The following are the details of the process as they have been described among Mollusca :

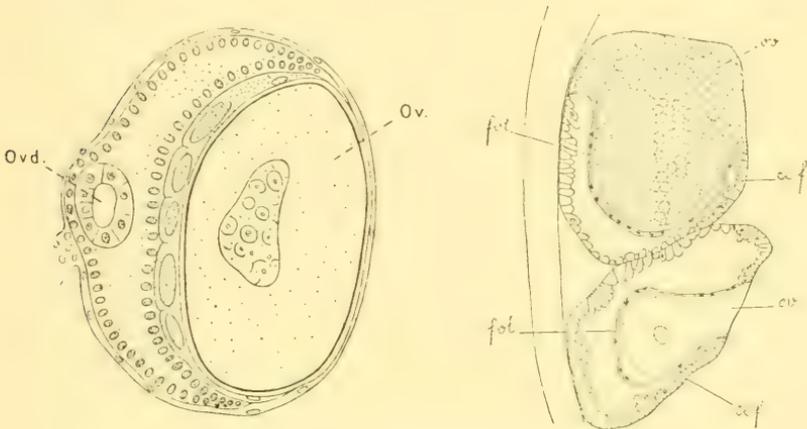
In Chiton, Haller observed the young ova to sink below the ovarial epithelium, and then growing larger bulge it out, so as to form a follicle. By a process of growth each egg-cell gets carried out into the perigonadial cœlom on the end of a stalk made by the follicular epithelium.

In the Cephalopoda the same process obtains. In Nautilus the result is exactly as in Chiton. In Octopus, Argonauta, and others a higher grade of complexity is reached by the formation of branches from the simple egg-stalks, resulting in the production of "egg-trees." In the Oigopsidæ the region that bears the egg-stalks projects right across the genital cavity as a "spindle-shaped body beset all over with stalked eggs."

In Sagitta the process is as follows : The genital cells in the embryo lie close to the longitudinal partition, bedded in mesoblast, one behind the other (Doncaster, figs. 14, 17). At a particular stage they become enclosed in a sort of cellular envelope of mesoderm, which does not yet form a definite epithelial sheath ; a condition to be compared with the stage in Chiton and other Molluscs before stalk-formation has commenced. Fig. 15 of Doncaster's paper shows some of these follicular nuclei closely adpressed to a genital cell. About the fourth day genital cells which have lain quiet for some time "move slowly across the cœlomic cavity until they reach the body-wall on each side." While traversing the cœlom the male and female cells on each side move together, and during their progress the transverse septum is formed between them by the cells, which we homologise with the follicular cells of Mollusca. The process of the formation of the transverse septum is therefore absolutely distinct and unlike the process of the growth of septa in Annelids, and although it is impossible to say whether the genital cells themselves or the follicle cells which lie about them are the veræ causæ of the movement, yet the fact of its occurrence in the two groups we are considering is significant.

The origin of the genital cells side by side from the walls of the same cavity, and the subsequent division of this cavity into anterior and posterior parts by a septum of quite peculiar growth, may be given as reasons against the view that the so-called trunk and tail cavities of Chætognatha are indications of metamerism.

Again, if the trunk and tail cavities were homomeric, we should expect to find their ducts also homomeric. This is certainly not the case, for the efferent genital ducts of the Chætognatha are essentially different both in structure and



TEXT-FIG. 9.—Ova and follicle cells.

A. Chætognath (after Hertwig). B. Lamellibranch (after Pelseneer).

in development. The sperm-ducts being ciliated, and the oviducts being devoid of ciliation, was clearly established by Hertwig in 1880, and should have made later writers hesitate before adopting a theory of metameric segmentation; but quite recently the embryological studies of Doncaster have indicated that the two ducts are derived from different germ-layers—the sperm duct from epiblast, and the oviduct from mesoblast.

We suggest that the oviduct of Chætognatha is merely a gonocœl, partly lined by the follicular epithelium covering the egg-cells, which has acquired an opening of its own to the exterior (text-fig. 9).

In the absence of complicated copulatory organs the Chætognatha resemble the Amphineura.

The spermatozoa are pin shaped, as in many Mollusca.

When we come to compare the arrangement of body cavities, genital organs, and their ducts in the Chætognatha with the various arrangements of these organs among the Mollusca, we find that the same structural plan is common to both groups, and that in the Chætognatha certain primitive features are retained (text-fig. 10).

The ancestor common to both Chætognatha and Mollusca had, we imagine, paired but unsegmented cœlomic spaces. Gonad mother-cells developed in each half, and were passed to the exterior through paired cœlomoducts, which also served for the discharge of renal products. This ancestor we believe to have been hermaphrodite (text-fig. 10, A).

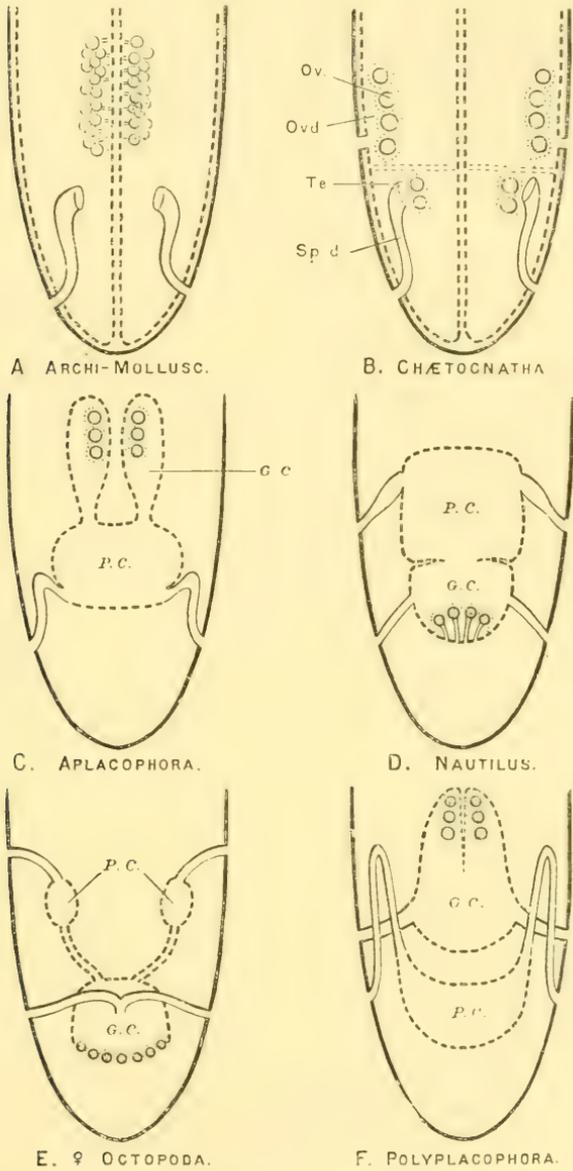
In the Aplacophoran *Proneomenia*, Chætognatha, and Cephalopoda certain of these primitive conditions persist, but not all in the same form.

In *Proneomenia* the cœlom has become restricted to a relatively small portion of the whole body. The anterior ends of the paired cœlomic spaces remain separate as gonocœls; the posterior portions have become confluent, forming the pericardium, which communicates with the exterior by right and left cœlomoducts, which serve the double purpose of genital and renal ducts (text-fig. 10, c).

In Cephalopoda the posterior portion of the cœlom is the gonocœl; it is undivided, and communicates with the exterior by a pair of gonad ducts (D). The anterior portion of the cœlom is paired in Octopoda, and forms the two pericardial spaces, each of which has its own duct to the exterior (E).

In *Chiton* the gonadial cœlom has become separated from the posterior pericardial cœlom, and each has its own pair of ducts to the exterior (F).

The universal occurrence of paired ducts in these primitive types is, we think, explained by the theory that in the common ancestor the cœlomic spaces were divided by a median longitudinal partition.



TEXT-FIG. 10.—Body cavities.
 The dotted line represents the cœlomic epithelium. *pc.* Pericardial cœlom; *gc.* Perigonadal cœlom.

In *Sagitta* this primitive independence of right and left cœlomic spaces is retained, but the male and female portions of the hermaphrodite gland have become separated by the transverse genital septum, and a second pair of gonad ducts has come into existence in consequence (text-fig. 10, B).

See Addendum, page 394.

DEVELOPMENT.

It now remains to be demonstrated that the developmental histories of the Chætognatha and Mollusca are not so diverse as has been usually supposed, for many close comparisons are possible between them.

In the larval stage the Chætognatha have no velum or other trace of external ciliation, and, in this respect, they resemble the Cephalopoda. The late appearance of cilia may be accounted for by the early development taking place inside an egg-shell, and by the larva, on hatching, being already so advanced as to have its adult swimming organs sufficiently developed. The typical Mollusc, on the other hand, hatches at an earlier stage, and goes through a period of swimming by cilia before settling down to the method of locomotion peculiar to its adult condition. So, although we have no evidence that the Chætognath ancestor was provided with any ciliary swimming organ, such a thing is very probable; and Grassi's suggestion that the ciliated olfactory ring is the survival of a larval organ (although he did not indicate the right one) may not be far from the truth.

We will now pass to the chief features of resemblance in the embryology. The invaginate gastrula is common to both. The mouth has been proved to develop by a stomodæal invagination at the pole opposite the blastopore both in those Gastropoda which have little food-yolk and in the Chætognath, and the same statement is true of the Scaphopoda and Lamellibranchia and of *Dondersia*, though not of *Chiton*.

In Mollusca the mesoblast cells usually arrange themselves in two lateral masses, in which paired cœlomic cavities subsequently appear as schizocœlic spaces. Erlanger was

certainly in error in attributing an enterocœlic origin to them in *Paludina vivipara*, and his statement has been refuted by Tönniges—and this is practically identical with what happens in *Sagitta*, for even here the lateral mesodermal pouches lose their enterocœlic lumen, according to Doncaster, and the permanent cœlomic spaces appear as splits at a later stage. The only important point of difference is that the paired lateral cœlomic spaces of *Sagitta* remain separate throughout life, the splanchnic mesoblast giving rise to the dorsal and ventral mesenteries, whereas in the Mollusca they generally become confluent, although in every case indications of their paired origin may be perceived in the fact that all organs derived from them are typically paired.

The four large cells which lie at the posterior end of mesoblastic pouches in a young *Sagitta* larva remind us of the first large mesoderm cells which are so conspicuous in the larvæ of *Chiton*, *Teredo*, and other Mollusca. Both ultimately give rise to the genital cells, but in the Chætognath these cells do not form anything but ova and certain ovarian structures (perhaps the oviduct). In the Mollusca the large cells give rise to other mesodermic structures as well.

The resemblances between Chætognath and Molluscan nervous systems have already been described; it remains but to observe that in their development they are absolutely identical. The dorsal and ventral ganglia appear as independent local thickenings of the ectoderm in which a considerable proliferation of nuclei takes place. A fact of importance is that the "ventral ganglion" of *Sagitta*, like the visceral ganglia of Mollusca, is paired in its origin, being derived from two cell-bands on the ventro-lateral regions of the body (Doncaster).

The buccal nervous system was said to be mesodermal by Hertwig, but this statement needs further confirmation. The development of the buccal ganglia from the stomodæal ectoderm has been demonstrated in *Paludina*, and a similar origin is possible in *Sagitta*.

The origin of the hood from two lateral ectodermal thickenings on either side of the mouth has already been described.

The fate of the mesoderm and of its paired cavities is of the highest importance. In *Sagitta*, after a transient stage, in which the body cavities are entirely obliterated, two bilaterally symmetrical pairs of cavities appear as schizocœls, the head cavities and the gonadial cavities respectively. The chief function of the mesoblast of the anterior or head pair of cavities is apparently to give rise to the musculature of the pharyngeal apparatus. The cavities themselves during the process of growth become extended into the base of the hood thus proving this organ not to be purely ectodermal in origin, a fact which is of considerable importance from the point of view of homology with the interbrachial membrane of the Cephalopoda.

The fate of the perigonadial cœlomic cavities is very similar in *Chaetognatha* and *Mollusca*, and, in our opinion, the *Chaetognath* type of cœlom, gonads and their ducts represents the ancestral Molluscan condition with greater exactitude than any scheme hitherto proposed.

In spite of the great variations in the arrangement of the Molluscan cœlom it is generally agreed that the most primitive condition is that in which the gonadial and reno-pericardial cavities are united as in the Cephalopoda and in the Aplacophoran *Amphineura*, as in a modified degree in the more archaic *Gastropoda* (*Trochus*) and *Lamellibranchia* (*Solenomya*), and as is indicated by the origin of the gonads from the wall of the pericardial space in *Paludina* and *Dreissensia*.

This view has been expressed by Dr. Pelseneer in a series of diagrams indicating the probable transformations of the genital duct (*Mollusca*, fig. 5 bis, p. 14), but in delineating the "ancestral hypothetical form" Dr. Pelseneer has not carried the history as far back as is now possible, for he has omitted to emphasise the real cause of the bilateral symmetry of the parts, namely, the primitive independence of right and left cœlomic cavities, necessitating the development of independent right and left ducts whether genital or renal.

When, as is typically the case in Mollusca, the right and left cœlomic cavities become confluent, one of the original pair of ducts may be dispensed with. Confirmatory evidence for the primitive separation of the cœlomic cavities into right and left among Mollusca is to be found in the paired structure of the gonads of Aplacophora and Lamellibranchia, in the paired origin of the pericardial cœlom in Paludina, and in the very widely-distributed, paired, renal cavities. In the Chætognatha the primitive division of cœlomic spaces into right and left halves is retained throughout life.

Thus far our comparison lies on certain ground. The next point to be considered is the significance of the division of the perigonadial cœlom by the transverse genital septum in the Chætognatha, and this is capable of more than one interpretation. If we regard this genital septum as a division between two homomeric segments we shall adopt the simplest explanation. The condition of transversely-divided cœlomic spaces in Chiton would be evolved from the Chætognath condition on the assumption that the posterior cavity has lost the power of developing gonads, and that its ducts have become purely renal in function.

The genital cœlom of Chiton would, on this theory, be the homologue of the anterior pair of body cavities of Sagitta.

Unfortunately the observed facts of development do not support the view of a metameric repetition of these cavities. The genital rudiments in Mollusca first appear as ridges on the pericardial walls, and therefore in a cavity which is not metamERICALLY repeated, and grave doubts have been recently thrown upon the view that the genital septum of Sagitta is an indication of metameric segmentation by the above-mentioned observations of Doncaster.

We are thus led to the conclusion that Sagitta and primitive Mollusca, like the Aplacophora, had but a single pair of gonadial-renal-cœlomic cavities, into which the hermaphrodite gonads were dehiscend and from which they passed to the exterior by a single pair of ducts, together with the nitrogenous waste. By the differentiation of the walls of this

cavity and its ducts into excretory and gonad-bearing portions we arrive at the condition typical of the higher types of Mollusca in which a second pair of ducts is developed in connection with the discharge of the genital products. Such is the condition in the Cephalopoda in which the gonadial pericardial cavity remains undivided, but in the Polyplacophora the gonadial cœlom has become completely separated from the renal cœlom. In the Chætognatha the gonadial cœlomic spaces have become separated for the purpose of dividing the male and female sexual cells (text-fig. 10).

Both Chætognatha and Mollusca are hermaphrodite. The parent germ-cells originate at the same time, and are indistinguishable. It might be suggested that the determining factors of sex only come into operation after the germ-cells have migrated into separate cavities in which different nutritive conditions prevail.

CONCLUSIONS.

The very numerous and close resemblances which exist between the Chætognatha and every class of Mollusca show that there is no single structure, or absence of structure, of importance in the Chætognath anatomy which is not capable of being developed somewhere within the limits of the Molluscan phylum, and that many apparently insignificant features have their exact counterparts therein. In short, granted similar conditions of life, a Chætognath type might be expected to arise from the Molluscan stock. On the other hand, the claims which have been put forward in favour of the alliance of the Chætognatha with the Annelida or Nematoda are not capable of being supported by so extensive or consistent an argument.

Can the Chætognatha be definitely associated with any one class of Mollusca, or are their relationships more general?

The following morphological characters are among those obviously of importance :

1. The original bilateral symmetry of the Mollusca is presented by the Chætognatha in its most perfect form, especially in respect of the body cavities.

2. The Chætognatha resemble many Mollusca of undoubtedly primitive type, in the absence of apparent segmentation.

3. The vermiform shape of the body, recalling that of the *Amphineura aplacophora*.

4. No extensive hæmocœl has been hitherto identified.

5. The alimentary canal is straight; the anus opens in front of part of the visceral sac.

6. There is no evidence of a radula, either in the Chætognatha or in their ancestors. The buccal armature is otherwise very like that of many Mollusca.

7. The nervous system is of the Molluscan type.

8. The "hood," the suggested homologue of the circumoral Cephalopod "foot."

9. The growth of the genital cells within a follicular epithelium and upon stalks. Hermaphroditism.

10. The two pairs of openings from the perigonadial cœlom to the exterior. These are believed to be the homologues of the two pairs of ducts leading from the pericardial-gonadial cœlom of the more primitive Mollusca.

11. The cephalic endo-skeleton and lateral fins.

12. The preoral ciliated ring; the suggested homologue of the velum.

The characters of the Chætognatha are, on the whole, just those of the more archaic types of Mollusca rather than of the Gastropoda or Lamellibranchia. Their affinity to the *Aplacophoran Amphineura* is indicated by the vermiform shape and bilateral symmetry of the body, by the straight alimentary canal, by many negative characters, such as absence of shell, foot, and radula. The *Amphineura* may have a more primitive nervous system, but in the Chætognatha the paired arrangement of body cavities is the more primitive. Molluscan nephridia and Chætognath sperm-ducts would appear to be homologous structures, but the genital

ducts of Chiton may only be the analogues of the oviducts of Sagitta.

The resemblance to certain Cephalopoda is also surprisingly close. The body-cavity of the Chætognatha is in the more primitive condition, in that it remains divided longitudinally throughout life, but owing to the fact that the Cephalopoda are diœcious, there is not the same need for a separation of the male and female gonads by a genital septum that there apparently is among Chætognatha. The nervous system of Nautilus and Chætognatha are both modifications of the same plan. Endocephalic skeletons, germ-cells mounted on stalks, and last, but not least, the bilateral "foot," hood-like and surrounding the mouth, are common to both Nautilus and Chætognatha.

Another very remarkable fact is that the peculiar characteristics of the Leioglossal Octopods—no radula, arms united by a membrane, fins developed on the sides of the body—are all characteristics of Sagitta.

Although a few naturalists have already suggested that the nervous system of the Mollusca and the Chætognatha may indicate relationship, no one has hitherto pointed out how perfect is the resemblance between the other organs of the two groups.

We have in the Chætognatha the key to many Molluscan mysteries, not the least important of which is the development of the Cephalopoda, which, owing to the presence of a large mass of inert food-yolk, has been profoundly modified. In fact, in the matter of developmental history, Sagitta would seem to bear the same relationship to the Cephalopoda that Amphioxus bears to the higher Vertebrata.

If our theory be correct the Chætognath type of development may be that of the Pre-Silurian ancestors of Nautilus, and probably of the earlier straight-bodied Cephalopoda, such as the Orthoceratidæ and the Bactritidæ.

A further inference of far-reaching importance is that the crawling Archimollusc of Lankester was not the common ancestor of the entire Molluscan phylum, but only of the

Polyplacophor-Gastropod-Lamellibranch section, and that the Cephalopoda have never had a creeping ancestor at all; and, in our opinion, the Creeping Archimollusc itself has been derived from a Swimming Archimollusc from which the Amphineura, Aplacophora, and Cephalopoda have been independently evolved, and of which the Chætognatha are the nearest living representatives.

Several groups of Mollusca descended from the creeping Archimollusc have again taken to a predatory life among the Necton and Plankton, and have apparently thrown back to the earlier swimming Archimolluscan ancestor in several respects. It is thus that characteristics common to the Chætognatha and the Pteropoda are to be explained. The hereditary taint of asymmetry, however, is never lost; even in *Phyllirhoe* the anus and the genital organs remain on one side of the body.

In the light of the undoubtedly primitive nature of the Chætognatha, the negative characteristics of the Aplacophora may well be reconsidered. Pelseneer and others consider that the Polyplacophora present the most archaic characters among the Amphineura, but until we have good evidence to the contrary there is at least equal justification for the view that certain structures in the Aplacophora are in an incipient rather than in a reduced condition. Foot, radula, and shell may never have attained to a higher grade of development among their ancestors than that which they have reached among the living Aplacophora.

The association of the Chætognatha with the Mollusca does not throw much light on the position of *Rhodope*. The absence of heart, shell, radula, and foot are not, in our opinion, insuperable obstacles to including this curious minute form in the Molluscan phylum, but the fact of the presence of flame cells opens a vista of new difficulties.

PHYLOGENY AND CLASSIFICATION.

By the inclusion of the Chætognatha within the Molluscan phylum no new suggestion is indicated as to the connection

of the phylum with any other division of the animal kingdom, but the classes within the phylum will require some re-adjustment.

Our phylogenetic speculations are influenced by the following facts :

A. Mollusca typically pass through a free-swimming, bilaterally, symmetrical, "veliger" stage.

B. In creeping and sessile forms the foot and shell reach their highest development.

C. In free-swimming marine forms the shell tends to atrophy, and the creeping foot tends to become either a swimming organ or to disappear.

It may therefore be fairly argued that if a race of Mollusca had always been free swimming, either foot or shell might be absent.

The Chætognatha may therefore be fairly regarded as the living adult representatives of the phyletic stage indicated by the veliger larva, and it is from such an ancestor that we conceive the creeping Polyplacophora, the worm-like Aplacophora, and the swimming Cephalopoda to have been independently derived.

A scheme of classification which would represent this view would be—

Phylum.—MOLLUSCA.

Grade A.—Nectomalacia or Mollusca natantia.

Mollusca in which the primitive free-swimming habit has been retained : "foot" circumoral, a propodium developed from paired lateral Anlage.

Class 1. Chætognatha.

Without shell.

„ 2. Cephalopoda.

With shell.

Grade B.—Herpetomalacia or Mollusca reptantia.

Mollusca in which a creeping habit has been developed : foot postoral, a metapodium, developed as an unpaired median structure.

- Class 3. Amphineura Aplacophora.
 Without shell.
 „ 4. Amphineura Polyplacophora.
 Shells octuple.
 „ 5. Lamellibranchia.
 Shell bivalve.
 „ 6. Gastropoda.
 Shell single.
 „ 7. Scaphopoda.
 Shell single, tubular.

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ADDENDUM TO PAGE 380.

Conant states that there is no longitudinal partition between the spermatic chambers in *Spadella schizoptera*, and that the right and left oviducts are in communication with one another by branches which meet and form a small blind tubule lying on the mid-ventral line beneath the intestine. This union is believed to be a means for helping a single copulation, affecting one side only, to fertilise the ova of both sides of the body. (MAY, 1907.)

The Structure, Development, and Bionomics of
the House-fly, *Musca domestica*, Linn.

Part I.—The Anatomy of the Fly.

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With Plates 22—26.

CONTENTS.

	PAGE
I. Introduction	395
II. Methods	399
III. External Structure.—1. Head	400
2. Thorax	406
3. Abdomen	414
IV. Internal Structure.—1. Muscular System	415
2. Nervous System	416
3. Alimentary System	420
4. Respiratory System	424
5. Vascular System and Body Cavity	429
6. Reproductive System	430
V. Internal Structure of Head	435
VI. Summary	439
VII. Literature	442

I. INTRODUCTION.

THIS paper is intended to be the first of a series of three dealing with the anatomy, development and bionomics of the House-fly, *Musca domestica*, L. The second part will

include an account of the anatomy of the larva, its development and the breeding habits of the fly; the series will be concluded with an account of the bionomics of the fly with special reference to its relations with man.

The term "House-fly" to the zoologist refers only to one insect—*Musca domestica* of Linnæus, but to the popular mind it includes insects, not different species only, but different families of Diptera. The Root Maggot fly (Pl. 22, fig. 2), *Anthomyia radicum*, L., sometimes occurs commonly in houses. *Homalomyia canicularis*, L. (fig. 3), often called the Small House-fly, is a very common inhabitant of houses. The latter species is smaller than *M. domestica*, and on this account they are frequently supposed to be young specimens of the latter species by persons who are ignorant of the fact that growth takes place during the larval stage and not after the exclusion of the imago. *Stomoxys calcitrans*, L. (fig. 4), is found in houses, especially in the autumn. It is frequently mistaken for *M. domestica*, and as it is one of the blood-sucking species (See Austen, 1906), the pernicious habit is attributed to the harmless *M. domestica* either on account of the supposed ill-nature of the latter or the influence of some change in the weather.¹

In addition to these, other species of flies occur in houses but these will be considered in a later part. Reference has been made here to the various species inhabiting houses to show that the term "House-fly" as ordinarily used is rather an inclusive one.

The House-fly has received some attention from naturalists in all ages. Reaumur (1738), De Geer (1752-78) and Bouche (1834) have all included a short account of this insect in their classical memoirs. They do not contribute much to our knowledge of the anatomy and development of the fly. The

¹ *Stomoxys calcitrans* can be readily distinguished from *M. domestica* by the awl-like proboscis which projects forwards from beneath the head. It has a more robust general appearance, a dark spotted abdomen, and its flight is more steady.

most complete of these early accounts is that of Keller (1790) which is illustrated by several striking plates. He gives an interesting account of the development and breeding habits, but in attempting to describe the anatomy he was not so successful as exemplified by his mistaking the brown testes for kidneys. In 1874 Packard wrote what is up to the present time the most complete account of the development of this species, and in 1880 Taschenburg, in his 'Praktische Insektenkunde' gave a good popular account of the insect. Howard has more recently (1898 and 1902) contributed to our knowledge of the developmental history.

No complete account of the anatomy of this insect has yet been published. A short popular account by Samuelson and Hicks (1860) though interesting is very superficial, and contains much that is inaccurate. Macloskie (1880) has published an account of the proboscis of *M. domestica*, and the foot has been made an object of study by several workers, chief of whom are Hepworth (1854), and Merlin (1895 and 1905), who correctly described the glandular hairs of the pulvilli. Wesche has recently (1906) described the genitalia of both sexes, but his description and figures are inaccurate. An interesting account of the copulation of the fly has been published by Belese (1902), in which he briefly describes the reproductive organs, his work will be referred to later. Lowne's monograph (1895) on the Blow-fly (*Calliphora erythrocephala*), which is an elaboration of his previous memoir (1870) is the only complete account which has been published on Muscid anatomy. The result of my study of the anatomy of *M. domestica*, which was begun in 1905, and is being continued in the Zoological Laboratories of the Manchester University, has been to make it apparent that much of Lowne's work needs confirmation.

Musca domestica was first described by Linnæus (1758), his description is as follows:—

"Antennis plumatis pilosa nigra, thorace lineis 5 obsolete abdomine nitidulo tessellato: minor. Habitat in Europæ

domibus, etiam Americæ. Larvæ in simo equinæ. Pupæ parallele cubantes.”

Later Fabricius described it more fully in his ‘Genera Insectorum.’ The House-fly, together with the Blowfly, and the blood-sucking flies *Stomoxys* and *Glossina* belongs to the family *Muscidæ*, which is characterised by having the terminal joint of the antenna—the arista always combed or plumed and by the absence of large bristles or macrochætæ on the abdomen. The *Muscidæ*, together with the *Anthomyidæ* and *Tachinidæ* constitute the group *Muscidæ calypteratæ* are characterised by the possession of squamæ, small lobes at the bases of the wings which cover the halteres. In the *acalyptrate* muscids the squamæ are absent or rudimentary. These two groups belong to the suborder *Cyclorhapha*, one of the two primary divisions of the *Diptera*. The *Cyclorhapha* have *coarctate* pupæ, the pupal case being formed by the hardening of the last larval skin, and the flies escaping through a circular orifice formed by the fly pushing off the end of the pupa by means of an inflated sac-like organ—the *ptilinium* which is afterwards withdrawn into the head, its presence being marked by a frontal crescentic opening the *lunule*. The other sub-order the *Orthorrhapha* have *obtectate* pupæ.

The most complete specific description of *Musca domestica* has been given by Schiner (1864), of which the following is a free translation :—

“Frons of male occupying a fourth part of the breadth of the head. Frontal stripe of female narrow in front, so broad behind that it entirely fills up the width of the frons. The dorsal region of the thorax dusty grey in colour with four equally broad longitudinal stripes. Scutellum grey, with black sides. The light regions of the abdomen yellowish, transparent, the darkest parts at least at the base of the ventral side yellow. The last segment and a dorsal line blackish brown. Seen from behind and against the light the whole abdomen shimmering yellow, and only on each side of the dorsal line on each segment a dull transverse band. The

lower part of the face silky yellow, shot with blackish brown. Median stripe velvety black. Antennæ brown. Palpi black. Legs blackish brown. Wings tinged with pale grey with yellowish base. The female has a broad velvety black, often reddishly shimmering frontal stripe, which is not broader at the anterior end than the bases of the antennæ, but becomes so very much broader above that the light dustiness of the sides is entirely obliterated. The abdomen gradually becoming darker. The shimmering areas on the separate segments generally brownish. All the other parts are the same as in the male."

The mature insects measure from 6-7 mm. in length and 13-15 mm. across the wings. Flies which have been starved during the larval stage or subjected to adverse conditions are generally smaller in size.

II. METHODS.

All the details of the anatomy which are about to be described have been studied by means of dissections. The dissections were made on both fresh and preserved material under a Ziess' binocular dissecting microscope with magnifications varying from 25-65 diameters. Serial sections have been made to confirm the dissections and to study the histological details.

Perfect series of sections of the whole fly were hard to obtain on account of the somewhat brittle nature of the internal chitinous structures. These internal chitinous skeletal elements caused the greatest trouble as they were apt to damage the internal anatomy. Celloidin sections were not a great improvement on those cut in paraffin. The best results were obtained by fixing the flies from 12-24 hours in Henning's solution, which is—Nitric acid 16 parts, chromic acid (.5 per cent.) 16 parts, corrosive sublimate saturated in 60 per cent. alcohol 24 parts, picric acid saturated in water 12 parts, and absolute alcohol 42 parts, washing out with iodine alcohol. This not only fixes, but to a certain extent, though not completely, softens the chitin. They should not

be imbedded too long or the chitin becomes brittle again. Serial sections made of recently emerged imagines before the chitin has hardened give good results. Other fixing agents used were Perenyi, Rabl's Chromoformic, Picro-formal (Boum's solution), Glacial acetic acid, and absolute alcohol. Of the various stains which were used the most satisfactory were Heidenhain's Iron-hæmatoxylin, Brazilin,¹ and Delafield's Hæmatoxylin. With the last stain perfect results were obtained by overstaining and differentiating with acid-alcohol.

The structure of the thoracic ganglion was studied by means of reconstructions. The method employed was as follows:—The sections were drawn by means of the camera lucida on Bristol board of a thickness proportional to the magnification. They were afterwards cut out and seccotined together. The resulting model was trimmed and soaked in melted paraffin, taken out and dipped several times till a thin coating of paraffin covered the model. This was then trimmed down to the original size, all the interstices having been filled by the paraffin. After a coating of graphite it was electrotyped with copper. In this way a permanent model was obtained.

III. EXTERNAL STRUCTURE.

1. The Head Capsule.

The head capsule of *M. domestica* presents great modifications when compared with the typical insect head. Considerable difficulty is experienced in explaining its structure in the morphological terms employed in the simpler orders of insects. Lowne did not lessen the difficulty in describing the head of the blowfly by the invention of new terms of little morphological value. The head of the fly is strongly convex in front (Pl. 23, fig. 1), the posterior surface being almost flat and slightly conical. For the sake of clearness the

¹ See Hickson, S. J., "Staining with Brazilin," 'Quart. Journ. Micr. Sci.,' vol. 44, pp. 469—471, 1901.

composition of the head capsule will be described from behind forwards. The occipital foramen occupies a median slightly ventral position on the posterior surface. It is surrounded by the occipital ring, the inner margin of which projects into the cavity of the head. From the sides of the inner margin of the occipital ring two short chitinous bars bend inwards and approach each other internally, forming a support—the jugum for the tentorial membrane. On each side of the occipital ring below the jugum a small cavity occurs into which a corresponding process from the prothorax fits, forming a support for the head.

The occipital ring is surrounded by the four plates, which make up the sides and back of the head capsule. On the ventral side, between the occipital ring and the aperture from which the proboscis depends, a median basal plate, the gulo-mental plate, represents the fused gula and basal portions of the greatly modified second maxillæ. The occipital segment is bounded laterally by the genæ (Lowne's paracephala) and dorsally by the epicranium. These parts have been divided by systematists into so many regions that a somewhat detailed description will be necessary to make their boundaries clear.

The genæ bear the large compound eyes which occupy almost the whole of the antero-lateral regions of the head. On the posterior flattened surface of the head the genæ are flat, and extend from the gulo-mental plate to the epicranial plate, the sutures of the latter being vertical. On the dorsal side each sends a narrow strip between the inner margin of the eye and the epicranium; this strip surrounds the eye and meets the ventral portion of the gena; it is of a silver to golden metallic lustre. On the ventral side below the eye each gena bounds the proboscis aperture laterally; a number of stout bristles arise from this margin and also from its antero-lateral region, which is often spoken of as the "jowl." In the anterior region, where the genæ are in contact with the clypeus, there are two prominent ridges bearing strong setæ; these are usually known as the "facialia."

The epicranium (epicephalon of Lowne) on the posterior surface of the head is flat. On the anterior surface it is convex, and divided into a number of regions. On the top of the head between the eyes it is called the vertex. This contains the three ocelli situated on a slightly raised ocellar triangle, which is surrounded by a second triangle, the vertical triangle. The median region in front of and below the vertex is the frons. In the middle of this there is a black frontal stripe. In the male the eyes are only narrowly separated by the frontal stripe. In the female the frontal stripe widens out on the vertex. This character provides a ready means of distinguishing the male from the female, as the result of it is that in the male the eyes are close together on the dorsal side being separated by about one fifth of the width of the head, whereas in the female the space between the eyes is about one third the width of the head. The edges of the genæ bordering on the frons bear each a row of stout setæ—the fronto-orbital bristles. The antennæ arise from the lower edge of the frons. Each antennæ consists of three joints and the arista. The two proximal joints are short and compose the "scape." The third joint, the flagellum, is longer, somewhat cylindrically prismatic in shape, and hangs vertically in front of the clypeus. It is covered with sensory setæ, and contains two pits of sensory function (olfactory, I believe). From the upper side the plumose arista arises. This probably represents the terminal three joints of the antenna. The lower edge of the frons represents the anterior margin of the epicranium.

The rest of the facial region is composed of the clypeus or, as it is usually called, the face—a convenient term, but one which hides its true morphology. The face is depressed, and is covered by the flagellæ of the antennæ. Between the upper and lateral edges of the face and the lower edge of the epicranium a crescentic opening, the lunule, marks the invagination of the ptilinum. The epistomium is a narrow strip below the face bounding the anterior edge of the proboscis aperture.

The Skeleton of the Proboscis.—The proboscis of *M. domestica* is very similar to that of the blowfly, which has been described by Kraepelin (1880) and Lowne (1895), though the results of these authors differ in many details. My study of *M. domestica* confirms Kraepelin's results, and as Lowne's is the only complete account of the muscid head a full description of its internal and external anatomy will be given in this paper.

Lowne regards the greater part of the proboscis as being developed from the first maxillæ and not from the labium or fused second maxillæ, which is the usually accepted view and one which I support on morphological grounds. On account of his exceptional conclusion he refuted the commonly accepted terms for the various parts and invented new ones. It will be necessary for the sake of descriptive clearness to refrain from constant reference to these or any discussion as to their value.

The proboscis consists of two parts, a proximal membranous conical portion—the rostrum and a distal half the proboscis proper which bears the oral lobes. The term haustellum is also used for this distal half (minus the oral lobes), and as a name it is probably more convenient, as the term proboscis is used for the whole structure—rostrum, haustellum, and oral lobes.

The rostrum (fig. 13, *Ros.*) is attached to the edges of the proboscis aperture, that is to the epistomium, genæ, and the gulo-mental plate. It has the shape of a truncated cone, and bears on the anterior side a pair of palps, which bear sensory setæ of two sizes.

The haustellum (fig. 13, *H.*), or proboscis proper, is attached to the distal end of the rostrum. The posterior side is formed by a convex, somewhat heart-shaped sclerite—the theca (figs. 1 and 3, *th.*) which probably represents a portion of the labium. The lower angle of the theca is incised by a semicircular sinus. By means of this the theca rests on a triradiate chitinous sclerite—the furca, which consists of a median, slightly convex rod (fig. 1, *f.*), from the

anterior end of which two arms diverge and form the chief skeletal structures of the oral lobes. The lower end of the theca rides on this structure, the bottom of the sinus resting on the median rod, and the two-pointed lateral terminations of the theca rest on the arms. In this manner these processes, in a state of repose, keep the arms of the furca closely approximated. The result of this will be seen later in studying the musculature of the proboscis.

The sides of the haustellum are membranous. On its anterior face, in a groove formed by the overlapping membranous sides, lie the labrum-epipharynx and labium-hypopharynx. The labrum-epipharynx (figs. 1 and 3, *lep.*) is attached at its proximal end to the membranous rostrum, but is incapable of a labral-like movement on account of its close connection with the labium-hypopharynx. Two slightly-curved, hammer-shaped apodemes (fig. 1, *ap.*) are attached to the proximal end of the labium-epipharynx. They assist in folding the proboscis during retraction, as will be shown later. The labium-epipharynx is shaped like a blunt arrow-head; the external surface is somewhat flattened. It is composed of two pairs of sclerites, an outer pair enclosing an inner pair, which form the pharyngeal channel. The edges of the inner tube are connected by a groove with the hypopharyngeal portion of the labium-hypopharynx, as shown in fig. 3. The labium-hypopharynx (fig. 3) represents the fusion of the hypopharynx with the greatly modified and fused second maxillæ or labium. It consists of a sclerite, curved in section, having the chitinous hypopharyngeal tube (fig. 3, *hp.*) fused to it along the upper half of its length. The edges of the hypopharyngeal tube engage with those of the inner pair of sclerites of the labium-epipharynx, as mentioned before. Distally the hypopharyngeal tube becomes free from the labium, as shown in fig. 3, and ends in a point where the lingual salivary duct opens.

Down each side of the labium-hypopharyngeal sclerite a rod-like thickening runs. Distally these thickened margins (paraphyses of Lowne) articulate with the discal sclerites.

The discal sclerites (fig. 1, *ds.*) are united at the posterior end to form, when the oral lobes are expanded, a U-shaped structure, with the limbs constricted in the middle where the ends of the thickened margins of the labium-hypopharynx articulate. They are sunk in deeply between the two oral lobes at the base of the oral pit with the free ends of the U anterior, these being spatulate and curved anteriorly.

The two oral lobes are normally connected by a bead and groove attachment along their anterior edges, but under pressure the connection is severed, and the oral disc presents a heart-shaped instead of the normal oval appearance. The oral lobes are covered on their upper aboral surfaces by sensory setæ, the large marginal setæ being different in structure from the rest. On the lower or oral surface a large number of channels, the pseudotracheæ (fig. 1, *ps.*) run from the internal margins of the oral lobes to the external borders. The channels of the pseudotracheæ are kept open when the lobes are extended by means of small incomplete chitinous rings, which give the channels a tracheal appearance, hence their name. Each of these incomplete rings has one end bifid, and as the bifid ends alternate the opening into the channel has a zigzag appearance. The number of pseudotracheæ on each lobe is generally thirty-six, and they are grouped in three sets. The anterior set of twelve all run into a single large pseudotracheal channel running along the anterior inner margin of the lobe, and a posterior set of twenty-one all run into a channel running along the posterior inner margin; between these two sets three pseudotracheæ run direct into the oral aperture. The oral aperture lies at the base of the small oral pit, which is a space kept open between the oral lobes by means of the discal sclerites. The pseudotracheæ do not extend as far as the discal sclerites, but on entering the oral pit the rings cease and the sides of the channels are covered by overlapping teeth, which extend back to the discal sclerites. Between the pseudotracheæ the membranous surface of each oral lobe is thrown into two longitudinal sinuous ridges, and projecting up from the

bottom of the furrows are several papillæ, generally four or five to each interpseudotracheal area, of a gustatory nature—the gustatory papillæ (figs. 1 and 18, *gp.*).

The Fulcrum.—This chitinous portion of the pharynx (fig. 1, *F.*) lies on the lower part of the head and in the rostrum. Kraepelin describes it as being shaped like a Spanish stirrup iron. Its structure will be best understood by referring to the figures. It consists of an outer portion, which is U-shaped in section; the basal portion, which is posterior and forms the floor of the pharynx (which Lowne, unfortunately, terms the hypopharynx) is vertical when the proboscis is extended. This basal portion is evenly rounded at both ends, and at the sides of the upper end there is a pair of processes—the posterior cornua (fig. 1, *pc.*) which serve for the attachment of muscles. The sides of the fulcrum are somewhat triangular in shape; their upper anterior portions are produced to form the anterior cornua (*a.c.*); here the sides bend inwards at right angles, and meet below the epistomium, upon which the fulcrum is hinged. The fulcrum is therefore quadrilateral in section at the upper proximal end, and trilateral at the lower distal end. The basal portion (fig. 2, *b.p.*) forms the floor of the pharynx; the roof of the pharynx is formed by another chitinous piece (*r.p.*) with a median thickened raphe. This roof lies parallel with the basal piece, and is fused with the sides of the fulcrum. On the membranous wall of the pharynx, between the labium-hypopharynx and the fulcrum, a small chitinous sclerite (fig. 1, *k.*) is developed, which Lowne terms the hyoid sclerite. It is U-shaped in section, and serves to keep the lumen of the pharynx in this region distended.

2. The Thorax.

As in all Diptera the possession of a single pair of wings has resulted in the great development of the mesothorax at the expense of the other thoracic segments, consequently the thorax is chiefly made up of the sclerites composing the

mesothorax. The prothorax and metathorax compose very small portions on the anterior and posterior faces respectively. Seen from above the thorax is oviform with the blunt end anterior and slightly flattened. Three transverse sutures on the dorsal side mark the limits of the prescutum, scutum, and scutellum of the mesothoracic segment; the mesothoracic scutellum forms the pointed posterior end, and slightly overhangs the anterior end of the abdomen.

The Prothorax.—The prothoracic segment has been reduced to such an extent that it is hopeless to attempt to homologise all the separate sclerites with those of a typical thoracic segment. To obtain a complete view of the prothorax it is necessary to examine it from the anterior end after the removal of the head. The following sclerites can then be recognised. The prosternum is a median ventral plate, quadrilateral in shape having the anterior end rounded and broader than the posterior end. It does not occupy the whole of the prosternal area, but is bounded by the prosternal membrane. Internally a ridge runs to the posterior end of the prosternum and bifurcates, each ridge running to the posterior corners, to which two strong processes (the hypotremata of Lowne) are attached. In front of the prosternum there is a small saddle-shaped sclerite which, on account of its position, may be called the interclavicle (the sella of Lowne). Two lobes at its anterior end are covered with small processes, probably sensory in function. A pair of small sclerites is situated in front of these lobes; these sclerites with the interclavicle no doubt belong to the prosternum. The interclavicle is ventral to the cephalothoracic foramen. The jugulares (3me jugulaires of Kunckel d'Herculais) are two prominent pocket-shaped sclerites lying one on each side of the cephalothoracic foramen, and having their convex faces external. Lying immediately below each of the jugulares is a small rod-like sclerite—the clavicle. The dorsal region of the prothorax the pronotum (fig. 6 *pr.n.*) is formed by two sclerites united in the median line, their dorsal sides being curved. From the ventral side of the pronotum a pair of

chitinous apodemes project into the thoracic cavity. The lateral regions of the pronotum are in contact with the humeri and the prothoracic episterna. The humeri (*hu.*) are a pair of strongly convex sclerites situated in the antero-lateral region of the thorax. They are bounded above by the prescutum of the mesothorax, internally and below by the episterna of the prothorax, and externally by the lateral plate of the mesosternum and the anterior thoracic spiracle. Its inner concave surface serves for the attachment of the muscle of the prothoracic coxa. The episterna (*eps.*) (epitrochlear sclerites of Lowne) are comparatively large sclerites forming the lateral regions of the prothorax. They overhang the attachments of the prothoracic limbs. The internal skeleton of the prothorax consists of the two stout hollow apodemes—the hypotremata mentioned previously. They arise from the postero-lateral edges of the prosternum, and run obliquely across the ventral edge of the anterior thoracic spiracle where the hypotreme divides, the posterior branch runs up the posterior margin of the spiracle, between the lateral plate of the mesosternum and the peritreme (the chitinous ring surrounding the spiracle), the anterior branch fuses with the prothoracic episternum.

The Mesothorax.—The notum of the mesothorax occupies the whole of the dorsal side of the thorax. It is composed of the four sclerites to which Audouin (1824) gave the name of prescutum, scutum, scutellum, and postscutellum. The prescutum (*prs.*) forms the anterior part of the dorsal region of the thorax. Its anterior portion bends down almost vertically to unite with the pronotum. The anterior edge of the prescutum is inflected after the pronotal suture, and is produced in the median line into a small bifurcating process. The prescutum is bounded laterally by the humerus and a membranous strip—the dorso-pleural membrane. The scutum (*se.*) is the largest of the mesonotal plates. It occupies the whole of the median dorsal region of the thorax. Anteriorly it is bounded by the prescutum, laterally by the alar membrane and the lateral plate of the postscutellum, and posteriorly by

the scutellum. From the lateral region of the scutum a process projects forwards and downwards, and articulates with the posterior portion of the wing-base (the metapterygium). The scutellum (*sc.tl.*) is a triangular pocket-shaped sclerite which overhangs the postscutellum and the base of the abdomen. The posterior surface of the thorax is chiefly composed of the large postscutellum. This is made up of three pieces, a median escutcheon-shaped plate (*mpsc.*) strongly convex to the exterior, and two convex lateral plates (*lp.sc.*). The lateral plates are bounded below by the metasternum and spiracles, and anteriorly by the pleural region of the mesothorax.

The mesosternum is a sclerite of considerable size and forms the keel of the thorax. It consists of a median ventral portion (*ms.*) which is produced laterally to form two large lateral plates (*lp.*). The median portion is bounded in front by the prosternum and the foramina of the anterior coxæ, and behind by the median coxal foramina. A short distance behind the anterior end a depression in the mid-ventral line extending to the posterior edge indicates a median inflection forming the entothorax. The lateral regions of the posterior margins of the mesosternum are inflected on each side to form the entopleura. The lateral plates of the mesosternum form the whole of the anterior portion of the pleural region; each is bounded in front by the humerus, spiracle, and prothoracic episternum, and above by the dorso-pleural membrane, and behind by the mesopleural membrane. The ventral side of the lateral plate is continuous in front with the median plate of the mesosternum, and behind is united by means of a suture. The remaining portion of the mesopleural region is made up of the episternum, epimeron, and two small sclerites connected with the wing-base—the parapteron and costa. The episternum (*eps.*) is situated behind the mesopleural membrane and below the alar membrane, below and behind it is bounded by the epimeron. Its surface is marked by two convexities, the ampullæ, the upper of the two corresponding to Lowne's great ampulla of the blowfly. The dorsal side of

the episternum is intimately connected with the sclerites¹ of the anterior portion of the wing-base.

The epimeron (*ep.*) is a triangular sclerite, and is bounded below by the mesosternum and metasternum, behind by the lateral plate of the postscutellum, and above by the episternum and alar membrane. The parapteron (*pt.*) is a sclerite situated at the top of the mesopleural membrane. The greater portion of it is internal, only a small triangular portion can be seen externally. Internally this is continued as a cruriform sclerite to which are attached important muscles controlling the wings. The costa (*ca.*) is a small sclerite situated on the dorsal margin of the epimeron. The internal skeleton of the mesothorax consists of the entothorax, entopleura, mesophragma, and the inflected edges of the episterna and epimera. The entothorax is composed of a median vertical plate subtriangular in shape, on the top of which a median plate produced laterally into wing-like processes rests. On this structure the thoracic nerve-centre lies. The entopleura and the inflected edges of the episterna and epimera all serve for the attachment of wing muscles. The mesophragma (*mph.*) is a convex sclerite fused with the lower edge of the postscutellum. Its posterior edge is incised in the middle and forms the dorsal arch of the thoraco-abdominal foramen.

The Metathorax.—The largest sclerite of the greatly reduced metathorax is the metasternum (*mts.*). It is a wing-shaped sclerite with the narrow transverse portion situated between the coxal foramina of the median and posterior pairs of legs; the expanded lateral portions form the wall of the thorax above the insertions of these legs. The edges of the narrow transverse strip are inflected, and unite the lateral portions of the metasternum. A trough-shaped longitudinal fold—the metafurca rests on the narrow transverse portion of

¹ In this account the individual sclerites which compose the wing base will not be described. Lowne has described them at great length for the blowfly, and although the wing-base sclerites of *M. domestica* differ slightly in shape from those of *Calliphora*, Lowne's description of their relations holds good for the former insect.

the metasternum. The posterior end of the metafurca bends downwards and articulates with the posterior coxæ on each side. The metafurca serves for the attachment of the thoraco-abdominal muscles. The pleural region of the metathorax is a narrow triangular space situated behind the lateral portion of the metasternum and the posterior coxæ. It is composed of a narrow triangular episternum and epimeron. The former (*eps.*"') is bounded in front by the metasternum, the posterior thoracic spiracle and the base of the haltere, below by the posterior coxal foramen, and behind by the epimeron. The epimeron (*ep.*"') is also bounded below by the coxal foramen and behind by the narrow dorsal arch of the metathorax and the first abdominal segment, its apex comes in contact with the base of the haltere. The dorsal region of the metathorax has practically disappeared, all that can be recognised as metanotum is a narrow chitinous strip (*mn.*) on each side between the apex of the metapleural area and the dorsal edge of the first abdominal area.

Wings.—The wings are situated at the sides of the scutum on the alar membrane, to which are attached the sclerites of the wing base. They are covered with very fine hairs.

In describing the neuration of the wings the nomenclature proposed by Comstock and Needham (1898) for the wings of the whole group of insects will be employed.

The nervures of the wing are ocreaceous. The anterior edge of the wing (fig. 16) is formed by a stout nervure, the costa (C_1), which is very setose. The second longitudinal nervure, the subcostal (Sc_1), joins the costal about half way along its length. A small transverse nervure, the humeral (*h.*), divides the costal cell into costal (*C.*) and first costal ($1C.$) cells. The next main nervure—the radial—divides into a number of branches (in the typical insect five); some of these have coalesced in the fly. A nervure joining the costal just past the middle is the first radial (R_1) cutting off the subcostal cell. The next nervure, which joins the costal on the apical curve, represents the fused second and third radial nervures

(*R.* 2 + 3). This cuts off the first radial cell (1 *R.*). The last nervure, which joins the costal almost at the apex of the wing, represents the fused fourth and fifth radial nervures (*R.* 4 + 5), and so cuts off the third radial cell (3 *R.*). The fourth main longitudinal nervure is the median, which, in the typical insect, divides into three, but in the fly the nervures have undergone coalescence, as will be shown. The first and second median nervures have coalesced (*M.* 1 + 2), and do not run direct to the margin of the wing, but bend forwards and almost meet *R.* 4 + 5 on the costa. About half way across the wing a transverse nervure, the radio-medial (*rm.*) unites *R.* 4 + 5 and *M.* 1 + 2, and cuts off the fifth radial cell (5 *R.*) from the radial (*R.*). The next longitudinal nervure represents the coalesced third medial and cubital nervures (*M.* 3 + *Cu.* 1). It runs to the posterior margin of the wing about half way along the length of the latter. The nervures *M.* 1 + 2 and *M.* 3 + *Cu.* 1 are united by two transverse nervures. The proximal nervure—the medio-cubital (*m.cu.*) cuts off the small triangular medial cell (*M.*); the distal transverse nervure (*m.*) cuts off the first second medial cell (2 *M.*¹) from the second second medial cell (2 *M.*²). The last longitudinal nervure—the anal (*A*₁)—is undivided, and does not reach the margin of the wing, thus incompletely separating the first cubital (1 *Cu.*) and anal (*A.*) cells. A small transverse nervure, the cubito-anal (*cu.a.*), slightly more proximal than the medio-cubital, cuts off the small triangular cubital cell (*Cu.*) from the first cubital cell (1 *Cu.*). Running parallel with, and posterior to, the anal longitudinal nervure, there is apparently another nervure. This, however, is not a true nervure, but is merely a chitinised furrow giving additional strength to the posterior angle of the wing. The posterior edge of the base of the wing is divided into a number of lobes. These are the anal lobe, and, as Sharp (1895) proposed, the alula, antisquama, and squama. The squama is thicker than the rest, and is attached posteriorly to the wing root between the mesoscutum and the lateral plates of the

postscutellum. It covers the haltere, as in all "calyptrate" Muscidae.¹

The Halteres.—The halteres or balances (fig. 6, *hal.*) are generally considered to represent the rudimentary metathoracic wings. They are covered by the squamæ, and are situated on the sides of the thorax above the posterior thoracic spiracles. Each consists of a conical base on which are a number of chordonotal sense-organs, and on this base is mounted a slender rod, at the end of which a small spherical knob is attached. The wall of the distal half of this sphere is thinner than the proximal half, and in preserved specimens is generally indented. Experiments show that the

¹ The nomenclature of Comstock and Needham has not yet been adopted by dipterologists in general; but, on account of its great morphological value, it will no doubt in course of time replace the present confused system. It may therefore be useful if the nomenclature employed in the foregoing description be compared with those most usually employed.

Longitudinal nervures.—*C*₁. Costal. *Sc*₁. Mediastinal, auxiliary. *R*₁. Subcostal, 1st longitudinal. *R*. 2 + 3. Radial, 2nd longitudinal. *R*. 4 + 5. Cubital, 3rd longitudinal; ulnar (Lowne). *M*. 1 + 2. Median, 4th longitudinal; discal (Verrall). *M*. 3 + *Cu*₁. Submedian, 5th longitudinal; postical (Verrall). *A*₁. Anal, 6th longitudinal. Pseudonervure, axillary, 7th longitudinal.

Transverse nervures.—*h*. Humeral, 1st transverse; basal cross vein (Verrall). *rm*. Discal, 2nd transverse; middle cross vein (Verrall); medial transverse; anterior transverse (Austen). *m-cu*. Anterior basal transverse (Austen); lower cross vein (Verrall); postical transverse (Lowne). *m*. Posterior transverse (Austen); postical cross vein (Verrall); discal transverse (Lowne). *cu-a*. Posterior basal transverse (Austen); anal cross vein (Verrall); anal transverse (Lowne).

Cells.—*C*. Costal. 1 *C*. Second costal. *Sc*. Third costal (Lowne correctly calls this "sub-costal"). 1 *R*. Marginal. 3 *R*. Sub-marginal; cubital (Lowne). 5 *R*. First posterior cell (Austen); sub-apical (Lowne and Verrall). 2 *M*². Second posterior cell (Austen); apical. 1 *Cu*. Third posterior cell (Austen and Verrall); patagial (Lowne). 2 *M*¹. Discal (this term is used also in Lepidoptera, Trichoptera, and Psocoptera, and in each family refers to a different cell!). *R*. Anterior basal cell (Austen); upper or 1st basal or radial (Verrall); prepatagial (Lowne). *M*. Posterior basal cell (Austen); middle or 2nd basal or radical (Verrall); anterior basal (Lowne). *Cu*. Anal cell (Austen); lower or 3rd basal or radical (Verrall); posterior basal (Lowne).

halteres are organs of a static function. They are not balancing organs in the sense that they are equivalent to the balancing pole of a rope-walker. They also have probably an auditory function. They are innervated by the largest pair of nerves in the thorax.

The Legs.—The three pairs of legs are composed of the typical number of segments. Each consists of coxa, trochanter, femur, tibia, and tarsus. The coxæ are the only segments which show any considerable difference in the three pairs of legs. The anterior coxæ are comparatively large and boat shaped, the intermediate coxæ are smaller and their separate sclerites more marked; the coxal plates of the intermediate coxæ are shown in fig. 6 (*cp.*). The coxal joints of the posterior pair of legs are almost similar to those of the intermediate pair. The anterior femora are shorter and stouter in the middle than those of the intermediate posterior pairs of legs. The anterior tibiæ are also shorter than those of the succeeding legs. The anterior tibiæ are covered on their inner sides with closely-set, orange-coloured setæ which serve as a comb by means of which the fly removes particles of dirt adhering to the setæ which clothe its body; the first tarsal joints of the posterior legs are also similarly provided. The tarsi consist of five joints, the terminal joints bearing the "feet." These organs about which so much has been written consist of a pair of curved lateral claws or "ungues" which subtend a pair of membranous pyriform pads—the pulvilli. The pulvilli are covered on their ventral sides with innumerable, closely-set, secreting hairs by means of which the fly is able to walk in any position on highly polished surfaces. A small sclerite lies between the bases of the pulvilli. The tarsal joints and the other segments of the legs are covered with a large number of setæ.

3. The Abdomen.

The abdomen is oviform with the broad end basal. The total number of segments which compose the abdomen is eight in the male and nine in the female. The visible portion con-

sists of apparently four segments in the male and female, in reality there are five as the first segment has become very much reduced, and has fused with the second abdominal segment forming the anterior face of the base of the abdomen (see fig. 8). The segments succeeding the fifth are greatly reduced in the male, and in the female they form the tubular ovipositor which, in repose, is telescoped within the abdomen. The second, third, fourth, and fifth abdominal segments are well developed, and consist of a large tergal plate, which extends laterally to the ventral side. The sternal plates are much reduced, and form a series of narrow plates lying on the ventral membrane along the mid-ventral line. The spiracles are situated on the lateral margins of the tergal plates. The sclerites of the abdomen which are exposed are strongly setose, especially the fourth and fifth dorsal plates, but they do not bear macrochæbæ.

IV. INTERNAL STRUCTURE.

1. The Muscular System.

The muscular system of the fly is similar to that of *Volucella*, described by Kunckel d'Herculeis (1881), and of the Blow-fly, described by Lowne and Hammond, and consequently they will be but briefly described. The muscles may be divided into the following groups: 1. Cephalic, 2. Thoracic, 3. Segmental, 4. Those controlling the thoracic appendages, and 5. Special muscles.

1. The cephalic muscles will be considered in the detailed description of the head.

2. The thoracic muscles are enormously developed and almost fill the thoracic cavity. They are arranged in two series. The dorsales (figs. 13 and 15, *do.*) are six pairs of muscle-bands on each side the median line, attached posteriorly to the postscutellum and mesophragma, and anteriorly to the prescutum and anterior region of the scutum. The sternodorsales (*st.do.*) are vertical and external to the dorsales and are arranged in three bundles on each side. The first

two pairs have their upper ends attached to the prescutum and scutum, and their lower ends inserted on the mesosternum, the third pair is attached dorsally to the scutum and ventrally to the lateral plate of the postscutellum above the spiracle. As Hammond has shown in the blowfly (1881) all these muscles are mesothoracic. The dorsales by contraction loosen the alar membrane and so depress the wing, the sternodorsales have the opposite effect.

3. The segmental muscles. These muscles, which are so prominent in the larva, have almost disappeared in the imago. They are represented by the cervical muscles, certain small thoracic muscles, the thoraco-abdominal muscles, and the segmentally-arranged abdominal muscles together with the muscles controlling the ovipositor and male gonapophyses.

4. The muscles controlling the thoracic appendages, the wings, legs, and halteres. There is an elaborate series of muscles controlling the roots of the wing, but in order to avoid too much detail they will not be described here. The flexor muscles of the anterior coxæ have their origin on the inner surfaces of the humeri, a fact supporting the prothoracic nature of these sclerites; the flexors of the middle pair of legs have their origin on the sides of the posterior region of the prescutum. The internal muscles of the leg are similar to those of the blowfly and *Volucella*.

5. Special muscles. These are the muscles controlling the spiracular valves, the penis, and other small muscles.

2. The Nervous System.

The central nervous system (fig. 11) consists of (1) the brain or supracæsophageal ganglia which are closely united with the subcæsophageal ganglia, the whole forming a compact mass which I propose to call the cephalic ganglion (fig. 1, *C.G.*), perforated by a small foramen for the passage of the narrow cæsophagus, and (2) the thoracic compound ganglion which is composed of the fused thoracic ganglia with the abdominal ganglia. The two compound nerve-centres are

united by a single median ventral cord running from the subœsophageal ganglia to the anterior end of the thoracic nerve-centre.

The cephalic ganglion consists of the supracœsophageal ganglion and the subœsophageal ganglia so closely united that the commissural character of the circumœsophageal connectives is quite lost. Externally, on the dorsal side of the brain three longitudinal fissures can be seen, a median fissure and two lateral fissures marking the origin of the optic lobes.

The supracœsophageal ganglia. The characters of the ganglia composing the brain are hidden by the sheath of cortical cells which fills up the spaces between the ganglia, the characters of these can be ascertained by the serial sections. The median mass the procerebrum is formed by the fusion of the procerebral lobes. These are united before and behind, and enclose a central ganglionic mass—the central body. Behind the procerebrum two pairs of fungiform bodies arise. On the anterior face of the procerebrum the antennal or olfactory lobes which represent the deutocerebrum are situated laterally. Each sends a nerve (figs. 1 and 11, *an.n.*) to the antenna. Above these and on the dorsal side are a pair of lobes—the frontal lobes contiguous with each other in the median line—these belong morphologically to the tritocerebrum. Posterior to these in the median dorsal line of the cerebrum a single median nerve, the ocellar nerve (figs. 1 and 11, *oc.n.*), arises; this runs vertically to the ocelli. A pair of lobes which correspond to Lowne's thalami of the blowfly are situated external to and between the frontal and antennal lobes. The peduncles of the optic lobes have their origins from the sides of the procerebrum. Each optic peduncle (fig. 11, *O.P.*) contains three ganglionic masses which Hickson (1885) has termed from the brain peripherally the opticon, epipticon, and periopicon (fig. 1, *P.O.*) respectively.

The subœsophageal ganglia (fig. 1, *S.O.*). The commissures uniting the supracœsophageal ganglia to the œsophageal mass cannot be recognised as such, owing to the extreme state of cephalisation of the cephalic ganglia. They are

represented by the regions lateral to the œsophageal foramen, and from the anterior side of each of them arises a pharyngeal nerve (figs. 1 and 11, *ph.n.*). From the ventral side of the subœsophageal ganglia a pair of nerves—the labial nerves (fig. 1, *lb.n.*)—arise and run down the proboscis, innervating the muscles of that organ; on reaching the oral lobes they bifurcate and branch freely, supplying the numerous sense organs in those structures. The cortical cells (Leydig's "Punktsubstanz"), which fill up the spaces between the ganglia and form an investing sheath round the whole ganglionic mass, are of two kinds. The smaller cells are rounded, their nuclei are large in proportion to the protoplasm, and their protoplasmic fibres anastomose with each other. Among these smaller cortical cells, and also occasionally in the ganglionic substance, larger ganglionic cells occur, their protoplasm taking the stain very readily. Unipolar, bipolar, and tripolar ganglion cells are found.

The eyes. Each eye contains about 4000 facets. They are similar in all respects to the eyes of the blowfly, which have been fully described by Hickson (*loc. cit.*), whose results my study confirms; consequently, a description of their structure will not be given. It should be noted that, in spite of the fact that Hickson corrected many mistaken views held by Lowne in his memoir (1884), these are repeated in his monograph of the Blowfly.

The cephalo-thoracic nerve cord (fig. 11, *c.n.*) unites the cephalic and thoracic ganglia. Near its junction with the thoracic ganglion a pair of cervical nerves (*cer.n.*) arise, innervating the muscles of the neck.

The thoracic ganglion (figs. 12 and 14) is pyriform, with the broad end anterior, and rests on the entothoracic skeleton of the mesothorax. As in the cephalic ganglion, the component ganglia are ensheathed in a cortical layer, which is of the same nature. The nerves of the three pairs of legs (*pr.cr.*, *ms.cr.*, *mt.cr.*) arise from three large ganglia, which are the prothoracic (*Pr.G.*), mesothoracic (*Ms.G.*), and meta-thoracic (*Mt.G.*) ganglia. These are united by a median

longitudinal band of nerve tissue, which runs dorsal to them, and behind the metathoracic ganglia swells out into a ganglionic mass (*A.G.*), which represents the abdominal ganglia. In this median dorsal band there is a median dorsal fissure stretching posteriorly from above the middle of the mesothoracic ganglia. The dorsal regions of the mesothoracic and metathoracic ganglia show ganglionic swellings. From the antero-dorsal sides of the prothoracic ganglia a pair of prothoracic dorsal nerves (*pr.d.*) arise and supply the muscles of that region, including those of the anterior thoracic spiracle. The nerves supplying the mesothoracic legs (*ms.cr.*) arise from the postero-ventral sides of the mesothoracic ganglia. Between the mesothoracic ganglia there is a median ganglionic mass, situated slightly dorsal, from the middle region of which the nerve-fibres of the large pair of dorsal mesothoracic nerves (*m.s.d.*) arise; Lowne, in the blowfly, calls these prothoracic. The roots of these nerves are broad dorsoventrally. These nerves innervate the sterno-dorsales muscles of the middle region. In this median mesothoracic nerve centre, posterior to the origin of the dorsal mesothoracic nerves, the fibres of a pair of nerves, the accessory dorsal mesothoracic nerves (*ac.ms.*), have their origin; these appear externally to arise dorsal to the roots of the mesothoracic crural nerves. The dorsal metathoracic nerves (*mt.d.*), which innervate the halteres, and are the largest pair of thoracic nerves, have their origin from the median dorsal band in front of the metathoracic ganglia, so that they appear to be almost mesothoracic in origin. The metathoracic crural nerves (*mt.cr.*) arise from the posterior-ventral sides of the metathoracic ganglia. Posterior to these a pair of slender nerves, the accessory dorsal metathoracic nerves, have their origin, and innervate the muscles at the posterior end of the thorax.

The dorsal band becomes much thinner posterior to the abdominal ganglion, and runs into the abdomen as a median abdominal nerve (*ab.n.*). In the thorax two pairs of abdominal nerves arise. In the abdomen the abdominal nerves

arise alternately and irregularly from the median abdominal nerve. The median abdominal nerve finally terminates in the genitalia.

3. The Alimentary System.

The alimentary canal of the house-fly is shorter than that of the blowfly, and also than that of *Glossina* described by Minchin (1905), and slightly longer than the alimentary tract of *Stomoxys* described by Tulloch (1906). It serves as a good example of the Muscid digestive canal. It is of a suctorial character, and consists of pharynx, œsophagus, crop, proventriculus, ventriculus or chyle stomach, proximal and distal intestine and rectum.

The pharynx has already been described, and will be further referred to in the detailed description of the head. At the proximal end of the fulcrum, where the œsophagus arises, there is usually a small mass of cells, which Kraepelin has described as glandular, but which I believe to be simply fat-cells.

The œsophagus (figs. 1, 17, 20, *œs.*) commences at the proximal end of the pharynx, and describes a curve before passing through the œsophageal foramen in the cephalic ganglion, where it narrows slightly. It then passes through the cervical region into the thorax in the anterior region, of which it opens into the proventriculus (figs. 17, 20, *Pv.*), continuous with, and in the same line as the œsophagus, the duct leading to the crop (fig. 20, *d.cr.*) passes along the thorax dorsal to the thoracic nerve-centre, and entering the abdomen it leads into the crop, which lies on the ventral side of the abdomen. The œsophagus has a muscular wall, enclosing a layer of flat epithelial cells, and is lined by a cuticular intima, which is thrown into several folds at the anterior end.

The crop (fig. 17, *Cr.*) is a large bilobed sac, capable of considerable distension, and, when filled with the liquid food, it loses its bilobed shape, and occupies a large portion of the

antero-ventral region of the abdomen. Its walls exhibit muscular (unstriped) fibres; the flat epithelial cells have a very thin cuticle.

The proventriculus (*Pv.*) is circular and flattened dorso-ventrally. Its structure will be understood by reference to fig. 20. In the middle of the ventral side it opens into the œsophagus, and on the dorsal side the outer wall is continued as the wall of the ventriculus (*Ven.*). The interior is almost filled up by a thick circular plug (*Pv.p.*), the cells of which have a fibrillar structure, and it is pierced through the centre by the œsophagus. The neck of the plug is surrounded by a ring of elongate cells, external to which the wall of the proventriculus begins, and, enclosing the plug at the sides and above, it merges into the wall of the ventriculus. I do not agree with Lowne in regarding the proventriculus as "a gizzard and nothing more," but its structure suggests a pumping function and also that of a valve. On the dorsal side of the œsophagus, at its junction with the proventriculus, a small ganglion, the proventricular ganglion (*Pv.g.*), lies, communicating by a fine nerve with the cephalic ganglion.

The ventriculus, or chyle stomach (figs. 17, 20, *Ven.*), represents the anterior region of the mesenteron, the posterior region of the latter being formed by the proximal intestine. It is narrow in front, and widest in the posterior region of the thorax, where it again narrows in passing through the thoraco-abdominal foramen into the abdomen to become the proximal intestine. Except in the anterior and posterior regions, where columnar cells compose the digestive epithelium, the walls of the ventriculus are thrown into a number of transverse folds, which are again subdivided longitudinally, the result being the formation of small crypts or sacculi, which are lined by large cells. These sacculi correspond to the digestive cœca of other insects.

The proximal intestine (figs. 17, 21, *p.int.*) is the longest region of the gut. It varies in length considerably. In the normal-sized condition its course is as follows:—Beginning at the anterior end of the abdomen it runs dor-

sally beneath the heart to the posterior region, where it curves downwards, turns to the left, and runs forward for a short distance, curving to the right, where it doubles back transversely to the left. Here it doubles sharply back to the right, from whence it runs forward for a little way, and crosses over to the left. Curving, it runs posteriorly to become the distal intestine. Its walls are lined by an epithelium of large columnar cells.

The distal intestine (*d.int.*). The junction of this with the proximal intestine is marked by the entrance of the ducts of the malphigian tubes. It runs posteriorly, and curves dorsally and forwards to become the rectum, from which it is separated by a cone-shaped valve—the rectal valve, the position of which is marked externally (fig. 21, X.). The epithelium of the distal intestine consists of small cubical cells, which project into the lumen, and are covered by a fairly thick chitinous intima. The epithelial wall of the distal intestine is thrown into usually about six longitudinal folds.

The rectum (*rect.*) is composed of three parts, an anterior region, an intermediate region which is swollen to form the rectal cavity, and a shorter region posterior to this which opens externally by the anus. The anterior region is lined by cubical cells, whose internal faces project into the lumen of the rectum, and give the chitinous intima a tuberculated structure. The intermediate region which forms the rectal cavity contains the four rectal glands (*rect.gl.*). Its walls are lined by a thin cuticle supported by a flattened epithelium. The posterior portion of the rectum is short, and has thick muscular walls. The cuticular intima is continuous with that of the external skeleton.

Salivary Glands.—There are two sets of salivary glands—a pair of labial and a pair of lingual glands. The structure of the labial glands will be described in the account of the anatomy of the head.

The lingual glands (fig. 17, *sl.g.*), though considerably longer than the total length of the body, are of the simplest

tubular type. They are of uniform width throughout their whole length, except the slightly swollen blind termination. These blind ends lie one on each side of the ventral and posterior region of the abdomen, generally embedded in the fat-body. They take a sinuous course forwards through the abdomen into the thorax, where they run alongside the ventriculus. At the sides of the proventriculus they are thrown into several folds, which appear to be quite constant in character. They pass forwards at the sides of the œsophagus and on entering the cervical region the ducts lose their glandular character, and assume a spiral thickening; before leaving the cervical region the two ducts unite below the œsophagus, and the single median duct enters the head ventral to the cephalothoracic nerve cord, and runs direct to the proximal end of the hypopharynx, at the end of which it opens. A short distance before entering the hypopharynx the salivary duct (fig. 1, *sal.d.*) is provided with a small valve controlled by a pair of fine muscles (*s.m.*), which serves to regulate the flow of the salivary secretion. The glands are composed of glandular cells (fig. 22), which are convex externally, and have a fibrillar appearance in section. No vacuoles have been found in the cells.

The Malpighian Tubes.—A pair of malpighian tubes (fig. 21, *malp.*) arises at the point of junction of the proximal and distal intestines, that is, where the mesenteron joins the proctodæum. Each malpighian tube shortly divides at an angle of 180° into two malpighian tubules. The malpighian tubules are very long and convoluted, and intimately bound up with the diffuse fat-body, so that it is a matter of considerable difficulty to dissect them out entire. They have a moniliform appearance and are of uniform width throughout; never more than two cells can be seen in section. They are generally yellowish in colour. As in most insects they are undoubtedly of an excretory nature, as the contents of the cells and tubules show. Lowne's view that, in the blowfly, they are of the nature of a hepato-pancreas is untenable morphologically and physiologically.

The Rectal Glands.—The four rectal glands (*rect.gl.*) are arranged in two pairs, two on each side of the rectal cavity. Each rectal gland (fig. 25) has a conical or pyriform apex with a swollen circular base. It is composed of a single layer of large columnar cells (*r.gl.*), the papilla being hollow, with the cavity in communication with the general body cavity. It is covered externally by a perforate chitinous sheath (*sh.*), which is continuous with the intima of the rectum. A number of tracheæ (*tr.*) enter the cavity of each gland, and fine tracheæ may be seen penetrating the wall. The cavity of the gland is filled with a loose tissue of branching cells. As the gland is capable of pulsation there is no doubt a constant interchange of blood between the cavity of the gland and the body cavity (which is a hæmocœl). By this means waste products may be extracted from the blood by the large gland cells and excreted into the rectum through the pores on the external sheath of the gland. The rich supply of tracheæ probably assists the cells in the process of excretion, as we find the tracheæ very numerous, and intimately connected with the malpighian tubules.

4. The Respiratory System.

The respiratory or tracheal system is developed to a very great extent in the fly and occupies more space than any other anatomical structure. Only by dissection of the freshly-killed insect can one obtain a true conception of its importance. It consists of tracheal sacs of varying size having extremely thin walls and tracheæ which may arise from the sacs, or, in the case of the abdominal tracheæ, independently from the spiracles.

The Anterior Thoracic Spiracles (figs. 6 and 13, *a.th.*).—Each is a large vertical opening behind the humeral sclerite and above the anterior legs. It is surrounded by a chitinous ring, the peritreme and the opening is guarded by a number of dendritic processes which prevent the entrance of dust and other foreign bodies. It leads into a shallow chamber or

vestibule which communicates with the rest of the spiracular system through a valvular aperture.

The anterior thoracic spiracles supply the whole of the head, the anterior and median regions of the thorax, the three pairs of legs, and by means of the abdominal air-sacs a large part of the viscera.

Internal to the valve the tracheal system divides. The tracheal sacs springing from the posterior side are as follows: Ventrally a rather narrow tracheal duct leads into a sac—the anterior ventral thoracic sac (fig. 13, *a.v.s.*) situated at the side of the thoracic ganglion which it supplies. Above the origin of this another tracheal duct leads to a vertical sac supplying the anterior sterno-dorsales muscles. Dorsally the ducts of two sacs take their origin; the smaller and more dorsal is a flat sac closely apposed to the anterior ends of the dorsales muscles (*do.*) which it supplies; the more ventral of the two is one of the two most important branches of the anterior thoracic spiracle (the other being the branch supplying the head). In the thorax it takes the form of an elongated sac lying below the dorsales muscles, and by side of the alimentary canal. From the dorsal side of this the longitudinal thoracic sac (*l.tr.s.*) a number of branches arise which supply the lower dorsales muscles. It is constricted about the middle of its length and anterior to the constriction; a branch is given off which supplies the ventral portion of the median sterno-dorsales muscles. In the posterior region of the thorax another ventral branch is given off from which branches arise, one supplying the ventral portions of the posterior sterno-dorsales muscles, the other opening into the posterior ventral thoracic sac (*p.v.s.*), which supplies the intermediate and posterior legs. The longitudinal thoracic sac then narrows, and passes through the thoraco-abdominal opening into the abdomen. In the adomen it immediately dilates to form one of the large abdominal air-sacs (*a.b.s.*). The pair of abdominal air sacs in some cases occupy about half the total space of the abdomen. When the fat-body is not greatly developed they occupy almost the whole of the

basal portion of the abdomen. They give off internally a large number of tracheæ which ramify among the viscera and provide a large portion of the contents of the abdomen with air.

From the anterior side of the anterior thoracic spiracle a flattened sac arises. On its ventral side this gives off a branch which supplies the muscles of the neck and the anterior leg. The sac then narrows into a rather thick-walled cervical tracheal duct (*c.tr.*), which passes through the neck alongside the cephalo-thoracic nerve-cord and enters the head.

Tracheal Sacs of the Head.—The tracheal sacs of the head occupy the greater portion of the head capsule. They entirely fill up all the space which would otherwise be hæmo-cœl. These tracheal sacs are supplied by the cervical tracheal ducts which, on entering the head capsule, curve dorsally behind the cephalic ganglion. Before curving upwards each gives off a large ventral duct (fig. 4), which spreads out beneath the cephalic ganglion forming a structure of a tentorial nature upon which the ganglion rests. The dorsal cephalic ducts unite behind the cephalic ganglion above the œsophagus. From the point of junction three ducts arise, two lateral ducts and a median dorsal duct. The median dorsal duct (*m.d.*) opens into a large bilobed dorso-cephalic sac lying on top of the ganglion, and occupying the dorsal region of the head capsule. It gives off branching tracheal twigs supplying the antero-dorsal portion of the optic ganglion (periopticon). Each of the lateral ducts (fig. 4, *l.d.*) supplies the posterior cephalic sacs. It first communicates with a sac (fig. 13, *p.c.s.*) lying behind the dorsal portion of the optic ganglion to which it gives off a large number of tracheal twigs. This sac opens into an elongate vertical sac which occupies the ventro-posterior region of the head capsule. The remaining tracheal sacs of the head are supplied by the tentorial tracheal ducts (*tr.d.*), which spread out beneath the cerebrum in a fan-shaped manner, and are bilaterally distributed. Each half, in addition to giving off internally tracheal twigs to the optic

ganglia, communicates with two tracheal sacs. An internal duct leads into a large spherical sac, the anterior cephalic sac (*a.c.s.*) situated in the anterior region of the head dorsal to the fulcrum. From the dorsal side of this sac a branch is given off which supplies the antenna of its side; the ventral side is continued down the fulcrum as a narrow tracheal sac. The lateral portion of the tentorial tracheal duct opens into the ventro-lateral cephalic sac (*v.c.s.*) situated posterior to the optic ganglion. The lower end of this sac gradually narrows as it enters the rostrum which it traverses, giving off half-way along its length a trachea which supplies the palp of that side. On reaching the haustellum it takes the form of a trachea proper, having annular thickenings. Shortly after entering the haustellum it gives off two branches to the muscles of this region. The main trachea is continued into the oral lobe of its side where it divides into anterior and posterior branches, and these again divide into numerous small tracheæ running to the edges of the oral lobes. Lowne, in his description of the tracheal system of the blowfly, describes and figures the tracheal supply of the proboscis as being of the nature of tracheal sacs and capable of distension; he also describes a trefoil-shaped tracheal sac at the base of the oral lobes giving off very regular branches, the dilation of which causes the inflation and tension of the oral lobes. The mechanism of the proboscis will be discussed later (p. 439), but it may be noticed here that in *M. domestica* there is no trace of a trefoil-shaped sac at the base of the oral lobes, and that all the tracheal structures of this the haustellum region are definite annular tracheæ, and therefore incapable of distension.

The posterior thoracic spiracle (figs. 6 and 15, *p.th.*) is triangular in shape and guarded by dendritic processes. It possesses a vestibule which leads into a distributing tracheal sac. The tracheal sacs of this system (fig. 15) have not the extended range of those supplied by the anterior thoracic spiracle, but are confined to the thorax, chiefly in the median and posterior regions which are not aerated to any great

extent by those of the other system. They supply chiefly the large muscles of the thorax. Laterally a series of sacs (*l.th.s.*) extends antero-dorsally in an oblique direction, external to the sterno-dorsales muscles to the humeral region. From the first of these sacs a large number of tracheal twigs arise and supply the muscles of the wing and the anterior sterno-dorsales muscles. Ventral to this sac a large sac (*m.v.s.*) penetrates internally between the anterior and median sterno-dorsales muscles and supplies the lower dorsales muscles. From the dorsal side of the distributing sac a number of sacs arise, some of which penetrate between the sterno-dorsales muscles and supply the upper dorsales muscles. A more posterior set supplies the posterior regions of the dorsales muscles, ramifying between them in a very extensive manner, some ultimately terminating in the tracheal sacs beneath the scutum and the scutellar sac (*sc.s.*).

The abdominal spiracles differ in number in the two sexes. In the male there are seven pairs of abdominal spiracles; in the female I have only been able to find five pairs. In both sexes each of the large tergal plates which cover the abdomen has near its lateral margin a small circular spiracle. The first abdominal segment which has fused with the second has a pair of small spiracles (see fig. 8) slightly anterior to those of the second (apparent first) abdominal segment. In addition to these the male possesses two pairs of spiracles in the membrane at the lateral extremities of the rudimentary sixth and seventh abdominal segments (see fig. 5). In the female I have been unable to find any additional spiracles. Each of the abdominal spiracles is provided with a vestibule and atrium which are separated by a valve controlled by a minute chitinous lever. All the spiracles of the abdomen communicate with tracheæ which ramify among the viscera and fat-body; there are no tracheal sacs in connection with these spiracles.

5. The Vascular System and Body-cavity.

By the great development of the tracheal sacs in the head, the muscles in the thorax, and the fat-body and air sacs in the abdomen, the hæmocœlic space in the fly is greatly reduced. The blood is colourless, and is crowded with corpuscles, mostly containing substances of a fatty nature.

The fat-body varies greatly in the extent of its development. In some cases it may almost fill the body-cavity, pushing the intestine back into a postero-dorsal position: this is generally the case in flies before hibernating; in other cases it may be only moderately developed. The fat-body receives a very rich tracheal supply, and stores the products of digestion which are conveyed to it by the blood with which it is bathed. It consists chiefly of very large cells, both uninucleate and multinucleate; the fat-cells of the head are not so large.

The dorsal vessel or heart lies in the pericardial chamber, immediately beneath the dorsal surface. It extends from the posterior end to the anterior end of the abdomen, and four large chambers, corresponding to the four visible segments, and a small anterior chamber can be recognised; the last represents the chamber of the first abdominal segment. The chambers are not separated by septa, but each has a pair of dorso-lateral ostia situated at its posterior end where the alar muscles of the pericardium arise. The walls of the heart are composed of large cells. The pericardium contains fat-cells and tracheæ, and its floor is composed of large cells of a special nature. The alar muscles run laterally in the floor of the pericardium to the sides of the dorsal plates where they are inserted. The anterior end of the heart is continued as a narrow tube (fig. 20, *d.a.*) along the dorsal side of the ventriculus, where it terminates in a mass of cells (*l.g.*), which are usually considered to be of a lymphatic nature.

6. The Reproductive System.

The two sexes are slightly different in size, the females being larger than the males; the sexual dimorphism of the width of the frontal region of the head has already been noticed (p. 402). There does not appear to be any great disparity in the numerical proportions of the sexes; near breeding places there is naturally a preponderance of females.

The Female Reproductive Organs.—The generative organs of the female consist of ovaries, spermathecæ or vesiculæ seminales, accessory glands and their ducts.

The ovaries, when containing mature ova, occupy the greater part of the abdominal cavity (fig. 23, *ov.*). They lie ventral to the gut, occupying the whole of the ventral and lateral regions, the gut resting on the V-shaped hollow between them. Each ovary contains about seventy ovarioles, in each of which ova in various stages of development can be seen. The two short thin-walled oviducts (*ov.d.*) unite on the ventral side of the abdomen to form the common oviduct (*c.o.d.*). The walls of the common oviduct are muscular, and when the ovipositor is in a state of rest, retracted into the abdominal cavity, the oviduct curves forwards and dorsally to enter the ovipositor (*ov.p.*) ventral to the rectum (*rect.*). Here it swells slightly to form a sacculus (fig. 26, *sac.*) which leads into the muscular vagina (*vag.*). The vagina opens into the ventral side of the ovipositor immediately behind the sub-anal plate.

The spermathecæ (*sp.*) or vesiculæ seminales are three in number, two on the left side, and a single one on the right. Each consists of a small, black, oviform, chitinous capsule, the lower half of which is surrounded by a follicular investment continuous with the cellular wall of the duct, the whole having the appearance of an acorn with a long stalk. The ducts of the spermathecæ are lined by a thin chitinous intima continuous with the chitinous capsule, and they open at the posterior end of the sacculus on the dorsal side.

There is a single pair of accessory glands (*ac.g.*), which are fairly long, and on nearing the vagina they become narrower to form a slender duct, which opens on the dorsal side of the vagina immediately behind the ducts of the spermathecae. The accessory glands are closely united with the fat-body. They probably secrete the adhesive fluid which covers the eggs when they are laid, and causes them to adhere to each other and to the material upon which they are deposited. Behind the accessory glands there is a pair of thin-walled transparent vesicles (*tasche dell' ovidutto* of Berlese), which I propose to name the accessory copulatory vesicles (*a.c.v.*) on account of the part they take in ensuring firm coitus with the male during copulation, during which process they expand to a much greater extent.

The ovipositor (fig. 8). The terminal abdominal segments of the female are much reduced to form a tubular ovipositor, the chitinous sclerites being reduced to form slender chitinous rods. When extended it equals the abdomen in length. It is composed of segments vi, vii, viii, and ix, each being separated from the adjacent segments by an extensible inter-segmental membrane, which is covered with fine spines. When the ovipositor is retracted (fig. 23, *ovp.*) it lies in the interior of the posterior end of the abdomen, the segments being telescoped the one within the other, so that only the terminal tubercles are visible from the exterior. The dorsal arch of the sixth abdominal segment is reduced to a Λ -shaped sclerite (vi, *d.*), lying on the dorsal side of the segment. The ventral arch of this segment is reduced to a slender chitinous rod (vi, *v.*) in the mid-ventral line. The dorsal arch of the seventh segment is represented by two slightly-curved sclerites (vii, *d.*), with their concave faces opposite; the ventral arch (vii, *v.*) is similar to that of the sixth segment. At the junction of the posterior ends of the sixth and seventh segments with the inter-segmental membranes succeeding them there are several setose tubercles arranged more or less in pairs, but they vary in development in different individuals. The dorsal arch of the eighth

segment consists of two parallel and slender sclerites (viii, *d.*), not so narrow as those of the two preceding segments. A pair of slender sclerites (viii, *v.*) also represents the ventral arch. The terminal anal segment, which I consider represents the reduced ninth segment, has a dorsal chitinous sclerite, the sub-anal plate (*su.p.*), which is triangular in shape, and a ventral sub-anal plate of the same shape. The female genital aperture is situated at the anterior end of the latter plate, between the eighth and anal (ninth) segments. A pair of terminal setose tubercles is situated laterally at the apex of the anal segment.

The Male Reproductive Organs.—The male reproductive organs (fig. 24) are situated ventral to the alimentary canal, and lie within the fifth abdominal segment. They consist of a pair of testes, vasa deferentia, ejaculatory duct and sac, and the terminal penis. There are no accessory genital glands in the male.

The testes (*te.*) are a pair of brown pyriform bodies, with their long axes placed transversely, and their pointed ends facing. In young males they have a bright red appearance. They are covered with a follicular investment of cells, which varies in thickness apparently according to age. The thin brown chitinous capsules contain the developing spermatozoa. The pointed end of each testis is continued as a fine vas deferens (*v.d.*), which meets that of the other testis in the median line, where they open into the common ejaculatory duct (*d.e.*). This runs forwards for a short distance, and then bends to the left ventrally, and, after several convolutions on the left ventral side of the abdomen, the duct narrows considerably, forming a narrow ejaculatory duct. This crosses over the dorsal side of the rectum to the right side, where it runs forwards for a short distance and then curves back in the median ventral line, opening into a pyriform ejaculatory sac (*e.s.*). The walls of this ejaculatory sac are muscular, longitudinal muscles, giving the walls a striated appearance. It contains a phylliform, chitinous sclerite—the ejaculatory apodeme (*e.a.*), which has a short handle at the broad end.

This sclerite is, no doubt, of great assistance in propelling the seminal fluid along the ejaculatory duct during copulation. A short distance behind the ejaculatory sac the duct opens into the penis.

The Male Gonapophyses.—The extremity of the abdomen in the male (fig. 10) has undergone considerable modification in the formation of the external genitalia. The visible portion of the abdomen, as seen from above, consists of the first five abdominal segments; the remaining three segments are slightly withdrawn into the fifth segment, and, on looking at the abdomen from the posterior end, only the terminal segment, the eighth, surrounding the anus, can be seen. The sixth and seventh segments have been greatly reduced. The sternal portion of the fifth segment consists of a cordiform sclerite (*V.v.*), the apex of which is directed forwards, and each of the lateral margins of the base is produced to form a short process, swollen at the tip—these lateral processes form the primary forceps (*p.f.*), and lie at each side of the aperture of the male genital atrium (*g.a.*), of which the posterior edge of the sclerite forms the lower or anterior lip. The dorsal plates of the sixth and seventh segments lie on the membrane, which is tucked underneath the posterior edge of the fourth abdominal segment. The dorsal plate of the sixth segment (*vi, d.*) is a narrow, transverse sclerite; its lateral edges, which do not extend down the sides, are slightly produced anteriorly. The ventral plate of the sixth segment (*vi, v.*) is asymmetrical, and, with the dorsal plate of the seventh segment, produces a pronounced asymmetry of the posterior end of the male abdomen. It consists of a spatulate plate on the left side, the anterior or ventral side of which is produced into a narrow bar extending across the ventral side of the aperture of the genital atrium, its distal extremity bifurcating. The dorsal plate of the seventh segment (*vii, d.*) is asymmetrical. It consists of a narrow sclerite, which, on the dorsal side, is similar to the sixth dorsal plate, but the left side (see fig. 5) extends down the side, and broadens out into a somewhat

triangular-shaped area; the anterior edge of this is incised, and receives the seventh spiracle (vii, *a.sp.*); the ventral edge is internal to the spatulate portion of the sixth ventral plate. The ventral arch of the seventh sclerite has been completely withdrawn into the abdomen, and consists of a pair of curved sclerites (fig. 9, vii, *v.*), somewhat rhomboidal in shape, lying dorsal to the fifth ventral arch and ventral to the penis (*P.*); they form the secondary forceps. Their lateral edges, which are thickened articulate with the alar processes of the body of the penis (*c.pe.*), and with the dorsal arch of the eighth abdominal segment (viii, *d.*). Their inner edges are curved, and almost meet in the mid-ventral line. The dorsal arch of the eighth and last abdominal segment (viii, *d.*) forms the apex of the abdomen. It consists of a strongly convex sclerite, deeply incised on the ventral side; in this incision the vertical slit-like anus (fig. 10, *an.*) lies. The ventral portion of the segment is completed by a pair of convex sclerites (viii, *v.*), which are united in the mid-ventral line, forming the ventral border of the anal membrane and the dorsal side of the entrance to the genital atrium.

All the sclerites of the posterior segments except the sixth and seventh are setose.

Berlese (1902) in his account of the copulation of the House-fly describes the genitalia. From his account of the male genitalia he appears to have missed the narrow dorsal arch of the sixth segment, or, what is very probable, he may have mistaken it for the fifth dorsal arch, as he terms the seventh dorsal arch the sixth, and describes what I have called the ventral arch of the seventh as the dorsal arch of that segment. This mistake in nomenclature has probably arisen from the fact that he considered the visible portion of the abdomen as consisting of four segments instead of five, in which case the narrow dorsal arch of the sixth segment would naturally be taken for that of the fifth.¹

¹ Berlese describes a sinistral asymmetry of the posterior segments, but his figures show a dextral asymmetry, a mistake probably in the reproduction of his figures which has escaped the author's notice.

The penis (figs. 7 and 9) lies internally on the ventral side of the abdomen, dorsal to the ventral arches of the fifth and seventh segments. It is composed of several sclerites. A median sclerite (*c.pe.*), the anterior and ventral edge of which is roughly semicircular in outline, forms the body of the penis. This is produced laterally to form two alar processes; at the bases of these processes the lateral extremities of the dorsal arch of the eighth segment articulate with the body of the penis; the extremities of the processes are attached to the lateral extremities of the ventral sclerites of the seventh segment, the secondary forceps. The penis proper consists of a hollow cylindrical tube, the theca, which receives the ejaculatory duct. The theca articulates with the body of the penis by means of a pair of small chitinous nodules ("cornetti" of Berlese); posterior to the attachment the theca is constricted slightly. Below the aperture for the entrance of the ejaculatory duct, the theca is produced into a ventrally directed curved process, the inferior apophysis (*i.ap.*); above the aperture a short cylindrical process, the superior apophysis (*s.ap.*), arises. The anterior end of the theca is continued as a slightly inflated hyaline structure, the glans (*p.gl.*), at the curved extremity of which the ejaculatory duct opens.

V. THE INTERNAL STRUCTURE OF THE HEAD.

The skeletal framework and tracheal system of the head have already been described. It remains, therefore, to give an account of the musculature of the head and pharynx, and also an account of the oral lobes.

The posterior region of the head (fig. 1) not occupied by tracheal sacs is usually filled up with small multinucleate fat-cells (*f.c.*), which are also occasionally found in the proboscis. The frontal sac or ptilinum (*Pt.*) fills up the anterior portion of the head not occupied by air-sacs. Its crescentic opening, the lunule, has already been described. It is attached to the

wall of the cephalic capsule by muscles which vary considerably in the extent of their development. In recently emerged flies the muscle-supply of the ptilinum is considerable, as they have served to retract the sac after it has been inflated to assist the exclusion of the imago, but in older specimens it becomes less. The walls of the ptilinum are muscular and lined by a chitinous intima covered with small broad spines.

The Musculature of the Proboscis.—The chief muscles controlling the movements of the pharynx and proboscis are these :

The Dilators of the Pharynx (figs. 1 and 2, *d.ph.*)—This pair of muscles occupies the interior of the fulcrum. Each muscle is attached to the antero-lateral regions of the fulcrum and inserted into the dorsal plate of the pharynx (*r.p.*). These muscles are the chief agents in pumping the liquid food into the œsophagus, and in drawing it up through the pharyngeal tube.

The Retractors of the Fulcrum (fig. 1, *r.f.*)—These muscles are attached to the internal anterior edges of the genæ, and are inserted into the posterior cornua (*p.c.*) of the fulcrum. Their contraction causes the rotation of the fulcrum on the epistome as a hinge in the retraction of the proboscis.

The Retractors of the Haustellum (*r.h.*)—These muscles have their origin on the dorso-lateral regions of the occiput. They are long and narrow, and running on each side of the common salivary duct are inserted into the dorsal margin of the theca.

The Retractors of the Rostrum (*r.r.*)—This pair of muscles has its origin at the sides of the occipital foramen, and is inserted into the posterior side of the membranous rostrum about half-way down its length. In the retraction of the proboscis these muscles draw in the rostrum.

The last two pairs of muscles acting together assist in the retraction of the whole proboscis.

The Flexors of the Haustellum (*f.h.*) have their origin close to that of the retractors of the rostrum at the

sides of the occipital foramen. They are inserted into the base of the labral apodeme (*ap.*), and serve to flex the haustellum on to the anterior face of the rostrum.

The Extensors of the Haustellum (*ex.h.*).—Each of these muscles arises from the distal cornu of the fulcrum, and is inserted into the head of the labral apodeme.

The Accessory Flexors of the Haustellum (*a.f.h.*) are attached to the lower (distal) anterior margin of the fulcrum, and inserted with the extensors into the head of the labral apodeme.

The Flexors of the Labium-epipharynx (*f.l.*).—These muscles have their origin on the anterior and upper edge of the fulcrum, and are inserted into the proximal end of the labium-epipharynx. The first pair of the last three sets of muscles serve to extend the haustellum in the extension of the proboscis, and the remaining two pairs assist in the retraction of the proboscis by flexing the haustellum on to the rostrum.

A pair of very fine muscles (*s.m.*) have their origin at the base of and internal to the posterior cornua of the fulcrum. They are inserted into the dorsal side of a small valve (*s.v.*) on the common salivary duct which regulates the flow of the secretion of the lingual salivary glands.

The muscles of the haustellum are—

The Retractors of the Furca (*r.f.u.*).—A pair of muscles having their origin on the upper part of the theca. Each is inserted along the upper proximal half of the lateral process of the furca. When the muscles contract the lateral processes of the furca, which, in a state of repose are brought together by the elasticity of the ventral cornua of the theca, are diverged, and thus cause the divergence and opening of the oral lobes.

The Retractors of the Discal Sclerites (*r.d.s.*).—These muscles have their origin on the lateral edges of the upper part of the theca, and are inserted upon the sides of the discal sclerites. They work together with the retractors

of the furca, their contraction causing the divergence of the discal sclerites, and the consequent opening of the oral pit.

The Dilators of the Labium-hypopharynx (*dil.*).—These fan-shaped muscles arise in the middle region of the theca on either side the median line, and diverging are inserted in the lateral edges of the labium-hypopharyngeal sclerite. By their contraction they will widen the channel of the labium-hypopharynx.

The Dilators of the Labium-epipharynx (*dil.*)—These form a series of short muscles attached to the anterior and posterior walls of the labium-epipharynx. The size of the pharyngeal channel will be regulated by these muscles.

The Oral Lobes.—The external structure of the oral lobes has already been described. Their internal structure and histology will be given here, as it seemed preferable to do so rather than postpone it to a future communication.

The setigerous cuticle and the pseudo-tracheæ lie on a hypodermis of cubical cells (fig. 18, *hy.*). Beneath the hypodermis of the aboral surface is another layer of cells containing a large amount of dark pigment. Each of the large marginal sensory bristles (*g.s.*) of the aboral surface has a fine channel running down the whole length of the seta. This channel communicates with the cavity of a pyriform mass of nerve-end cells (*s.p.*), consisting of five or six cells. These masses of cells occupy a large part of the interior of the oral lobes. As these gustatory bristles are exposed and directed ventrally when the proboscis is retracted, they may assist the fly in testing the nature of its food before extending its proboscis. On the oral side of the oral lobes the nipple-like gustatory papillæ (figs 1 and 18, *gp.*) have already been described. The aperture at the end of the papilla leads into a fine duct, which ends in a pyriform sensory bulb (*s.g.p.*). The tracheæ (*tr.*) can be seen running through the cells, some of which contain several nuclei, and from their appearance are probably derived from the fat-body. No tracheal sacs could be found either in the oral lobes or at their bases, but the annular tracheæ are continuous with those of the proboscis. The

hæmocœl of the oral lobes is well developed. This supports the view set forth by Kraepelin, and with which I agree that the inflation of the oral lobes is due to the blood. I consider that the extension of the proboscis is due to the inflation of the tracheal sacs of the head. The proboscis having been protruded the oral lobes are then diverged by the contraction of the retractor muscles of the furca and discal sclerites, and distended by the inrush of blood which keeps them turgid, and causes the openings into the pseudo-tracheal channels to remain open.

The Labial Salivary Glands (figs. 19 and 1, *lb.sl.*).—These salivary glands lie in the haustellum at the base of the oral lobes. The glands, which are spherical in shape, are composed of a large number of gland cells somewhat triangular in shape. Each gland cell is $40\ \mu$ in size, and possesses a large nucleus ($12\ \mu$), and internal to this a permanent circular vacuole (*vac.*), which is $16\ \mu$ in size, and is lined by a thin chitinous intima. The duct of each gland cell opens into the side of the vacuole (*od.*). The ducts (*ic.d.*) are intracellular, and run from the centre of the gland, some of them uniting, to form a number of fine ducts on the ventral sides of the discal sclerites, which unite and open into the oral pits by a median pair of pores. Kraepelin, in his description of the proboscis of the blowfly, described the labial glands and their ducts (but not their histology) of that insect, his description being similar to the condition I find in *M. domestica*. Lowne, however, states that in the blowfly he traced the ducts of the gland cells through the oral lobes to the apertures of the gustatory papillæ, which he regarded therefore as the apertures of the labial salivary glands.

The secretion of the labial salivary gland serves to keep the surface of the oral lobes moist.

VI. SUMMARY.

1. The exoskeleton of the head capsule and of the pharynx is described in detail; the relations of the parts in the terms

generally employed by dipterologists to the morphological divisions of the insect head capsule are shown. On morphological grounds, the view that the distal portion of the proboscis represents the modified second maxillæ or labium is adopted, as opposed to that of a first maxillar derivation put forward by Lowne for the blowfly.

2. After a detailed description of the external and internal skeletal structures of the thorax, the neuration of the wings is described in the terms proposed by Comstock and Needham in their valuable memoir; and to facilitate their more general adoption for the wings of the Muscidæ and other Diptera, a comparison is made between their nomenclature and the several systems employed in describing the muscid wing.

3. The abdomen is shown to consist of eight segments in the male and nine in the female, in both cases the first five segments form the visible portion of the abdomen; the external genitalia of the two sexes are described under another section.

4. As the muscular system does not differ from that of *Volucella* described by Kunckel d'Herculeis and the blowfly described by Hammond and Lowne, it is briefly described. The cephalic muscles, however, are fully described in the detailed description of the head (V).

5. The nervous system, which is of the normal muscid type, is described, but for the sake of clearness a very detailed description of the composition of the cephalic ganglion is not given. The structure of the optic tract is similar to that of the blowfly as described by Hickson. The structure of the thoracic nerve-centre is found to differ slightly from that of the blowfly as described by Lowne.

6. The alimentary canal is similar in its structure to those of *Stomoxys* and *Glossina*, only differing in a few details. The mesenteric region, which is represented by the ventriculus or chyle, stomach, and proximal intestine, is well developed. The lingual salivary glands, rectal glands, and

Malpighian tubes are described; the function of the rectal glands is believed to be of an excretory nature.

7. As the tracheal systems of the Diptera have not received much attention a detailed account of the tracheal system is given. There are two thoracic spiracles, the first of which supplies the whole of the head, the anterior and median regions of the thorax and the three pairs of legs, and by means of a pair of large abdominal air-sacs a large part of the viscera. The posterior thoracic spiracle supplies the muscles of the median and posterior region of the thorax, especially the large dorsales muscles. There are seven pairs of abdominal spiracles in the male and five pairs in the female all of which are connected with tracheæ only.

8. The dorsal vessel or heart is found to consist of five incomplete chambers, each with a pair of ostia. The anterior end is continued forwards along the dorsal side of the ventriculus, and terminates in a glandular mass in the anterior margin of the proventriculus.

9. The reproductive organs of the male are simple, consisting of a pair of testes, vasa deferentia, and common ejaculatory duct; there are no accessory glands such as are found in many other Diptera. The terminal abdominal segments of the male exhibit a sinistral asymmetry.

The ovaries of the female, when mature, occupy the greater portion of the abdominal cavity. There are a pair of accessory glands (probably of a "gum" or "glue" nature), three spermathecae, and a pair of vesicles used during copulation. The ovipositor is about as long as the abdomen, and is composed of segments six to nine.

10. The musculature of the head is described in detail, and it is found that the House-fly agrees with the blowfly in the number and relations of its cephalic muscles, though in a few cases the attachments are slightly different. In the haustellum and oral lobes of the House-fly no tracheal sacs similar to those described and figured by Lowne for the blowfly occur, but only annulated tracheæ are found, and, as these are incapable of distension, the view that the oral lobes are

distended by the action of inflated air cannot be held. The extension of the proboscis I believe is due to the inflation of the tracheal sacs of the head and rostrum, and I agree with Kraepelin that the distension of the oral lobes is effected by blood-pressure.

Two kinds of gustatory sense-organs are found on the margin of the aboral and on the oral surfaces respectively. The latter were described in the blowfly by Lowne as the openings of the ducts of the labial salivary glands, but Kraepelin's correct description of their structure in the blowfly is confirmed by this study of the House-fly. The labial salivary glands are described in detail. They consist of large cells containing permanent vacuoles, which communicated with intracellular ducts. These open by a pair of pores into the oral pits, the secretions of the glands serving to keep the surface of the oral lobes moist.

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EXPLANATION OF PLATES 22—26,

Illustrating Mr. C. Gordon Hewitt's paper on "The Structure, Development, and Bionomics of the House-fly (*Musca domestica*, Linn.). Part I. Anatomy of the Fly."

PLATE 22.

- FIG. 1.—*Musca domestica*. Female.
 FIG. 2.—*Anthomyia radicum*. Female.
 FIG. 3.—*Homalomyia canicularis*. Male.
 FIG. 4.—*Stomoxys calcitrans*. Female. The halteres of this species have been drawn too far back, and in this and the other species the nervures of the wings have been made thicker than they naturally are.
 These figures are not drawn to the same scale.

PLATE 23.

FIG 1.—Interior of the head of *M. domestica*. In this figure the left side of the head capsule and of the proboscis have been removed and the compound eye of the same side, leaving the optic ganglion (periopticum). All the tracheal structures have been omitted.

a.c. Anterior cornu of fulcrum. *a.f.h.* Accessory flexor muscles of haustellum. *ap.* Apodeme of labrum. *an.n.* Antennal nerve. *C.G.* Cephalic ganglion. *di.l.* Dilator muscles of labium hypopharynx. *d.ph.* Dilator muscles of pharynx. *d.s.* Discal sclerite. *ex.h.* Extensor muscle of haustellum. *F.* Fulcrum. *f.* Furca. *f.c.* Fat-cells. *f.h.* Flexor muscle of haustellum. *f.l.* Flexor muscle of labrum-epipharynx. *g.p.* Gustatory papillæ of oral lobes. *h.* Hyoid sclerite of pharynx. *lb.n.* Labial nerve. *lb.sl.* Labial salivary gland. *l.hp.* Labium-hypopharynx. *lep.* Labrum-epipharynx. *max.p.* Maxillary palp. *œs.* Œsophagus. *oc.n.* Ocellar nerve. *ph.n.* Pharyngeal nerve. *p.c.* Posterior cornu of fulcrum. *P.O.* Periopticum. *ps.* Pseudotrachea. *Pl.* Ptilinium. *r.d.s.* Retractor muscles of the discal

sclerites. *r.f.* Retractor muscle of the fulcrum. *r.fu.* Retractor muscle of the furca. *r.h.* Retractor muscle of haustellum. *r.r.* Retractor muscle of rostrum. *S.O.* Sub-œsophageal ganglion. *sal.d.* Common duct of the lingual salivary glands. *s.v.* Valve of the common salivary duct. *s.m.* Muscle controlling the valve of salivary duct. *th.* Theca.

FIG. 2.—Transverse section through the lower portion of the head-capsule, showing the muscles and tracheal sacs in this region and the fulcrum in section. (Camera lucida drawing.)

bp. Floor of pharynx. *r.p.* Roof of pharynx. *tr.s.* Tracheal sac. Other lettering as in Fig. 1.

FIG. 3.—Transverse section through the lower half of the haustellum, where the hypopharynx (*hp.*) has become free from the labium. (Camera lucida drawing.)

di.l. Dilator muscles of the labium-epipharynx. *tr.* Trachea. Other lettering as in Fig. 1.

FIG. 4.—Posterior view of the tracheal ducts which supply the cephalic sacs and tracheæ.

c.tr. Cervical tracheæ which fuse above the œsophagus on the posterior side of the cephalic ganglion. *l.d.* Lateral duct. *m.d.* Median dorsal duct. *tn.d.* Tentorial tracheal ducts which spread out beneath the cephalic ganglion.

FIG. 5.—Lateral view of the terminal segments of the abdomen of the male after their removal from the fifth segment.

vi, a.sp. and *vii, a.sp.* Sixth and seventh abdominal spiracles. Lettering as in Fig. 10.

FIG. 6.—The thorax seen from the left side. The insertions of the larger setæ are shown; for the sake of clearness the sclerites of the wing-base are omitted.

a.th. Anterior thoracic spiracle. *ca.* Costa. *cp.* Intermediate coxal plates. *ep', ep''.* Epimera of the meso- and meta-thoracic segments. *eps', eps'', eps'''.* Episterna of the pro-, meso-, and meta-thoracic segments. *hal.* Haltere. *hu.* Humerus. *lp.* Lateral plate of mesosternum. *lp.sc.* Lateral plate of postscutellum. *mpl.* Mesophragma. *mpsc.* Median plate of postscutellum. *mn.* Metanotum. *ms.* Mesosternum. *mts.* Metasternum. *p.th.* Posterior thoracic spiracle. *pt.* Parapterm. *pr.n.* Pronotum. *prs.* Pre-scutum of mesothorax. *sc.* Scutum. *scll.* Scutellum.

FIG. 7.—Penis seen from the right side after it has been removed from within the terminal abdominal segments.

i.ap. Inferior apophysis. *th.p.* Theca of penis. *p.gl.* Glans. *s.ap.* Superior apophysis. Other lettering as in Fig. 9, etc.

FIG. 8.—Abdomen of female showing the extended ovipositor.

V, d. to *ix, d.* Fifth to ninth dorsal arches or plates of the abdomen. *V, v.*

to viii, *v.* Fifth to eighth ventral plates or arches. *su.p.* The suranal plate (ninth dorsal arch).

The anus is situated between the two lateral terminal tubercles.

FIG. 9.—Dorsal view of the penis and the ventral half of the terminal abdominal segments. The median portion of the eighth dorsal arch has been removed, leaving the lateral portions attached to the body of the penis (*c.pe.*) and the ventral arch of the seventh segment (*vii, v.*).

Lettering as in Fig. 10.

FIG. 10.—The posterior end of the abdomen of the male seen from behind, showing the pronounced sinistral asymmetry.

v, d. to *viii, d.* Fifth to eighth dorsal plates or arches. *v, v.* to *viii, v.* Fifth to eighth ventral plates or arches. *an.* Anus. *g.a.* Aperture of genital atrium. *p.f.* Primary forceps.

PLATE 24.

FIG. 11.—Nervous system. The very fine nerve which runs along the dorsal side of the œsophagus to the proventricular ganglion (*Pv.g.*, Fig. 20) has been purposely omitted.

ab.n. Abdominal nerve. *ac.ms.* Accessory mesothoracic dorsal nerve. *ac.mt.* Accessory metathoracic dorsal nerve. *cer.n.* Cervical nerves. *cn.* Cephalothoracic nerve cord. *O.P.* Optic peduncle. *pr.cr., ms.cr., mt.cr.* Pro-, meso-, and meta-thoracic crural nerves. *pr.d., ms.d., mt.d.* Pro-, meso-, and meta-thoracic dorsal nerves.

FIG. 12.—Thoracic compound ganglia. Left aspect.

Lettering as in Figs. 11 and 14.

FIG. 13.—The tracheal sacs supplied by the anterior thoracic spiracle (*a.th.*). In this figure the tracheal sacs supplied by the posterior thoracic spiracle and the sterno-dorsales muscles of the left side have been removed. The left side of the head and proboscis have also been removed. The first abdominal segment has been removed to show the large abdominal air sacs (*ab.s.*) and an abdominal trachea which is supplied by the second abdominal spiracle (*a.sp.*).

a.c.s. Anterior cephalic sac. *a.v.s.* Anterior ventral thoracic sac. *c.tr.* Cervical tracheal duct. *d.c.* Dorsal cephalic sac. *do.* Dorsales muscles. *H.* Haustellum. *l.tr.s.* Longitudinal tracheal sac. *p.c.s.* Posterior cephalic tracheal sacs. *p.v.s.* Posterior ventral thoracic sac. *p.op.* Periopticton. *Ros.* Rostrum. *v.c.s.* Ventral cephalic sac.

FIG. 14.—Thoracic compound ganglion after the removal of the cortex. Seen from the ventral side. This and Fig. 12 were drawn from models reconstructed from sections.

Pr.G., Ms.G., Mt.G. Pro-, meso-, and meta-thoracic ganglia. *A.G.* Abdominal ganglion. Other lettering as in Fig. 11.

FIG. 15.—The tracheal sacs supplied by the posterior thoracic spiracle.

In this figure the left side of the thorax has been removed, together with the wing muscles and the posterior sterno-dorsales. It must be imagined that this figure is superimposed on Fig. 13.

do. Dorsales. *l.th.s.* Lateral thoracic sac. *m.v.s.* Median ventral sac. *v.th.* Posterior thoracic spiracle. *sc.s.* Scutellar sac. *st.do.* Sterno-dorsales.

FIG. 16.—Wing. The nervures are drawn slightly thicker than they naturally are.

an. Anal lobe. *al.* Alula. *as.* Antisquama. *A.* Anal cell. *A₁*. Anal nervure. *Cu.* Cubital cell. *1 Cu.* First cubital cell. *cu-a.* Cubito-anal transverse nervure. *C₁*. Costa. *C.* Costal cell. *1 C.* First costal cell. *M.* Medial cell. *m.cu.* Medio-cubital transverse nervure. *m.* Medial transverse nervure. *2 M¹, 2 M².* First and second second medial cells. *M1+2.* Medial longitudinal nervure. *M3+Cu.* Medio-cubital longitudinal nervure. *R.* Radial cell. *R1 to R4+5.* Radial longitudinal nervures. *Sc.* Subcostal cell. *Sc₁.* Subcosta.

PLATE 25.

FIG. 17.—The alimentary canal as it is seen on dissection from the dorsal side. The malpighian tubes have been omitted, and also the distal portion of the lingual salivary gland (*s.lg.*) of the right side. The duct of the crop (*Cr.*) is shown by the dotted line beneath the proventriculus (*Pv.*) and ventriculus (*Ven.*).

p.int. Proximal intestine. *d.int.* Distal intestine. *rect.* Rectum.

FIG. 18.—Portion of a transverse section of the oral lobes, showing the two types of gustatory sense organ, etc.

g.s. Gustatory seta. *g.p.* Gustatory papilla. *hy.* Hypodermis under which lies a pigmented layer. *p.s.* Pseudo-trachea in section. *s.g.p.* Sensory bulb of gustatory papilla. *sp.* Sensory bulb of gustatory seta. *tr.* Trachea.

FIG. 19.—Transverse section of labial salivary gland, to show the structure of the gland cells (*g.c.*). (Camera lucida drawing.)

hy. Hypodermis. *ic.d.* Intracellular duct. *p.s.* Pseudo-trachea. *od.* Opening of intracellular duct into the permanent vacuole (*vac.*) of the gland cell.

FIG. 20.—Section through the proventriculus and the anterior end of the ventriculus, to show the structure of the proventricular plug (*Pv.p.*) and the ducts of the œsophagus (*œs.*) and crop (*d.cr.*). (Camera lucida drawing.)

FIG. 21.—The posterior region of the alimentary canal, to show the rectal glands (*rect.gl.*) with their tracheal supply, the origin of the malpighian tubes (*malp.*), and the position of the rectal valve indicated at \times .

FIG. 22.—Transverse section of the lingual salivary gland, showing the fibrillar character of the gland cells. $\times 220$. (Camera lucida drawing.)

PLATE 26.

FIG. 23.—Female reproductive organs in situ; the left ovary and the viscera have been removed. The ovipositor (*ovp.*) is shown retracted, in which state the common oviduct (*c.o.d.*) is doubled back.

ac.g. Accessory gland. *a.c.v.* Accessory copulatory vesicle. *ov.* Ovary composed of about seventy ovarioles, and containing ova in various stages of development. *ov.d.* Oviduct. *retr.m.* Retractor muscles of the ovipositor. *Sp.* Spermatheca or vesiculae seminales.

FIG. 24.—The male reproductive organs. They have been slightly spread out, and the rectum (*rect.*) has been turned over to the right side.

d.e. Ejaculatory duct. *e.a.* Ejaculatory apodeme. *e.s.* Ejaculatory sac. *te.* Testis. *v.d.* Vas deferens.

FIG. 25.—Vertical section of one of the rectal glands, to show its structure. $\times 56$. (Camera lucida drawing.)

sh. Perforate chitinous sheath. *rgl.* Gland cell. *tr.* Trachea.

FIG. 26.—Terminal region of the female reproductive organs, showing the accessory glands, etc.

sac. Sacculus. *vag.* The muscular vagina which evaginates during copulation; a pair of retractor muscles are shown. Other lettering as in Fig. 23.

Trichomastix serpentis, n.sp.

By

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Scholar of Trinity College, Cambridge.

With Plate 27, and 2 Text-figures.

THE subject of this paper is a parasitic flagellate which I have obtained from the rectum of *Boa constrictor*, L. Owing to the kindness of Mr. W. A. Harding, of Histon, Cambridge, I was enabled to examine one of these snakes shortly after it had died of canker of the mouth. In the rectum there was about 30 c.c. of a brownish, almost odourless, alkaline fluid, containing many organic particles in suspension. This fluid was transferred to a glass dish, and prevented as far as possible from evaporating by covering with a thick glass plate fixed down by vaseline. It was by examination of this culture from time to time that the observations here recorded were made.

The first examination was made upon October 30th, 1906, one day after the death of the snake. *Trichomastix* was found to be present in small numbers. The parasites increased in numbers in the culture, and reached a maximum at the beginning of December, 1906. A decline in the number of the organisms present in the fluid then followed, accompanied by several curious changes in the animals themselves. At the end of February, 1907, there were very few specimens to be found after a very careful search, and by March 2nd, 1907, all the parasites had died out. Alto-

gether I was able to keep the organisms alive in this manner for a period of 120 days. All attempts to grow the organisms in other fluids (solutions of peptone, albumen, etc.) were unsuccessful. The death of the culture was not due to increase in the number of bacteria or to a change in the reaction of the medium, both of which remained very much as they were in the beginning. The numbers of the bacteria were somewhat reduced however. Death resulted, I believe, from a too great increase in the amount of katabolic products.

The method of examination was as follows:—A few drops of the culture were drawn up in a fine pipette (used exclusively for this purpose), and examined fresh either in a hanging drop preparation or under a coverslip with wax feet, and waxed round the edges. In this latter anærobic condition I have been able to keep the animals alive and active for thirteen days. For examining the living animals I used almost exclusively a 2.5 mm. apochromatic water immersion objective by Zeiss, with compensating oculars 2, 6, 12, and 18. Most of the observations here recorded were made from the living animal. Good permanent preparations were exceedingly hard to obtain owing to the small numbers of the parasites and the large amount of gritty foreign matter in the fluid. However, a few successful preparations were made, the stains employed being Delafield's hæmatoxylin, Heidenhain's iron hæmatoxylin, and Giemsa's stain. Observation of the living animal was often facilitated by intravital staining with neutral red. Brillanteresyblau (Grübler) and methylene blue were also tried, but proved to be of but little use.

From the morphological characters of this parasite there can be no doubt that it is properly referable to the genus *Trichomastix*, Blochmann. Hitherto this genus has been represented by but a single species, *T. lacertæ*, Bütschli. The life-history of this form has been fully worked out by Prowazek. It is, I think, highly probable that the form which I am about to describe will prove to be quite common

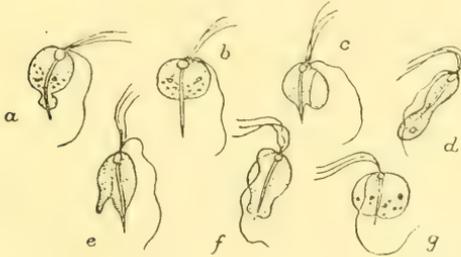
in Ophidia generally. Unfortunately I have not been able to obtain suitable material for deciding this point up to the present. I venture, however, to give the specific name *serpentis* to this parasite.

It is possible that the form under consideration is identical with that described by Grassi under the name *Monocercomonas coronellæ* from the gut of *Coronella austriaca*. The description given is, however, not sufficiently accurate for the identity to be established. The same may be said of an organism described by Hammerschmidt in the cloaca of *Tropidonotus natrix*, and named by him *Cercomonas colubrorum*. This form appears to be the same as *Monocercomonas colubrorum* (Hammerschmidt), Doflein, and Bodo *colubrorum* (Hammerschmidt), Saville-Kent.

STRUCTURE, ETC.

Trichomastix serpentis is usually of a more or less oval or pyriform shape. It is subject to considerable variations however. Some of the more commonly occurring modifications of its form are seen in text-fig. 1 *a-g*.

TEXT-FIG. 1.



Most of these shapes are only temporary, the animal usually returning after a time to a condition more or less

closely corresponding with that depicted in Pl. 27, fig. 1. This shape may therefore be considered as "normal." The length varies from about $8\ \mu$ to $17\ \mu$, an average-sized animal being about $14\ \mu$. At the anterior end [see Pl. 27, fig. 1] are inserted three anteriorly directed flagella, in length about one and a quarter times that of the body. From the same basis arises another and longer flagellum, which is directed backwards (Schleppgeissel). The spot from which these flagella arise appears in the living animal as a refractive granule, situated immediately above the nucleus, which is placed anteriorly. In stained preparations this basal granule is seen to be composed of a chromatic substance which stains like the nucleus. Running through the whole length of the body of the animal is a somewhat flexible axial rod (Achsenstab), which terminates anteriorly in the basal granule of the flagella, and posteriorly is drawn out into the caudal process of the body. The axial rod either traverses the nucleus, in order to reach the base of the flagella, or else lies in close contact with it. It is impossible to be quite certain of the exact relationships of these structures. The rod is, I believe, skeletal in function. Its general aspect and relations to the nucleus recall the axial rods of the pseudopodia of the Heliozoa. Near the base of the flagella is to be seen a well-marked cell mouth or cytostome.

The creatures exhibit great activity of movement, which does not appear to be affected in the very least by light, as is the case with some flagellates. Warming appears to increase their activity, but no critical experiments on the effects of temperature were made. The food consists of the small micro-organisms abounding in the medium.

Division.—Division is, as in most flagellates, longitudinal, and presents certain features of interest. Briefly, the process is as follows [see Pl. 27, figs. 2—10]:—An ordinary individual [fig. 2] becomes more or less globular [fig. 3]. At the same time the axial rod is absorbed, that is to say, it disappears. The flagella are now seen as a very rapidly vibrating bunch, springing from a refringent spot—the

basal granule. This latter loses its connection with the nucleus, and divides, so that two bunches of flagella are formed. It has been found impossible to count the flagella at this stage, or to determine how the new ones arise. Concomitantly the nucleus becomes dumb-bell shaped [fig. 4]. The organism then assumes a roughly triangular appearance, but is rapidly drawn out, and becomes somewhat sausage shaped. A constriction appears in the middle [fig. 6], and it is now possible to make out that there are four flagella at either end, one in each case directed away from the other three. The nucleus is still dumb-bell shaped, and situated mesially. The daughter-cells now rapidly draw apart [fig. 7], and remain connected by only a small bridge of protoplasm, at either end of which is a nucleus, the daughter nuclei being still connected by a very fine protoplasmic strand. Further separation now takes place, finally resulting in the snapping of the connecting strand of protoplasm [figs. 8, 9]. In the daughter individuals which are thus separated, it will be seen that the nuclei lie at the extreme posterior end of the body, and there is no axial rod. Up to this stage the process usually occupies about twenty minutes. If one of these daughter cells be carefully watched, it will be seen that the nucleus travels forwards, and as it does so the axial rod is seen to be developed in the region immediately posterior to it. The rod appears to be formed by the nucleus in its track as it passes forward [figs. 9, 10]. After some time—usually one to two hours—the nucleus reaches the anterior end of the body, and enters once more into relation with the basal granule of the flagella. In some way the axial rod becomes connected with the basal granule, but it is impossible to see how this is effected. The cytostome appears to be lost prior to division, and to be re-formed in each of the daughter cells, but I am not quite clear on this point.

This account of the division of *T. serpentis* differs from that of *T. lacertæ* as described by Prowazek. If I understand this author correctly, the axial rod would appear to function as a kind of division centre; for it retires towards

the nucleus, arranges itself there at right angles to its original position, and the chromatin passes along it forming a nucleus at each end.

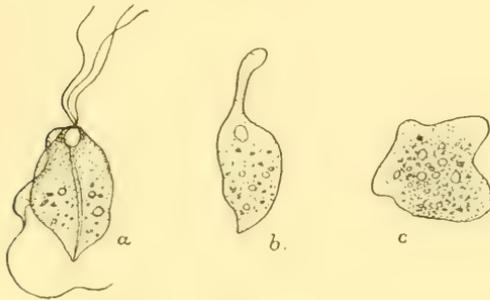
Unfortunately, in spite of much careful observation, I was unable to ascertain whether conjugation occurred or not. In *T. lacertæ* only autogamy occurs, and has been very carefully worked out by Prowazek in his excellent paper on parasitic flagellates. I have frequently seen two individuals apply themselves to one another for a short time, but they always separated without any conjugation taking place. In some cases a very delicate cyst wall was formed, the animal becoming globular, and a number of refractive bodies were developed inside. In the course of a few hours, however, these forms always disintegrated.

DEGENERATION AND DEATH.

As already mentioned, it was found possible to keep the organisms in cultures for 120 days, but no longer. The animals all underwent degenerative changes, and finally died. A very great variety of forms occurred among those animals which were degenerating. The following is the course of events which usually took place, though many variations were seen. First, the animals cease to move from place to place. Movements of the flagella continue, but at a slower rate, the animal remaining approximately in the same position. At this period the posterior flagellum frequently shows a tendency to become adherent to the body, a delicate membrane uniting them together [see text-fig. 2, *a*]. In this condition the creature is almost indistinguishable from a *Trichomonas*—a genus which differs from *Trichomastix* in possessing an undulating membrane (often with a short free flagellum) in place of the posterior flagellum. This adhesion of the posterior flagellum soon becomes more extensive, and finally the flagellum is completely merged in the protoplasm of the body. The move-

ments of the anterior flagella are meanwhile becoming slower, and they very frequently become completely fused into a single finger-like process [text.-fig. 2, *b*], which continues to contract in the same direction as did the flagella. This state may continue for hours, or even days. The axial rod disappears before long, and finally the whole organism becomes an amœboid mass of protoplasm [text.-fig. 2, *c*]. In this condition no movement away from the original position takes place, the protoplasm being simply thrust out and then rapidly withdrawn. Undulating movements, involving the

TEXT-FIG. 2.



whole amœba, frequently and rapidly occur. The creature remains in this condition sometimes for days. Before death the movements become slower, and finally cease. The animal then becomes rounded off, and, after a varying period of time, disintegrates. Not uncommonly two or more creatures, degenerating side by side, run together and fuse before death. This is even more remarkable in the case of the degenerate forms which attempt to divide. All the stages of division up to that corresponding with fig. 7, Pl. 27, may be gone through, when suddenly both individuals, instead of separating, become amœboid and fuse. Death follows, as in the case of the ordinary amœboid forms. In all cases disintegration of the nucleus appears to occur before that of the cytoplasm.

A very remarkable fact was sometimes observed in the involution forms. An individual, instead of dividing when it reached a certain size, continued to grow. In this way giant individuals arose, which reached the enormous length of $30\ \mu$, i. e. about twice the normal length. Very few of these were observed, but quite a number reached a length of $20\ \mu$ — $24\ \mu$. In these exceptionally large forms I was able, in an unexpected manner, to confirm my previous observations on the inter-relationships of nucleus, flagella, and axial rod. Structures which had been made out with great difficulty, and after many hours' watching, were here seen very plainly after an examination lasting a few moments. These giant forms divided abnormally, commonly giving rise to three or four daughter-cells [see fig. 14, Pl. 27]. Division rarely became complete, the whole usually fusing into a large amoeboid mass, which finally died. Unfortunately, owing to insufficiency of material, I was never able to obtain stained preparations of these stages. It could be seen in the living animals, however, that each of the daughter individuals possessed a nucleus, axial rod, and full complement of flagella. Sometimes the old axial rod was seen sticking out of the central mass of protoplasm [fig. 14].

A very similar atypical division has been described by Prowazek in the allied form, *Trichomonas lacertæ*, Prow. —apparently as a normal occurrence. I cannot think, however, that this is a normal process in *Trichomastix serpentis*.

Before concluding I may call attention to the resemblance between the basal granule of the flagellar apparatus and the blepharoplast of a trypanosome. As I find that a detailed comparison of these structures has been made already by Laveran and Mesnil in the case of the closely allied form *Trichomonas*, I refer the reader who may be interested in such speculations to their original paper.

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EXPLANATION OF PLATE 27,

Illustrating Mr. C. Clifford Dobell’s paper on “*Trichomastix serpentis*, n. sp.”

[The drawings from the living animal were all made under a Zeiss 2·5 mm. water immersion apochromatic objective (apert. 1·25). Those from stained preparations (Figs. 11, 12, 13, and 15) under a 3 mm. oil immersion (apert. 1·40) by the same maker. Compensating oculars, Nos. 2, 6, 12, and 18, were employed.]

FIG. 1.—Living *Trichomastix serpentis*, showing details of structure. At the upper end is seen the large vesicular nucleus, upon which lies the basal granule of the flagella. The axial rod is seen supporting the basal granule and flagella; and passing backwards, in relation to the nucleus, it traverses the whole length of the body and terminates in the caudal process.

To the left of the nucleus is seen the cystostome. Numerous food particles are seen in the posterior part of the body. This figure is drawn on a larger scale than the others.

Figs. 2—10 show division as observed in the living animal. (For the sake of clearness, the nucleus is represented as being more distinctly outlined than is really the case.)

FIG. 2.—Animal before division.

FIG. 3.—Animal becoming rounded posteriorly. Axial rod disappearing.

FIG. 4.—Nucleus has become dumb-bell shaped, and there are now two bunches of rapidly vibrating flagella, at the base of which a refringent spot is visible, no longer attached to nucleus.

FIG. 5.—Flagella separating—likewise the nuclei. These latter are still connected by a protoplasmic strand, however.

FIG. 6.—Body has become elongated, and a constriction has appeared. Nuclei still connected; and flagella now distinctly seen to consist of two groups of four each, one being in each case directed away from the other three.

FIG. 7.—Separation of daughter-cells from one another. Nuclei still connected, and lying between the two individuals.

FIG. 8.—Daughter-individuals nearly drawn apart.

FIG. 9.—One of the daughter-individuals soon after separation. The nucleus, which lay at the extreme posterior end, is now making its way anteriorly, and the axial rod is seen behind it.

FIG. 10.—Later stage of same individual. The nucleus has not yet reached the base of the flagella, and the growth of the axial rod is therefore not yet complete.

FIG. 11.—Preparation, showing nucleus, basal granule, and flagella, etc.

FIG. 12.—Stage in division, corresponding with Fig. 4. The chromatin masses at the bases of the groups of flagella are well shown.

FIG. 13.—Specimen at about the stage seen in Fig. 10.

FIG. 14.—Large individual dividing into three. Living animal.

FIG. 15.—Degenerate animal which has become amoeboid. The nucleus is breaking up, and there are, in addition, many food particles in the cytoplasm.

The specimens reproduced in Figs. 11, 12, 13, and 15 were stained by Giemsa's method.

Notes on Common Species of Trochus.

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With Plate 28.

WHILE the present work was in progress a paper on Trochus was published by Randles (10), and stated several results to which our study had also led us. In view of these published results, the present paper has been shortened as far as possible, and includes only some supplementary observations on special points not particularly studied by Randles.

Our observations, in the main, confirm those he has described. We find, for example, the pericardial communications of the kidneys just as he has stated, and are thus led to the belief that the Rhipidoglossa possess, typically, a pericardial canal for each kidney, the gonad communicating with the pericardial canal of the right kidney.

Randles rightly refers to the unsatisfactoriness of Pilsbry's subdivision of the old genus Trochus or the family Trochidæ from the anatomical point of view, and we have found the two commonest species of Trochus of interest in this connection. In Pilsbry's work Trochus lineatus, Da Costa, often, and perhaps better, styled *T. crassus*, Montagu, becomes *Monodonta crassa*, Montagu, and belongs to the group of the Trochininæ. In discussion we shall use for it the name *T. crassus*, Montagu. Pilsbry makes *T. umbilicaris*,

Montagu, into *Gibbula obliquata*, Gmelin, belonging to the group of the *Gibbulinæ*. For the purposes of this paper we call it *T. obliquatus*, Gmelin. The fault of the Pilsbry system is that it is based too exclusively on conchological differences, too little being known about detailed structure to make an anatomical survey possible. In the definition of the group *Trochininæ*, however, we find that members of this group do not possess jaws, and, as those structures are present in *T. crassus*, Montagu, just as in *T. obliquatus*, Gmelin, the unsatisfactoriness of the system is further demonstrated. These jaws also show very interesting adaptations in their structure and relations.

They are paired chitinous plates formed in the same way as those of *Haliotis* (3). Here, as in *Haliotis*, the anterior and downward-pointing ends of the jaw-plates do not lie against the actual gut wall, but against mouthward-outgrowths of the latter, so that the anterior edges of the plates are to some extent free (fig. 1). The jaw-plates in these species are much smaller and thinner than those of *Haliotis* and the *Docoglossa*. In *T. crassus*, Mont., they are merely a pair of dorso-lateral plates on projections of the wall of the buccal cavity, continuous with one another across the median line. The tissue beneath them, instead of being primarily muscular, as in *Haliotis*, has a structure (fig. 2) rather like that of the superficial parts of the odontophore cartilages. It shows muscle-fibres running in small strands through what is practically a mass of "cartilage" of loose texture. The contrasts in structure point to functional differences between the jaws of *Trochus*, and those of *Haliotis* and other *Rhipidoglossa*. In *Haliotis* the jaws are distinctly lateral, and their strong brush-like free edges help actively in the work of bringing food fragments into the mouth. In *Patella* the two jaws are connected dorsally, and the edges form a strong arch which leaves space enough beneath for the protrusion of the unusually broad and solid odontophore cushion; the dorsal part of the jaws is fairly flexible, and the lateral edges are thus able to help in cutting food when the animal is browsing on a

seaweed. The jaws in the species of *Trochus* mentioned are not strong enough to be useful in these directions. They probably protect and give firmness to the upper lip, leaving the work of drawing in food particles to the odontophore and the lip papillæ.

Randles states that there is close agreement in anatomical characters between the various species of *Trochus* studied by him, and this agreement is very marked in the two species already named. Still, there remain differences between *T. crassus*, Mont., and *T. obliquatus*, Gmel., which it seems possible to correlate with the differences in their habits. This must not be taken to imply, however, any belief that the two species are specially related to one another, we have no evidence at present on this point.

T. obliquatus, Gmel., occurs in abundance where there is a reasonable amount of plant life and adherent growth on rocks washed by the tide, and it frequents both the upper and the under sides of boulders, often retiring beneath when it is exposed to strong tide wash, or to heat, strong light, or possibilities of drought when the tide is away. It crawls over and browses on a great variety of Algæ, but has hitherto been classed too exclusively as a vegetable feeder. It is almost omnivorous, and in early summer may often be found attacking the egg-fringes and chains of Nudibranchs and other forms. Remains of eggs and other animal matter are frequently seen in sections of the gut.

T. crassus, Mont., is found, to some extent, with the previous species, but it lives, for the most part, nearer high-tide level, so much so that specimens may remain for a considerable time in corners washed only by high spring-tides. It crawls over the rocks chiefly above half-tide level, but is more lethargic than *T. obliquatus*, and less inclined to browse on the larger Algæ. During stormy periods, especially in winter, numbers may be found huddled in sheltered nooks, often with a number of *Littorina littorea* as companions.

As is well known, the spire of *T. obliquatus* is typically much lower than that of *T. crassus*, and this is probably

correlated with the greater activity of the former in the shore-zone where a high spire would give too much purchase to a side blow from a wave.

BRANCHIAL CAVITY.

(a) Mucus Gland.—The mucus gland of *T. obliquatus* is developed on the right side of the rectum in ridges and furrows running towards the anus (fig. 5). This tissue is evidently the equivalent of the mucus gland of the right side in *Haliotis* and *Pleurotomaria*, and its disposition reveals its primary function of coating rough excreted fragments (expelled from the right kidney or the anus) with slime, so that they may be less likely to injure the gill-leaflets and other delicate organs before being washed away. There is also a special aggregation of this tissue around the aperture of the right kidney (fig. 5). The mucus gland of the left side is not nearly so well developed in *Trochus obliquatus* as in the more primitive *Rhipidoglossa*. It forms ridges which coat the transverse pallial vein just in front of the external aperture of the left kidney, and stretches forward along the rectum to some extent; it is developed also along the afferent axis of the ctenidium, where this axis unites with the roof of the branchial cavity, and especially at the front end of the uniting fold. This indicates that its special function is to protect the main blood channels of this region and the gill leaflets hanging in the branchial cavity from the damage due to grit or hard fragments which may have wandered into the cavity or may have been expelled from the kidneys or the anus.

In *T. crassus* the mucus gland epithelium is found in the corresponding places, but is also present on ridges in the roof of the branchial cavity between ctenidium and rectum to a far greater extent than in *T. obliquatus* (fig. 4). This comparatively greater development of the mucus gland in a

high tide form contrasts with the reduction of the gland observed by Pelseneer (6) in the high tide *Littorinas*. There can be no doubt, however, that the roof of the branchial cavity in the latter, with the prolongation of the ctenidial leaflets across it, is much more specialised for respiratory purposes. The mucus gland of *T. crassus* preserves the condition found in many other species of the genus, and, in spite of its approach to a high-tide habitat, there is greater need for damp protecting slime here than in *Littorina*. *T. crassus* keeps its foot in contact with the rock throughout a period of exposure, while a high-tide *Littorina* withdraws deep into its shell, the edge of which remains adherent to the rock by means of a dried film of mucus. In other words, the branchial cavity of *T. crassus* cannot be nearly so completely protected (by retraction) from drying during a period of exposure as can that of *Littorina*, and the greater development of mucus gland may be a compensation for this. *T. crassus* is found in shadier and less exposed corners than the high-tide *Littorinas* often manage to occupy, and not usually so far up the shore.

(b) Gill.—The gill is built on the same lines in the two species under discussion, but shows several specialisations on the condition found in more primitive *Rhipidoglossa*. The loss of the right gill and the migration of the anus and excretory openings towards that side has been discussed by Ainsworth Davis (1) as a device for more complete separation of incurrent and excurrent streams through the branchial cavity. The surviving left ctenidium in *Trochus* remains biseriata, as in *Haliotis* and *Pleurotomaria*, but the details of its disposition are very different, as is well known. In those more primitive types a median axis between the two series of leaflets contains the longitudinal afferent and efferent blood channels of the ctenidium, and that axis is attached to the wall of the branchial cavity on the side of the efferent channels only. Right at the back of the cavity the afferent axis is also attached to the roof on either side (fig. 8), and the basi-branchial sinus, which feeds the afferent ctenidial

channels, runs across the roof of the cavity in the region of these attachments.

In *Trochus* this connection of the afferent side of the gill axis with the roof of the cavity has extended much farther forwards. This has raised the lower series of gill-leaflets so that they hang in the cavity instead of almost, if not quite, resting on its floor. In this new position they are more efficiently bathed by the incoming water, and are less likely to impede its course, and they are also more easily kept from "packing together." The dorsal series of leaflets is, however, necessarily enclosed in a pocket through this development, and, in the species considered, this series is reduced as compared with the more freely hanging ventral leaflets.

In detailed structure the ctenidial leaflets of *Trochus* resemble in the main those of *Haliotis* (fig. 6). They are epithelial folds with a foundation of elongated cells below the epithelium of each surface, and bridges of cells across the cavity, which is a blood space. Between the epithelium and the cells which more or less line the blood space, we find, as in *Haliotis*, a development of chitinous substance, the ends of the chitin plates towards the "efferent" border of ctenidial leaflet being thickened. Away from this thick part, the chitinous layer thins out, and is soon no longer observable; but, where the efferent border of the leaflet meets the efferent side of the gill axis, it can be seen that, as in *Haliotis*, the chitinous plate of one side of a leaflet is continuous with that of the opposite face of the next leaflet. A nerve runs along each border of the leaflet beneath the epithelium. The epithelium (fig. 6) along the efferent border is mostly ciliated, and it is composed of high and narrow cells, some of which have basal nuclei, and apparently nerve connections, so that they are very probably sensory cells. A little way in from this border the epithelium is a good deal higher, very regular, and close set, and the surfaces of these extra high cells have a thick covering of uncertain nature (fig. 6). In this way the height of the band is much increased, and, as the bands of successive leaflets are opposed

to one another, they must act as cushions to keep the leaflets from packing together, the thickened chitinous plates already mentioned contributing to stiffen the leaflet.

The surface of the leaflet is thrown into folds, which run fairly parallel with the line where the leaflet unites with the gill axis. The epithelium of this part of the gill varies very much in appearance according to the exact direction in which it happens to be cut, but it is not nearly so high as that along the efferent border.

The afferent border of the leaflet is the one which is most directly exposed in the branchial cavity. Along this edge the epithelium is fairly high and regular, and includes mucus-secreting cells; we do not think it is ciliated (fig. 6). There are no supporting chitinous plates and no cushions near this border; if the topographically lower efferent edges of the leaflets are stiffened and held apart that suffices to keep the leaflets from packing against one another. The mucus-secreting cells of the afferent border must help to keep the leaflet from injury due to rough fragments rubbing against this exposed part. In *Trochus*, but not in *Haliotis*, we find the afferent border somewhat expanded (fig. 6), and this may be a cushion arrangement, so the statement above made perhaps needs modification. The appearance of odd fragments in the mucus outside the expanded border suggests that the swelling may have the added value of hindering the entry of these fragments into the chinks between the leaflets.

NOTES ON THE KIDNEYS.

The kidneys of *Trochus* were described by Perrier (8) and Haller (4), and Thiele (12) added the observation of a "nephridial gland" along the left side of the left kidney. Randles (10) and Pelseener (7) have both described the pericardial communications of the kidneys, and Randles has added correct sketches of the cells typical of the right kidney. We

give sketches illustrating the types of cell found in both kidneys (figs. 10—12), with the corresponding cells from *Haliotis* (figs. 14 and 15) to show the close agreement between these two types; our figures do not agree with those of Perrier and Haller.

The nephridial gland of *Trochus* (cf. Perrier's *Glande nephridienne* of *Monotocardia*) is composed of a set of branching tubules opening into the kidney cavity, and embedded in a tissue rich in blood vessels (Perrier's *Glande hématique* of *Monotocardia*). The cells lining the tubules of this gland (fig. 12) are much lower than those of the left kidney. The function of this tissue, as, indeed, the function of the whole left kidney, is quite unknown, but it is, perhaps, important to notice that it occurs along that side of the kidney from which blood channels go to join the efferent ctenidial vein, and to form with it the left auricle. If the nephridial gland of *Trochus* is really the homologue of the nephridial gland in the *Monotocardia*, this is an argument in favour of the view of Lankester (5), Pelseneer (7), and the embryologists that the kidney of *Monotocardia* is equivalent to the left kidney of the *Diotocardia*. Perrier, Woodward (13), and one of ourselves have urged the opposite view on other grounds which cannot be altogether set aside, and the question must be further discussed after more comparative and embryological research, some of which is now being undertaken.

Circulation.—In its main features, the circulatory system of the species of *Trochus* considered agrees with that described by one of us for *Haliotis* (3). Blood leaves the ventricle by the aorta, the communication being guarded by a simple valvular flap. The aorta bifurcates soon after leaving the heart, and one branch goes to the visceral hump while the other runs forward and ensheathes the radular sac. This is as in *Haliotis*, but here the blood channel is practically embedded in the side of the shell muscle for some distance. Still surrounding the radular sac it reaches the head where a great deal of its blood seems to be directed into spaces

surrounding the pedal ganglia and pedal cords. Thence blood is distributed throughout the foot. It appears to gather again in a median sinus above the nerve-cords, which opens in front into the general cavity of the head. The blood in the head is thus in part returned from the foot, and in part supplied from the branch of the aorta which surrounds the radular sac. The muscles of the odontophore are probably mainly supplied from the latter channel, while the blood from the former source is very likely partly aërated in the head when the animal is extended and active.

The blood from the general cavity of the head is collected into a fairly definite channel which carries it back to the right kidney. These species of *Trochus*, however, differ from *Haliotis* in that the anterior lobe of this kidney is much reduced, so that the blood channel does not run nearly all the way in the kidney wall, as is the case in *Haliotis*. The right kidney also receives blood from the visceral hump further back. In fact the blood flow from the head is by no means so intimately connected with the right kidney as in *Haliotis*. That organ must, in *Trochus*, be mainly a purifier of blood from the visceral hump.

An afferent channel from the right kidney takes blood from these two sources into the roof of the branchial cavity at the back, and runs across beneath the rectum to the left kidney, but first gives off a channel into the branchial roof. We look upon this last channel as a probable homologue of the afferent channel of the right ctenidium of *Haliotis* which has been lost in *Trochus*. The efferent channel from the right kidney thus corresponds with a part of the basi-branchial sinus of that type.

The blood reaching the neighbourhood of the left kidney, as just described, goes in part to that organ, but the main flow runs forward in a subrectal sinus, and then turns to the left, crossing the roof of the branchial cavity, as the transverse pallial vein of most authors (figs. 4 and 5).

Before reaching the left kidney, however, it communicates with the right auricle, which also receives a channel from the

branchial roof. Following Thiele, we homologise the latter channel with the efferent vein of the right ctenidium (lost in *Trochus*) of *Haliotis*.

In *Haliotis* the right auricle is supplied from the right efferent ctenidial vein, and also connects with the basi-branchial sinus, so conditions are essentially similar in *Haliotis* and *Trochus*, allowance being made for the loss of the right ctenidium in the latter.

The channel from the right to the left kidney in *Trochus* together with the forwardly running subrectal sinus and the transverse pallial vein, may be homologised with the basi-branchial sinus of *Haliotis*. The changes in detail are connected with the large size of the left kidney and its position in the roof of the branchial cavity.

As already stated, the blood spaces beneath the epithelium of the left kidney are supplied from the channel arriving from the right kidney, and so some of the blood (particularly that from the visceral hump) may be purified from nitrogenous waste. That from the head is, however, by no means so completely purified as it has really only skirted the right kidney.

The transverse pallial vein shows interesting differences in the two species considered. It is equally obvious on dissection in both, mainly owing to the covering ridges of the mucous gland, but the blood space is relatively larger in *T. obliquatus*, Gmel., than in *T. crassus*, Mont. In the former, also, its anterior and posterior branches are essentially afferent ctenidial channels sending blood through the gill axis into the leaflets. In *T. crassus* the gill does get some blood in this way, but a great part of the contents of the transverse pallial vein goes out into the roof of the branchial cavity which also receives blood from the sub-rectal sinus. There seems, therefore, ground for the opinion that the roof of the branchial cavity in this mid- to high-tide form is of considerable importance in respiration, while the ctenidium cannot have quite so much importance as in the other species.

The blood from the ctenidium together with that brought

by various channels from the roof of the branchial cavity goes back along the efferent ctenidial channel to the left auricle, which also receives important supplies from the left kidney. The left kidney thus gets its blood from the efferent channel of the right kidney, and passes it on to the left auricle, but its blood spaces are intimately associated with those of the neighbouring part of the roof of the branchial cavity, so perhaps a good deal of what it contributes to the left auricle has been aërated.

As the efferent channel from the right kidney communicates with the right auricle before reaching the left kidney, past which blood flows to the left auricle, it follows that right and left auricles are not so very indirectly connected with one another. This is another point of resemblance between *Trochus* and *Haliotis*.

Reference has been made to the possible respiratory activity of the roof of the branchial cavity, especially in *T. crassus*, Mont., and it is interesting to notice that the corresponding tissue seems to have acquired respiratory functions in *Patella*, which is also often a mid- to high-tide form.

In a recent paper Spillman (11) incidentally mentions the course of the circulation, but the vagueness of the statements made renders a discussion of his views unnecessary. He has evidently not given any special attention to this particular subject.

Bionomical Notes.—*T. crassus*, Mont., is often found in fairly large numbers huddled together in protected gullies and corners during the winter, especially when the weather is stormy. In summer it is interesting to watch these animals, often following one another's tracks as if the fresh trail of mucus were a help in progression. They also have the habit of mounting on one another's shells, where they apparently browse the small Algæ, etc., on the surface. As *Trochus* has no mating habits (in common species, at any rate), these peculiarities in behaviour may be thought of as the indistinct beginnings of that mutual recognition, perhaps

mainly through chemical sense impressions, which must be a first step in the evolution of the mating tendency.

Probably some of the ancestors of the *Montocardia*, which have mating habits, went through somewhat similar stages in the course of their evolution. Prof. Ainsworth Davis has suggested to us that the egg-eating habit of certain species of *Trochus* should also be considered in connection with the development of mating habits.

Our material was obtained from the shores of Cardigan Bay, south of Aberystwyth, which show for many miles as a plane of marine denudation formed by fairly evenly worn ridges of Silurian grits and slaty shale. The old valleys in the Silurian rocks, of which most of the coast cliffs are built, are filled in some cases by the boulder clay of the glacial epoch. The small streamlets which run down from the cliffs thus drain sometimes off the boulder clay, and sometimes off the shales and grits. We find that in several cases *T. crassus* occurs in abundance where the shore is washed by streams from the boulder clay, but disappears sometimes almost abruptly when the boulder clay makes way for the Silurian rock. The same thing is true, though in a lesser degree, for *Trochus obliquatus*, and the fact seems to be of sufficient interest to be chronicled, though we have not yet been able to approach an explanation. The boulder clay is found on analysis to be rich in salts of potash and magnesium. *Trochus* is not found much within three miles of Aberystwyth at present; the boulder clay in this immediate neighbourhood has probably been carried by a different set of glaciers from that flowing seawards further south. The pollution of the Ystwyth and Rheidol rivers by lead works may also be a determining factor.

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EXPLANATION OF PLATE 28,

Illustrating the paper by Dr. H. J. Fleure and Miss Muriel M. Gettings on “Notes on Common Species of *Trochus*.”

REFERENCE LETTERS.

Aff. Afferent side or axis of the ctenidium. *Ch.* Chitinous plate. *Ct.* Ctenidium. *Ct.Aff.* Afferent blood channel of the ctenidium. *Ct.Eff.* Efferent blood channel of the ctenidium. *Eff.* Efferent side or axis of the ctenidium. *L.Au.* Left auricle. *L.K.Eff.* Efferent channel from the left kidney. *L.K.* Left kidney. *Muc.gl.* Mucus gland. *N.G.* Nephridial gland. *Osph.* Osphradium. *R.* Rectum. *R.K.* Excretory channel of the right kidney. *R.Au.* Right auricle. *S.R.S.* Subrectal sinus. *St.* Strengthening tissue beneath the jaw-plates.

- FIG. 1.—The jaw-plates of *Trochus crassus*, Mont.
FIG. 2.—A section of the jaw-plates.
FIG. 3.—Cells from the strengthening tissue beneath the jaw-plates.
FIG. 4.—The roof of the branchial cavity in *Trochus crassus*, Mont.
FIG. 5.—The roof of the branchial cavity in *Trochus obliquatus*, Gmelin.
FIG. 6.—An oblique transverse section of a gill leaflet of *Trochus*.
FIG. 7.—Section of the roof of the branchial cavity and gill of *Trochus*.
FIG. 8.—Section of the roof of the branchial cavity and gills of *Haliotis*.
FIG. 9.—Section of the left kidney of *Trochus* in the region of the nephridial gland.
FIG. 10.—Epithelium of left kidney of *Trochus*.
FIG. 11.—Epithelium of right kidney of *Trochus*.
FIG. 12.—Epithelium of nephridial gland of *Trochus*.
FIG. 13.—Section of pericardium of *Trochus* showing the blood channels which feed the left auricle.
FIG. 14.—Epithelium of the right kidney of *Haliotis*.
FIG. 15.—Epithelium of the left kidney of *Haliotis*.

Note on the Formation of the Skeleton in the Madreporaria.

By

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WITHIN the last few days there has been brought under my notice a short account by M. Armand Krempf¹ of the mode of origin of the calcareous skeleton in Madreporarian Corals.

M. Krempf, whose observations were made on a species of the genus "Seriatopora," writes as follows:

"It is easy to verify that the corallum ("polypier") of this animal, in conformity with the investigations of Heider, is formed by the imbrication of a multitude of small calcareous scales, themselves constituted by a small bundle of fibres of lime carbonate.

"I have been able, in addition, to demonstrate by means of a decalcification conducted with great caution, the substratum of organic matter in the midst of which the calcareous part of this scale has taken solid form.

"A very fine membrane limits each of these elements, tending thus to give it the physiognomy of a small cell. After suitable treatment of the fresh skeleton with decalcifying reagents, the whole of the delicate organic meshwork (trames agglomérées) forms a light translucent mass, similar to jelly and of extreme fragility.

¹ A. Krempf, "Sur la formation du squelette chez les Hexacoralliaires à polypier." Note by M. Armand Krempf, presented by M. Yves Delage ('Comptes Rendus,' 21st January, 1907).

"Thus there exists, contrary to the opinion of Gardiner (1900), who was not able to disclose it in his preparations, an organic substratum in the skeleton of the "Coralliaries."

"Contrary again to the opinion of Duerden (1904), who admits the existence of a fundamental substance, but who considers it as homogeneous, this substance has a histological constitution, well characterised, which realises all the appearances of a cellular structure.

"These facts much depreciate in value Koch's hypothesis of extracellular secretion. They seem to fully justify Heider, who considers the 'polypier' as formed by the accumulation of calcified cells.

"At the same time this is not so; the calcareous scales whose juxtaposition forms the corallum are not the skeletons of calicoblasts. They have not the value of cells."

Before proceeding further with M. Krempf's description a few remarks may be made regarding these preliminary statements.

The first statement that M. Krempf "finds it easy to verify that the skeletal parts are formed by the imbrication of a multitude of small calcareous scales" is a corroboration of observations which were unknown to zoologists previous to the publication of my work on the "Madreporaria."¹

That paper for the first time gave a description and illustrative figures of the minute skeletal elements which everywhere compose the corallum. I described these elements as "calcareous scales," and showed the imbricated manner of their apposition with one another, and their various shapes and positions at different parts of the corallum (l. c., pp. 114, 115, 127; figs. 7 A—C; 8 A—C, etc.).

The references made by M. Krempf to von Heider's work are somewhat ambiguous, as they lead one to infer that von

¹ M. M. Ogilvie, "Microscopic and Systematic Study of Madreporarian Types of Corals," 'Phil. Trans. Roy. Soc.,' London, vol. 187 (1896), B, pp. 83—345. Also see my monograph on the "Stramberger Korallen" (Palæontographica, Stuttgart, 1897, Suppl. ii, No. 7, pp. 73—282, pls. vii—xviii).

Heider had deciphered this structure in 1881. Full references to the papers of von Heider and Koch are made in my work (l. c., pp. 91—97). Von Heider made a special study of the soft parts, and observed here and there certain cells in whose organic contents small groups of calcareous fibres were included, and the nuclei were either shrunken or vanished. To these cells he gave the name of “calicoblasts,” and thought they occurred in the mesoderm, but must, in some way, accumulate to build the corallum. As I explained in my paper, Heider’s surmise was that the individual skeletal parts in the Madreporaria were formed in a manner analogous with the skeleton of Alcyonarians, but that in the former the calicoblasts produced a solid structure at the outer limit of the mesoderm, whereas the analogous cells in the Alcyonarian polyp formed calcareous spicules, which remained isolated throughout the whole life of the polyp (l. c., p. 93, and cf. Heider, “Korallenstudien,” 1886). Heider admitted he could not trace the connection between the groups of fibres as he observed them in cells of the embryonic skeletal disc, and the various complex structures of the mature skeleton.

Subsequent observers said that the cells referred to by von Heider were components of the ectodermal tissue, and that they never contained inorganic material as he depicted; that the calcareous material of the corallum was originally of the nature of a secretion thrown out by the cells of the ectoderm (ref. Ogilvie, 1896, l. c., pp. 101—2).

This was the position when my work was published by the Royal Society of London, and I there demonstrated that the whole corallum was composed of a series of calcareous lamellæ, each of which was primarily an organic tissue comprising innumerable minute cellular parts, in each of which lay a group of calcareous fibres. As these parts corresponded in appearance to the “calicoblast” described by von Heider, I applied this term and wrote: “Each lamina (average width .003 to .005 millim.) is a deposit of calicoblasts, the wavy outline corresponding to originally separate cells” (l. c., p. 123; pp. 114—117, etc.).

I demonstrated by descriptions and illustrations (p. 123, 124, 138; figs. 13, 14, 17, etc.) that several layers of calicoblasts were shed from the ectoderm of the polyp during each period of active growth, and that these adhered more closely with one another than with the previously or subsequently formed series of layers, yet that each layer was complete in itself; farther, that each individual calcifying part originally limited by organic walls (which I termed cell-walls), retained its individuality during its transformation into a "calcareous scale," and could be obtained apart from its neighbours as an individual entity.

It creates rather a confusion of ideas when M. Krempf implies that von Heider had held the view that the "calcareous scales" were the "skeletons of calicoblasts"—since von Heider was not aware that the "juxtaposition of calcareous scales formed the corallum," and he could not possibly have claimed for these skeletal elements, as I did, that they were transformed cell-products, and represented the "fibre-containing cells," which he had observed in the cellular tissue, and termed calicoblasts (cf. Ogilvie, l. c., p. 125).

The second part of M. Krempf's account in the 'Comptes Rendus' describes a series of observations which bear upon the relationship of the ectoderm to the layer of skeletal elements immediately external to it. Before quoting from M. Krempf, I shall indicate the direction followed in my investigations of this subject.

My sections of the soft parts corroborated the observations of Dr. Fowler and Dr. Bourne (cf. Ogilvie, l. c., pp. 101-2). Comparing my sections of the soft parts with my preparations from the corallum, I was able to observe farther that in these positions, such as the periphery of the calyx, which corresponded to the most closely nucleated and actively-dividing parts of the ectodermal tissue, the calcareous scales were most thickly piled in the corallum, and their organic remnants ("dark points") most closely grouped—sometimes in little rings, sometimes in rows.

"In all cases we have simply to do with centres and axes

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" (l. c., p. 128; refer to p. 133, fig. 18; p. 148, fig. 29, etc.).

The wider spacing or closer massing of the unit elements in the skeletal layer was thus shown to depend upon localisation of "centres of calcification" in the ectoderm, and the number produced in a single growth-period to be greatest where fissional activity was most marked in the ectodermal layer. Careful measurements conducted over many species farther showed that the size of the "calicoblast cells" in the ectoderm was the same as the size of the unit elements in the skeletal layer (p. 117, 136, etc.). Upon such grounds "I took the nuclear fission to be associated with the separation of the organic outer layer" containing the skeletal elements, and concluded that, in virtue of fissional processes in the cellular tissue of the ectoderm, calcifying calicoblasts were constantly being eliminated, and the vitality of the ectoderm itself renewed.

"The fibre-containing calicoblasts which lie next the skeleton are shed off, so to speak, from the polyp, new cells constantly taking their place in the ectoderm by cell-division" (Ogilvie, l. c., p. 102).

"At the beginning of any particular growth-period the calcification goes on only locally at certain points of the calicoblastic layer of the colony" (id., l. c., p. 131; cf. p. 143).

"The calicoblasts remain adherent to one another in dense groups or may be more uniformly distributed. And in this manner they are gradually left behind on the skeleton, and completely calcify, while active cell-division develops constantly new ectodermal cells. The calicoblasts adherent to the skeleton represent such as were already in course of losing living continuity with the polyp at the time when the polyp was removed from the skeleton" (id., l. c., p. 116).

"The scale-like arrangement on the surface presents irregularities—sometimes like thicker zones, sometimes thicker

patches. Those are doubtless due to irregular disposition of the calicoblasts, as they originally separate from the ectodermal polypal layer" (id., l. c., p. 116).

Mr. Duerden's work,¹ referred to by M. Krempf, brought the first confirmation of my description of the primarily organic nature of the layer external to the ectoderm in which the skeletal elements developed, but Mr. Duerden described it as homogeneous, and thought it took origin from the ectoderm probably by a process of secretion, and that the calcareous groups of fibres which made their appearance must be regarded as "ectoplastic" in origin. Against this I wrote as follows:

"On Mr. Duerden's interpretation, if I understand it aright, we are to believe that the exceedingly particular 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." ('Quart. Journ. Micr. Sci.,' 1905.)

"Each individual lime-forming part or 'calicoblast' of the skeletal 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" ('Quart. Journ. Micr. Sci.,' vol. 49, pt. 1, Oct., 1905, pp. 207, 208).

M. Krempf now recognises the distinct partitioning of the groups of calcareous fibres by organic strands, but says although these simulate cell-walls in their appearance, the contained

¹ J. E. Duerden, "The Coral *Siderastraea radians* and its Post-larval Development," Washington, U.S.A., publ. by Carnegie Institution, Dec., 1904.

portions have not morphologically the value of cells, as nuclei can only be shown to be present in some of them. The following is M. Krempf's description :

"In the ectoderm which carpets the skeleton, and which consists of a protoplasmic layer with scattered nuclei, in which it is somewhat difficult to define cellular limits, one sees the skeletal element appear in one place and another under the form of a small mass, readily stainable with nucleus dyes.

"It presents distinct fibrous structure. Its fibres, clearly individualised, are in general regularly parallel with one another and most often are disposed perpendicularly to the surface of the calicoblastic epithelium. Its shape varies according to the points of the skeleton where one observes it. Sometimes the shape is that of a small parallelepiped, fairly regular and flattened, sometimes it recalls that of a Lepidoptera scale, sometimes even that of a cup compressed parallel with its vertical axis and hollowed by a not very deep cavity."

The setting of the fibres in the layer external to the ectoderm and in all the skeletal lamellæ was shown in my illustrations and was very frequently described by me, as also the varieties of form displayed by the skeletal elements at different parts of the corallum (l. c., pp. 113, 121, 125, 128, 136, 137, 138, 144, etc.). To continue M. Krempf's observations :

"There is always a small nucleus, homogeneous and highly chromophil, placed at one of the sides of the mass.

"This element, arrived at its complete development, calcifies entirely. It then ceases to form part of the still living cell within which it is developed and adheres to the skeleton with which henceforth it is embodied. But the nucleus of the cell which formed it is not carried away with it. It withdraws on the contrary into the midst of the protoplasmic layer which remains living, and after a period of rest, the duration of which I cannot fix, it presides at the elaboration of a new element similar to the preceding.

"It is only after having assisted at the formation of a

certain number of these elements that it becomes embodied in the last-formed, finally removed from the living layer and incorporated in the skeleton where good preparations enable it to be recognised. It has then almost completely lost its sensitiveness to colour, but it has preserved almost without alteration its characteristic shape and its dimensions.

“In the preparations which I have examined and which up to the present only concern a single species, I have found an average of 150 scales unprovided with nuclei for one nucleated scale. If one is justified in supposing that none of the nuclei disseminated in the skeleton can escape observation, the necessary conclusion would be that one determinate calicoblast can, during its life, produce a total of about 150 calcareous scales.”

M. Krempf, in the foregoing description, appears to reserve the term “calicoblast” for that evanescent stage in the production of a skeletal element which immediately precedes its actual separation from the ectoderm, in fact to limit it to the nucleated fibre-containing body in the ectoderm. It must, however, be remembered that von Heider gave the term to fibre-containing bodies in which, as a rule, the nucleus was shrunken or vanished. Thus so far as terminology is concerned, I was strictly accurate when in my work I applied the term to organic, fibre-containing bodies, whether nucleated or non-nucleated; calcareous scales are most certainly what I defined them to be—“calcified calicoblasts.”

It is another question whether each of them may be looked upon as the morphological equivalent of a cell, or cellular part.

Until M. Krempf's evidence is given in full, it is impossible to form any opinion regarding his statement of the oft-repeated withdrawal of a nucleus into the ectoderm. Meantime, from the brief description he now puts before us, I see no reason to infer that the presence of a spent nuclear body in a skeletal element marks out that particular element as the morphological equivalent of a cell, whereas the other skeletal elements have not that morphological value. Each time that

there is a definite aggregation of protoplasmic material with reference to the nucleus, a living cell takes form, and each time that, by an act of fission, an organic, fibre-containing unit is added to the skeletal layer, that unit may be termed the structural equivalent of a cell, although it is not itself living. Some proportion of the nuclear vitality has been employed in the making of each fibre-containing unit. In a word, each calcareous scale represents the product of a nucleated body.

The most important morphological point in M. Krempf's description is his statement that the nucleated cell is finally transferred "from the living layer to the skeleton." This has been the real point of controversy. To quote from my work in 1896:—"Fowler and Bourne have observed the specialised character and large size of the calicoblastic cells at the growing points of the skeleton in several corals, among others *Galaxea*. Both these authors regard the calicoblasts as ectodermal cells, which merely secrete calcareous substance without being themselves calcified." . . . "I find that the cells themselves become incorporated in the skeleton." . . .

On M. Krempf's own showing, this transferred nucleated cell becomes a calcareous scale, undistinguishable from its neighbours; in the farther processes of calcification there is no difference between nucleated and non-nucleated skeletal elements.¹

M. Krempf has given his attention especially to the histological processes, and we may expect a valuable addition to our knowledge of these. The outstanding features in his

¹ It may be of interest to add that in my preparations I sometimes observed traces of nuclei with the organic remnants in calcareous scales; in some, the reaction to stains was quite marked; in others, very faint; and in still others, no nucleus could be discerned by means of stains. I felt I could not draw any secure conclusion as to the absence or presence of the nuclei, and knowing how irregularly they were distributed in the ectodermal tissue, I did not follow this line of observation, but in my published work limited myself to the statement that each "scale" contained organic remnants.

results are his recognition of the controlling agency of ectodermal nuclei, and of divisional processes in ectodermal tissue as the means of origin of the external organic layer in which are contained the primary groups of calcareous fibres. These, together with his observation of the marked individualisation of each calcareous group, confirm the interpretation I gave of the relationship between the ectoderm and the skeletal parts.

Studies in Spicule Formation.

VII.—The Scleroblastic Development of the Plate-and-Anchor Spicules of *Synapta*, and of the Wheel Spicules of the *Auricularia* Larva.

By

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With Plates 29 and 30, and 6 Text-figures.

Part I.—The Spicules of *Synapta inhærens* and *S. digitata*.

INTRODUCTORY.

THE complete morphogenesis of the curious aggregate (8) plate-and-anchor spicules of *Synapta inhærens* has been previously described and figured by Semon (6), but, with the exception of my description of the disposition of the scleroplasm associated with the adult spicules (7), no account of the part played by the living tissues of the organism in the production of these wonderful structures has hitherto been published.

These plate-and-anchor spicules are so well known that it is not necessary for me to do more than briefly mention their more obvious characteristics. Each spicule, as seen in the integument of *Synapta inhærens*, e. g., consists of two parts—the anchor and the plate—and these two parts are quite separate from each other. The anchor consists of a shaft, a bow (with its two arms), and a handle—to use the current terminology; the plate, on the other hand, has several large perforations, and is somewhat more pointed in shape where it comes into apposition with the handle of the anchor than at the opposite side (fig. 23). This more pointed region

of the plate I shall term the base, and the opposite, more obtuse region the apex. In side aspect it can be seen that the handle of the anchor and the base of the plate form a distinct joint, the one with the other (fig. 25), and the anchor and the plate rotate upon each other at this joint, the angle included between the two in consequence varying in magnitude (Östergren, 4). It can also be observed in side view that the arms of the anchor bow do not lie in the same plane as the shaft, but in a plane slightly more inclined towards the dermal layer, i. e. more nearly parallel with the plane of the plate (text-fig 1).

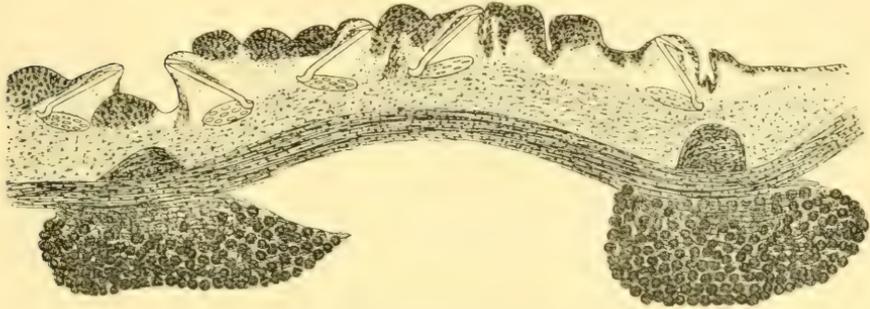
Respecting the general arrangement of the spicules, it is an obvious and noteworthy fact that the major axes of these plate-and-anchor spicules, as they lie in the body-wall, are disposed transversely to the long axis of the animal, so that in a transverse section of *Synapta* the spicules are seen in side aspect (text-fig. 1). Further, although the spicules never assume, as regards their major axis, other than a transverse, or approximately transverse, disposition in the body-wall, it is quite a matter of chance as to whether the bow of the anchor and apex of the plate of each spicule lie to the right or to the left of the observer; in other words, they are disposed pretty equally in both positions.

A few words concerning the preparation of *Synapta* material for the study of the spicule development are necessary. My material was obtained from Naples, and consisted of specimens of the two species—*Synapta inhærens* and *S. digitata*. My specimens of *S. inhærens* averaged 1 cm. to 1.5 cm. in length, and they were specially prepared for me by the osmic acid and picro-carminic method described in Study IV, e. g.¹

¹ I am indebted to Dr. P. Mayer for recommending me his picro-magnesia carminic stain as a substitute for the Ranvier and Weigart preparations. The objection to these latter is their inconstancy of composition—the free ammonia evaporating and the carminic being precipitated; an excess of free ammonia of course tends to macerate the tissues. I must say, however, that with one exception I have always used Ranvier and Weigart with perfect success, and so long as free ammonia is absent there is little objection to them, at least in practice.

I may mention that after fixation it is well to slit open the wall of the *Synapta* so as to stain the integument from the inner side as well as the outer. The integument is subsequently cut into convenient pieces before mounting, and, like the *Cucumarias* of Study IV, immersed for about five minutes in a saturated solution of lichtgrun¹ in absolute alcohol, then washed well in absolute, cleared in xylol, and mounted in balsam, the inner side of the wall being placed uppermost on the slide. To observe the spicules in side view, I cut transverse sections of the undecalcified² body-wall about 12 μ to 20 μ in thickness or slightly thicker. Numerous sections must be cut, since

TEXT-FIG. 1.



Camera lucida drawing of a portion of a transverse section through the body-wall of the adult *Synapta* in hærrens, showing the spicules in position. The longitudinally-wrinkled condition of the dermal epithelium and the dermal anchor-pockets are observable. These latter are more apparent in a surface-view of the integument. The thickenings of the dermal epithelium are the sense-organs. (Reprinted from 'Quart. Journ. Micro. Sci.,' vol. 49, p. 557.)

the majority of spicules are fractured during the process of cutting, and it is only occasionally that the knife happens to fall just on each side of the (usually young) spicule instead of on to it.

My specimens of *S. digitata* were considerably older, none being less than 5 cm. in length; nevertheless, I have been able to observe all stages in development (though

¹ In the plates a grey tint has been substituted for green.

² Sections of the decalcified body-wall I found to be useless.

naturally a far greater amount of material had to be examined) excepting the initial granule. I may here remark that the numerous specimens labelled "*Synapta digitata*" which have been forwarded to me differ considerably in their spiculation—in the forms and sizes of the spicules—and I cannot help suspecting that the *S. digitata* examined by me includes several distinct species, or at least sub-species. The specimens of *S. inhærens* and *S. hispida*, on the other hand, have always been constant in their spicule characters. In most specimens of *S. digitata* the spicules differ considerably in size and form in the same animal—a feature I have not noticed in the other two species.

THE DEVELOPMENT OF THE SPICULE IN *SYNAPTA INHÆRENS*.

The first sign of the future spicule is the multiplication of the cells of the dermal or outer layer at one point. I say "cells," but, strictly speaking, it is a multiplication of nuclei to form a syncytium, since cell-outlines are rarely, if ever, distinguishable. This preliminary formation of a syncytium is best seen in a section of the body-wall (fig. 1). It will be observed in fig. 1 that in these young *Synaptas* the dermal layer is already quite separate from the circular muscle layer, the intervening space containing, at this stage, irregular strands of nerve- and muscle-fibres. These initial syncytia are easily distinguishable in section from the numerous thickenings of the dermal layer which form the sense-organs, since the elongated cell-outlines and pigment deposits are conspicuous features in these latter. It is also easy to distinguish them in surface view under a low magnification after a little practice. The sense-organ thickenings are, needless to say, much the more numerous.

The actual spicule first appears as a small spherical granule situated in the centre of the syncytium, but more internally than externally, so that the majority of the nuclei are situated on the other side of the spicule when this is viewed from the internal side of the body-wall (fig. 2). This spherical granule

next elongates on one side (figs. 3 and 4) in a direction transverse to the long axis of the animal. Thus, remarkable as it may seem, the minute rod which the granule gives rise to, and which, in its turn, becomes the anchor of the adult spicule, is from the very first orientated in the direction assumed by the full-grown structure. The process formed from the side of the initial granule (situated to the right or to the left of the observer, as the case may be) continues to grow (figs. 5-10) until there is produced a stick-like structure, equal in length to the future anchor. One end of this stick-spicule is somewhat swollen to form a knob, and represents the unmodified half of the original granule—the other half having grown out to form the stick. The stick is also usually of slightly greater diameter mid-way in its length than towards its extremities; in other words, the stick tapers somewhat, both towards the knobbed and the pointed ends. Also it possesses, at this stage, a distinct axial thread. The nuclei of the syncytium, up to this period of growth, and until the knobbed end of the stick has produced the recurved arms of about half the adult length, remain, for the most part, on the outer side of the stick—i. e. towards the external dermal layer—although one or two may be situated more internally (figs. 9, 10, 11). I may also mention that the syncytium surrounding the spicule usually remains in connection with the epithelium from which it originated by means of a few irregular protoplasmic strands.

The next stage in the development of the anchor spicule is the protrusion of both sides of the knobbed extremity of the stick or shaft to form the arms of the future bow of the anchor (fig. 10). These incipient arms elongate, and very soon become recurved; at the same time this lateral extension of the knobbed extremity stretches the substance of the syncytium enveloping the spicule so as to produce the appearance shown in figs. 16 and 18.

When the arms of the anchor have become half-grown a very remarkable phenomenon occurs in connection with the syncytial nuclei, situated a little bow-ward of the middle of

the shaft. A number of these (usually from six to ten), which up to the present have been situated either at the sides of the shaft or on its external aspect, now migrate on to its internal side, and form a small cluster in that position (fig. 12). This fact seems to me to be most remarkable and I am quite unable to account for it, at least in an adequate manner. This cluster of nuclei is always easily distinguishable from the rest of the syncytium, and it is this specialised part which gives rise to the plate spicule. Thus, although the anchor and plate portions of the entire aggregate spicule are quite separate, and without doubt equivalent to two spicule individuals—to two echinoderm plate-spicules, to be precise—yet they are both developed from the same syncytium, though in distinct parts of it.

The plate-spicule, like other spicules, first originates as a granule, and this is situated in the centre of the internal cluster of nuclei (fig. 13). This granule next elongates on opposite sides and at right angles to the length of the anchor-shaft (cf. the development of the plate-and-anchor spicule of *Synapta digitata* described below) to form a rod or rhabdus (figs. 14, 15, 16) which is at first pointed at both ends. The nuclei of the cluster are situated on both sides of this rod, and in fact the entire development of the plate spicule is identical with that of the plate spicules of the Cucumaridæ described by me in Study IV, with the exception that the number of nuclei initially concerned is greater in the present instance. The rod thickens at its extremities (fig. 16), then bifurcates (fig. 18), these primary bifurcations elongate and themselves bifurcate (fig. 20), and the processes of bifurcation and fusion of the extremities continue (figs. 21, 22), as in the Cucumarian plate-spicule, until the adult plate is produced (fig. 24). The *Synapta* plate-spicule is obviously different in several respects from the Cucumarian plate-spicule, but the plan of structure is the same. The *Synapta* plate possesses extremely large perforations, which diminish in size towards the more pointed "basal" extremity, and the edges of these perforations develop small processes which

add considerably to the picturesque effect of the whole. As the spicule increases in size, the nuclei of the portion of syncytium concerned in its formation distribute themselves more uniformly over its area. Apparently very few, if any, nuclei are subsequently added to those initially present in the internal cluster which gives rise to the plate-spicule. It must also be remarked that the special internally-situated portion of the syncytium which produces the plate becomes almost quite separated from the rest of the syncytium enveloping the anchor as the growth of the plate proceeds, since the anchor and the plate gradually diverge to include the angle previously mentioned (text-fig. 1); the syncytia of the anchor and of the plate in fact alone remain continuous at the joint formed between the handle of the anchor and the base of the plate, and this continuity of the two syncytia in this region probably serves to some extent both to keep the two structures in apposition and to render the joint a true joint, i. e. a mutual centre of rotation (see fig. 44, in Study IV, 7).

To return to the later development of the anchor. Up to the time when the arms of the anchor-bow are but half-grown, the syncytium in this region is, as before mentioned, stretched to form two "patagia," so to speak, on the two sides of the shaft (figs. 16, 18). But on further elongation of the arms the central portions of the patagia apparently give way, with the result that the scleroplasma remains in the neighbourhood of the shaft as a thin layer, and away from the shaft as "two elongated strands of protoplasm containing many nuclei which run on either side from the arms of the bow to the handle" (Study IV). When the arms are fully formed, these two strands (figs. 20-24) are thickened at the bow end, forming "blobs," and the nuclei at this end of the anchor are almost entirely collected in these two thickenings. There is also in each strand another such collection of nuclei situated in the vicinity of the handle. Nuclei do occur in other parts of the strand, but they are principally aggregated in these two regions; they also occur, of course, on the shaft, bow, and handle, though not in clusters. Thus the formation of

these conspicuous strands of protoplasm joining the extremities of the arms with the handle is due to the presence of these arms and not vice versâ, as I suggested when I first observed the scleroplasm associated with the fully-formed spicule (7). The strands are not muscular and exercise no "tractive action" which can account for the recurved shape of the arms, i. e. they do not exert an active pull producing this effect, though it is possible that they have a slight passive influence in this connection.

THE DEVELOPMENT OF THE SPICULE IN *SYNAPTA DIGITATA*.

As might be anticipated, the development of the plate-and-anchor spicules of *Synapta digitata* proceeds on very much the same lines as that of *Synapta inhærens*, but there is one difference which, though slight, is yet remarkable on account of its striking incomprehensibility. In the last section I described the plate of the spicule as arising in the form of a rod disposed transversely to the length of the anchor-shaft (figs. 16, 18, e.g.). In *S. digitata* the plate also arises as a rod, but curiously enough it is disposed parallel with the anchor-shaft and not transversely to it (figs. 17, 19). I have observed scores of young plates of both species of *Synapta*, but I have never observed a single exception to this rule, that the plate-rod of *S. inhærens* is disposed at right angles to, and the plate-rod of *S. digitata* parallel with, the length of the shaft.

THEORETICAL CONSIDERATIONS.

The distribution and disposition of the plate-and-anchor spicules in the body-wall of *Synapta* are subjects which first demand our attention. Respecting the former there is little to say, since the spicules are uniformly spread over the entire area of the body-wall. However, it is worth remarking that the production of these relatively few but huge spicules in *Synapta* involves the concentration of numerous scleroblasts

in a limited number of centres—i. e. involves the formation of syncytia, whereas in the Cucumariidæ, e. g. in which the spicules are relatively numerous but small, the scleroblasts either remain independent or only associate in pairs. In other words, given a certain number of scleroblasts in the organism, these can either aggregate in some degree to produce a relatively few huge spicules, or can remain independent and produce numerous small spicules. What factor determines the choice of these alternatives in any given instance I do not pretend to know, and I can only remark, on Spencerian grounds, that the Synapta condition is the more highly evolved.

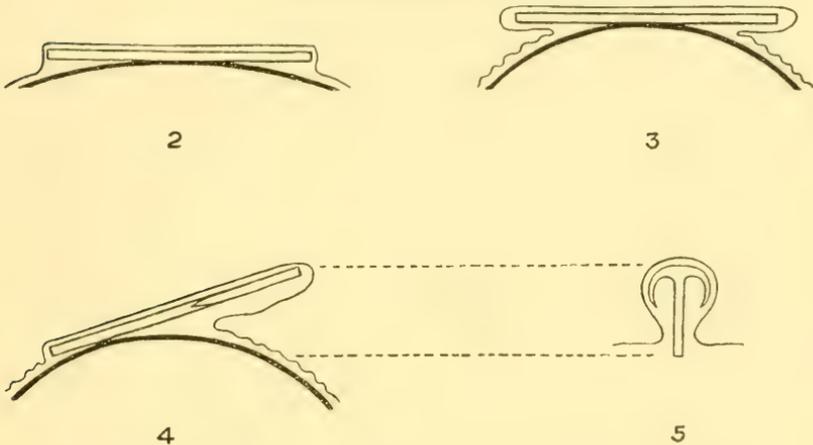
With regard to the conspicuously definite disposition of the spicules in the body-wall, the explanation of this feature is, I believe, not difficult to find. In the first place it must be remembered that the body-wall of Synapta is highly contractile, the creature being able by means of its powerful longitudinal muscles to contract itself with ease to a considerable fraction of its normal length. This longitudinal contraction must and does involve a transverse wrinkling of the body-wall, and this contraction not being an infrequent expression of the animal's activity, it seems clear that the initial granule of the spicule will elongate in the transverse grooves formed in the body-wall during such contractions. It is only necessary to mount and examine a contracted portion of the body-wall of Synapta to see that rigid structures like the spicules must lie in the grooves so formed, and no objection can be taken to this view on the ground that the outgrowth of the initial granule is appropriately orientated from the very first, since the grooves may be assumed to be quite capable of determining the direction of the initial as well as of the later growth of the spicule. Whether the granule elongates on one side or the other I believe to be purely a matter of chance, the spicules being, as before-mentioned, pretty equally disposed in both directions, as they should be, in the absence of any determining factor. Why the granule only elongates on one side and not on both I am unable to

say. The contractility of the animal is then in all probability the cause of the definite transverse disposition in the body-wall of the long axes of the anchors.

The shape of the anchor can also, I believe, be associated to some extent with the contractility of the body-wall. The body-wall of *Synapta* not only possesses bands of longitudinal muscle, but also a continuous sheet of circular muscle, the presence of which latter implies that the body-wall can diminish in diameter. Now the body-wall consists in the main of two layers: the external thin dermal layer and the internal, comparatively thick, sheet of circular muscle, and between these two layers the elongated spicules lie, on a bed of fibres, in a transverse position. If the circular muscle-layer be imagined to contract, then it is evident that the dermal layer, which at first is uniformly attached to the muscle-layer, will be thrown into longitudinally-disposed folds (see text-fig. 1), and that the position of these longitudinal folds will, to some extent, be determined by the spicules. In other words, the dermal layer will adhere to the contracted muscle-layer in those parts of the circumference which are devoid of spicules, but that, where spicules are present, the dermal layer will, by the inevitable protrusion of elongated spicules situated transversely on a diminished circumference, be separated from the muscle-layer in the form of pockets pushed out by the anchors. Consider the mechanical aspect of the matter. Rods lying between two connected layers forming the wall of a cylinder and situated transversely with regard to its long axis will, if their length be adapted to the circumference of the cylinder, not project on the surface at their extremities in any appreciable degree (text-fig. 2). If, now, the inner layer only of the cylinder contract in a considerable degree, it is evident that the extremities of the rods will project on the exterior and that the outer layer will be thrown into pocket-like folds enveloping the extremities of the rods, but that in the portions of the circumference situated between the rods it will still remain more or less attached to the contracted inner layer, though in a longitu-

dinally-wrinkled condition (text-fig. 3). If, further, we suppose that, in some way, the rods are caused to project on the exterior by one extremity only, the pocket-like folds will be rendered still more conspicuous (text-fig. 4). These conditions are those found in the case of the adult spicules of *Synapta*, the last possibly being brought about by the continued attachment of the knob end of the primary anchor rod to the dermal epithelium from which it originated, and by the connection of the handle extremity of the shaft with the

TEXT-FIGS. 2, 3, 4, and 5.



These diagrams illustrate the formation of the dermal pocket which envelops the anchor-bow described in the text. In fig. 2 the rod is lying tangentially on the uncontracted circular muscle-layer of the animal; in fig. 3 the circular muscle-layer has contracted, and in consequence the outer dermal layer is thrown into longitudinal folds and forms two pockets at the extremities of the rod; in fig. 4 the anchor protrudes on the surface at one extremity only, with the result that one large dermal pocket is formed; in fig. 5 the anchor and dermal pocket enveloping it are viewed from the surface, though they are supposed to be foreshortened.

plate, which lies internally and parallel with the muscular layer (see text-fig. 1).¹ The knobbed extremity of the anchor

¹ I cannot guarantee that the position of the spicules relative to the dermal- and muscle-layers represented in figs. 11, 12, and 15 is exactly that which obtains in nature, since the process of section-cutting is liable to shift the spicules.

supporting a pocket-like protrusion of the dermal epithelium, it seems probable that the lateral extension of the knob to form the recurved arms of the anchor-bow is, after all, but an illustration of the deposition of skeletal matter in the direction of least resistance. It is an elementary fact that the anchor-bow does lie in a closely-enveloping dermal pocket, which is identical in general outline with the bow, and as fresh calcareous matter has for some reason to be deposited at the knobbed extremity of the anchor-shaft, the suggestion that the form which this additional deposit of calcareous matter takes is largely determined by the enveloping dermal pocket is not a very bold one.

The objection that, were the form of this further deposit of calcareous matter merely determined by mechanical pressure this calcareous matter would simply assume a more or less spatulate form and not the arms of an anchor, may be met by the reply that the bow-form, which the calcareous matter does assume, is not supposed to be solely due to the mechanical contact of the dermal pocket. As is well known, rod-structures in echinoderms generally have, for some unknown reason, a tendency to bifurcate terminally, and though the shaft of the *Synapta* anchor is abnormally large owing to the large syncytium concerned in its production, yet this is no reason why this rod should prove an exception to the general rule. If this be the case, then the two arms of the anchor-bow of *Synapta* must be regarded as the echinoderm rod-bifurcations, which in this case have been secondarily reflected and otherwise modified by the special conditions obtaining. Similarly, the handle of the anchor may be regarded on this hypothesis as an abortive attempt of the shaft to bifurcate at its internal extremity.

It seems useless to attempt any further explanations in connection with the form of the *Synapta* spicule, since such explanations must necessarily, in our present state of knowledge, be vague and unsatisfactory. There is, of course, one obvious question which every intelligent observer of these spicules has asked himself, viz. why are the plates and

anchors developed in connection with each other? But to assume that this question is capable of being answered implies that we know the answers to several questions which naturally precede it. Grant that the huge anchor-shaft owes its size, shape, and disposition to the factors already mentioned, grant also that the relatively small rod of the incipient plate is small because of the small syncytium concerned, yet we are quite ignorant either as to why the six to ten nuclei should migrate in a bunch from the mass of the syncytium on to the internal side of the shaft, or as to why this cluster of nuclei when produced should give rise to a separate plate-spicule. Why should not the internal cluster of nuclei merely deposit an outgrowth of the shaft on its internal aspect in the same manner as the gastral actinoblast of the calcareous sponge deposits the gastral ray on the triradiate basis? Personally, I am as yet quite unable to suggest solutions to these problems.

Before concluding I may mention that occasionally no plate is developed in connection with the anchor, the anchor remaining solitary (fig. 24). For some reason or other, the migration of nuclei on to the internal surface of the shaft has (presumably) not taken place. Whether plate structures are ever developed in *Synapta*, apart from the anchor, I cannot say for certain. I have observed several fully-formed solitary plates, but, although no signs of disturbance were visible, yet I suspect that the anchors had, in every case, been detached. It is, indeed, hard to suppose that the plates would assume the normal shape (which is modified in connection with the anchor¹) when developed by themselves. I have also observed two or three instances of what appear to be young plates developing on their own account, but, again, I cannot be quite certain that they had not become detached from anchor counterparts. Anchors can develop without plates (I think the instances are too numerous to suppose that the plates had merely become detached), but it is very doubtful if normal plates can develop apart from anchors.

¹ This is conspicuously shown in the abnormalities figured by Hérouard (2).

NOTE ON SOME POINTED OBLONG BODIES PRESENT IN THE WALL
OF *SYNAPTA INHÆRENS*.

While searching the internal surface of the body-wall of *S. inhærens* for young spicules, I not infrequently encountered the curious bodies depicted in fig. 26. They were stained a dark green, and were apparently in every case enclosed each in a single cell, which was one of a cluster. The surrounding cells (which often contained large vacuoles not shown in the figures) did not appear to be in any way connected with the cell containing the body, and, so far as I observed, only one of these bodies was contained in each cluster. I did not detect any internal structure in the interior of these bodies. Beyond offering the somewhat trite suggestion that they may be parasites, I am unable to explain their nature.

SUMMARY.

(1) The first sign of the future spicule is the multiplication of the nuclei of the dermal epithelium at one point to form a syncytium.

(2) In this syncytium a calcareous granule is deposited on its internal aspect.

(3) This granule elongates on one side either to the right or to the left of the long axis of the animal to form the shaft of the future anchor; the other side of the granule persists for a while as the knobbed extremity. During development the knobbed extremity remains in apposition with the dermal epithelium, but the opposite end comes into connection with the subjacent fibrous layer, so that the shaft is not situated tangentially in the body-wall but is inclined. The shaft, like the initial granule, lies on the internal aspect of the syncytium, i. e., nearly all the nuclei lie on the external side of the shaft.

(4) When the shaft has attained its full length, and the

knobbed extremity has commenced by lateral growth to give rise to the arms of the anchor-bow, six to ten nuclei of the syncytium travel round in a cluster to the internal side of the shaft about mid-way in its length, and there produce, quite independently of the rest of the syncytium, a granule which elongates and bifurcates and finally becomes the plate much in the same way as that already described for Cucumarian spicules (Study IV). The shape of the Synapta plate is modified in connection with the anchor. Also the portion of the original syncytium devoted to the production of the plate becomes entirely separate from the rest of the syncytium except in the region of the anchor-handle and plate-base, where the anchor and the plate are in contact and include a joint.

(5) In *Synapta inhærens* the rod producing the plate lies transversely to the length of the anchor, whereas in *S. digitata* it as constantly lies parallel. No explanation is offered in connection with this curious fact.

(6) The arms of the anchor-bow are perhaps to be homologised with the almost universal terminal bifurcations of the echinoderm rod-spicule, and their recurved form is possibly due to contact with the dermal epithelium which forms a pocket for their reception. The syncytium in connection with the anchor is stretched by the formation of the arms into "patagia," which subsequently form the conspicuous strands of protoplasm joining the extremities of the arms with the handle. These strands are, as shown by their development, not muscular, and exercise no tractive function which can account for the recurved shape of the arms.

(7) The transverse disposition of the Synapta spicules is attributed to longitudinal contractions of the body-wall.

(8) The shape of the anchor is to be largely attributed to contact with the body-wall, which, it must be remembered, is contractile transversely as well as longitudinally.

(9) The very usual association of anchor and plate is a physiological problem at present insoluble.

Part 2.—The Spicules of the Auricularia Larva.

INTRODUCTORY.

The morphogenesis of the Auricularian wheel-spicule has been described and figured by Semon (5). An unillustrated account of the scleroblastic development of the Auricularian wheel has also been published by Chun (1) in 1892, but, as I surmised in Study IV, Chun's remarkable statements concerning this subject are totally misleading, owing to the fact that he unconsciously studied decalcified material. As will be seen in the quotation given below, Chun described the spicule as being deposited within a mould which is formed for its reception, so to speak, in the substance of the scleroplasm. I am able to say that this supposed mould is nothing but the space contained within the scleroplasm which was occupied by the spicule before it was dissolved away by acid reagents. I have recently observed many of these "moulds" in improperly preserved (decalcified) material, and I am able to make the above statement concerning their real nature because I also possess properly preserved (undecalcified) material.

My material consisted of numerous Auricularia larvæ in different stages of growth obtained at Naples and specially fixed with absolute alcohol. I stained them for a week in a saturated solution of Grüber's safranin in absolute alcohol, and washed out the superfluous stain by keeping the larvæ in warm 90 per cent. alcohol¹ (several changes) for a week; they were then dehydrated, cleared in cedar-wood oil and mounted in balsam in the ordinary way.

THE DEVELOPMENT OF THE SPICULES OF THE AURICULARIA LARVA.

The wheels and "globes," as I shall term the other class of calcareous deposits found in the Auricularia larva, are found

¹ Absolute alcohol with the required percentage of distilled water to ensure neutrality.

in the pair of processes situated at the anal, i. e. lower, extremity of the larva. Both wheels and globes abut against the ectoderm of the larva, though they arise from cells internally situated, i. e. from the mesenchyme cells. But before describing the development of the spicules I will quote the relevant passages of Chun's account of this subject, which, though incorrect as regards the main point, yet contains many true observations. "At the time of the appearance of the first calcareous wheels the cellular elements of the gelatinous substance (filling the interior of the larva) are sharply differentiated into skeletogenous and connective-tissue cells. The latter possess several long processes, which are much ramified, and are interwoven almost after the manner of felt; the skeletogenous cells, on the contrary, are spherical and surrounded by a distinct membrane, in consequence of which they emit no pseudopodia. The sharp histological differentiation of the mesoderm cells, which was certainly preceded by an indifferent stage, may be essentially due to the fact that the calcareous bodies originate at a remarkably late period in comparison with what is found to be the case in other Echinoderm larvæ. The skeletogenous cells accumulate . . . close beneath the ectodermal epithelium." "A richly vacuolate plasma at once distinguishes the skeletogenous cells, the average size of which is 0.01 mm. They rapidly grow to twice and thrice this bulk, while simultaneously the number of the cell-nuclei increases. In the same *Auricularia* we meet with all intermediate stages between uni- and multinucleate cells, which at first still retain a rounded contour, but subsequently flatten out on one side and become cup-shaped. The nuclei measure from 0.003 mm. to 0.004 mm. in length, and originally (so long as only from two to four are present) occupy a peripheral position; they afterwards increase to from six to eight in the case of the Mediterranean *Auricularia*, and to from twelve to eighteen in that of those from the Canary Islands, and form a central nuclear cluster. When the cells have 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. The tubular rays of the star which grow out are equal in calibre and meet the external membrane, arching forwards somewhat at the points of contact. The longitudinal extension of the radially-arranged outgrowths keeps pace with the increase in the size of the cell, and finally, when the cell attains a size of from 0·06 mm. to 0·07 mm., 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; the central portion with the cluster of nuclei corresponds to the nave, the tubes running out like the rays of a star represent the spokes, and the peripheral ring takes the place of the circumference (the felly) of the future calcareous wheel. Moreover the calx is actually secreted into this organic matrix formed by the skeletogenous cell, 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. It is likewise in accordance with the theories which have recently been formulated as to the share of the nuclei in the vital processes of the cell that, corresponding with the centrifugal progress of the calcification, the majority of the cell-nuclei also separate from one another in a centrifugal direction, and in the case of the *Auriculariæ* from the Canary Isles come to lie in the acute angles between the spokes. In rare instances they advance as far as the middle of the spokes, or even to the periphery. No secondary multiplication of the spokes of the wheel takes place; their number corresponds exactly with that of the undulating evaginations of the newly-formed internal membrane, which develop into radiating tubes. As is well known, the number of the spokes varies; in the case of the *Auriculariæ* from the Canaries, we find from thirteen to eighteen. Since the diameter of the fully-formed calcareous wheels is found to be from 0·09 mm. to 0·1 mm., it follows that a ten-fold enlargement of the diameter of the skeletogenous cells takes place, since the latter in the stage

with a single nucleus only measure 0.01 mm. Nevertheless, after the secretion of the calcareous wheels they expand still further; for if we examine the wheels in alcoholic preparations . . . we can distinguish a distinct periphery formed by a delicate membrane, from which, alternating with the spokes and almost equalling them in length, membranous tubes arranged in the shape of a star run to the periphery of the wheel, where they usually exhibit flask-shaped expansions. On careful decalcification of the wheels by means of weak chromic acid it is easy to show the nuclei and the contour of the wheel in the shape of a delicate membranous envelope within the skeletogenous cell. The above statements as to the formation of the wheels in the *Auricularia* reveal a mode of development which at first appears to be unique. While the skeletal pieces of Echinoderms were hitherto essentially regarded as intercellular structures, the formation of which was due to several mobile amoeboid cells (I am well aware that more recent observers are inclined to attribute the shape of the skeletal elements without hesitation to directly mechanical influences), we now find that the form of the calcareous wheel is traced out within a multinucleate cell by means of an organic membrane which assumes complex folds, and that in this definitely circumscribed mould the casting of the hard parts ensues."

I have quoted Chun at length because his remarks show how carefully most of his observations were made and how purely accidental his mistakes were. I will now describe the mode of formation of the wheels and globes in the *Auricularia* larva as it really occurs. I am unable to say for certain as to whether the syncytium which deposits the spicule arises from one cell solely by the simple multiplication of its nucleus as Chun describes, or as to whether it is also formed in part by the fusion of originally separate scleroblasts. I have certainly seen cells containing two and three nuclei, but I have also seen numerous clusters of separate scleroblasts which, unless they take on some other function, must coalesce

with each other to produce the few spicules found; probably both processes occur. The syncytium of the *Auricularia* larva differs from the syncytium of *Synapta* in that in the former each nucleus is almost entirely surrounded by a distinct layer of protoplasm, i. e. cell-outlines are to a large extent visible, each nucleus occupying a distinct subspherical portion of the syncytium, whereas in the latter the individual cells are so fused together as to constitute merely a mass of protoplasm containing numerous nuclei. The syncytium of the *Auricularian* spicule contains, in the specimens I studied, from five to nine nuclei.

The spicule first appears in this syncytium as an endoplastic spherical granule (figs. 27, 28), which is usually situated on the side of the syncytium next to the adjacent body-wall (not indicated in most of the figures). This granule flattens and gradually becomes disc-shaped with a slightly concave internal surface, the centre of which at first bears a slight eminence, and a convex external surface (figs. 31, 32) which is in contact with the body-wall. All the scleroblasts (i. e. the nuclei with their subspherical masses of protoplasm) are situated on the concave side of the disc, i. e. remote from the body-wall, and are clustered together (figs. 31, 32, 34), though of course the entire disc (and all later stages of the spicule) is enveloped by the scleroplasm. The next step in the development is the formation of processes (variable in number, ranging from about fourteen to eighteen) at the margin of the disc or nave of the future wheel (fig. 35), and these processes, which vary in number in different spicules, elongate, and ultimately form the spokes (figs. 36-38). By the development of these spokes (the exact shape of which can be seen in fig. 41) the convexo-concave disc assumes more the shape of a cup. Finally these spokes, by lateral extension, join up to form the felly of the wheel (figs. 39-41). The adult structure then, as shown by the figures, forms a cup-shaped structure, in the concavity of which are lodged the scleroblasts—somewhat like eggs in a basket (fig. 41).

In every case the extension of the scleroplasm which is

necessary for the envelopment of the entire spicule is effected by the growth of the spicule itself, the spokes, e. g. pushing out the peripheral scleroplasm before them as they increase in length. The syncytium no more enlarges itself for the accommodation of the spicule, as Chun imagines, than an adipose tissue corpuscle swells out to make room for the secreted oil.

Unlike Chun I have not observed that "corresponding with the centrifugal process of the calcification, the majority of the cell-nuclei also separate from one another in a centrifugal direction." On the contrary, in the *Auriculariæ* observed by me, they do not desert the nave portion of the wheel. In short, the development of the *Auricularian* wheel resembles that of all other echinoderm spicules in that they are endoplastic deposits, the form of which bears little or no relation to the disposition of the nucleus or nuclei. The study of spicule formation clearly shows that the proximity of a nucleus is not essential to the deposition of skeletal material at any given point, and that deposition goes on in any given region of a cell independently of the distance of the nucleus or nuclei from that region. Nuclear material is in all probability a *sine quâ non* where spicular deposition is concerned, but, as in the political constitution, the exact position of the governing centre is of little or no account in regulating the activities of the various areas governed—work proceeds in all quarters, and not merely in the vicinity of the nucleus or parliament as the case may be.

The development of the calcareous globes of the *Auricularia* is quite simple. The globe originates as a granule in the syncytium and simply increases in diameter by the uniform deposition of calcareous matter on its surface. The globe protrudes on the surface of the body-wall, at least half of its area being in contact with the wall, and the nuclei of the syncytium, probably in consequence of the contact, become restricted to the surface of the internal hemisphere (fig. 42).

THEORETICAL.

With regard to the cause or causes determining the form of the Auricularian spicules, little can be said. It seems probable that the flattened disc- and later cup-forms are at least in part due to contact of the spicule with the body-wall, unless these are indeed to be attributed to the presence of the nuclei on the inner side of the plate only, the nuclei, contrary to the usual assumption, preventing deposition in their immediate vicinity.¹

But whatever forms the spicules may assume, it is clear that one of their inevitable attributes, viz. weight, may play an important part in the life of the larva. In the Auriculariæ I have studied, the wheels and globes are all situated, as before stated, at the lowest extremity of the larva, and it seems certain that this extremity is lowest because of the presence of the relatively weighty spicules in this region. In other words, the larva maintains a certain position in the water because it is appropriately weighted. The so-called baguettes de corps of the pluteus larva doubtless serve a similar function.

SUMMARY.

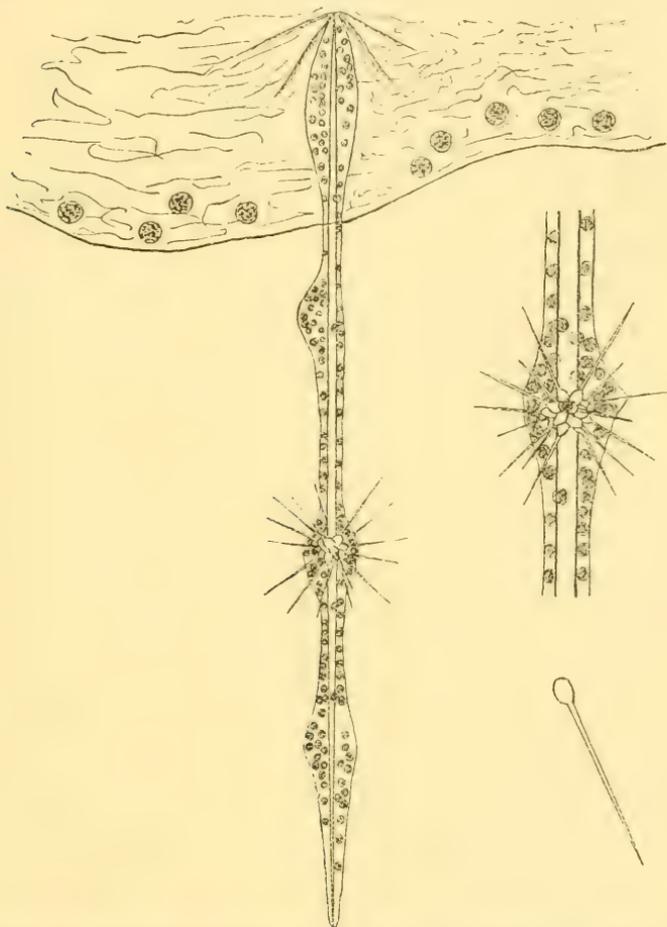
(1) The spicule first appears as a granule contained in a syncytium, in which, however, the scleroblasts retain their individuality to some extent, which is not the case in the syncytium of *Synapta*.

(2) The spicule becomes disc- and then cup-shaped, develops the spokes as outgrowths from the margin of the disc, and finally forms the felly of the adult wheel, the spicule during the whole of its development being enclosed by the syncytium in which all the nuclei (scleroblasts) are situated on its internal side, i. e. away from the body-wall against which the spicule lies.

¹ I may point out in this connection that Hérouard (3) attributed, though quite erroneously, the perforations of the holothurian plate to the presence of nuclei. See Study IV.

(3) The extension of the scleroplasma depositing the spicule is determined by the growth of the spicule itself, and is not

TEXT-FIG. 6.



The abnormal siliceous spicule found inserted into the body of an *Auricularia* larva. The whole spicule is enveloped in a large syncytium. Nearly midway in its length is situated the cluster of curious radiating spines with swollen bases attached to the main shaft. The figure of the entire spicule is magnified just over 500 diameters.

the result of a mould-forming tendency on the part of the protoplasm, as Chun asserted.

(4) The situation of the heavy wheel and globe spicules at one (the lower) extremity of the larva determines the position which the larva assumes in the water—the spicules weight the larva.

NOTE ON A CURIOUS TYPE OF SILICEOUS SPICULE.

The curious spicular structure represented in text-fig. 6 was found inserted for a short distance into the body of one of the *Auricularia* larvæ which I examined. As it is not doubly refractive under the polariscope, I conclude that it is siliceous. It is well clothed in a thick layer of scleroplasm containing numerous nuclei. Being quite unable to identify this spicule I should say that it is probably a freak.

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EXPLANATION OF PLATES 29 & 30,

Illustrating Mr. W. Woodland's "Studies in Spicule Formation. VII.—The Scleroblastic Development of the Plate-and-Anchor Spicules of *Synapta*, and of the Wheel Spicules of the *Auricularia* Larva."

Figs. 1, 9–25 \times 800 diameters; Figs. 2–8, 26–42 \times 1600 diameters.

PLATE 29.

The Development of the *Synapta* Plate-and-Anchor Spicules.

FIG. 1.—Portion of a transverse section through the body-wall of *Synapta nhærens* showing the multiplication of nuclei at one centre on the internal side of the dermal epithelium to form a syncytial mass. To the right lies the circular muscle layer and between this and the dermal epithelium some muscle- and nerve-fibres.

FIG. 2.—A syncytium viewed from the internal side of the body-wall. The initial granule is deposited on its internal aspect—i. e. the majority of nuclei are situated on its external side. These syncytia have very definite outlines, being quite distinct and usually distant from all surrounding structures. It must also be remarked that, owing to difficulties of observation, the exact number of nuclei figured cannot be guaranteed as having been the actual number present. It can, however, be guaranteed that every nucleus figured was carefully observed and correctly placed relative to the spicule, but, as is self-evident, it is impossible to be certain that every nucleus present was observed.

FIG. 3.—The granule has elongated slightly towards the observer's left. It is possible that the syncytium is in part formed by the fusion of at-first-separate scleroblasts as well as by the multiplication of nuclei; this latter, however, is the principal mode of formation of the syncytium. Some of the nuclei are larger than others, a feature probably denoting approaching nuclear division.

FIG. 4.—The initial granule has here elongated to the observer's right hand.

Figs. 5–8 illustrate the further elongation of the granule to one side or the other. The axis of the future shaft is first discernible when the full length is nearly attained. The majority of the nuclei, it will be noticed, lie on the external side of the shaft.

FIG. 9.—The nearly adult shaft.

FIG. 10.—The fully-elongated shaft. The terminal knob (representing the unelongated portion of the initial granule) is now extending laterally. The axis is conspicuous at this stage.

FIG. 11.—The fully-elongated shaft seen in a transverse section of the body-wall. The external position of the majority of the nuclei relative to the shaft is well shown.

FIG. 12.—Adult shaft, in which the arms of the anchor are about half formed, seen in a transverse section of the body-wall. In this figure there is shown in the clearest manner the external position of the majority of the nuclei, and the internal position of the half-dozen or so nuclei which have migrated internally to form a separate cluster in this position. All stages of this migration can be seen in the actual preparations.

FIG. 13.—Spicule viewed from the internal aspect. A granule has been deposited in the internally-situated cluster of nuclei. The formation of the arms of the bow is distending the general syncytium in this region (formation of the "patagia").

FIG. 14.—The granule is elongating on both sides in a direction transverse to the length of the anchor-shaft.

FIG. 15.—The same stage of growth of another spicule viewed in a transverse section of the body-wall.

FIG. 16.—Spicule viewed from the internal aspect. The granule has become a distinct rod with rounded extremities and swollen centre. In this species—*S. inhærens*—it is, as remarked in the text, situated transversely to the length of the anchor-shaft.

FIG. 17.—The same stage in *S. digitata*. It will be noticed that in this species the rod is situated parallel with the shaft and not at right angles to it. This difference between the two species of *Synapta* is quite constant.

FIG. 18.—*S. inhærens*. The extremities of the transverse rod have bifurcated. The "patagia" are well shown here.

FIG. 19.—*S. digitata*. The extremities of the parallel rod have bifurcated.

PLATE 30.

FIG. 20.—*S. inhærens*. The bifurcated extremities of the transverse rod have themselves bifurcated. The "patagia" are giving place to the "strands" uniting the apices of the anchor arms and the handle.

FIGS. 21—23 illustrate the further formation of the plate and the protoplasmic strands with their aggregates of nuclei.

FIG. 24 illustrates an anchor, in connection with which no plate has been formed.

FIG. 25.—Side aspect of the anchor-handle and plate-base to show the joint formed between the two.

FIG. 26.—The problematic bodies found in the body-wall.

The Development of the Auricularian Wheel-Spicule.

FIG. 27.—The initial granule in the syncytium. Its proximity to the body-wall is indicated.

FIGS. 28—30 illustrate the growth of this granule (surface aspect).

FIG. 31.—The plate-like form which this granule assumes and the clustering of the nuclei on its inner aspect (the aspect remote from the wall).

FIG. 32.—The plate or dish in side view. The inner position of the cluster of nuclei is well shown.

FIG. 33.—Plate viewed from its inner aspect.

FIG. 34.—Lateral aspect of full-sized plate.

FIG. 35.—Plate viewed from its outer aspect (nuclei situated on its remote side). The edge of the plate is crenate—the first indication of the spokes.

FIGS. 36—38.—Plate in all cases viewed from its inner aspect (nuclei on the near side). The formation of the spokes and the corresponding distension of the scleroplasm of the syncytium is shown. The nuclei with their accompanying scleroplasm lie in the concavity of the plate.

FIG. 39.—The spokes extend circumferentially.

FIG. 40.—The felly of the wheel formed. The entire spicule is enveloped by the scleroplasm.

FIG. 41.—The wheel viewed from the side in optical section showing the "eggs-in-a-basket" appearance.

FIG. 42.—One of the calcareous globes of the Auricularia larva with its enveloping syncytium situated in a shallow pocket of the body-wall.

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CONTENTS OF No. 204.—New Series.

MEMOIRS:

	PAGE
The Development of the Head-muscles in <i>Gallus domesticus</i> , and the Morphology of the Head-muscles in the Sauropsida. By F. H. EDGEWORTH, M.B., D.Sc., Professor of Medicine, University College, Bristol. (With 39 Text-figures)	511
The Development of <i>Ophiothrix fragilis</i> . By E. W. MACBRIDE, M.A., D.Sc., F.R.S., Professor of Zoology in McGill University, Montreal. (With Plates 31—36, and 4 Text-figures)	557
On the Segmentation of the Head of Diplopoda. By MARGARET ROBINSON, University College, London. (With Plate 37, and 6 Text-figures)	607
The Fixation of the Cypris larva of <i>Sacculina carcini</i> (Thompson) upon its Host, <i>Carcinus mænas</i> . By GEOFFREY SMITH, M.A., New College, Oxford. (With 6 Text-figures)	625
Physiological Degeneration in <i>Opalina</i> . By C. CLIFFORD DOBELL, B.A., Scholar of Trinity College, Cambridge. (With Plate 38, and 2 Text-figures)	633
Some Facts in the Later Development of the Frog, <i>Rana temporaria</i> .—Part I. The Segments of the Occipital Region of the Skull. By AGNES I. M. ELLIOT, B.Sc., Associate of Newnham College, Cambridge. (With Plates 39 and 40)	647

WITH TITLE, CONTENTS, AND INDEX TO VOL. 51.

The Development of the Head-muscles in Gallus domesticus, and the Morphology of the Head-muscles in the Sauropsida.

By

F. H. Edgeworth, M.B., D.Sc.,

Professor of Medicine, University College, Bristol (from the Anatomical Laboratory).

With 39 Text-figures.

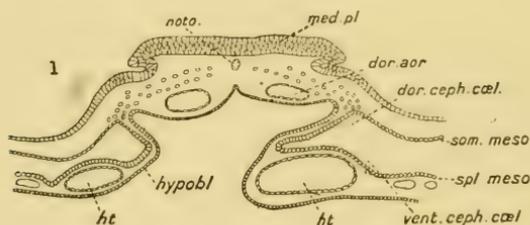
A FEW papers have been published on the early stages of development of the head-muscles of Gallus and other birds, and on the insertion of the mandibular muscles into Meckel's cartilage and the lower jaw; but as yet there has been no description of the formation of all the muscles in any one bird which might serve as a basis for a discussion of their morphology. The following is a contribution to the subject founded on an investigation of Gallus.

I found it was impossible to understand these head-muscles without comparing them with those of Reptiles, but the difficulty of doing so was accentuated by the almost complete absence of knowledge of their development; for although Corning has described the early stages in *Lacerta*, the later ones are not known, and no other Reptiles have been investigated. Several friends, however, were good enough to lend me sections or embryos of the later stages of *Chelone*, *Alligator*, *Sphenodon*, *Agama*, and *Chamæleon*, and I was able to procure those of *Tropidonotus natrix*, so that it was possible to follow the growth and changes in the muscles which take place and give each group its special characteristics.

The treatises of Gadow and Hoffman have been taken as guides for the adult anatomy of the head-muscles of the Sauropsida. Some changes in the nomenclature have been made in an attempt to apply identical names to homologous muscles, for examination of their development shows that different names have been given to homologous muscles,¹ and the same name to muscles of different origins and morphology.² A list of the terms employed is given in an appendix.

VISCERAL CARTILAGES.

The development of the visceral cartilages in the various groups of the Sauropsida has already been described by



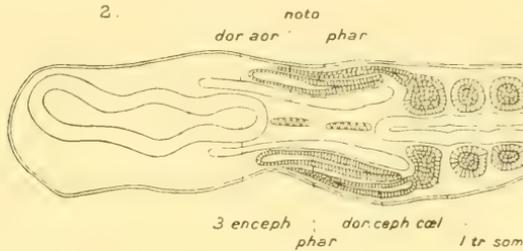
TEXT-FIG. 1.—Transverse section through embryo of *Gallus*, with 11 trunk somites. (For explanation of lettering see p. 555.)

Parker, Howes and Swinnerton, and by Broom; and I have very little to add. An interesting point is, that in early stages of *Tropidonotus* there is a long pterygoid process projecting forwards from the upper end of the quadrate (text-fig. 37, p. 543). By the time chondrification has taken place this pterygoid process has atrophied, and there is only its proximal

¹ E. g. the anterior part of the ventral longitudinal muscles has been called "cerato-mandibularis" (*Sphenodon* and *Lacertilia vera*), "genio-hyoid" (*Chelonia* and *Rhoptoglossa*), "maxillo-hyoideus" (*Ophidia*), and "anterior belly of maxillo-coracoideus" (*Crocodylia*).

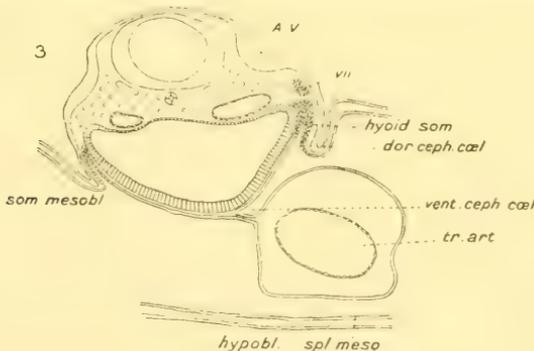
² E. g. the name "cerato-hyoideus" has been given to a muscle developed from the first branchial myotome of *Sphenodon* and *Lacertilia vera*, to the hyoglossus in *Crocodylia* and *Rhoptoglossa*, and to a muscle developed from the posterior mylohyoid in Birds.

end left—as a stump, which Parker (who, however, did not see the earlier stage) erroneously homologised with the “pedicle” in the frog.



TEXT-FIG. 2.—Coronal section through embryo of Gallus, with 14 trunk somites; outside the dorsal aorta, on either side, is seen the upper lateral part of the pharynx (cp. text-fig. 3), and outside this the upper part of the dorsal cephalic coelom, which is continuous behind with the first trunk somite. (For explanation of lettering see p. 555.)

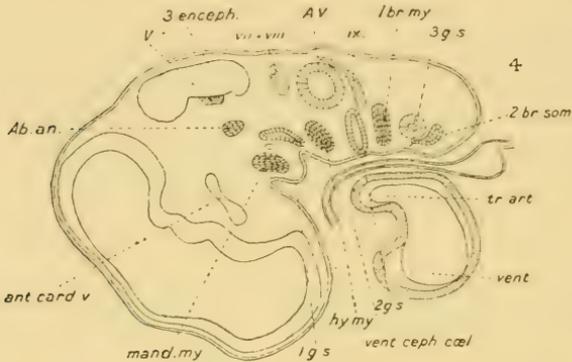
In all the Sauropsida, consequently, there is a long pterygoid process to the quadrate in early stages of development.



TEXT-FIG. 3.—Transverse section through embryo of Gallus, with 18 trunk somites. (For explanation of lettering see p. 555.)

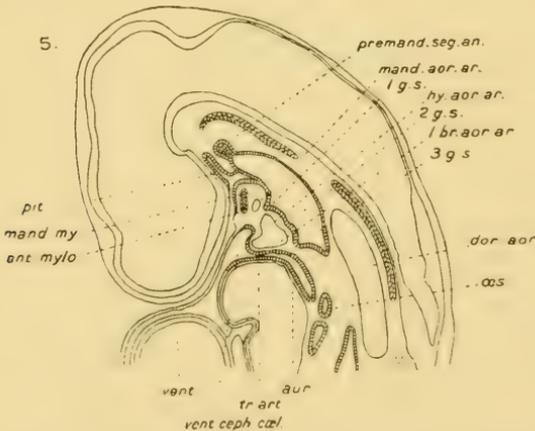
In Sphenodon, Lacertilia vera, Rhiptoglossa, and Crocodilia this pterygoid process has a processus ascendens, or epipterygoid, which is developed very early in Sphenodon (vide Howes and Swinerton), late in the Alligator (text-fig. 22, p. 527).

There are three median hyobranchial cartilages in Gallus. Parker called them the cartilago entoglossa, basihyal, and



TEXT-FIG. 4.—Sagittal section through embryo of Gallus, with 2 fully formed and 1 partially formed gill-pouch. (For explanation of lettering see p. 555.)

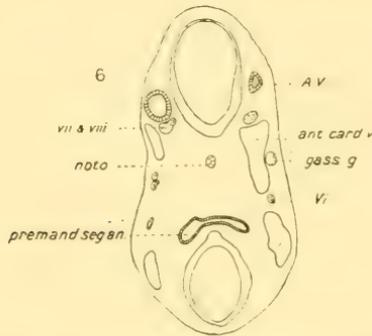
urohyal, and stated that the first named was formed by lateral fusion of the lower ends of the ceratohyals. Geoffrey Smith



TEXT-FIG. 5.—Sagittal section through embryo of Gallus, with 3 gill-pouches. From the anterior portion of the gut a hollow pouch projects laterally, the wall of which is cut by this section (the Anlage of the premandibular segment). (For explanation of lettering see p. 555.)

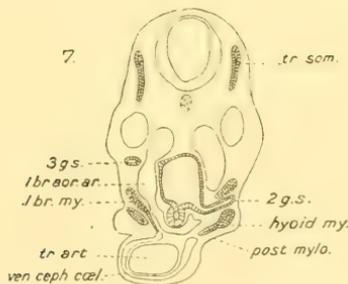
subsequently stated that in a five-day-old chick each ceratohyal is continuous below with a "branchial blastema."

Transverse sections show that whereas the lower ends of



TEXT-FIG. 6.—Transverse section through an embryo of Gallus, showing fully formed Anlage of premandibular segment. (For explanation of lettering see p. 555.)

the first branchial bars meet each other in the middle line, the lower ends of the ceratohyals are separated from one another

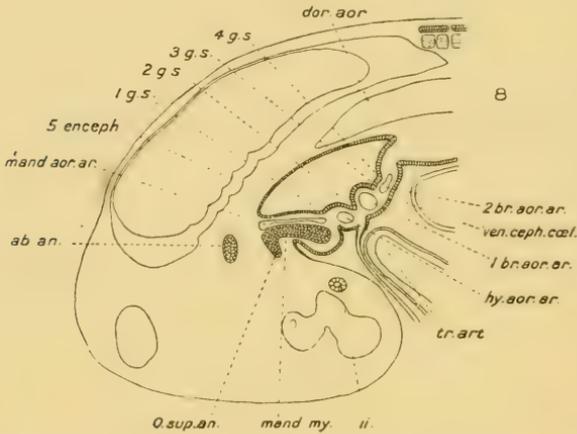


TEXT-FIG. 7.—Transverse section through an embryo of Gallus, with 3 fully formed gill-pouches, in hyoid and first branchial segments. (For explanation of lettering see p. 555.)

by a median procartilagenous structure (text-fig. 12, p. 519), which is continuous with a median structure in front and behind. Chondrification takes place, the middle portion of

the ceratohyal disappears,¹ and the lower end forms a short cornu to the cart. entoglossa, which is, therefore, a median basihyal or glossohyal with ceratohyal cornua. The middle piece (Copula i, of Gaupp) is a first basibranchial, and the urohyal (Copula ii) a second basibranchial.¹

This conclusion is supported by comparison with the conditions found in Reptiles. In *Sphenodon*, *Lacertilia vera*, *Rhoptoglossa*, *Chelonia*, and *Crocodylia* there is a continuous basihyobranchial cartilage, and in the first four groups there are ceratohyal cornua homologous with those in *Gallus*. In



TEXT-FIG. 8.—Sagittal section through an embryo of *Gallus*, with 4 gill-pouches. (For explanation of lettering see p. 555.)

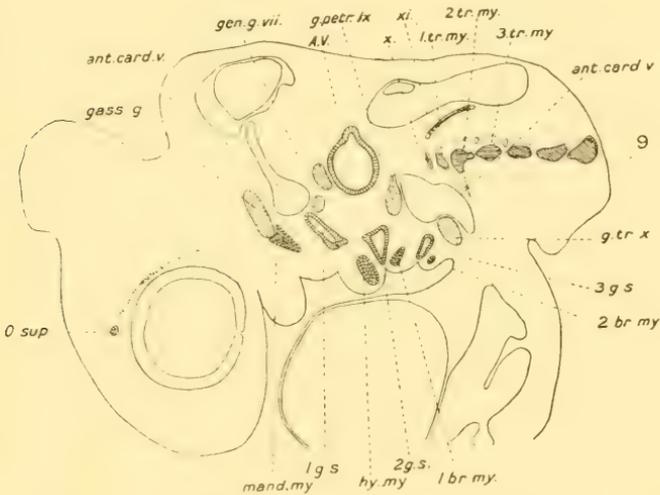
Sphenodon these ceratohyal cornua are formed by ceratohyal bars which are continuous above with the hyomandibulars, in *Agama*, *Chamaeleon*, and *Chelone* they are the lower ends of ceratohyal bars which, in embryonic stages, were continuous with the hyomandibulars. In the *Crocodylia* there are no ceratohyal cornua to the basihyobranchial, and the single

¹ The small bit of cartilage which is described by Geoffrey Smith as separating from the lower end of the infrastapedial on the eighth day is probably homologous with that called "stylohyal" by Parker in *Chelidon urbica*.

embryo I had was too far advanced in development (chondrification was complete) for one to see this stage—of a ceratohyal continuous from the hyomandibular above to the basi-hyobranchial below.

A first branchial bar is present in all groups of the Sauropsida, a second branchial in *Chelonia*¹ (vide text-fig. 19, p. 525), *Sphenodon*, and *Lacertilia vera*, but is absent in Birds, *Crocodylia*, *Rhoptoglossa*, and *Ophidia*.

The main differences, consequently, between Birds and



TEXT-FIG. 9.—Sagittal section through an embryo of *Gallus*, with 4 gill-pouches. It shows (partially) the formation of the ventral longitudinal muscles, which originate from the first (half) myotome and the four succeeding ones. Owing to the spiral twist of the embryo no single section shows all the five downgrowths. (For explanation of lettering see p. 555.)

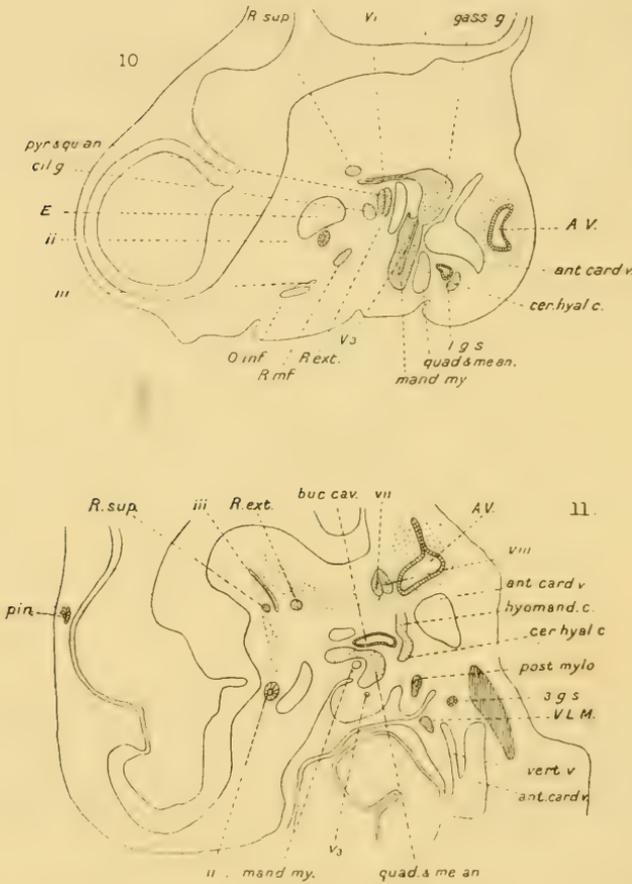
Reptiles in the state of the hyobranchial skeleton, are that in the former a second basibranchial is developed² and the median cartilages become jointed. There are correlated

¹ The suggestions of Gaupp in regard to the nature of the cart. entoglossa in Birds and the existence of a second branchial bar in *Chelone* are thus confirmed.

² It is absent in some few Birds, e. g. *Rhea*, *Platalea*.

differences in certain muscles of the head which will be described later on.

Parker, who did not investigate any embryonic stages,

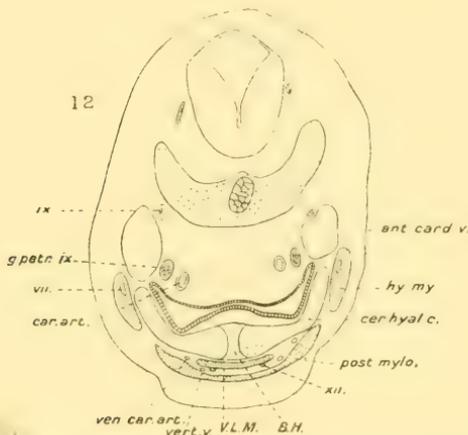


TEXT-FIGS. 10 AND 11.—Sagittal sections through an embryo of Gallus at the end of the fifth day. 10 is the more external. (For explanation of lettering see p. 555.)

described the columella of the adult Chamæleon as consisting of stapedial, mediostapedial, extrastapedial, and supra-

stapedial portions, the extra- and supra-stapedial portions lying internal to the quadrate. Study of the development, however, shows that his "extrastapedial" is the persisting upper end of the ceratohyal which lies at first posterior to the quadrate (text-figs. 32 and 33, p. 536) in the hyoid segment. A true extrastapedial is absent.

The hyoid bar of *Tropidonotus* similarly lies at first in the hyoid segment (text-fig. 37, p. 543). The "tuberculum" (of Parker) attached to it is probably a suprastapedial element; it is developed as an outwards and upwards projecting process of



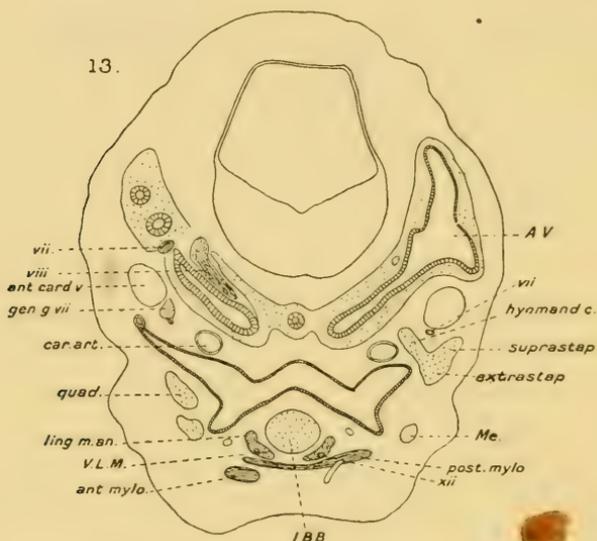
TEXT-FIG. 12.—Transverse section through an embryo of *Gallus* at the beginning of the sixth day. (For explanation of lettering see p. 555.)

the hyoid bar (at a stage later than that shown in text-fig. 37, p. 543), and only subsequently rotates forward. Parker stated that the "tuberculum" became adherent to the stapedial plate, but so far as my observations go it simply disappears. The "stylohyal," of Parker, which becomes adherent to the inner face of the quadrate is the separated lower end of the hyoid bar.

In both *Chamaeleon* and *Tropidonotus* the hyoid bar thus assumes a secondary position internal to the quadrate,

and, probably consequent on this, a suprastapedial is not developed.

The first branchial bar of *Tropidonotus*¹ is not developed as a dorsi-ventral bar which subsequently rotates into its adult position with lower end forward, but as a longitudinal one (text-fig. 38, p. 543). Its position and elongation backwards, together with the carrying back of the origin of the hyo-



TEXT-FIG. 13.—Transverse section through an embryo of *Gallus* at the middle of the sixth day. (For explanation of lettering see p. 555.)

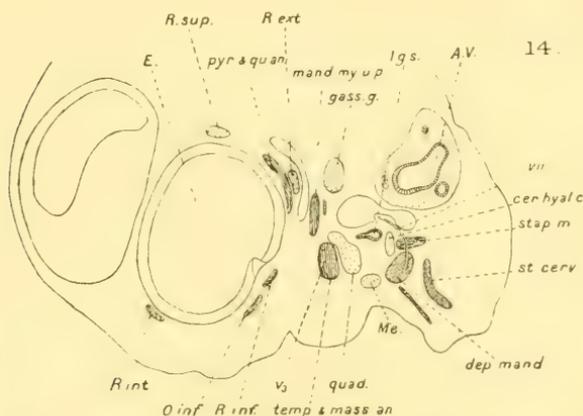
glossus muscle, are evidently correlated with the development of the (secondary) protrusible tongue.

On the Number of Vertebrae coalescing with the Skull.—Suschkín² described, in *Tinnunculus*, four vertebrae coalescing with the skull, each of which possessed, as temporary procartilaginous structures, a pair of “cranial ribs.”

¹ It cannot be the ventral portion of the hyoid bar as Rathké (cited by Gaupp) thought, for, on its first appearance as a procartilaginous structure, it lies behind the second gill-pouch, i. e. in the first branchial segment.

² Cited by Gaupp.

In *Sphenodon* there are similarly four vertebræ visible (text-fig. 26, p. 529). In the other Sauropsida investigated only three were visible—probably due to a dropping out, in development, of the most anterior. In *Tropidonotus*, for instance, the first two hypoglossus roots pass out together, i. e. the intervening vertebra is not developed, even as procartilage. Costal processes or cranial ribs were only seen in *Sphenodon* (text-fig. 26, p. 529), and then only over the hindermost coalescing vertebra; these persist, forming on each side the structure known as the “proatlas.”¹ In this embryo chondrification



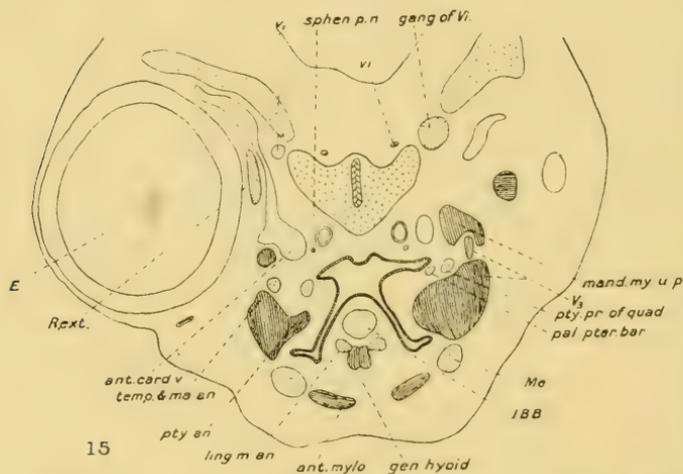
TEXT-FIG. 14.—Sagittal section through an embryo of *Gallus* at beginning of seventh day. (For explanation of lettering see p. 555.)

had already taken place in the basis cranii, so that possibly in an earlier stage a greater number of costal processes was present. A similar explanation probably holds for the “proatlas” of the Alligator.

¹ Howes and Swinnerton did not see the posterior part of the basis cranii in stage P, and by stage Q the evidence of coalescence of vertebræ has disappeared. They consequently did not express any opinion as to the nature of the “proatlas.”

MUSCLES.

The premandibular segments are formed in *Gallus* by hollow lateral outgrowths from the anterior end of the fore-gut, which, a little later, is constricted off, forming a canal connecting the vesicles (text-figs. 5 and 6, pp. 514, 515). The lumen in the canal disappears, and the resulting column of cells atrophies. The vesicle, on either side, becomes a solid



TEXT-FIG. 15.—Transverse section through an embryo of *Gallus* at beginning of seventh day. (For explanation of lettering see p. 555.)

clump by proliferation of its lining cells, and develops into the eye-muscles innervated by the third nerve.

In the Lizard¹ and Duck² the premandibular segment is also formed from the hypoblast, though in a slightly different way—by solid lateral outgrowths from the dorsal wall of the foregut, which are cut off, and develop a cavity extending from side to side. In the Lizard the eye-muscles begin to

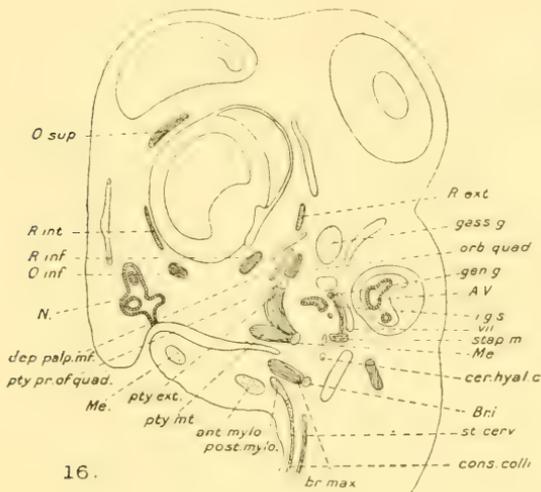
¹ Corning (loc. cit.).

² Rex (loc. cit.).

develop from the walls of each lateral vesicle before its cavity is obliterated.

The muscles developed from the premandibular segment are exceedingly uniform throughout the Sauropsida—a rectus superior, rectus inferior, rectus internus, and obliquus inferior. In Birds and Crocodilia a levator palpebræ superioris is also developed from the rectus superior.

An orbicularis palpebrarum is developed in Birds, Spheno-



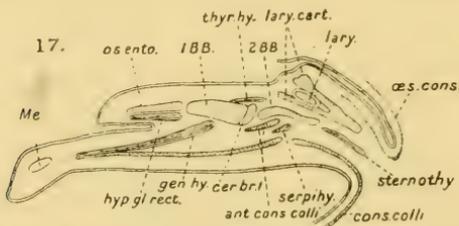
TEXT-FIG. 16.—Sagittal section through an embryo of Gallus at end of seventh day. (For explanation of lettering see p. 555).

don, Lacertilia vera, and Rhiptoglossa—probably from the mesoblast of the mandibular segment.

The myotomes of the head in Gallus are developed from the epithelium lining the dorsal cephalic cœlom on either side of the forepart of the alimentary canal (text-figs. 1 and 2, pp. 512, 513). The layers disappear at the sites of the gill-pouches, but thicken in the intervening portions, and form the somites of the head—the corresponding head cavities (dorsal cephalic cœlom) disappearing (text-fig. 3, p. 513).

The cells forming the somites are at first undifferentiated, then those lying centrally become converted into the myotomes, whilst those lying peripherally become part of the general mesoblast. The lower ends of the myotomes are continuous with the epithelium lining the ventral cephalic cœlum (text-fig. 7, p. 515). In the case of the mandibular and hyoid segments the lower ends of the myotomes become continuous with the mylohyoids, which are formed from the epithelium lining the obliterated portions of the ventral cephalic cœlum in those segments (text-fig. 7, p. 515). The lower ends of the first and second branchial segments separate from the epithelium lining the ventral cephalic cœlum.

The formation of the myotomes of the head of Gallus is



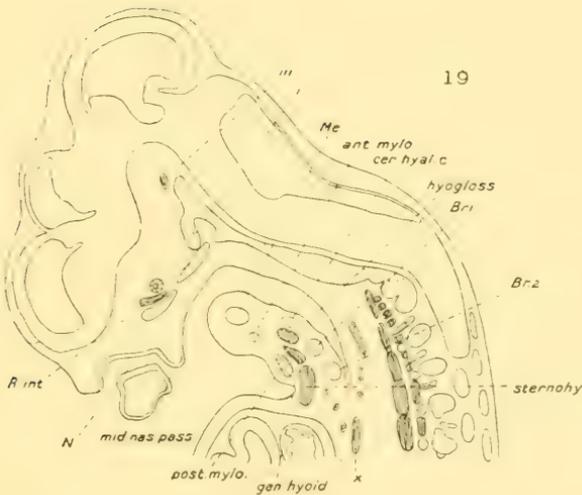
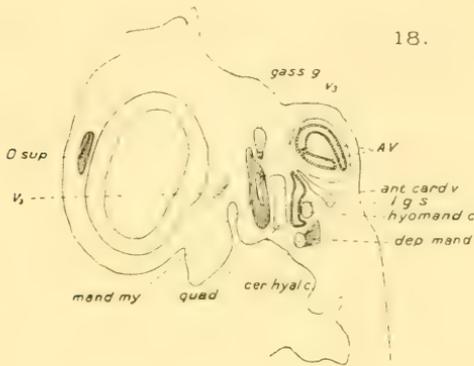
TEXT-FIG. 17.—Sagittal section through an embryo of Gallus at end of eighth day. (For explanation of lettering see p. 555.)

thus similar to that of Scyllium. The difference is that the head cavities in Gallus are not nearly so marked as in Scyllium. In Gallus, too, some of the general mesoblast is proliferated from the epithelium (from both splanchnic and somatic layers) before the formation of the head somites (text-fig. 1, p. 512).

In Gallus four myotomes are formed—those in the mandibular, hyoid, first and second branchial segments (text-fig. 4, p. 514); and there are similarly four in Lacerta.¹ In reference to this point, it should be remarked that Corning, following v. Wijhe's views, described them as "Seitenplatten," i. e. as visceral structures. As, however, I have shown that Balfour's

¹ Corning.

view—that the muscle plates of the head are serially homologous with those of the trunk and somatic—is probably the

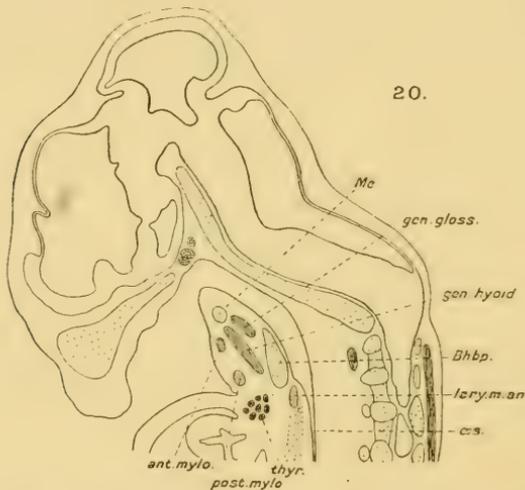


TEXT-FIGS. 18 and 19.—Sagittal sections through an embryo of *Chelone*, in "stage 4" of Parker. 18 is the more external. (For explanation of lettering see p. 555.)

true one, I shall assume that those of *Lacerta* are also somatic. It will be seen later that the changes undergone

by the myotomes of *Lacerta* and *Gallus* are closely similar to those of *Scyllium*.

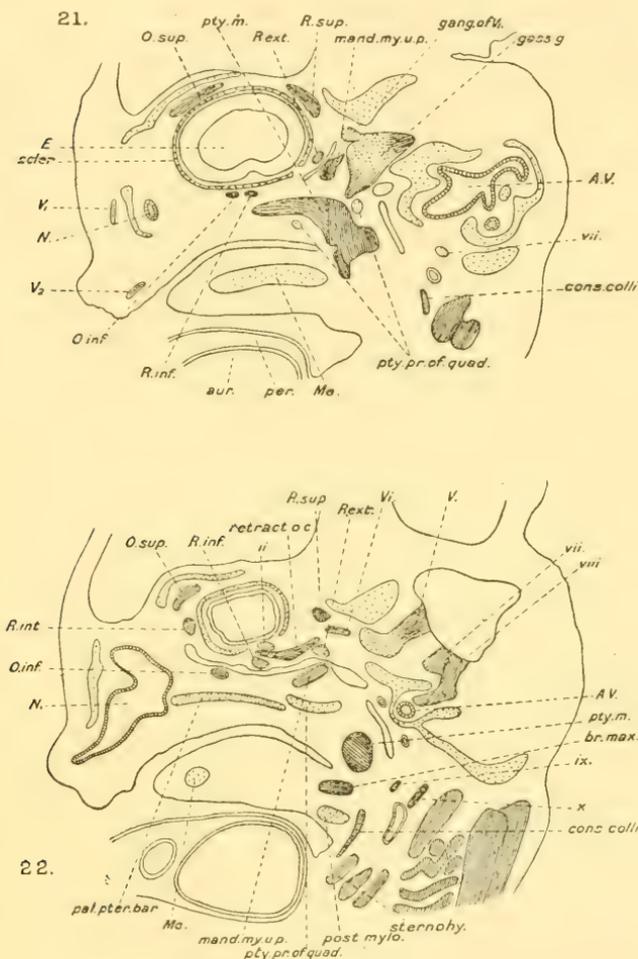
Myotome of the Mandibular Segment.—The superior oblique muscle of *Gallus* is developed as a forward and upward directed off-shoot of the upper end of the mandibular myotome (text-fig. 8, p. 516), just as in *Scyllium*. It never contains a lumen. In these respects it resembles the superior oblique of *Lacerta* (Corning). In some Birds—*Anas*, *Larus* (v. Wijhe), it has a transitory lumen—possibly owing to a relatively earlier formation.



TEXT-FIG. 20.—Sagittal section through an embryo of *Chelone*, in "stage 4" of Parker. (For explanation of lettering see p. 555.)

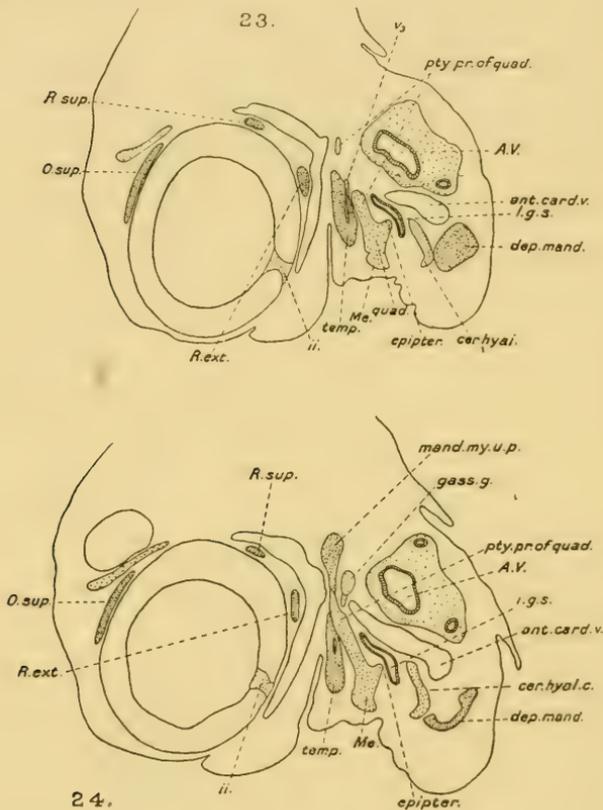
In *Scyllium* the mandibular myotome (after separation of the Anlage of the superior oblique) forms a vertical strip lying across and outside the horizontal palato-pterygoid process of the quadrate. It divides into an upper part above the palato-pterygoid process, and a lower between this and Meckel's cartilage—the upper part forming the levator maxillæ superioris, and the lower the adductor mandibulæ. A portion, however, of the strip does not so divide, and forms the first dorsal constrictor.

In the Sauropsida the mandibular myotome (after separation of the Anlage of the superior oblique) grows upwards to



TEXT-FIGS. 21 and 22.—Sagittal sections through an embryo of Alligator, slightly younger than "stage 2" of Parker. The pterygoid muscle can be seen extending upwards outside the pterygoid process (which has as yet no processus ascendens) of the quadrate, and excluding the upper part of the mandibular myotome from its insertion into the pterygoid process. 21 is the more external. (For explanation of lettering see p. 555.)

abut on,¹ or even reach a higher level than (text-fig. 32, p. 536), the Gasserian ganglion, and similarly forms a strip lying across and outside the pterygoid process of the quadrate (text-figs. 10, 18, 32, 36, pp. 518, 525, 536, 541), and then divides into an upper and a lower part.

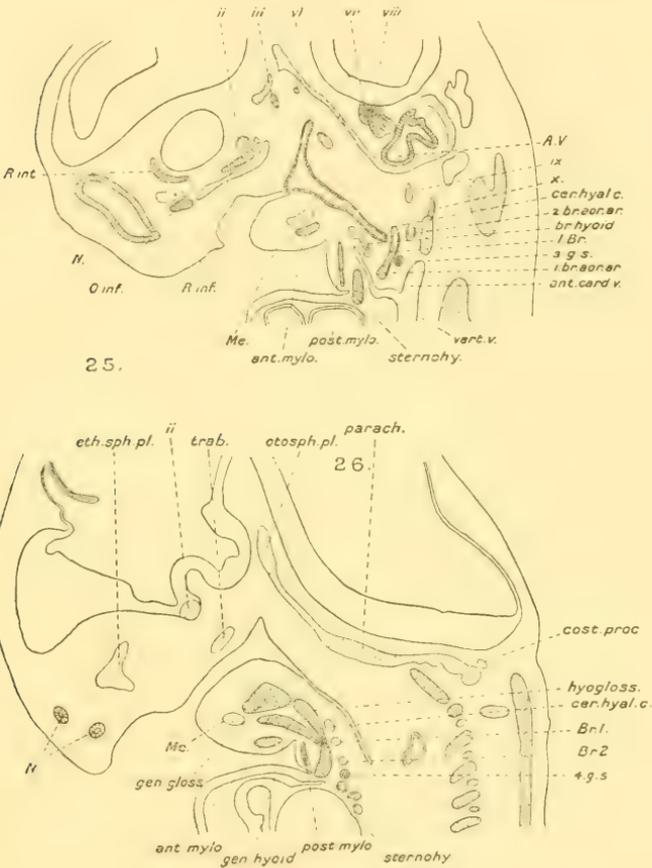


TEXT-FIGS. 23 and 24.—Sagittal sections through an embryo of *Sphenodon*, in "stage P.Q" of Howes and Swinnerton. 23 is the more external. (For explanation of lettering see p. 555.)

In *Gallus*, which preserves a movable pterygo-quadrate, the upper part of the myotome, after separation of a strip to form the depressor palpebræ inferioris (text-fig. 16, p. 523),

¹ First described by Corning in *Lacerta*.

develops into the orbito-quadratus—an elevator of the pterygoid process of the quadrate (text-fig. 16, p. 523)—though in later periods of development the insertion shifts in part to the

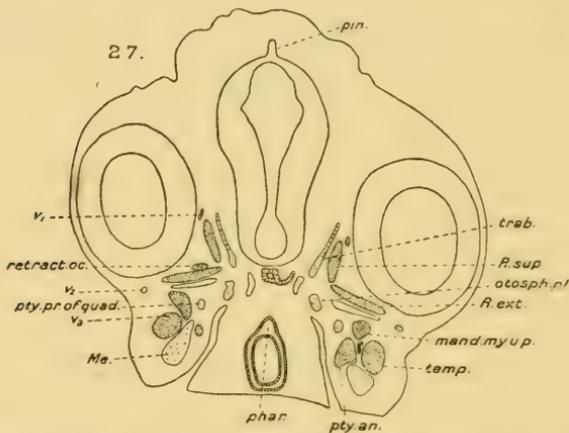


TEXT-FIGS. 25 and 26.—Sagittal sections through an embryo of *Sphenodon*, in "stage P-Q" of Howes and Swinnerton. 25 is the more external. (For explanation of lettering see p. 555.)

body of the quadrate, and in part to the hind end of the pterygoid bone.

In Reptiles the upper part of the myotome (after separa-

tion of a strip to form the depressor palpebræ inferioris in *Sphenodon*,¹ *Agama*, *Chelone*, and *Alligator*) either forms a muscle tying the pterygoid process to the side of the skull, or is inserted into the (membranous) palato-ptyergoid bar or atrophies. An insertion into a membrane bone must be a secondary feature. Reptiles must therefore be descended from forms which, like *Selachians* and *Birds*, had a movable pterygo-quadrato. *Birds* preserved this feature, and with it the primitive insertion of the upper part of the mandibular myotome, whereas in *Reptiles* the pterygo-quadrato became



TEXT-FIG. 27.—Transverse section through an embryo of *Sphenodon*, in "stage P" of Howes and Swinnerton. The upper part of the section is more anterior than the lower. (For explanation of the lettering see p. 555.)

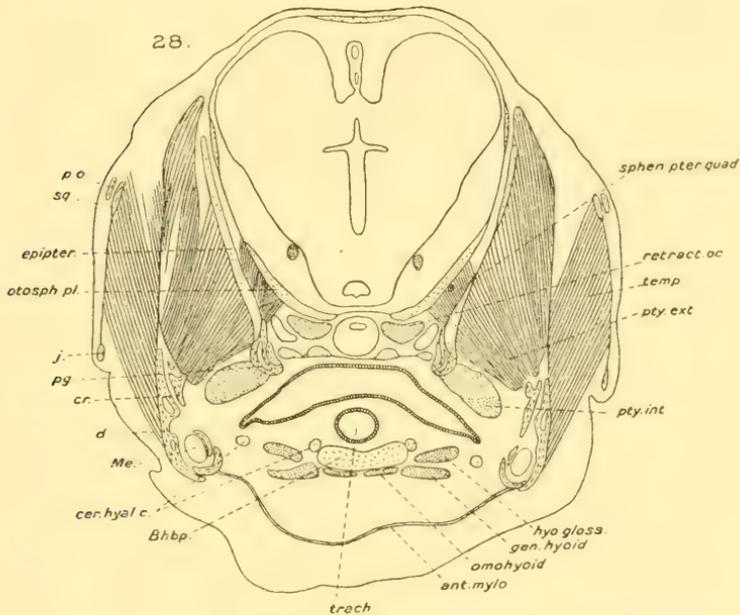
fixed, and the upper part of the myotome can be seen, during development, undergoing secondary modifications.

In *Alligator* (text-figs. 21, 22, p. 527) and *Chelone* the upper part of the myotome atrophies without ever having had an insertion into the palato-ptyergoid bar; the pterygoid process, either in continuity with the quadrato (*Alligator*) or after

¹ The depressor palpebræ inferioris of *Sphenodon* is inserted into the reflected angle of the conjunctival sac (text-fig. 29, p. 532), that of *Chelone* and *Alligator* into both lids.

separation from it (*Chelone*), becomes a part of the skull, and the quadrate becomes immovably fixed.

In *Sphenodon* the upper part of the myotome is at first an elevator of the pterygoid process (text-fig. 24, p. 528); it subsequently loses this function on fixation of the pterygo-quadrata, and ties its pterygoid and epipterygoid processes to the side of the skull, with an insertion of a very few of its lowest



TEXT-FIG. 28.—Transverse section through an embryo of *Sphenodon*, in "stage R" of Howes and Swinnerton. (For explanation of lettering see p. 555.)

fibres into the fixed palato-ptyerygoid bar (text-figs. 28 and 29, pp. 531, 532)—forming the sphenop-ptyerygo-quadratus of Fürbringer.

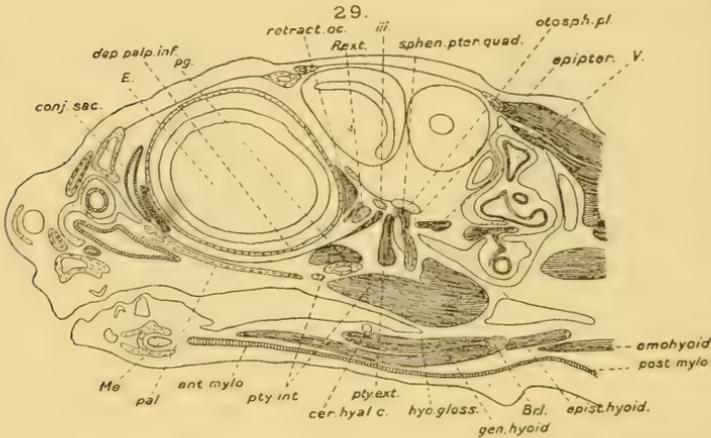
In *Agama* the upper part of the myotome, after a temporary insertion into the pterygoid process (text-fig. 30, p. 534), becomes inserted into the pterygoid bone forming the pterygo-parietalis and protractor pterygoidei¹ muscles. In some

¹ Of Versluys.

Lizards, e. g. *Hemidactylus*, *Gephyra*, *Varanus*, some of the fibres are inserted into the quadrate (Fürbringer).

In *Chamaeleon* there is no depressor palpebræ inferioris developed, and the whole of the upper part of the myotome, after a temporary insertion into the pterygoid process, becomes divided into the pterygo-sphenoidalis anterior, pterygo-parietalis,¹ and protractor pterygoidei;¹ of these the first and third arise from the processus anterior inferior and adjacent parts of the otic capsule; the second from the parietal bone.

In *Tropidonotus* there is also no depressor palpebræ infe-



TEXT-FIG. 29.—Sagittal section through an embryo of *Sphenodon*, in "stage S" of Howes and Swinnerton. (For explanation of lettering see p. 555.)

rioris, and the upper part of the myotome, after a temporary insertion into the pterygoid process (text-fig. 37, p. 543), becomes divided into the vomero-sphenoideus, pterygo-sphenoidalis anterior, pterygo-parietalis, and pterygo-sphenoidalis posterior.

In the *Lacertilia vera* and *Ophidia* the quadrate—after separation or atrophy of its pterygoid process—is movable;

¹ Of Versluys. Bradley states that these muscles are absent in the adult. The condition in my embryos (one of which is fairly advanced in development) supports the opinion of Versluys; they also show that a pterygo-sphenoidalis anterior is present.

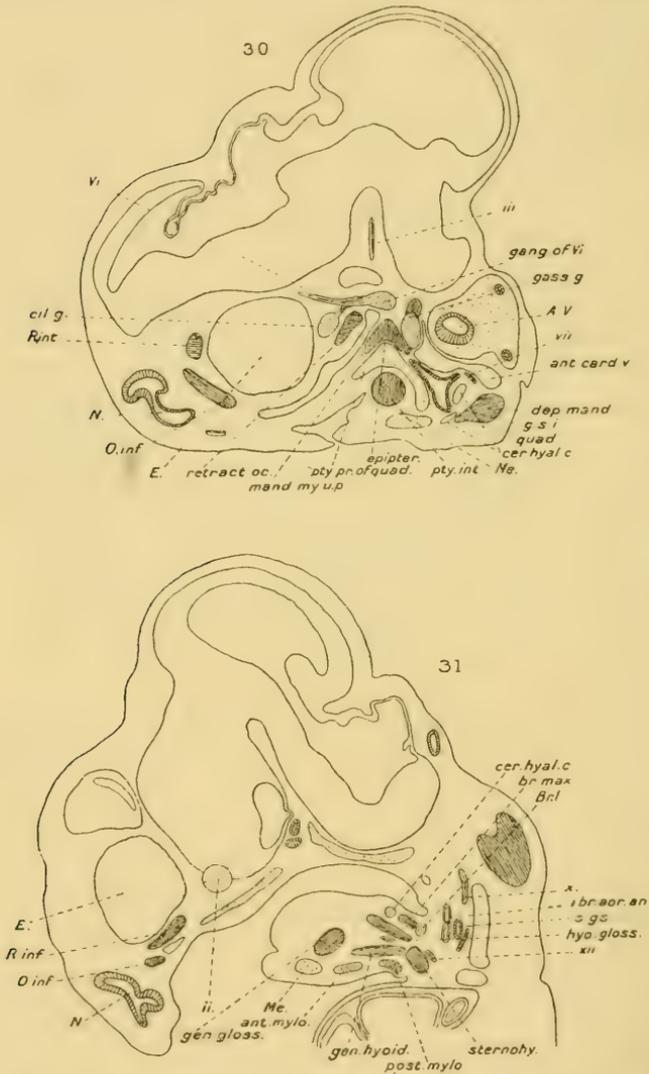
in *Chamæleon* it is fixed. It is probable that the movability of the quadrate in the two former groups is a secondary phenomenon and not a primitive Sauropsidan condition. According to Broom it was initiated by loss of the quadrato-jugal bone.

The formation of the *processus ascendens* (or *epipterygoid*) from the pterygoid process in *Crocodilia*, *Rhyncocephalia*, and *Lacertilia* was probably a development subsequent to fixation of the pterygo-quadratus. Its development is intimately associated in the *Crocodilia* with the upgrowth of the pterygoid muscle; in the *Rhyncocephalia* and *Lacertilia vera* with the upgrowth of the external pterygoid muscle. It is not developed in *Birds*, *Chelonia*, or *Ophidia*. The "epipterygoid" of *Chelonia* is merely the anterior end of the pterygoid process; on the other hand, in *Rhoptoglossa* the anterior end of the pterygoid process, which also becomes a part of the cranial wall, contains the basal part of a *processus ascendens* (Broom).

Fürbringer, from an analysis of the adult anatomy, homologised the *levator maxillæ superioris* of *Selachians* with the *spheno-ptyerygo-quadratus* of *Lacertilia* and the *orbito-quadratus* of *Birds*, and deduced the theory that the monimostylic (with fixed quadrate) condition of *Crocodilia* and *Chelonia* is a secondary one, and that the streptostylic (with movable quadrate) condition found in *Lacertilia*, *Ophidia*, and *Birds* is the primary one. The theory sketched above—based on comparison of developmental and adult features—separates the streptostylic condition into two forms: a primitive streptostylic pterygo-quadratus (*Birds*), and a secondary streptostylic quadrate (*Lacertilia vera* and *Ophidia*). The original Sauropsidan stock thus probably possessed a movable pterygo-quadratus and a fixed (membranous) palatopterygoid bar; the former has been retained only by *Birds*, the latter only by *Chelonia*, *Crocodilia*, and *Rhyncocephalia*.

The lower part of the mandibular myotome in *Gallus* passes through three stages of development. At first, like the adductor mandibulæ of *Scyllium*, it forms a single muscle

passing from the pterygoid process to the lower jaw; it then divides into an inner and outer part—pterygoid and temporal



TEXT-FIGS. 30 and 31.—Sagittal sections through an embryo of *Agama*. 30 is the more external. (For explanation of lettering see p. 555.)

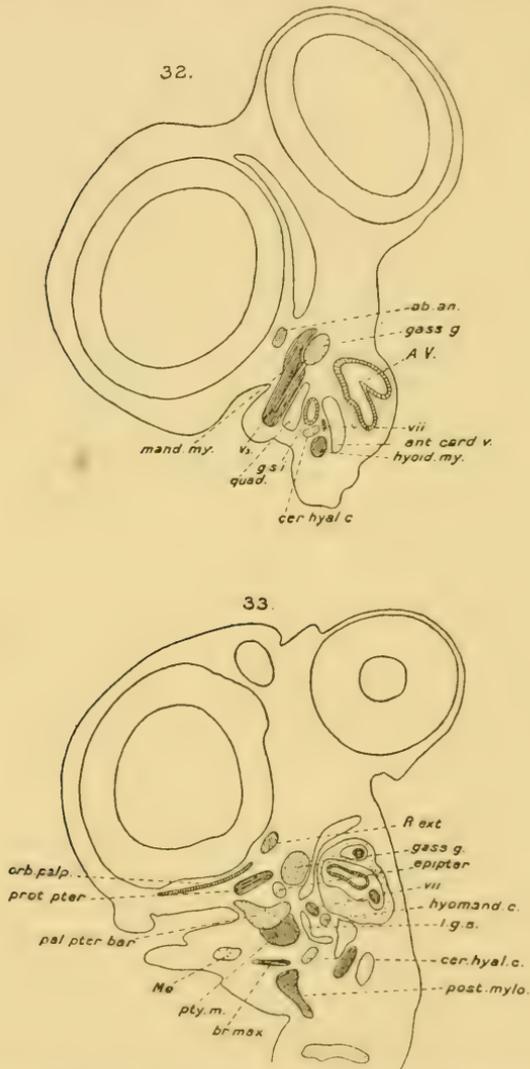
(text-fig. 15, p. 522). The outer part divides into the quadrato-maxillaris which keeps its primitive origin from the pterygoid process, and the temporal which grows up to gain an origin from the skull. The inner part divides into the external pterygoid which keeps its pterygoid-quadrate origin (text-fig. 16, p. 523), and the internal pterygoid which arises from the hind end of the palato-pterygoid bone (text-fig. 16, p. 523).

The lower part of the mandibular myotome in Reptiles similarly divides into inner and outer portions—pterygoid and temporal (text-fig. 27, p. 530). The development of the outer portion is much the same as in Birds. It is at first a muscle passing from the pterygoid process to the lower jaw (text-fig. 24, p. 528); subsequently, whilst retaining an attachment to the quadrate, it also extends up to the side of the skull. The part, however, which retains its original upper attachment is not differentiated as a separate muscle (quadrato-maxillaris) lying internal to the temporal as in Birds. In *Chamæleon* the development is a little exceptional: the outer portion separates into a temporal (which becomes digastric in condition) and a quadrato-mandibularis which arises from the front of the quadrate (text-fig. 35, p. 539); the position of the latter muscle, below and not internal to the temporal, suggests that it is not homologous with the quadrato-maxillaris of Birds, but is merely the lower part of the temporal—that part which does not become digastric.

The development of the internal, pterygoid portion is very diverse in the various groups of the Reptilia. The simplest condition is present in *Chelone*, where there is during development a single undivided pterygoid muscle with two heads, one (inner) attached to the hind end of the palato-pterygoid bar, and the other (outer) attached to the pterygoid process of the quadrate. These two heads extend upwards, the former gaining an additional origin from the descending plate of the parietal, the latter an additional one from the prootic. The muscle does not become divided into two bellies.

In *Sphenodon* and *Agama* the pterygoid Anlage becomes

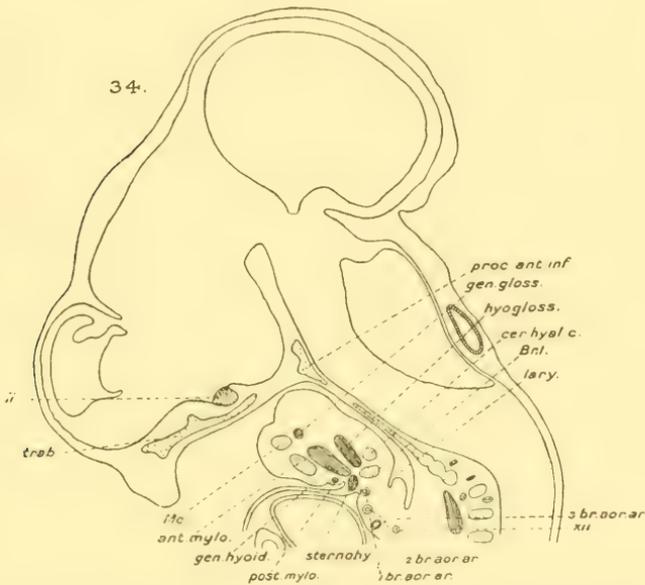
divided into an internal pterygoid muscle arising from the palato-ptyergoid bar (text-fig. 28, p. 531), and an external



TEXT-FIGS. 32 and 33.—Sagittal sections through an embryo of Chamæleon. 32 is the more external. (For explanation of lettering see p. 555.)

pterygoid arising from the pterygoid process of the quadrate; subsequently, the upper end of the external pterygoid muscle extends upwards outside the pterygoid process, and gains attachments to the epipterygoid and side of the skull (text-figs. 28 and 29, pp. 531, 532).

In *Chamæleon* and *Tropidonotus* the pterygoid Anlage remains single, it loses its attachment to the pterygoid pro-



TEXT-FIG. 34.—Sagittal section through an embryo of *Chamæleon*. (For explanation of lettering see p. 555.)

cess and takes origin from the palato-pterygoid bar (text-figs. 33 and 37, pp. 536 and 543).

In *Alligator* the pterygoid Anlage also remains single, it grows up outside the pterygoid process to the side of the skull (text-figs. 21 and 22, p. 527).

It may be concluded that the fixation of the palato-quadrate in the *Crocodylia* was intimately associated with the upgrowth of the pterygoid muscle, and in the *Rhynchocephalia* and

Lacertilia vera with the upgrowth of the external pterygoid muscle, to the side of the skull. Investigation of the process of development of the head-muscles did not throw any light on the causation of the fixation of the pterygo-quadrate in Chelonia, on the fixation of the quadrate in Rhiptoglossa, or its movability in Ophidia.

The above differences, however, in the condition of the upper part of the mandibular myotome and of the pterygoid, suggest that fixation of the pterygo-quadrate was brought about by different processes in, and so was independently acquired by, ancestors of (1) Chelonia, (2) Crocodilia, (3) Rhynchocephalia and Lacertilia vera, (4) Rhiptoglossa and Ophidia.

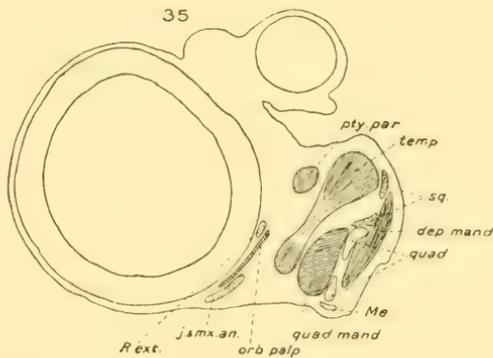
Myotome of the Hyoid Segment.—The abducens musculature of Gallus originates as a small clump of cells which grows forward and then separates from the upper end of the hyoid myotome. It passes forwards internal to the Gasserian ganglion (text-fig. 4, p. 514), and divides into an anterior and a posterior mass (text-figs. 10 and 14, pp. 518, 521); the anterior is the Anlage of the pyramidalis and quadratus nictitantis muscles, the posterior that of the external rectus. In Reptiles the abducens Anlage similarly divides into an anterior and a posterior mass; the former (except in Tropidonotus, where it atrophies) develops into the retractor oculi,¹ the latter into the external rectus (text-figs. 22, 29, 30, pp. 527, 532, 534).

The myotome of the hyoid segment, after giving off the abducens Anlage, develops into the depressor mandibulæ. The primary insertion of this muscle is into the ceratohyal (text-figs. 12, 18, 24, 30, and 32, pp. 519, 525, 528, 534, 536), but this is soon lost, and one into the hind end of the lower jaw acquired. In Gallus (text-fig. 14, p. 521), Alligator, and some Lizards (Parker), a stapedius muscle is also formed

¹ The retractor oculi is greatly developed in Sphenodon, its origin extending backwards (text-fig. 28, p. 531). Its insertion varies, and accordingly Huxley calls it "pyramidalis" in Chelonia and Crocodilia, "bursalis" in Lizards.

from the upper part of the myotome; this may be regarded as that portion of the hyoid myotome which retains its primitive insertion, whereas the main mass has gained a secondary one. The upper end of the depressor mandibulæ is attached to the postero-lateral aspect of the skull; in *Chamæleon* and *Tropidonotus* (text-fig. 35, p. 539) it has additionally an origin from the quadrate.

Myotome of the First Branchial Segment.—The upper part of the first branchial myotome of *Gallus* disappears, whilst the lower part develops into the branchio-maxillaris muscle which extends forwards from the first branchial bar to the lower jaw (text-fig. 16, p. 523).



TEXT-FIG. 35.—Sagittal section through an older embryo of *Chamæleon*. (For explanation of lettering see p. 555.)

The muscle is present in all the groups of the Sauropsida. Its primary condition is that of a muscle—the branchio-hyoid—which connects the first branchial bar with the ceratohyal (text-figs. 25 and 31, pp. 529, 534). This condition is the permanent one in *Sphenodon*, and is found as a temporary one in *Gallus*, *Chelone*, *Agama*, *Chamæleon*, and *Tropidonotus*. (The embryo of the Alligator was too far advanced in development for this stage to be seen.) In all these groups the muscle subsequently extends forwards to the lower jaw. This extension forwards is not dependent on loss of the continuity of the

ceratohyal, for it occurs in *Chamæleon* before this takes place. Probably it is related to the extension backwards of the jaw into the hyoid segment. In *Chelonia* the anterior attachment of the branchio-maxillaris muscle is to the hind end of the lower jaw, in the other groups the muscle extends still further forwards—in *Rhoptoglossa* even as far as the symphysis.

The branchio-hyoid or branchio-maxillaris muscle of *Sauropsida* is homologous with the most anterior of the coraco-branchiales muscles of *Scyllium*.

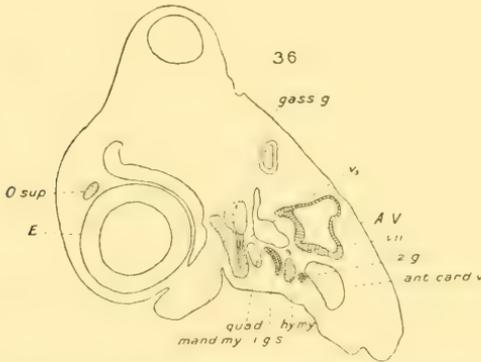
Myotome of the Second Branchial Segment.—A myotome is developed in the second branchial segment in *Gallus* (text-fig. 4, p. 514), but disappears leaving no trace. In *Lacerta*, too, a second branchial myotome is described by Corning, but there are no muscles present in the embryos of *Agama* investigated or in adult *Lacertilia* which might have developed from the myotome. And in the other Reptilian embryos the stages were likewise too far advanced for any rudiments of this myotome to be seen. In the *Sauropsida*, then, a second branchial myotome, if formed, soon disappears and does not develop into any muscle.

Its existence, however, though only as a temporary structure in *Gallus* and *Lacerta*, coupled with that of a second branchial bar in *Chelonia*, *Rhynchocephalia*, and *Lacertilia vera*, suggests that ancestors of *Sauropsida* may have possessed a longitudinal muscle connecting the second to the first branchial bar.

Cephalic Section of the Cœlom and Mylohyoid Muscles.—There are certain similarities and differences between *Scyllium* and *Gallus* in the relations of the ventral cephalic cœlom to the heart and visceral muscles of the mandibular and hyoid segments. In early stages of *Scyllium* there is, in the mandibular and succeeding segments of the head, a ventral section of the cœlom which bears the same relation to the ventral aorta as does the anterior portion of the body section of the cœlom to the truncus arteriosus and the heart. Subsequently, the developing heart bulges into the hinder portion of the cephalic cœlom. Still later, the heart and

cœlom gradually retreat from the head. The mandibular and hyoid mylohyoid muscles are formed from the obliterated sections of the ventral cephalic cœlom in those segments.

In early stages of *Gallus* the cephalic section of the cœlom similarly exists in the mandibular and succeeding segments (text-fig. 3, p. 513), but its relations to the vascular system are a little different. The heart is situated more anteriorly, lying in part in the cephalic and in part in the body portion of the cœlom; the truncus arteriosus is in the first branchial segment, and there is a very short ventral aorta between the origins of the hyoid and mandibular aortic arches, the three



TEXT-FIG. 36.—Sagittal section through an embryo of *Tropidonotus*. (For explanation of lettering see p. 555.)

branchial aortic arches being subsequently given off from the posterior end of the aorta. A little later the ventral aorta, mandibular, and hyoid, aortic arches disappear, the ventral end of the hyoid aortic arch being left as the ventral carotid artery. The mylohyoids are formed, as in *Scyllium*, from the walls of the obliterated portions of the ventral cephalic cœlom in the mandibular and hyoid segments. The subsequent retreat of the heart and cœlom from the head are also exactly parallel events to those occurring in *Scyllium*. The condition of the heart, truncus arteriosus, and ventral aorta in the early embryo of *Gallus* is evidently a secondary one.

The anterior or mandibular mylohyoid of Sauropsida preserves the form existing in Scyllium—a transverse sheet of muscle passing from one Meckel's cartilage to the other (text-figs. 15 and 28, pp. 522, 531).

The posterior or hyoid mylohyoid in Scyllium forms a transverse band passing from one ceratohyal to the other. The primary attachment of the muscle in Sauropsida is also to the ceratohyal, but this is lost in all groups with the possible exception of *Sphenodon*.¹ It gains, in all Sauropsida, a secondary attachment to the lower jaw, becoming more or less continuous with the anterior mylohyoid, and spreads down the neck forming the constrictor colli. This posterior part, which is attached laterally to the fasciæ of the neck (in *Chelonia* also to the first branchial bar, in *Rhoptoglossa* also to the crista occipitalis) is continuous with the anterior part.

In *Gallus* secondary changes take place in the posterior mylohyoid. After a transitory stage of attachment to the ceratohyal (text-fig. 12, p. 519) it becomes attached laterally to the hind end of the lower jaw (text-fig. 13, p. 520) and first branchial bar, and spreads down the neck. The portion attached to the first branchial bar becomes a separate muscle—the anterior constrictor colli²—forming a muscular sling for the second basibranchial (text-fig. 17, p. 524), whilst that in front forms the serpihyoid and stylohyoid, that behind the constrictor colli. The part attached to the lower jaw in the *Lamellicostren*, however, forms a simple transverse band (Gadow). The differentiation of an anterior constrictor colli is evidently related to the existence of a second basibranchial—it is absent in Reptiles, and in those Birds, e.g. *Rhea* (Gadow), where a second basibranchial is absent, and even when this is present is not always developed.

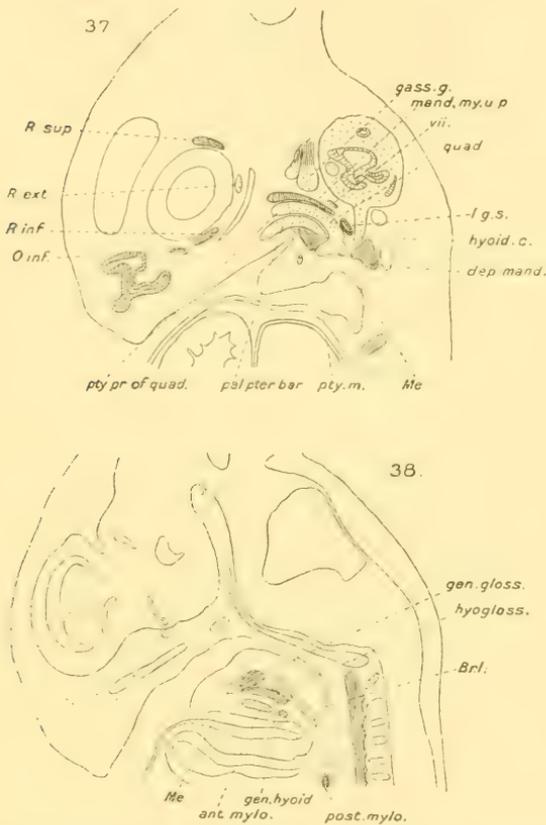
Laryngeal and Syringeal Muscles.—The Anlage of the laryngeal muscles in *Gallus* is developed in the splan-

¹ It was present up to stage S (of Howes and Swinnerton), but is not mentioned by Osawa.

² The "ceratohyoid" of Gadow, who included it among the lingual muscles as a differentiated part of the ceratoglossus.

nic mesoblast of the second branchial segment on the sixth day. A day later each lateral mass has divided into an apertor and its lateral half of the sphincter laryngis.

The laryngeal muscles of *Sphenodon*, *Agama*, *Chelone*,

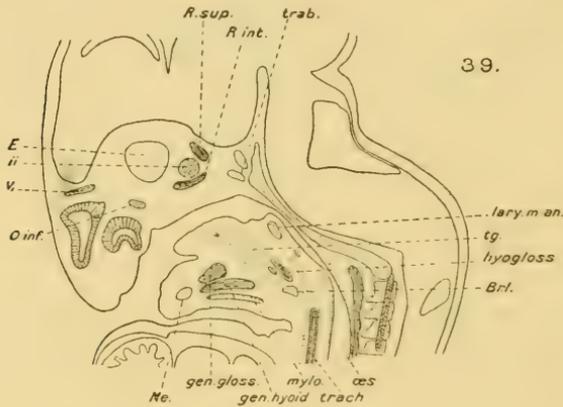


TEXT-FIGS. 37 and 38.—Sagittal sections through an older embryo of *Tropidonotus*. (For explanation of lettering see p. 555.)

Alligator, Chamæleon, and *Tropidonotus* are formed similarly from an Anlage in the second branchial segment.

The syringeal muscles of *Gallus* are formed from mesoblast cells which have spread down the trachea, and their Anlage is visible on the eighth day.

The Ventral Longitudinal Muscles are developed in *Scyllium* from the five trunk-myotomes (4th to 8th), in *Gallus* from the first five (text-fig. 9, p. 517), though the front part of the first (cf. text-fig. 2, p. 513) has by then disappeared; in *Lacerta* (Corning) from the second to the fifth, the first having previously atrophied. Ventral downgrowths take place from each contributing myotome in the form of a curve, with concavity forwards, round the branchial region of the head. The ventral ends of these downgrowths fuse together, separate from the rest of the myotomes, and form an elongated



TEXT-FIG. 39.—Sagittal section through an older embryo of *Tropidonotus*. (For explanation of lettering see p. 555.)

mass of cells in the ventral region of the branchial segments dorsal to the cephalic portion of the coelom. The mass extends forwards and backwards to form the Anlage of the ventral longitudinal muscles of the head and neck.

In *Scyllium* it forms two parallel muscles—the coraco-hyoideus and coraco-mandibularis. In the Sauropsida the long column divides into an anterior and a posterior part—the genio-hyoid and the sterno-hyoid. The division takes place opposite the first branchial bar, and the primary condition of the genio-hyoid is that of a muscle passing from the anterior end of Meckel's cartilage to the ventral end of the

first branchial bar, whilst the sterno-hyoid extends from this down the neck to the sternum (text-figs. 19, 26, 31, 34, 38, pp. 525, 529, 534, 537, 543).

This condition of the genio-hyoid persists in the Chelonia, Sphenodon, Lacertilia vera, Rhiptoglossa, and Ophidia. In the Alligator the muscle, after passing through this stage, becomes connected by tendon with the anterior end of one of the muscles into which the sterno-hyoid divides. In Gallus the primary stage—of insertion of the genio-hyoid into the first branchial bar—is rapidly passed through, and the hind end becomes inserted into the under surface of the first basibranchial (text-fig. 17, p. 524); later on the whole muscle atrophies and disappears.

The sterno-hyoid divides in all Reptiles, except Ophidia, into several parallel strips, some of which retain their original attachment to the first branchial bar, whilst others become inserted into the basihyobranchial. In the Alligator one of the strips becomes connected with the genio-hyoid by tendon.

In Birds the sterno-hyoid, which does not become divided into parallel strips, gains a new insertion into the dorsal surface of the first basibranchial (text-fig. 17, p. 524)); and in most Birds, though not in Apteryx and some others, it divides into anterior and posterior portions, e. g. in Gallus into sterno-thyroid and thyreo-hyoid (text-fig. 17, p. 524).

The lingual muscles of Reptiles are developed from the genio-hyoid, and consist of a genio-glossus and hyo-glossus (text-figs. 19, 20, 26, 31, 38, pp. 525, 526, 529, 534, 543). The former is attached in front to the anterior end of Meckel's cartilage, and the latter behind to the posterior branchial bar—just as is the muscle from which they originate.

The genio-glossus is attached posteriorly to the front end of the basihyobranchial in the embryos of Chelone (text-fig. 20, p. 526), Alligator, and Sphenodon; this condition persists in the two former, but in Sphenodon the muscle subsequently also spreads into the tongue. The muscle ends free in the tongue in Agama, Chamæleon, and Tropidonotus

(text-fig. 38, p. 543); in the last named a secondary protrusible tongue is subsequently formed (text-fig. 39, p. 544), and the genio-glossus becomes its protractor, and also gives rise to a genio-laryngeus.

The hyo-glossus is inserted into the side of the side of the basihyobranchial in *Chelone* (text-fig. 19, p. 525), and Alligator; it grows forward and ends free in the tongue in *Sphenodon* (text-fig. 29, p. 532), *Agama* (text-fig. 31, p. 534), and in early stages of *Chamæleon* (text-fig. 34, p. 537). In later stages of *Chamæleon* the anterior part of the muscle disappears (possibly developing into some of the many intrinsic lingual fibres), whilst the hinder part forms a muscle passing from the first branchial bar to the ventral end of the ceratohyal cornu.¹ In *Tropidonotus* the hyo-glossus becomes a retractor of the protrusible tongue, and also gives off a hyo-laryngeus.

The lingual muscles of *Gallus* are developed from an Anlage which is homologous with the hyo-glossus of Reptiles (text-figs. 13, 15, pp. 520, 522);² it at first extends from the first branchial bar to the first basibranchial; it subsequently grows forward and then divides into the ceratoglossus, and hypoglossus rectus and obliquus. This differentiation of the hyo-glossus is probably related to the fact that the median hyobranchial cartilages in Birds, unlike Reptiles, become jointed.

In some Birds, e. g. *Procellaria*,³ though not in *Gallus* even as a temporary structure, there is also a genio-glossus passing from the anterior end of the jaw to the os entoglossum.

The lingual muscles of the Sauropsida are thus somatic in origin—arising from the anterior element of the ventral

¹ The resultant muscle looks a little like the branchiohyoid of *Sphenodon*, and the same name, "Ceratohyoid," has been given to it, but it is of quite different origin.

² The Anlage of the lingual muscles of *Gallus* begins to be formed before the genio-hyoid is quite separated from the sterno-hyoid.

³ The posterior insertion of this muscle—into the tip of the os entoglossum and not into the ventral surface of the first basibranchial—shows that it is probably not a persisting genio-hyoid.

longitudinal muscles; it would seem probable that their original function was to connect the basihyobranchial with the lower jaw and first branchial bar, and that their extension into a tongue was a secondary matter. Only by this supposition do the phenomena occurring in *Chelone*, *Alligator*, *Sphenodon*, *Gallus*, and *Procellaria* become explicable.

SOME THEORETICAL CONCLUSIONS.

The above observations suggest that some remote ancestor of the Sauropsida possessed the following features:—A movable pterygo-quadrate, fixed palate and pterygoid bones, a ceratohyal which formed a continuous bar extending from the hyomandibular above to a median unjointed basihyobranchial below, first and second branchial bars. The eye-muscles consisted of four recti, two obliqui, and the retractor oculi. The mandibular myotome had divided into an upper and a lower part; the upper formed the depressor palpebræ inferioris and an elevator of the pterygoid process of the quadrate; the lower consisted of an inner and an outer division, the inner forming the pterygoid muscle which arose by two heads—from the pterygoid bone and from the pterygoid process of the quadrate, the outer forming the temporal muscle, which arose partly from the quadrate and partly from the skull wall above. The hyoid myotome formed the depressor mandibulæ. The first branchial myotome formed the branchiohyoid muscle passing from the first branchial bar to the ceratohyal. The ventral longitudinal muscles were formed by a genio-hyoid and a sterno-hyoid, whose adjacent ends were attached to the first branchial bar. The lingual muscles—developed from the genio-hyoid—were the genio-glossus and the hyo-glossus, the former passed from the anterior end of the lower jaw to the anterior end of the basihyobranchial, the latter from the first branchial bar to the side of the basihyobranchial; neither ended free in the tongue. The anterior and posterior mylohyoids formed a continuous sheet and extended down the neck.

No one of the Sauropsida has preserved all these features, but in each group they exist (though sometimes masked) in embryonic stages, and the various changes which take place and bring about the characteristics of each can be followed in the subsequent development.

Birds differ from all living Reptiles in possessing the following features. The pterygo-quadrate is movable; there is a joint between the basihyal and the first basibranchial, and a second basibranchial is generally present; the retractor oculi divides into pyramidalis and quadratus nictitantis; the upper portion of the mandibular myotome, after giving off the depressor palpebræ inferioris, forms an elevator or elevators of the pterygoid process of the quadrate; a distinct quadrato-maxillaris is formed; the genio-hyoid, after having had a temporary insertion into the first basibranchial, atrophies; the sterno-hyoid becomes, secondarily, inserted into the dorsal surface of the first basibranchial, and in most Birds divides into anterior and posterior portions; the hyoglossus divides into several muscles; a genio-glossus is seldom developed; a portion of the posterior mylohyoid generally becomes a separate muscle—the anterior constrictor colli—forming a muscular sling for the second basibranchial; in most Carinatae syrinxal muscles are formed.

Birds resemble the Rhynchocephalia in possessing an upper portion of the mandibular myotome inserted into the pterygoid process, but the adult condition in the latter group is clearly a secondary modification correlated with a fixation of the pterygo-quadrate.

The pterygoid process of the quadrate of Birds resembles that of Chelonia in possessing no processus ascendens such as exists in Crocodilia, Rhynchocephalia, Lacertilia vera, and Rhiptoglossa, and such a structure (to which no muscles are attached in early stages of development) does not seem to be a part of a freely movable pterygo-quadrate, but rather a formation occurring concurrently with or subsequently to its fixation.

The condition of the pterygoid muscles in Birds might easily

be derived from that seen in the embryo of *Chelone* (i. e. a single muscle with two heads, one from the pterygoid process of the quadrate, and one from the hind end of the palatopterygoid bar), and which undergoes very little modification in subsequent development, but not from that found in *Rhynchocephalia* and *Lacertilia vera* (where the external pterygoid muscle grows up outside the pterygoid process to the side of the skull), nor from that found in *Crocodylia* (where an undivided pterygoid muscle grows up outside the pterygoid process to the side of the skull.)

The lingual muscles of Birds, in that they are only derivable from a *genio-glossus* and *hyoglossus*, which were attached to the *basihyobranchial* and did not end free in the tongue, are more like those of *Chelonia* and *Crocodylia* than any other Reptilian group.

These features of resemblance suggest at first sight a very distant Chelonian relationship for Birds, but are in reality only ancestral traits, which are also present in embryonic stages of other Sauropsidan groups. The secondary fixation of the pterygo-quadrate and atrophy of the elevator of the pterygoid process, which occur in *Chelonia*, are strongly marked differences from Birds.

The *Crocodylia* resemble the *Chelonia* in the atrophy of the upper part of the mandibular myotome and in the fixation of the pterygo-quadrate, but these features have come about in different ways in the two groups. They also resemble one another in the condition of the lingual muscles.

The *Rhynchocephalia* have preserved two features more archaic than are found in any other Sauropsidan group—the continuity of the ceratohyal, and the condition of the branchiohyoid muscle—but in the upgrowth of the external pterygoid muscle and the condition of the lingual muscles are less primitive than the *Chelonia*. Like the *Chelonia* and *Crocodylia*, they have preserved a fixed pterygoid bone.

The *Lacertilia vera* present great resemblances to the *Rhynchocephalia* in the condition of the external pterygoid muscle and the lingual muscles. The chief differences are

that in the former the greater portion of the pterygoid process disappears, the quadrate becoming secondarily movable, the continuity of the ceratohyal is lost, and the brachio-hyoid muscle also extends forwards to the lower jaw. Further, whereas in the Rhynchocephalia the upper portion of the mandibular myotome is inserted into the pterygoid process with a very few fibres to the (fixed) pterygoid bone, in the Lacertilia it is inserted into the (movable) pterygoid bone, with a few fibres in some Lizards to the quadrate.

The Rhiptoglossa and Ophidia present the Lacertilian features of a movable pterygoid bone into which is inserted the upper part of the mandibular myotome and (in embryonic stages) lingual muscles ending free in the tongue. Though they differ markedly from one another in the adult form of the tongue and in the associated condition of the hyobranchial visceral cartilages and lingual muscles, yet they possess in common the following features which are not found in any other groups—the non-development of a depressor palpebræ inferioris, an undivided pterygoid muscle arising from the pterygoid bone, the partial origin of the depressor mandibulæ from the quadrate, and the secondary position of the ceratohyal internal to the quadrate.

In conclusion, I have the pleasure of thanking Prof. W. N. Parker for the loan of embryos of *Chelone viridis*, Prof. F. S. Clarke for sections of *Alligator mississippiensis*, Prof. Moore for an embryo of *Sphenodon*, Mrs. Howes for sections of *Sphenodon*, and Prof. Broom for embryos of *Agama* and *Chamæleon*. My thanks are also due to my colleagues, Profs. Fawcett and Kent, for innumerable kindnesses.

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ON THE NAMES EMPLOYED.

B = Bradley, F = Fürbringer, G = Gadow, H = Hoffman, K = Kingsley,
O = Osawa, P = Parker, V = Versluys.

Orbicularis palpebrarum :

Birds (G), Sphenodon, Lacertilia vera, Rhiptoglossa.

Mandibular myotome—

Upper part divides :

Birds	{	depressor palpebræ inferioris (G).
	{	orbito-quadratus (G).
Chelonia	{	depressor palpebræ inferioris et superioris (H).
	{	atrophies.
Crocodylia	{	depressor palpebræ inferioris et superioris (H).
	{	atrophies.
Sphenodon	{	depressor palpebræ inferioris.
	{	spheno-ptyerygo-quadratus (F).
Lacertilia vera	{	depressor palpebræ inferioris.
	{	ptyerygo-parietalis.
	{	protractor ptyerygoidei (V) = ptyerygo-sphenoidalis posterior (B).
Rhiptoglossa	{	ptyerygo-sphenoidalis anterior.
	{	ptyerygo-parietalis (V).
	{	protractor ptyerygoidei (V).
	{	vomero-sphenoideus (H).
Ophidia	{	ptyerygo-sphenoidalis anterior (H).
	{	ptyerygo-parietalis (H).
	{	ptyerygo-sphenoidalis posterior (H).

Lower portion, inner division—

Pterygoid (undivided):

- Chelonia—pterygo-maxillaris (H).
- Crocodylia—pterygo-maxillaris (H).
- Rhoptoglossa—pterygo-maxillaris (H).
- Ophidia—transverso-maxillo-pterygo-mandibularis (H).

Internal pterygoid:

- Birds—pterygoideus internus (G).
- Sphenodon—pterygoideus internus (O).
- Lacertilia vera—pterygoideus internus (H) = pterygoideus (V)
= pterygo-mandibularis (B).

External pterygoid:

- Birds—pterygoideus externus (G).
- Sphenodon—pterygoideus externus (O).
- Lacertilia vera—pterygoideus externus (H) = pterygoideus (B).

Lower portion, outer division—

Temporal and quadrato-maxillaris: Birds (G).

Temporal:

- Chelonia—occipito-squamoso-maxillaris (H).
- Crocodylia—temporale-maxillaris (H).
- Sphenodon—capiti-mandibularis (O).
- Lacertilia vera—temporalis (H) = capiti-mandibularis (B).
- Rhoptoglossa—temporo-mandibularis et quadrato-mandibularis (H).
- Ophidia—parieto-quadrato-mandibularis (H).

Hyoid myotome—

Stapedius: Birds (G), Alligator (K), *Lacerta agilis*, *L. viridis*, and *Zootoca vivipara* (P).

Depressor mandibulæ:

- Birds—digastricus (G).
- Chelonia—squamoso-maxillaris (H).
- Crocodylia—occipito-maxillaris (H).
- Sphenodon—parieto-mandibularis (O).
- Lacertilia vera—digastricus (H) = depressor mandibulæ, (V) = parieto-mandibularis (B).
- Rhoptoglossa—depressor mandibulæ (H).
- Ophidia—occipito-quadrato-mandibularis (H).

First branchial myotome—

Branchio-hyoideus, branchio-maxillaris:
Sphenodon—cerato-hyoideus (O).

- Birds—genio-hyoideus (G).
 Chelonia—cerato-maxillaris (H).
 Crocodilia—maxillo-hyoideus (H).
 Lacertilia vera—cerato-hyoideus (H).
 Rhiptoglossa—genio-ceratoideus (H).
 Ophidia—mylo-hyoideus (H).

Ventral longitudinal muscles—

Genio-hyoideus :

- Birds—atrophies.
 Chelonia—genio-hyoideus (H).
 Crocodilia—anterior belly of maxillo-coracoideus (H).
 Sphenodon—cerato-mandibularis (O).
 Lacertilia vera—cerato-mandibularis (H).
 Rhiptoglossa—genio-hyoideus (H).
 Ophidia—maxillo-hyoideus (H).

Sterno-hyoideus divides :

- Birds—in Gallus into thyreo-hyoideus and sterno-thyroideus (G).
 Chelonia—coraco-hyoideus and coraco-cerato-hyoideus (H).
 Crocodilia—coraco-ceratoideus, episterno-ceratoideus, and posterior belly of maxillo-coracoideus (H).
 Sphenodon—episterno-hyoideus and omo-hyoideus (O).
 Lacertilia vera—episterno-hyoideus profundus, sterno-hyoideus, and omo-hyoideus (H).
 Rhiptoglossa—sterno-hyoideus and omo-hyoideus (H).
 Ophidia—cervico-hyoideus (H).

Genio-glossus :

- Birds—in some, e. g. Procellaria (G).
 Chelonia—maxilla-glossus (H).
 Crocodilia—genio-glossus.
 Sphenodon }
 Lacertilia vera } present, but not mentioned by (O) or (H).
 Rhiptoglossa }
 Ophidia }it gives off the genio-laryngeus (H).

Hyo-glossus :

- Birds—divides into cerato-glossus, hypoglossus rectus and obliquus (G).
 Chelonia—cerato-glossus.
 Crocodilia—cerato-hyoideus (H).

Sphenodon }
 Lacertilia vera } present, not mentioned by (O) or (H).

Rhoptoglossa—the posterior part forms cerato-hyoideus of (H).

Ophidia—hyo-glossus (H); it gives off the hyo-laryngeus (H).

Mylohyoids:

Birds—anterior mylohyoid = mylohyoideus (G).

posterior mylohyoid:

Anterior portion—serpihyoideus and stylohyoideus (G); a simple band in Lamellicostren (G).

Anterior constrictor colli = ceratohyoideus (G); not always developed (G).

Constrictor colli (G).

Chelonia—anterior mylohyoid = intermaxillaris (H).

posterior mylohyoid = constrictor colli (H).

Crocodylia—anterior mylohyoid = mylohyoideus (H).

posterior mylohyoid = sphincter colli (H).

Sphenodon—anterior and posterior mylohyoids form a continuous sheet = subcutaneous colli (O).

Lacertilia vera }
 Rhoptoglossa } mylohyoideus (H) and sphincter colli (H).

Ophidia—intermaxillaris (H) and transverso-hyoideus (H).

LIST OF ABBREVIATIONS EMPLOYED IN THE TEXT-FIGURES.

ab.an. Abducens Anlage. *ant.cons.colli.* Anterior constrictor colli. *ant.card.v.* Anterior cardinal vein. *ant.mylo.* Anterior mylohyoid muscle. *A.V.* Auditory vesicle or involution. *aur.* Auricle of heart. *1 B.B.* 1st basibranchial. *2 B.B.* 2nd basibranchial. *B.H.* Basihyal. *Bhbp.* Basihyo-branchial plate. *1 br.aor.ar.* 1st branchial aortic arch. *br.max.* Branchio-maxillaris muscle. *1 br.my.* 1st branchial myotome. *2 br.som.* 2nd branchial somite. *buc.cav.* Buccal cavity. *cart.ento.* Cartilago entoglossa. *car.art.* Carotid artery. *cer.br.1.* Ceratobranchial of 1st branchial bar. *cer.hyal.c.* Ceratohyal bar. *cer.hyal.cornu.* Ceratohyal cornu. *cil.g.* Ciliary ganglion. *conj.sac.* Conjunctival sac. *cons.colli.* Constrictor colli muscle. *cr.* Coronoid bone. *cost.proc.* Costal process. *d.* Dentary bone. *dep.mand.* Depressor mandibulæ. *dep.palp.inf.* Depressor palpebræ inferioris. *dor.aor.* Dorsal aorta. *dor.ceph.cæl.* Dorsal cephalic cælom. *E.* Eye. *enceph.* Encephalomere. *epipter.* Epipterygoid or processus ascendens. *epist.hyoïd.* Episterno-hyoideus muscle. *extrastap.* Extrastapedial. *g.petr.iv.* Ganglion petrosum of ix nerve. *gass.g.* Gasserian ganglion.

gang.vi. That portion of gasserian ganglion from which the first portion of v nerve proceeds. *gen.gang.vii.* Geniculate ganglion of vii nerve. *gen.gloss.* Genio-glossus muscle. *gen.hyooid.* Genio-hyoideus muscle. *g.s.* Gill pouch. *ht.* Heart. *hy.aor.ar.* Aortic arch of hyoid segment. *hyogloss.* Hyo-glossus muscle. *hyoid.my.* Myotome of hyoid segment. *hyoid.som.* Somite of hyoid segment. *hyomand.c.* Hyomandibular cartilage. *hypobl.* Hypoblast. *hypogloss.rect.* Hypoglossus rectus muscle. *int.orb.s.* Interorbital septum. *j.* Jugal bone. *j.&mx.an.* Anlage of jugal and superior maxilla. *lary.* Larynx. *lary.m.an.* Anlage of laryngeal muscles. *lary.c.* Anlage of laryngeal cartilages. *ling.m.an.* Anlage of lingual muscles (in Gallus). *mand.aor.ar.* Aortic arch of mandibular segment. *mand.my.* Myotome of mandibular segment. *mand.my.u.p.* Upper portion of myotome of mandibular segment. *Me.* Meckel's cartilage. *med.pl.* Medullary plate. *mid.nas.pas.* Middle nasal passage. *N.* Nose. *noto.* Notochord. *O.inf.* Obliquus inferior muscle. *O.sup.* Obliquus superior muscle. *O.sup.an.* Anlage of obliquus superior. *æs.* Œsophagus. *æs.cons.* Constrictor muscle of œsophagus. *omohyooid.* Omo-hyoideus muscle. *orb.palp.* Orbicularis palpebrarum muscle. *otosphen.pl.* Otosphenoidal plate. *pal.* Palate bone. *pal.pter.bar.* Palatopterygoid bar. *per.* Pericardium. *pg.* Pterygoid bone. *phar.* Pharynx. *pin.* Pineal body. *pit.* Pituitary body. *p.o.* Post-orbital bone. *post.mylo.* Posterior myloheid muscle. *premand.seg.an.* Anlage of premandibular segment. *protr.pter.* Protractor pterygoidei muscle. *pty.an.* Anlage of pterygoid muscles. *pty.ext.* Pterygoideus externus muscle. *pty.int.* Pterygoideus internus muscle. *pty.m.* Pterygoideus muscle. *pty.pr.of quad.* Pterygoid process of quadrate. *pty.par.* Pterygo-parietalis muscle. *pyr.&qu.an.* Anlage of pyramidalis and quadratus nictitantis. *quad.mand.* Quadrato-mandibularis muscle. *quad.* Quadrate. *quad.&Me.an.* Anlage of quadrate and Meckel's cartilage. *R.ext.* Rectus externus muscle. *R.int.* Rectus internus muscle. *R.sup.* Rectus superior muscle. *retract.oc.* Retractor oculi muscle. *scler.* Sclerotic (cartilaginous). *serpihy.* Serpi-hyoideus muscle. *som.meso.* Somatic mesoblast. *sphen.p.n.* Sphenopalatine nerve. *sphen.pter.quad.* Sphenopterygo-quadratus muscle. *spl.meso.* Splanchnic mesoblast. *sq.* Squamosal bone. *stap.m.* Stapedius muscle. *st.cerv.* Sterno-cervicalis muscle. *sternohy.* Sterno-hyoideus muscle. *sternothy.* Sterno-thyroideus muscle. *suprastap.* Suprastapedial. *temp.* Temporal muscle. *temp.&ma.an.* Anlage of temporal and masseter muscles. *trab.* Trabecula. *tr.art.* Truncus arteriosus. *trach.* Trachea. *1 tr.som.* 1st trunk somite. *thyr.* Thyroid. *ven.car.art.* Ventral carotid artery. *ven.ceph.cæl.* Ventral cephalic cælom. *vent.* Ventricle of heart. *vert.v.* Vertebral vein. *V.L.M.* Anlage of ventral longitudinal muscles. *Roman numerals.* Cranial nerves.

The Development of *Ophiothrix fragilis*.

By

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With Plates 31—36, and 4 Text-figures.

CONTENTS.

	PAGE
Introduction	557
Historical Sketch	558
Material and Methods	567
Normal and Abnormal Development	570
The Development of the Full-grown Larva	575
Metamorphosis of the Larva into the Brittle-star	580
Comparison with Development in other Classes of Echino- dermata	590
Summary and Conclusion	596

INTRODUCTION.

THE present work, which has occupied my attention for the last four years, was begun with the object of extending to other classes of Echinoderms the researches which I had already made on the development of Asteroidea (18) and Echinoidea (19). It has proved to be a task of extraordinary difficulty owing to the minute and refractory character of the larvæ of Ophiuroidea. Nevertheless, the results obtained will, I think, bear fair comparison with those which I have already published concerning the development of Asteroidea and Echinoidea, whilst a number of new and unexpected facts have disclosed themselves which possess interest for a

wider range of students than specialists in the class Echinodermata. The most interesting result which was obtained from the study of the development of Asteroidea and Echinoidea was the discovery that the coelom of the larva showed distinct traces of metameric segmentation, a division into three somites being clearly indicated. The pioneer in this work is Bury, to whose stimulating papers (4, 5, and 6) I wish on this occasion, as formerly, to express my deep indebtedness. Bury, however, only recognised two somites, whilst the main point of my previous researches was that the hydrocoele, or rudiment of the water-vascular system, which Bury regarded as essentially a single organ, was in reality paired, the left rudiment developing whilst the right remained small and insignificant. This interpretation of the facts has been challenged by other observers such as Goto (11) and Masterman (20), who interpret what I regard as the right hydrocoele in a different manner. I may summarise the results of the present paper by saying that the study of the development of Ophiuroidea has afforded me a complete confirmation of my views. The state of affairs in the Ophiuroidea is much clearer and simpler than in the other classes of Echinodermata, and I hope to convince every unprejudiced reader of this paper that the Echinoderm larva really possesses three metameres.

HISTORICAL SKETCH.

Our knowledge of the development of Ophiuroidea dates back to Johannes Müller. At the meeting of the Academy of Sciences of Berlin, held on December 4th, 1845, this celebrated naturalist described a number of new marine animals which he had observed whilst at Heligoland in the autumn of that year. Amongst these was one which Müller named *Pluteus paradoxus*, from its fancied resemblance to a painter's easel. This description was published in the 'Archiv für Anatomie und Physiologie,' in 1846 (24), and on October 29th of that year Müller read before the Academy

of Sciences a paper entitled "Über die Larven und die Metamorphosen der Ophiuren und Seeigel" (25), in which he describes the metamorphosis of *Pluteus paradoxus* and shows that it is the larva of an Ophiurid. A somewhat similar organism also referred to the genus *Pluteus* is shown in this paper to be the larva of an Echinoid, and Müller adopted the regrettable custom of using the same word "*Pluteus*" to designate these two quite different larvæ; this custom has persisted until quite recently and has been the source of much confusion. In the case of *Pluteus paradoxus* the change into an Ophiurid is traced step by step, and Müller figures without describing it a specimen which has a five-lobed rosette—the form assumed by the rudiment of the water-vascular system—on the right as well as on the left side of the œsophagus. In this figure also the metameric division of the cœlom is beautifully shown, and, indeed, Müller's drawing could have been used to illustrate this paper. Of course, Müller was unable to interpret his results fully, and since the cœlomic rudiments have extremely narrow lumina it is not surprising that he did not recognise them as the forerunners of the body-cavities of the adult. A second form of Ophiurid *pluteus* with more slender arms is described in the same paper, and in a later paper (27) read before the Academy in 1851, Müller describes two further forms of Ophiurid larvæ which he discovered during a visit to Trieste, one of which he named *Pluteus bimaculatus* from the brown pigment spots with which it was ornamented, the other he recognised as the subject of the present paper—the larva of *Ophiothrix fragilis*. In *Pluteus bimaculatus* Müller discovered the larval anus of Ophiuroidea, thus showing that the absence of the anus in this class of Echinodermata is a secondary, not a primary affair. The hydrocœle or rudiment of the water-vascular system is described as the palmate organ, since it possesses five lobes like the five fingers of the human hand. Müller saw that it was formed late in larval life, and was at first round in shape. In its later stages it extends ventral to the œsophagus, and each lobe becomes

five-lobed, so that each somewhat resembles the whole organ in an earlier stage. Müller was led astray by this resemblance and gave to each primary lobe the name "palmate organ," and he imagined that the palmate organ of the earlier larva was identical with the first of these, and that the rest were formed *de novo*. He described also a semi-circular wavy fold of skin, from which the rudiments of the adult arms grew out, to which the "palmate organs" became co-adapted later. These latter gave rise to the radial canals and tentacles of the water-vascular system.

The larva of *Ophiothrix fragilis* was recognised by Müller owing to its possessing on the first tentacles of the developing brittle-star the papillæ characteristic of this species. Müller only obtained late metamorphosing stages, and his description concerns itself chiefly with the elaboration of the details of the brittle-star. The last Ophiurid larva described by Müller is one which he called the "worm-like larva," which he imagined to belong to the Asteroidea (28). It was re-discovered by Krohn (15), who proved that it was the larva of an Ophiurid. In 1900 Caswell Grave (12) found a similar larva on the American coast, and showed that it was the young stage of *Ophiura brevispina*. Owing to the opacity of this larva neither Müller nor Krohn made out much of its structure.

The next great advance in our knowledge of Ophiurid development was made by Metschnikoff in 1869 (21). In a paper on the development of Echinoderms and Nemertines he records the results of investigations on all four classes of Echinoderm larvæ, as well as on the embryos of the viviparous species, *Amphiura squamata*. Three kinds of Ophiurid plutei are described, two of which are identified with larvæ described by Johannes Müller. Of only one form, however, did he obtain sufficient stages to describe the development in any detail, and this is the *Pluteus bimaculatus* of Müller. In the youngest stage which he obtained he was able to make out the right and left cœlomic cavities lying at the sides of the larval œsophagus. In the next stage he discovered a

pair of discs lying at the sides of the stomach, and concluded that they had been segmented off from the œsophageal cavities, which he could still make out. This is the first assertion of a trace of metameric segmentation in any Echinoderm larva. In subsequent stages he discovered that the left anterior cavity assumed the five-lobed form characteristic of the hydrocœle, whilst the right grew smaller and ultimately disappeared. In the next stage he showed that the hydrocœlic rudiment had by growth changed its longitudinal position for a transverse one, and was, in fact, beginning to surround the larval œsophagus. Müller had supposed that, as in Echinoidea, the larval œsophagus disappeared, but Metschnikoff shows that it persists. He further corrected Müller's error about the "palmate organs," and showed that each of these corresponded to one lobe of the original palmate organ or hydrocœle. He showed also that the primary madreporic pore, although formed on the left side, rotates during metamorphosis to the right along with the left præ-oral arm of the Pluteus. In a word, Metschnikoff made out almost everything that it is possible to discover in a Pluteus without the use of sections.

The viviparous species, *Amphiura squamata*, offered an opportunity of studying the embryonic type of development in an Ophiurid. Its viviparity was discovered by Quatrefages in 1842. He communicated his results to Milne Edwards, by whom they were published. Krohn published in 1851 a paper on the subject (14), dealing with the relation of the embryo to the maternal tissues. In 1852 Schultze (30) discovered a transitory larval skeleton, which he compared to the skeleton of the Pluteus.

In the paper referred to above Metschnikoff records most valuable observations on the development of *Amphiura squamata*, although the younger embryos are difficult to obtain. He found a thick-walled blastula, and then a later stage, in which the cœlom was represented by two thick-walled bodies lying at the sides of the archenteron. In the next stage each of these bodies had divided into anterior and

posterior portions, and soon afterwards the left anterior portion had assumed the rosette form characteristic of the hydrocœle. Metschnikoff expressly states that the right anterior sac often likewise assumed the same form, and this is the first clear statement that I have been able to find in the literature that the hydrocœle rudiment is paired. Müller's figure alluded to above shows the same thing in *Pluteus paradoxus*, but, as already mentioned, he gives no description of it.

Further researches on the development of *Amphiura squamata* were made by Ludwig (16), Apostolides (2), Fewkes (10), Carpenter (7), Russo (29), and myself (17). Ludwig's work has reference to the development of the skeleton; amongst other things he showed that the ossicle termed a "vertebra" is formed by the concrescence of two calcareous plates, so that its homology with a pair of ambulacral plates in an Asterid is proved. Apostolides dealt with the whole development, and according to him the endoderm is formed by delamination, not, as in other Echinoderms, by invagination. This extraordinary statement is reaffirmed by Russo, who also deals with the whole development. Russo asserts further that the cœlomic cavities arise as spaces in the mesenchyme; but he utterly fails to confirm Metschnikoff's observations as to the division of the right cœlomic sac into anterior and posterior portions, and the occasional assumption of a five-lobed form by the anterior half. Russo's results are at variance with what is known of the early stages of development of all other Echinoderms; and since he failed to see what Metschnikoff was able to make out in the embryos of *Amphiura squamata* his results have been received with general scepticism, but cannot, of course, be formally denied till some other investigator has the patience to re-examine the early stages of development of this species. These stages are opaque and difficult to obtain, hence, no doubt, the unsatisfactory nature of our knowledge on the subject. Fewkes, who had very little material of the early stages at his disposal, deals chiefly with the development of the adult skeleton, but made out also the ectodermal origin of the

œsophagus. Carpenter (7) was chiefly occupied in establishing a fanciful homology between the dorsal plates of *Amphiura squamata* and the aboral plates of a Crinoid. My own work (17) had reference to the first origin of the genital cells, and dealt solely with post-larval stages. I discriminated for the first time between various spaces which had been confused under the name "axial sinus." The problematical organ lying along the stone-canal, which had been regarded as a heart by some zoologists, was shown to be of peritoneal origin, and to represent the first rudiment of the genital cells. This paper contains one serious blunder which I endeavoured to set right in my paper on *Asterina gibbosa* (18), viz. the vesicle representing the right fellow of the hydrocœle is confused with a space originating as a peritoneal invagination and labelled in my figures Sinus b.

Reviewing the narrative so far given, it will be seen that the only two investigators who advanced our knowledge of the early larval stages of Ophiuroidean development were Müller and Metschnikoff. To these it is now necessary to add a third name, viz. that of H. Bury. This talented investigator, who gave to zoology the first satisfactory account (4) of the development of the Crinoid *Antedon rosacea*, was led by his study of this species to revive the idea of a metameric segmentation of the Echinoderm larva. Taking up the investigation of the larvæ of other classes of Echinodermata, he found his ideas amply confirmed, and in 1889 he published a paper (5) in which he showed that a transverse division of the cœlom took place in the larvæ of both Echinoidea and Ophiuroidea. Bury was the first to discriminate an anterior cœlom from the hydrocœle, which he regarded as an essentially unpaired organ. This hydrocœle, as he showed, was formed comparatively late in development, and arose as an outgrowth from the left anterior cœlomic vesicle in Echinoidea, whilst in Ophiuroidea he felt himself compelled to assert that it was an outgrowth from the left posterior vesicle. During the interval between the appearance of the papers of Metschnikoff and Bury quite a number of authors had made

use of artificial fertilisation, and thus accumulated a certain amount of information about the earliest stages of development of Ophiurids other than *Amphiura squamata*. Kowalevsky, in a paper devoted to *Amphioxus* (13), had even before Metschnikoff's time noted the fact that the eggs of an undetermined Ophiurid formed a gastrula by invagination. Balfour had observed the same in the case of *Ophiothrix fragilis* (3), Selenka in *Ophioglypha lacertosa* (31), and Fewkes in *Ophiopholis aculeata* (9). These results naturally led to increased scepticism as to the supposed formation of the endoderm by delamination in *Amphiura squamata*. In 1895 Bury published another paper entitled "The Metamorphosis of Echinoderms" (6). This paper, although containing most valuable observations on the other groups of Echinoderms, as well as a general theory of the phylogeny of the group, contains few new observations on Ophiuroidea, the only notable one being a suggestion that the left posterior cœlom encircles the stomach in the later stages of development. This view Bury bases on a single section, which showed a horizontal mesentery.

Ziegler in 1896 published a short paper on the early stages of development of *Ophiothrix fragilis* (33). His account only reaches as far as the gastrulation, but he describes a vacuolated crest which is very characteristic of these larvæ.

In 1900 Caswell Grave published an interesting and valuable paper (12) on the development of *Ophiura brevispina*. This species lays comparatively large eggs and has a shortened development. It transpires, indeed, that the eggs develop into the "worm-like" larva described by Johannes Müller and Krohn, which is thus shown to be the larva of *Ophiura brevispina*, or of a closely allied species. Grave's material was, as he himself explains, decidedly scanty, a circumstance which renders it possible that some of his conclusions may require revision should more abundant material become available. The first stage which he describes is an oval larva, $1\frac{1}{2}$ days old, uniformly ciliated and swimming freely. In this stage an anterior, bilobed, cœlomic vesicle is being constricted

off from the apex of the archenteron. A large cellular plug projects from the apex of the cœlomic vesicle and extends into the cavity of the archenteron. From this circumstance Grave draws the somewhat rash conclusion that the archenteron has been formed by the hollowing out of the inner mass of a planula-like larva. In the next stage, which is only a few hours older, a remarkable state of affairs has come about. The first vesicle is completely constricted from the archenteron and divided into right and left halves. A second vesicle, however, has been budded from the archenteron and is still in open communication with it. This vesicle is already partially divided into two by a constriction, and its anterior portion shows the five lobes which proclaim it to be the hydrocœle, whilst its posterior portion gives rise to the hypogastric cœlom, i.e. the left posterior cœlom. This state of affairs is widely different from that which has been described in the case of any other Echinoderm. The very early appearance of a lobed hydrocœle shows that the first stages of development have been very much hurried over, and hence we must regard differences from the normal type of Echinoderm development as secondary modifications, not as indications of a primary state of affairs. In the next stage the ectodermal stomodæum has met the œsophagus. It passes under the arch of the hydrocœle, so that this forms a bridge half-way around it. The stone-canal is formed as a secondary connection between the left anterior cœlom and the hydrocœle. A so-called epigastric cœlom—corresponding to the right posterior cœlom of the free-swimming pluteus—appears in this stage, and after some discussion of the point, Grave concludes that it must have been formed from the right anterior cœlom. In the next stage the cilia are restricted to four transverse rings, which produce an appearance of metameric segmentation and give rise to the "worm-like" appearance of the larva. The posterior end of the larva is broadened, and contains practically all the organs of the Ophiurid; the anterior portion contains mesenchyme and forms a large præ-oral lobe. The hydrocœle has rotated to such an extent

that its most anterior lobe, numbered 1 by Grave, has not only passed over the œsophagus to the right, but has continued its revolution underneath it to the left again, so that it has been rotated through an angle of 180° , whilst the most posterior lobe, No. 5, has rotated through 90° only, the whole hydrocœle having grown longer. The left anterior cœlom, together with the madreporic pore which has now appeared, follow the rotation of the hydrocœle and pass over the œsophagus on to the right side. The lobes of the hydrocœle become trifid, the lateral lobes being the rudiments of the first paired tube-feet.

From the left posterior cœlom, termed by Grave the "hypogastric," there arise four out-growths, which, insinuating themselves between the lobes of the hydrocœle, form the rudiments of the radial perihæmal canals and of the perihæmal ring in the same manner as I have described in *Asterina gibbosa*. A fifth is given off from the left anterior cœlom. In later stages the nervous system makes its appearance as thickenings of the neutral ectoderm overlying the ring and radial canals. These thickenings are then invaginated into grooves and so the epineural canals of the adult are formed. The larval organ gradually disappears, but the young Ophiurids retain for a considerable period some of the larval transverse rings of cilia.

I have described Grave's work at some length, since it is the latest work of any importance on the formation of organs in an Ophiurid which has been published. Making allowances for differences in nomenclature, it will be seen that Grave confirms Bury in the opinion that the hydrocœle springs from the left posterior cœlom. The independent origin of this portion of the cœlom as a separate evagination of the gut from that which gives rise to the two anterior cœlomic vesicles is almost unique in the published accounts of Echinoderm development. It is true that Masterman (20) has described a somewhat similar state of affairs in a star-fish, *Cribrella oculata*, in which, according to him, the hypogastric cœlom (left posterior cœlom) does not arise from the common rudiment

of the anterior and epigastric (right posterior) cœloms, but has an independent origin from the gut ; in this case, however, the hydrocœle is derived from the anterior cœlom. In both cases we have to do with yolky eggs and a shortened development, and in both cases a re-examination of the facts is desirable, as Grave would be the first to admit, since throughout his paper he laments the scantiness of his material and the gaps between the stages. It is to be hoped that he will be able at no distant date to give the whole subject a thorough revision.

Mortensen in 1898 published a systematic review of all the forms of Echinoderm larvæ known up till that date (22). In this valuable paper he discusses the homology of the various larval appendages, and introduces a nomenclature which will be employed in this paper. Another service which he has rendered is to introduce the terms *Ophiopluteus* and *Echinopluteus* for the larvæ of *Ophiuroidea* and *Echinoidea* respectively, thus doing away with the confusion implied in the use of the term "Pluteus." In a second paper (23) he makes an addition to the number of varieties of Echinoderm larvæ known, and with this second paper our review of the knowledge so far gained may be said to be complete.

MATERIAL AND METHODS.

In the summer of 1898, whilst I was staying at Plymouth investigating the development of *Echinus esculentus*, I received a large number of specimens of *Ophiothrix fragilis*, which appeared to be sexually ripe. I therefore attempted artificial fertilisation, but as the developing eggs remained at the bottom I concluded that they were abnormal. About eight days afterwards, to my delight, I found a certain number of perfectly formed, free-swimming larvæ in the jar. These larvæ continued to develop, and at the end of twenty-six days had completed their metamorphosis. The jar in which the larvæ were placed was shaded from the light and fitted with a Browne plunger. During the summer of 1898 the Phytoplankton was particularly abundant, and so the de-

veloping larvæ obtained plenty of food. Returning to complete my work on *Echinus esculentus* in 1899, I again tried to rear the larvæ of *Ophiothrix fragilis*, but although I obtained ripe adults and fertilised the eggs I was unable to keep the larvæ living for longer than five days. Towards the end of my stay the larvæ became very abundant in the Plankton, and from July 14th to 19th, 1899, it might be fairly said to swarm with them. As I observed that the Echinoderm larvæ were in a somewhat pathological condition when the bottles containing the Plankton were brought into the laboratory, I went out in the steamer belonging to the station and preserved the Plankton immediately on its being brought to the surface. For this purpose I used 1 per cent. osmic acid followed by Müller's fluid—a method which has the advantage of giving an excellent preservation of the tissues, but it labours under two disadvantages: it makes the specimens brittle and it completely dissolves the calcareous matter, and hence no attention is paid in this paper to the development of the adult skeleton. The Plankton contained in all four varieties of Ophiurid larvæ, but the larvæ of *Ophiothrix fragilis* were in an enormous majority, and in all stages of development up to the completed metamorphosis. They were easily distinguishable by the greatly developed postero-lateral arms, which in this species are extraordinarily long. In this way a large amount of material was obtained, but I was unable to commence work on it till 1903, when I had completed the work on *Echinus esculentus*. In 1905 I paid another visit to Plymouth in order to fill the gap in the series of stages which I had obtained from the Plankton. It happened that the season was rather later, so far as the breeding habits of this species were concerned, than it had been in 1898 and 1899, and though I examined hundreds of adults I only succeeded in obtaining two females which were capable of fertilisation. Of these the first gave larvæ which pursued a development similar to that undergone by the larvæ which I obtained by artificial fertilisation in 1899, and which I believed to be the normal course of events, but I was unable to keep

them alive more than eight or nine days. The second female was placed in a half-gallon jar along with two males and spawned in a natural manner. The larvæ were exceedingly vigorous and developed with great rapidity; they differed in a marked manner from the result of previous cultures so far as the earliest stages were concerned. I was able to rear them for sixteen days, by which time the hydrocœle had made its appearance. Their eventual death was clearly due to starvation, as the Phytoplankton was extraordinarily scanty in that year. Reviewing the account just given, it will be seen that in 1898 a culture was obtained as the result of artificial fertilisation in which some larvæ completed the entire development, until the end of metamorphosis. In 1899 and 1905 similar cultures gave larvæ which were only able to carry on the development for about a week; whilst in 1905 a culture obtained by what may be termed natural fertilisation gave larvæ which grew and developed vigorously as long as food was available, and which in their earlier stages were different from those which resulted from artificial fertilisation. The main mass of the material was obtained from Plankton. If we take the successful culture of 1898 as giving an indication of the time required to effect complete development, it will follow that metamorphosis is finished in about a month, and the approximate time for the appearance of other organs is given in the following table:

Free-swimming blastula	24 hours.
Gastrula	36 „
Formation of cœlom	2 days.
Stomodæum joins gut	3 „
First transverse division of cœlom	8 „
Formation of hydrocœles	16 „
Left hydrocœle five-lobed	20 „
Encircling of œsophagus by hydrocœle	23 „
Absorption of larval arms complete	26 „

These times, however, are only approximate, and the rate of development varies very much with the amount of food present, and possibly from other causes too. In particular

it will be noticed that the culture obtained by natural fertilisation in 1905 developed at a quicker rate than that indicated by these figures.

The larvæ were observed and drawn living. Those preserved in osmic acid and Müller's fluid were sectioned. For this purpose the celloidin paraffin method was used, and the procedure adopted was similar to that employed in the case of *Echinus esculentus* and described in my paper on the subject (19). One additional caution, which it was found necessary to adopt, may be mentioned here. It transpired that if the sections, after being spread out on hot water in order to flatten them, were left to dry for longer than forty minutes on the top of the thermostat, great cracks developed in them owing to the shrinkage of the celloidin.

When it was necessary to supplement the information obtained from views of the living larvæ by whole mounts of preserved ones, these were cleared from osmic acid. This was done by placing them in water or weak alcohol. The vessel containing them was then placed (open) inside a larger vessel on the bottom of which was a layer of crystals of chlorate of potash on which strong hydrochloric acid was poured. The larger vessel was closed. The euchlorine gas evolved became dissolved in the fluid of the smaller vessel and oxidised the black deposit of metallic osmium in the tissues.

In the orientation of sections the postero-lateral arms of the larva were of the greatest assistance, for they persist until the metamorphosis is quite complete, so that they mark a constant plane amidst the varying position of the other organs. This plane will be called the frontal plane, and most of the sections were cut parallel to it. Sections parallel to the median sagittal plane of the larva were also employed as were transverse sections when they became necessary in order to elucidate special points.

NORMAL AND ABNORMAL DEVELOPMENT.

It has already been mentioned that eggs fertilised under different conditions give rise to larvæ which in their early

stages differ considerably from one another. From eggs artificially fertilised larvæ are obtained which are shown in Pls. 32 and 33, figs. 18-23, whilst when a male and female are brought together and allowed to spawn naturally the fertilised eggs develop into larvæ, the corresponding stages of which are shown in Pl. 31, figs. 1-5, and Pl. 33, figs. 24-28.

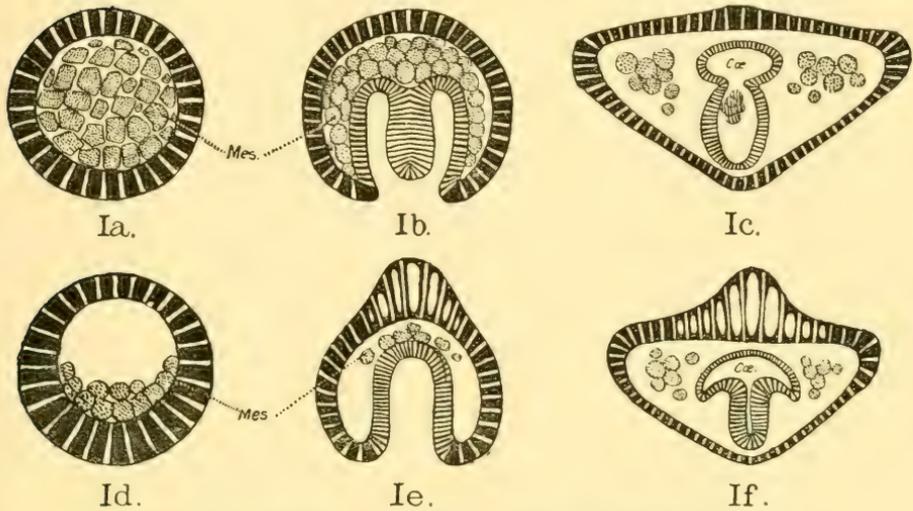
Considering first the normal development due to natural fertilisation we find that the eggs, which are small (about 1 mm. in diameter) and opaque, owing to the presence of a yellow yolk, develop in seven or eight hours into small thick-walled **blastulæ** (Pl. 31, figs. 1 and 2), from one side of which mesenchyme cells are budded off (Pl. 33, fig. 24, *mes.*). At the end of twelve to eighteen hours the embryo has escaped from the egg membrane and become ciliated and free-swimming, and is now henceforth to be designated a larva. The larva is egg-shaped (Pl. 31, fig. 3), the more pointed end, which is directed forwards, being formed of a great **crest** of vacuolated cells, which possibly acts as a hydrostatic apparatus (Pl. 33, fig. 25), whilst at the opposite pole the formation of mesenchyme is going on, and the invagination which is to form the primary gut or **archenteron** is beginning (Pl. 33, figs. 25-27). During the next fifteen or twenty hours the form of the larva changes from an egg-shape to a lozenge-shape, owing to the appearance of two lateral outgrowths, which are the earliest rudiments of the **postero-lateral arms** of the larva (Pl. 33, fig. 28, *pl.*). Into these outgrowths the most of the mesenchyme cells wander, where they form the skeletogenous basis of the calcareous rods which support these arms. The space between the archenteron and the ectoderm is of course identical with the **blastocœle** or cavity of the blastula; it has been termed the **archicœle** or **primary body-cavity** by German authors; both these names will be employed in this paper. The **secondary body-cavity** or **cœlom** (Pl. 33, figs. 28 and 29, *cœ.*), arises as a thin-walled bilobed outgrowth from the apex of the archenteron when the larva is from thirty to forty hours old. The postero-lateral arms grow rapidly and the vacuolated crest dwindles in similar

proportion. The characteristic **longitudinal ciliated band** by means of which locomotion is effected has already appeared as a transverse ridge, which rapidly becomes a horizontal loop (Pl. 31, figs. 4 and 5); as the postero-lateral arms grow, they receive extensions of this loop, and so the swimming powers of the larva are increased. It may be partly owing to this circumstance that the vacuolated crest disappears, but it is also partly replaced by a conversion of the ectoderm of the posterior end of the larva into an appendage of vacuolated cells, which persists until metamorphosis is complete (figs. 29 and 44, *vac.*).

Leaving the consideration of eggs which have received natural fertilisation, and turning to those which have been artificially fertilised, we find that they are distinguished by such an early and abundant formation of mesenchyme that sections through earlier stages (fig. 18) show a practically solid mass of blastomeres, and when segmentation is complete there is an outer covering of cylindrical ectodermal cells, and an inner mass of rounded cells, so that an appearance is presented which strongly recalls that of a Cœlenterate planula (Pl. 32, fig. 19). A flattening and thickening of one pole now follows, together with a renewed production of mesenchyme (fig. 20, *mes.*), and then invagination takes place in such a way as to leave a wedge of cells projecting into the cavity of the archenteron (fig. 21). This results from the fact that the endodermic plate of cells becomes in one meridian several cells deep before it is invaginated. In the next stage the postero-lateral arms make their appearance (fig. 22) and receive most of the primary mesenchyme, whilst soon after the cœlom appears as a vesicle at the apex of the archenteron. Part of the projecting tongue of cells is included within it and part within the lower portion of the archenteron, both parts being eventually absorbed. At no time is there the slightest trace of the vacuolated crest of cells so characteristic of normal development. Caswell Grave, in his paper on the development of *Ophiura brevis* (12), describes a tongue of cells projecting into the archenteron as

a normal feature of the development of that species, and he suggests (as he did not have the earlier stages) that it is an indication that the archenteron is formed, not by invagination, but by the hollowing out of an originally solid endoderm. It is but just to record that when I gave an account of the abnormal development of *Ophiothrix fragilis* before the American Society of Zoologists in Philadelphia in 1903, Dr. Grave, in commenting on my paper, stated that he had

TEXT-FIG. 1.



Ia.-Ic Three stages in development of eggs artificially fertilised.
 Id.-If. Three stages in development of eggs naturally fertilised.
Mes. Mesenchyme. *Cæ.* Cæomic rudiment. (From the 'Proceedings of the Royal Society,' B, vol. 79, 1907.)

obtained further material of *Ophiura brevis*, and that he had become convinced that the course of events in that species was identical with what I had described for *Ophiothrix fragilis*, in that form of the development which I at that period took to be the normal one.

The contrast between the normal and abnormal methods of development is well shown in text-fig. 1.

Turning now to the question of the cause of the difference between normal and abnormal development, it will hardly be

contended that the mere act of cutting out the ovary is competent to produce such an effect. This is negatived by the fact that in one culture raised from artificial fertilisation some larvæ were found showing a well-developed vacuolated crest. The much more probable assumption is that abnormal development results from the fertilisation of eggs which are not quite ripe.

Up till now development following on fertilisation has been taken as adequate proof of the ripeness of an egg, as anyone who reads the numerous papers of Driesch, Herbst, Loeb, and other workers on what is called "Developmental Mechanics" may readily see. But when working with *Echinus esculentus* I obtained some evidence that this is not necessarily the case. I was, however, so much astonished by the facts which came to light that I have not until now dared to publish them, and only do so now that a similar state of affairs has been discovered with reference to the development of *Ophiothrix fragilis*. I found that in order to obtain good cultures of the larvæ of *Echinus esculentus* it was necessary to choose for artificial fertilisation specimens which were thoroughly ripe. This thorough ripeness was evidenced by the condition of the ovary. When this organ at a touch dissolved into eggs, then the resulting culture was highly successful. When, however, I tried to fertilise eggs from half-grown specimens, I obtained indeed some larvæ, but these were small and had imperfectly developed arms, and lived only a few days.

Of course, as all know, an egg is incapable of fertilisation until it has formed its two polar bodies, but this process, in some animals at least, is hurried on by the mere presence of the spermatozoa. In the American oyster it is impossible by inspection under the microscope to discriminate between ripe and unripe eggs, for in this species the membrane of the nucleus of the oöcyte remains undissolved until it comes into contact with the spermatozoa. It seems, therefore, allowable to conclude that although in Echinoderms polar bodies are normally formed independently of the presence of sperma-

tozoa, yet they may be prematurely produced by the presence of these, or possibly by the mechanical irritation of shaking the ovary, and that in these cases the resulting development is abnormal. This abnormality must be due therefore to a difference in the chemical constitution of the egg, and the fact that one abnormal feature appears to be normal in another species seems to be an indication that very slight chemical alterations in the constitution of the fertilised egg may produce marked structural alterations in the organism which results from it, and that these alterations may be inheritable. Here, perhaps, is to be found the origin of those mutations which, according to recent speculation, constitute the raw material with which evolution works. But for the solution of this problem animals must be selected, which are easier to breed and in which the infant mortality is less than in Echinoderms.

THE DEVELOPMENT OF THE FULL-GROWN LARVA.

We left the consideration of the normal development at the point where the cœlom had arisen from the apex of the archenteron as a bilobed vesicle, and the vacuolated crest had diminished in size, whilst the postero-lateral arms were making their appearance. By the end of the second day the crest has entirely disappeared, whilst the postero-lateral arms have grown so much that the organism takes on the form of a **V** with a thickened point. The cœlom separates as a single vesicle from the archenteron, while the rest of the archenteron constitutes the definite gut (Pl. 33, fig. 29). Soon afterwards the single cœlomic rudiment divides into right and left halves, and the **stomodæum** makes its appearance as a wide, shallow pit just ventral to the place where the crest disappeared (Pl. 31, fig. 6). In fig. 30 the stomodæum is shown in the act of opening into the gut, and from a comparison of this specimen with older specimens, sections of which are shown in figs. 31 and 32, the conclusion is arrived at that, as in *Echinus esculentus*, the **V-shaped adoral ciliated band** which projects

into the œsophagus is formed partly from ectoderm and partly from endoderm cells (fig. 30, *Cil. ad.*). As in other Echinoderm larvæ the blastopore forms the **anus**, whilst the definitive gut becomes divided by constrictions into **intestine**, **stomach**, and **œsophagus**, the last-named of which joins with the stomodæum. On the third day the **antero-lateral arms** make their appearance (fig. 31, *a.l.*), and these are supported from their first inception by branches given off from the primary skeletal rods which support the postero-lateral arms. The posterior ends of these primary rods undergo a branching just inside the vacuolated posterior end of the larva, which is characteristic of *Ophiothrix fragilis*. Each rod branches into anterior and posterior spikes, and each of these divides into a dorsal and ventral branch, so that by the juxtaposition of right and left halves of the skeleton a kind of basket is produced. In none of the figures is the forking into dorsal and ventral spikes shown, but the division into anterior and posterior branches is shown in many of the figures (c f. figs. 6-12).

When once right and left cœlomic sacs have been established from their inner walls muscular fibres grow out and surround the œsophagus (fig. 31, *musc.*). Thus **constrictor muscles** are provided by means of which swallowing movements can be carried on and the larva becomes able to feed. At the same time the **madreporic pore** is formed. As shown in the transverse sections represented in figs. 35 and 36, an ectodermic ingrowth (*m.p.*) consisting apparently of two large clear cells, meets a short outgrowth (*p.c.*) from the left cœlomic sac, and by the fusion of the two a tube is established which leads from the left cœlomic sac to the exterior. This tube may be termed the **pore-canal**, whilst its opening may be called the **primary madreporic pore**. This pore, as in *Echinus esculentus*, is at first markedly on the left side of the larva, but subsequently shifts so as to reach the mid-dorsal line; indeed, in *Ophiothrix* it even passes beyond the mid-dorsal line. The left cœlomic sac has a wider lumen than the right, thus foreshadowing its eventual conversion into the so-called

“ampulla” of the stone-canal, the equivalent of the axial sinus in *Asterina gibbosa*. About the fourth day the **post-oral**¹ arms make their appearance and the ventral side of the larva becomes markedly concave. The new arms are supported by outgrowths from the primary skeletal rods, which arise at the same point as those which support the antero-lateral arms. The left cœlomic sac now grows out into a posterior tongue of cells at first solid (fig. 32, *l.p.c.*), which soon hollows out to form a vesicle (fig. 33). This vesicle becomes separated from the left cœlomic sac as the left posterior cœlom, and moves back so as to be at the side of the stomach (fig. 8, *l.p.c.*), whilst the original sac now termed the left anterior cœlom (fig. 8, *l.a.c.*) remains at the side of the œsophagus. About the fifth day the right cœlomic sac undergoes a similar series of changes. The solid posterior outgrowth is shown in fig. 34, whilst in fig. 8 is represented a specimen in which this outgrowth (*r.p.c.*) having become a hollow vesicle is in the act of separating from the original sac, now termed the right anterior cœlom (*r.a.c.*), which remains at the side of the œsophagus. In fig. 9, a slightly older specimen is shown in which the division is complete on both sides. Thus in *Ophiothrix fragilis* as in *Asterina gibbosa* and in *Echinus esculentus* the transverse division of the cœlom of the left side precedes that of the right, thus foreshadowing that predominance of the left side which plays such an important part in the metamorphosis.

The larva now continues to grow in size without undergoing any important changes for a day or two, but at a period varying from seven to ten days from fertilisation, according to the amount of food-supply present in the water, the last pair of arms, the **postero-dorsal**² (figs. 10 and 11, *p.d.*)

¹ Owing to an unfortunate misunderstanding of Mortensen's paper (22) the arms of the larva of *Echinus esculentus* are wrongly named in my paper on the subject (19). What I have called in that paper postero-dorsal arms are really post-oral and *vice-versâ*.

² These arms correspond to those arms of the larva of *Echinus esculentus*, wrongly named post-oral by me in my paper on the subject.

make their appearance. They are supported by branches from the rods which support the antero-lateral arms. At the same time the posterior ends of both the right and the left anterior cœlomic sacs become thickened and the thickenings are hollowed out so as to form vesicles (fig. 37 *a*). These vesicles are the rudiments of the right and left hydrocœles respectively. On the left side the vesicle becomes constricted from the left anterior cœlom (figs. 10 and 11); so that it is connected therewith by a narrow neck only, which is the rudiment of the stone-canal. On the right side no such constriction takes place, but the cavity of the right hydrocœle becomes entirely shut off from that of the right anterior cœlom (figs. 38 *a* and *b*). Bury supposed (5) that the left hydrocœle was formed by an outgrowth from the left posterior cœlom. This supposition is an exceedingly natural one, for it corresponds with the appearances presented by slightly older larvæ than those which we are at present discussing. The left posterior cœlom grows forward so as to become closely apposed to the rudiment of the future hydrocœle, and an observer who had not an unbroken series of stages before him might easily imagine that the hydrocœle was being nipped off from the posterior cœlom. With regard to the right hydrocœle, this is larger relatively to the left rudiment than it is in the case of either *Asterina gibbosa* or *Echinus esculentus*. In both the last-named forms it occupies from the beginning a position near the mid-dorsal line, for which reason Masterman (20) calls it the central cœlom and identifies it with the pericardial cavity of *Balanoglossus*. Goto (11) also on the same ground denies that it is the fellow of the left hydrocœle. But in *Ophiothrix fragilis* there can be no doubt on the subject; the structure in question is on the right side from the beginning and remains on the right side, and occasionally, as Müller (25) has figured and as is shown in fig. 53, takes on a five-lobed form. Shortly after this period when all the larval arms have attained their full length, the left hydrocœle begins to take on a five-lobed form (figs. 12 and 39). The five lobes soon

increase in length and become finger-shaped; they are at first arranged in a straight line antero-posteriorly, and will be numbered 1 to 5, commencing with the most anterior. It is a question of considerable interest where the stone-canal enters the hydrocœle. The latter organ, as it grows, extends forward beneath the anterior cœlom and eventually reaches beyond it, although the anterior cœlom was originally situated entirely in front of the hydrocœle.

The stone-canal was thought by Bury (6) to open into the hydrocœle between lobes 4 and 5. Bury regards the opening of the stone-canal as a fixed point when comparing one class of Echinoderms with another, and imagines that "the inter-radius in which the ring-canal closes" is the point which varies. I confess that I cannot follow this reasoning. In dealing with an organ like the hydrocœle, which at one period of development has an identical form in all groups of Echinoderms, viz. an open hoop with five projecting lobes, it seems to me impossible to evade the conclusion that the front and hind ends of the structure correspond throughout the phylum, and hence the point where they meet so as to "close the ring" must be identical throughout the group. The stone-canal opens on the inner surface of the hoop or ring, whilst the lobes project from its outer surface, and hence it is quite conceivable that the position of the opening of the stone-canal could shift without involving any such morphological impossibility as jumping a lobe. But before we have recourse to this modest hypothesis it would be well to satisfy ourselves that as a matter of fact the point where the stone-canal enters the hydrocœle is different in the different groups. In *Asterina gibbosa* this point is situated between lobes 1 and 2, and I have found that this is also true of *Ophiothrix fragilis*, as the sagittal sections shown in figs. 42*a* and 42*b* clearly demonstrate. The first figure shows the opening of the stone-canal and its oblique course backwards to join the anterior cœlom; the second shows the pore-canal opening to the exterior. This second figure shows how Bury's error arose, for he did not use sections, but based his

statement on views of living larvæ. Now, fig. 42*b* shows that the pore is opposite the interval between lobes 4 and 5, and if one were to imagine that the stone-canal led vertically downwards beneath the pore one would conclude that it must open between lobes 4 and 5. With regard to the other groups of Echinoderms, the evidence at present to hand certainly does suggest that the opening is placed in a different inter-radius from that in which it opens in Asteroidea and Ophiuroidea, but until this point has been thoroughly investigated by means of sections it would be premature to give up the hope that the rest of Echinoderms may be shown to agree in this respect with these two groups. The intestine, as fig. 41 shows, is a loose, baggy structure in the Ophiopluteus larva. The anus is kept closed, except during the emission of fæces; the cells lining the intestine differ in tone and size from those lining the stomach, these latter being more cylindrical and staining more deeply than those lining the intestine.

THE METAMORPHOSIS.

In the case of the larva of *Asterina gibbosa* the metamorphosis may be said to begin from the moment that the larva fixes itself. At this period the hydrocœle is an open hoop with five lobes, which are just beginning to show themselves externally by causing protrusions of the ectoderm. In the case of the larva of *Echinus esculentus* and that of *Ophiothrix fragilis*, which remain swimming until the metamorphosis is complete, it is impossible to fix in such a way a definite point which indicates the beginning of the change. In the larva of *Asterina gibbosa*, when it has just fixed itself, the hydrocœle is an open hoop; one may, therefore, regard the beginning of metamorphosis in the larva of *Echinus esculentus* as marked by the acquisition of lobes by the hydrocœle, which is at first a hoop but almost immediately passes into the ring form. In *Ophiothrix fragilis*, where the lobes appear whilst the hydrocœle is still straight, metamorphosis may be regarded as begun, by

the commencement of the encircling of the œsophagus of the larva by the hydrocœle; in a word, by the assumption of a hoop-like form by this organ. This stage is shown in dorsal and ventral views in figs. 13 *a* and 13 *b*, and in section in figs. 43 *a* and 43 *b*.

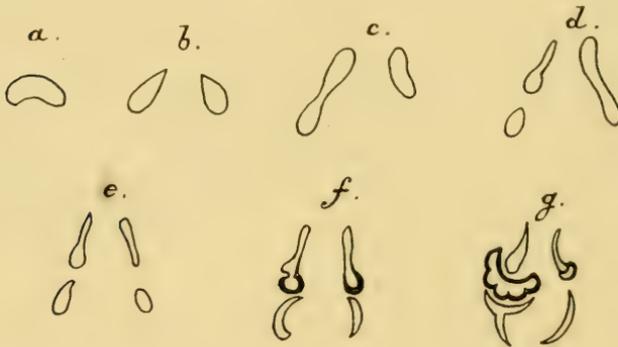
As yet the external symmetry, as shown by the larval arms, is unaffected, but the first lobe of the hydrocœle has extended towards the mid-dorsal line above the stomodæal portion of the œsophagus, whilst the fifth lobe has begun to pass beneath the œsophagus towards the right. Corresponding to this extension of the dorsal portion of the hydrocœle goes a corresponding extension of the thickened side of the œsophagus, which constitutes the left half of the adoral ciliated band. This pushes the dorsal, thin-walled, scoop-like portion of the œsophagus, which is part of the stomodæum, over to the right. The beginning of this shifting is shown in fig. 13 *b*. The outer wall of the left posterior cœlom becomes thickened and produced into five elevations, which are the rudiments of the arms of the adult brittle-star. Three of these are shown in fig. 13 *a*; besides these there are two ventral ones. These arm rudiments consist at first of mere conical thickenings, but each soon acquires a lumen which is an evagination of the cœlomic cavity (fig. 43 *a*, arm 1). This lumen forms the dorsal cœlomic canal of the adult arm. The greater proportion of the cells of the thickening seem to be destined to form the plates which ensheath the adult arm, but as I have already intimated, all trace of the adult calcareous structures is lost owing to the method of preservation.

As the metamorphosis proceeds the dorsal and ventral ends of the hydrocœle extend over to the right.

Soon the external features are involved, as shown in fig. 14 and in figs. 44 *a*, *b*, and *c* (which are sections of a specimen of the same age); the left antero-lateral arm is carried over to the right. In fig. 44 *a* it is seen that the madreporic pore and left anterior cœlom have shared in the movement, and have reached the mid-dorsal line; in fig. 44 *b* the left half of the adoral ciliated band is seen to have increased tremendously,

so that the thin-walled, scoop-like portion of the œsophagus has been pushed completely over to the right and very much diminished in size. In fig. 44 *c* the lobes of the left hydrocœle are seen to project as the **primary tentacles** into the outer and ventral part of the stomodæum. The left posterior cœlom has meanwhile developed two extensions which may be termed the dorsal and ventral horns (fig. 44 *a*, *l''*. *p''*. *c''*, and figs. 44 *b* and *c*, *l'*. *p'*. *c'*). Of these the first extends forward along the left side of the œsophagus outside the

TEXT-FIG. 2.



A series of diagrams to show the changes undergone by the cœlom. *a*. Single cœlomic rudiment. *b*. Right and left cœlomic sacs. *c*. Left cœlomic sac preparing to divide. *d*. Left sac divided, right preparing to divide. *e*. Both sacs divided. *f*. Formation of hydrocœles (indicated by heavy lines). *g*. Further development of hydrocœle, and of left posterior cœlom.

lobes of the hydrocœle, but only reaches as far forward as the second lobe. The second reaches across to the right side of the larva. Eventually the two meet and complete the ring form of the left posterior cœlom, outside and parallel to the ring of the hydrocœle. A precisely similar development takes place in *Asterina gibbosa*, and in *Echinus esculentus* the front end of the left posterior cœlom grows in a ring-form round the stone-canal and axial sinus. The intestine becomes diminished in size and pushed over to the right; meanwhile its cells commence to develop large

vacuoles and thus show obvious signs of degeneration. When matters have reached this point the animal may be said to be in the **first stage** of metamorphosis. The changes

TEXT-FIG. 3.

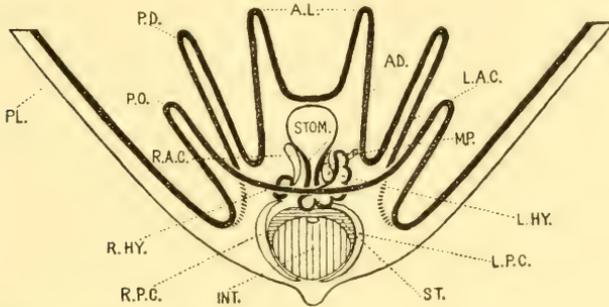


Diagram showing relations of organs in a full-grown larva of *Ophiothrix fragilis*. AD. Adoral ciliated band. A.L. Antero-lateral arms. INT. Intestine. L.A.C. Left anterior coelom. L.HY. Left hydrocoele. L.P.C. Left posterior coelom. M.P. Madreporic pore. P.D. Postero-dorsal arm. P.L. Postero-lateral arms. P.O. Post-oral arm. R.A.C. Right anterior coelom. R.HY. Right hydrocoele. R.P.C. Right posterior coelom. ST. Stomach. STOM. Stomodaeal portion of the oesophagus.

TEXT-FIG. 4.

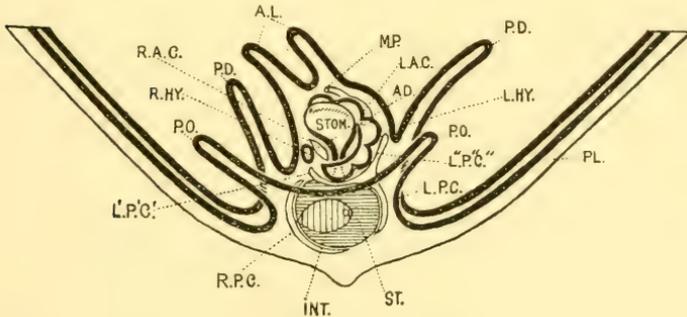


Diagram showing the changes which supervene during the first period of metamorphosis. Lettering as in preceding diagram; in addition: L'P.C.'. Ventral horn of the left posterior coelom. L''P.C''. Dorsal horn of the left posterior coelom.

which initiate metamorphosis are easily understood if the two following text-figures be compared with one another. Text-figure 3 shows the arrangement of organs in a larva

just before the metamorphosis has commenced, whilst text-figure 4 shows the changes which have supervened during the first period of metamorphosis.

Whilst these changes in the relative growth of the internal organs and in the external symmetry have been taking place, new structures have been making their appearance. From the inner wall of the left posterior cœlom four outgrowths are given off which alternate with the lobes of the left hydrocœle. These outgrowths have thick walls and a narrow lumen. At first their cavities are in open communication with the left posterior cœlom (cf. fig. 49), but this communication is rapidly narrowed (fig. 45), and soon entirely cut off. A similar outgrowth is given off from the anterior cœlom (fig. 44 A, *p.h.* 1 and 2), which insinuates itself between lobes 1 and 2 of the hydrocœle, whilst those originating from the left posterior cœlom intervene between lobes 2 and 3, 3 and 4, 4 and 5, and (when the hydrocœle ring is completed) lobes 5 and 1 respectively. These outgrowths are the rudiments of the **perihæmal** system of cavities and of the muscles and nerves which originate from their walls. As already mentioned, the tips of the hydrocœle lobes now appear as tentacles freely projecting into the outer part of the stomodæum. Alternating with these tentacles there appear inter-radial ridges of ectoderm (fig. 46, *ep.*), from which flaps grow out to the right and left. The flaps of adjacent inter-radii meet one another over the bases of the tentacles and in this way the **epineural canals** or closed ambulacral grooves characteristic of the Ophiuroidea are formed. I own that I was disappointed to find that in this respect Ophiuroidea so closely resemble Echinoidea, for the development which I have just described is identical with the process of formation of the epineural ridges in *Echinus esculentus* (19). We possess a fairly full geological history of the evolution of Ophiuroidea out of Asteroidea, and of the gradual conversion of the open ambulacral groove into a canal by the meeting of the edges, and I expected to find that the star-fish form would be definitely attained by the larva before the groove became closed. Such is, however, not

the case ; the process as repeated in ontogeny is pushed back to an earlier stage in development than that at which (to judge from palæontology) it actually occurred in the history of the race.

When by the meeting of the epineural flaps the epineural canals are formed, and when at the same time the hydrocœle ring is completed by the meeting of its dorsal and ventral ends, the second stage in the metamorphosis may be said to be reached. Its external appearance is shown in fig. 15, which represents a larva twenty-three days old raised from artificial fertilisation in 1898. It will be observed that the left antero-lateral arm has been carried over the œsophagus until it has come into close contact with its right fellow, by the double process of the preponderant growth of the organs of the left side, and the shrinkage of the thin-walled forehead of the larva which intervenes between the two antero-lateral arms. This forehead is the very much reduced representative of the præ-oral lobe of the *Asterina gibbosa* larva, and its shrinkage corresponds to the atrophy of the præ-oral lobe or stalk of Asteroidea. At the same time, all the larval arms except the postero-lateral become reduced in size. The two horns of the left posterior cœlom meet one another, and thus the arm rudiments are brought into juxtaposition with the hydrocœle lobes. This is, however, effected in such a way that arm rudiment 1 comes to rest on hydrocœle lobe 2, whilst hydrocœle lobe No. 1, which has been carried over the œsophagus, is supported by arm rudiment 5, which is an outgrowth from that horn of the left posterior cœlom, which has passed under the œsophagus. Here, again, the larva of *Ophiothrix fragilis* agrees with the larva of *Asterina gibbosa*.

Each hydrocœle lobe has given rise to two or three pairs of lateral lobes which are the rudiments of the first **paired tentacles**, the original lobe giving rise to the **terminal or azygous tentacle** of each arm. New tentacles are formed by acropetal buds from the median tentacle. The perihæmal cavities shut off from the left posterior cœlom have expanded

into V-shaped spaces, one limb of each V extending up each arm adjacent to the cavity beneath the lobe of the hydrocœle. From the wall of this extension of the cavity are derived the motor ganglion cells of the **cœlomic nervous system** (fig. 50, *n'c'*), whilst the cells of the **ectodermic nervous system** (fig. 50, *n. c.*) are derived from the ectoderm covering the tentacles themselves. The first nerve-fibres are seen in fig. 50 as fine wavy lines connecting the two sets of nuclei. Other outgrowths from the wall of this cavity, consisting of larger cells with sparser nuclei, are in all probability to be regarded as the rudiments of the **ventral intervertebral muscles**, though in the latest stages examined by me histological differentiation had not progressed far enough to be absolutely decisive on this point. Similarly, cellular outgrowths from the dorsal cœlomic canal (fig. 48, *musc.*) are in all probability the rudiments of the **dorsal intervertebral muscles**.

The intestine is reduced to a small solid vestige consisting of vacuolated cells. The dorsal thin-walled portion of the stomodæum has completely disappeared, and the whole œsophagus is now surrounded by a thick wall derived from the adoral ciliated band, chiefly from its left half. In Asteroidea and Echinoidea a new adult mouth is formed by a meeting of ectoderm and endoderm on the left side of the larva; in Holothuroidea, where the larval mouth persists, it moves (as Bury has shown [6]) at the decisive moment of metamorphosis to the left side; the outer thin-walled portion of the œsophagus becomes evaginated at a later period to form the buccal membrane or peristome, whilst the inner thick-walled portion persists as the œsophagus. *Ophiothrix fragilis* resembles in this respect the larva of Holothuroidea in that the permanent œsophagus is formed from the inner portion of the larval one, whilst the outer portion of the larval œsophagus by the shrinkage of the forehead is, so to speak, uncovered, and forms the **peristomial membrane** of the adult.

The right hydrocœle has, during this stage, a very variable

development. In one specimen (fig. 53), it had actually assumed a five-lobed form, presenting a resemblance to the shape assumed by its left fellow at an earlier period of development. Its normal form may be regarded as that shown in fig. 47, viz. a small vesicle with a comparatively thin wall. But quite often it is large, thin-walled, and conspicuous, sometimes, indeed, forming quite a projection on the external surface of the larva. Sometimes a projection of its inner wall is noticeable similar to that which gives it a crescentic form in the larva of *Asterina gibbosa*. The right anterior cœlom has disappeared with the alteration in form of the œsophagus whilst the left anterior cœlom forms, as it does in both *Asterina gibbosa* and *Echinus esculentus*, the axial sinus or "ampulla" of the stone-canal.

As is well shown in fig. 47, the madreporic pore has been brought close to the right hydrocœle, although it was at first widely separated from it. This is due to the rotation of the madreporic pore from its original position on the left side over the œsophagus and so on to the right side. Thus is eventually brought about a juxtaposition of organs which in the larvæ of *Asterina gibbosa* and *Echinus esculentus* exists from the first. The position of the right hydrocœle in these two species close to the mid-dorsal line of the larva, has led both Goto (11) and Masterman (20) to combat my view of its homology. But *Ophiothrix fragilis* is quite decisive on the point, and a little consideration will show why the Ophiurid in this respect retains a much more primitive arrangement than the other two forms. In it the larval mouth is gradually transformed into the adult mouth, and instead of the mouth moving to the left the stomach is displaced towards the right (fig. 47, *st.*); in the Asterid and Echinid, on the other hand, the permanent mouth is formed on the left, whilst the stomach retains its original position. The right hydrocœle in these two groups is orientated with respect to the eventual position of the permanent mouth, and "right" with respect to a mouth which is going to be

formed on the left side of the larva is practically "mid-dorsal" with respect to the median sagittal plane of the larva and to the larval mouth.

The madreporic pore in these two groups attains its adult position before the right hydrocœle is formed, so that when the latter appears it is close to the madreporic pore.

With the disappearance of all the larval arms except the two postero-lateral, the **third** and last stage of the metamorphosis is begun. Its external appearance is shown in fig. 16, and a frontal section is given in fig. 48. The adult arms have become longer and are folded ventrally over the disc—this is not general among Ophiuroidea, but is characteristic of *Ophiothrix fragilis*. The oral spines which eventually give rise to the so-called "teeth" have already appeared (fig. 16, *sp.*). In the internal anatomy the great point to be noted is the appearance of the **peri-oral cœlom** (fig. 48, *peri.*), as an outgrowth from the left posterior cœlom. The peri-oral cœlom closely surrounds the œsophagus (figs. 51 and 52, *peri.*), and corresponds to the similarly-named space in the larva of *Asterina gibbosa*. But whereas in *Asterina gibbosa* the wall of this space gives rise to the ten retractor muscles which restore the everted stomach to its place on the conclusion of a meal, in *Ophiothrix* the space remains as an apparently functionless vestige throughout life, as the stomach of an Ophiurid is not eversible.

The formation of the **primitive germ cells** commences at this period. As shown in the sagittal section represented in fig. 51, they make their appearance as a series of large nuclei in the wall of the left posterior cœlom overlying the stone-canal (fig. 51, *gen.*). The further history of these cells has been worked out by me in my paper on *Amphiura squamata* (17), to which the reader is referred. A section, however, of one of the embryos of *Amphiura squamata* is given in fig. 52. The single layer of cells shown in fig. 51 is here seen to have become a projecting nodule (fig. 52, *gen.*), the first rudiment, in fact, of the string called the **genital**

rachis which eventually surrounds the disc and from which the genital organs arise as buds. The **ampulla** of the stone-canal (*l.a.c.*) is seen in fig. 52 to be still in continuity with the perihæmal space to which it gave rise. This ampulla—the original left anterior cœlom—into which both pore-canal and stone-canal open (cf. fig. 51) is the equivalent of the axial sinus of Asteroidea and Echinoidea, but has nothing to do with the space which originally received that name in Ophiuroidea. This latter space, lettered *b* in my paper on *Amphiura squamata*, is not formed till the post-larval period, and hence is not described in this paper. Its outer wall separating it from the general hypogastric (i. e. left posterior) cœlom is a thin membrane, and the space itself appears to be due (as far as I can make out from a re-examination of my sections) to an invagination of the cœlomic cavity into the tissue lying above the stone-canal. Now in *Asterina gibbosa* the cells which give rise to the genital rudiment are likewise invaginated, but the cavity of the invagination is occluded. When we reflect also that the true axial sinus of Ophiuroidea is of limited extent, the confusion between the true axial sinus of Asteroidea and the wrongly named axial sinus of Ophiuroidea becomes explicable. The right hydrocœle is seen in fig. 51 as a closed space (*r. hy.*). When I commenced my work on the late development of *Amphiura squamata* I had made no researches on the early development of Echinodermata, and hence I was much puzzled by the appearance of this space, whose origin I could not trace. After long wavering I persuaded myself on the evidence of one or two sections that it, too, was derived from an evagination of the general cœlom, and so it is lettered as if it were part of sinus *b*, the wrongly denominated axial sinus. Of course, this is a mistake which I endeavoured to correct in my paper on *Asterina gibbosa*, and which receives renewed correction here.

As the metamorphosis nears its conclusion the ciliated epithelium covering the long postero-lateral arms begins to degenerate, and the animal sinks lower and lower in the water;

such larvæ are only captured when the tow-net is dragged along the bottom. Soon the animal rests on the bottom altogether, and then the ciliated epithelium is rapidly absorbed, leaving sometimes, as fig. 17 shows, a portion of one of the calcareous rods, which it originally covered, as a bare, projecting spine. The arms of the young Ophiurid are armed at the sides with hooks. These hooks strongly resemble the hooks which form the sole armature of the arms in such primitive genera as *Ophiohelus*, and it is interesting to find them recurring in the brephic stage of *Ophiothrix*. As a matter of fact these hooks persist in this genus throughout life, but as growth proceeds there is added to each one of them a vertical row of those delicate, thorny, lateral spines from which the genus takes its name.

COMPARISON OF THE DEVELOPMENT OF OPHIOTHRIX WITH THAT OF OTHER ECHINODERMS.

Before discussing the bearing of the facts which have just been laid before the reader, it is proper that a word or two should be spoken as to the difference between my results and those of Dr. Caswell Grave on *Ophiura brevis* (12). Some of these differences, such as the rapid development, are attributable to the fact that *Ophiura brevis* has yolky eggs and never develops a typical *Ophiopluteus* larva; but others are of greater import. The origin of the cœlom, as two distinct evaginations from the archenteron, of which the hinder forms the hydrocœle and the left posterior cœlom, is unparalleled among Echinoderms. We can only venture to suggest that, as Dr. Grave distinctly states that he had only an exceedingly limited supply of material, that his interpretation of the appearances presented to him is mistaken, owing to stages being missed out.

It is true Masterman has announced somewhat similar results in the case of *Cribrella oculata* (20). Here, according to him, the cœlom originates as two evaginations, of which one gives rise to the single anterior cœlom, characteristic of

Asteroidea, from which the right posterior cœlom, called by him the epigastric cœlom, arises as does the true right hydrocœle, termed by him the central cœlom. This tallies fairly well with *Ophiura brevis*, where the anterior evagination divides into right and left anterior cœloms, and from the right anterior cœlom Grave infers that the right posterior cœlom (the epigastric) is subsequently derived. In *Cribrella oculata* the posterior evagination, as in *Ophiura brevis*, gives rise solely to the left posterior cœlom (hypogastric cœlom). The difference between the two is that in *Cribrella oculata* the left hydrocœle originates from the anterior rudiment, whereas in *Ophiura brevis* it originates from the posterior vesicle. This irreconcilable difference between these two authors prevents us placing as much stress on the points of agreement between them as we should otherwise feel bound to do. When we recollect that Masterman's results would fall at once into line with what is known of the development of other Asteroidea, if we make the assumption that the supposed independent origin of the left posterior cœlom from the gut is founded on a mistaken observation, we shall, I think, be justified in provisionally assuming that this is the case. All that is known of the development of the cœlom in the classes of Echinoderms, apart from these two papers, can be subsumed under the formula that, in all cases, the cœlom arises as a single bilobed evagination from the apex of the archenteron. In the single Crinoid whose development is known (viz. *Antedon rosacea*) the whole archenteron is bodily transformed into the cœlom, which then divides into anterior and posterior portions, whilst from the anterior portion the definitive gut subsequently arises as a pair of out-growths. This late appearance of the gut is rendered possible by the fact that it is functionless in the embryo. In Holothuroidea the single out-growth also divides first into anterior and posterior portions, and whilst the anterior subsequently divides into anterior cœlom and left hydrocœle, the posterior portion divides into right and left halves. The right hydrocœle is not developed. In Asteroidea (*Bipinnaria*),

Ophiuroidea and Echinoidea, the cœlomic rudiment first divides into right and left halves, and then these subsequently divide into anterior and posterior portions. This has been doubted in the case of Asteroidea. From Agassiz's figures (1) some have drawn the conclusion that in the Bipinnaria larva the right and left cœlomic sacs originate as independent evaginations of the gut. But an inspection of these figures shows that Agassiz did not observe enough stages to warrant such a conclusion, and Driesch (8), who has used the larvæ both of *Asterias* and *Astropecten* for experimental embryology in all cases shows a single vesicle as the first rudiment of the cœlom. The course of development in *Asterina gibbosa* (and apparently in *Cribrella oculata* also) differs from that of the Bipinnaria larva in that the anterior portion of the cœlom never divides into right and left halves; whereas in the Bipinnaria it first divides into right and left cœlomic sacs, and then these reunite in the præ-oral lobe to form the single anterior cœlom. It is obvious that in *Asterina* and *Cribrella* the stage when the anterior cœlom becomes divided into right and left halves is missed out owing to the shortening of development.

If we regard the free-swimming larva as representing a bilaterally symmetrical ancestor we may then with confidence consider that the development of the cœlom, according to the scheme which I have now shown to obtain in Asteroidea, Ophiuroidea and Echinoidea, gives us the best idea of the condition of the cœlom in this ancestor. We must credit this ancestor with a long præ-oral lobe, which is not found in the Ophiurid because in Crinoidea and Asteroidea this lobe is used as a fixing organ or stalk, and a fixed stage in the development supplies us with the best idea of a transitional condition between a bilaterally symmetrical animal swimming freely and a radially symmetrical creeping animal. At the apex of this præ-oral lobe there was a brain in the form of a neuro-epithelial plate; such an organ I have not been able to find in the Ophiopluteus, but it exists in the Crinoid larva and in the larva of *Echinus esculentus* and as a thickened plate

of cells in the Bipinnaria. The cœlom on each side was three-lobed, and the central division in all probability was produced into tentacles, whilst locomotion was effected by means of a ciliated band. Now, if we survey the existing animal kingdom in order to find some type still living which will approximately answer to this description we are led to the group of the Ctenophora. Here we find a group of animals with an anterior neuro-epithelial plate, swimming by means of cilia. Connected with the endodermic stomach or archenteron, as we may term it, are right and left outgrowths. From each of these a canal runs forward into the anterior portion of the body to end near the brain (so-called excretory canal) whilst another runs backwards to near the mouth (paragastric canal). The middle division of the outgrowth leads into a tentacular canal which also gives rise to canals which open into the meridional canals lying beneath the ciliated ribs. Now I am very far from suggesting that the ancestor of Echinoderms resembled in details a living Ctenophore, but if Ctenophora are the remnants of a great group of pelagic animals with considerable variation in structure, the Echinoderm ancestor may well have belonged to that group. The Ctenophore represents a stage before the cœlom had been separated from the gut or the primitive mouth divided into mouth and anus. It is to me a matter of extreme interest to find that not only have the researches of Lang and the discoveries of Willey and others with regard to *Ctenoplana* led to the conclusion that *Turbellaria*, and therefore all the group of *Platyhelminthes*, are descended from a Ctenophore-like ancestor, but that Woltereck (32), working on the development of *Polygordius*, is led to the same conclusion with regard to this animal also. This conclusion, if justified, carries with it the consequence that the whole of the *Annelida* and *Mollusca*, which are bound together by the common possession of the trochophore larva, are also derivable from this root. When such diverse lines of research seem to converge upon one point, it really does seem as if we were within measurable distance of gaining some idea of what

the common ancestor of Cœlomate animals was like. I think it will be admitted that to call this ancestor a Ctenophore would be extremely ill advised, as this would suggest that in details of the arrangement of cilia, etc., it agreed with the Ctenophora. Better, I think, to adopt the name already suggested by me in my paper on *Asterina gibbosa* (18), viz. Protocœlomata, for the ancestral group from which Ctenophora, Platyhelminthes, Annelida, Mollusca, and Echinodermata have sprung. To this group Vertebrata are also to be traced back, since the Tornaria larva of *Balanoglossus* exhibits the three-fold division of the cœlom and the apical nervous plate, which we have determined as characteristic of the ancestor of Echinodermata, whilst the middle division of the cœlom in the allied form *Cephalodiscus* is produced into long tentacles, just as is the case with the hydrocœle in Echinodermata.

Turning now to the later development of *Ophiothrix fragilis*, the principal point to be noticed is that the ontogeny bears out the conclusion of comparative anatomy that the Asteroidea and Ophiuroidea are closely allied. Thus in the formation of one perihæmal rudiment from the axial sinus, in the apposition of arm-rudiment 1 and hydrocœle-lobe 2, in the opening of the stone-canal between lobes 1 and 2, in the development of dorsal and ventral horns from the left posterior cœlom, and finally in the development of the peri-oral cœlom, the development of *Ophiothrix* recalls that of the Asterid. The points in which it differs are, first, that the fixed stage is entirely missed out; second, that the cavities are in general smaller and their walls thicker; third, in the development of epineural folds; and fourth, in the persistence of the larval mouth. Taking these points in order, the missing out of the fixed stage is in accordance with the rule everywhere exemplified in the animal kingdom, that there is a tendency to reduce the number of larval stages. In each stage the organism is exposed to a special set of dangers; if, therefore, enough food can be accumulated during one stage to enable the necessary changes in structure to be made, so that the next stage can

be missed out, this will be done. So we explain the missing out of the Mysis stage in the embryology of the crab, for instance, and the passing over numerous larval stages within the egg-shell in other cases. The smallness of cavities and thickness of the tissues derived from their walls is a general feature of Ophiuroidea which runs throughout the entire class, and is an adult feature retrospectively affecting development. The epineural folds require, of course, no comment, as they are one of the features in which Ophiuroidea have progressed beyond Asteroidea. They made their appearance in ontogeny rather earlier than they should, and in so far are another example of adult features affecting the ontogeny. The retention of the larval mouth, however, cannot be viewed otherwise than as a primitive feature. We cannot imagine that in the history of the race a period intervened when the previously existing mouth disappeared and a totally new one made its appearance, but why the mouth should persist in Ophiuroidea and Holothuroidea and be lost in Asteroidea and Echinoidea is a mystery. We may notice, however, that the mouth is retained in Crinoidea also, for though the larval stomodæum, as Bury calls it (4), never opens into the yolky, functionless gut, yet there is no doubt from its position of its homology with the stomodæum of other Echinoderm larvæ. Now this stomodæum is shifted posteriorly and opens out to form the vestibule of the fixed larva, out of which the tentacles project. A similar fate befalls the stomodæum in Ophiuroidea. Owing to the shrinkage of the forehead it opens out, and the primary tentacles of the hydrocœle project through it. The same thing happens in the development of Holothuroidea. A difficulty may here occur in the minds of some that, in the development of *Synapta digitata*, the only Holothurid of which the whole ontogeny is known, the first tentacles to make their appearance are the buccal tentacles, not those terminating the radial canals. But from Metschnikoff's paper (21) it appears that as the radial canals grow back, extensions of the "atrium" (as the stomodæum is called), accompany them, and hence it may

be inferred that here, too, these terminal tentacles when they appear, project at first into the atrium. To the stomodæum or atrium, containing the primary tentacles, found in Ophiuroidea, Holothuroidea, and Crinoidea may be compared the amniotic cavity of Echinoidea and we must suppose that in the last-named group the original stomodæum is divided into two parts — one developed in the middle line in order to function for the larva, the other in the adult position to protect the first tentacles.

As to Asteroidea, there occurs in the text-books a statement, attributed to Agassiz, that in some Bipinnariae the larval mouth persists and moves to the left. Later researches on the development of Bipinnaria by Bury and Goto have failed to confirm this statement, but considering of how few types the development is known, it is quite possible that in some form the larval mouth does persist, and the detailed study of the development of such a form would be of rare interest. In the meantime we can only conclude that as the groups of Asteroidea and Ophiuroidea diverged from one another, the Asteroid larva, whilst retaining the fixed stage, lost the primitive features of the mouth, whereas the Ophiuroid larva retained these features whilst losing the fixed stage, just as the lower insects have lost the caterpillar larva although the higher insects have retained it.

SUMMARY.

The principal points which have been brought out in this paper may be summarised as follows :

(1) The early development of *Ophiothrix fragilis* varies with the condition of the egg at the moment of fertilisation, and the development of the unripe egg resembles in certain features that of *Ophiura brevis*.

(2) The cœlom originates as a single vesicle from the apex of the archenteron, and this appears to be true for all classes of Echinoderms.

(3) The segmentation of the cœlom proceeds along the same lines as those already elucidated for Asteroidea and

Echinoidea, viz. into three somites on each side; but the middle somite on the right side is not shifted dorsally as it is in Asteroidea and Echinoidea. This somite occasionally assumes a five-lobed form, proving beyond doubt that it is a right antimere of the water-vascular system.

(4) The left hydrocœle is budded from the anterior division of the left cœlom, not from the posterior division as Bury supposed (5), and its persistent connection with the left anterior cœlom constitutes the stone-canal; this opens into the hydrocœle between lobes 1 and 2, as in Asteroidea, not between lobes 4 and 5 as Bury asserted.

(5) The metamorphosis is initiated by a preponderant growth of the organs of the left side, which affects the larval arms and the sides of the œsophagus, and which not only carries the hydrocœle round the œsophagus but also the madreporic pore and the left anterior cœlom, so that these come to be near the right hydrocœle.

(6) The perihæmal system of canals originates as a series of five hollow evaginations, the cavities being small and the walls thick. The first originates from the left anterior cœlom, the other four from the left posterior cœlom. From their walls originate the motor ganglion cells and in all probability the ventral inter-vertebral muscles.

(7) The left posterior cœlom gives rise to a dorsal and a ventral horn, which eventually meet, causing it to assume a ring-shape. From it five evaginations give rise to the arm rudiments and the first of these comes to overlie hydrocœle lobe No. 2.

(8) A peri-oral cœlom closely surrounding the adult œsophagus originates from the left posterior cœlom.

(9) A series of epineural ridges, the tops of which grow out so as to form arch-like folds, are formed inter-radially alternating with the primary tentacles. By the union of adjacent arches the basal portions of the tentacles are covered, exactly as occurs in the Echinoid larva.

(10) The adult œsophagus is formed from the left inner portion of the larval one: its covering is made of the adoral

ciliated band, chiefly of the left half thereof, which grows *pari passu* with the left hydrocœle. The outer part of the larval œsophagus opens out in consequence of the shrinkage of the forehead to form an atrium into which the primary hydrocœle lobes project.

(11) The larval intestine slowly diminishes in size and degenerates, this process being accompanied by a vacuolisation of its cells. The larval stomach becomes pushed over to the right—a process which leads to the same result as the moving of the larval mouth to the left in Holothuroidea, or the formation of the adult mouth on the left in Asteroidea and Echinoidea.

(12) The primitive germ-cells originate from the left posterior cœlom covering the stone-canal.

In conclusion, I have to express my thanks, first to the University of Cambridge and to the British Association, who granted me the use of their tables at Plymouth; next to Dr. E. J. Allen, Director of the Marine Biological Laboratory at Plymouth, who in the most whole-hearted and generous manner placed the resources of the laboratory at my disposal; then to my friend and colleague, Mr. J. Simpson, B.Sc., Demonstrator of Zoology in McGill University, to whose skill I owe a large proportion of the drawings which illustrate this paper; and lastly to my wife, who also assisted with the more complicated drawings, with the text-figures, and with the general revision of the text.

LIST OF WORKS REFERRED TO IN THIS PAPER.

N.B.—It has not seemed to me desirable to encumber this list with references to works dealing with groups of animals other than the Echinoderms, such as that of Lang on the Turbellaria, or Willey on Ctenoplana, etc., since the facts referred to have been embodied in text-books and are the common property of all zoologists. Woltreck's recent paper forms the sole exception.

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EXPLANATION OF PLATES 31-36,

Illustrating Professor E. W. MacBride’s paper “On the Development of *Ophiothrix fragilis*.”

LIST OF ABBREVIATIONS EMPLOYED.

The Arabic numerals 1—5 are employed to denote the lobes of the left hydrocœle. When a dash is added to the numeral, thus—1', 2', etc.—a lobe of the right hydrocœle is indicated. The Roman numerals I—V are employed to denote the developing arms of the adult. In all cases the numbers commence with the lobe or arm which is situated in the larva farthest forwards and the enumeration proceeds backwards.

al. Antero-lateral arm of larva. *az. t.* Azygous tentacle of the young Ophiurid. *b. t.* Buccal tentacle of the young Ophiurid. *cil.* Longitudinal

ciliated band of the larva. *cil. ad.* Adoral ciliated band of the larva. *coe.* Undivided cœlomic rudiment. *ep.* Epineural fold. *ep. s.* Epineural sinus or canal. *gen.* Primitive germ-cells. *int.* Intestine. *l. a. c.* Left anterior cœlom. *l. coe.* Left cœlomic vesicle as yet undivided. *l. hy.* Left hydrocœle. *l. p. c.* Left posterior cœlom. *l'. p'. c'.* Ventral horn of left posterior cœlom. *l''. p''. c''.* Dorsal horn of left posterior cœlom. *mes.* Mesenchyme. *mes¹.* Primary mesenchyme. *mes².* Secondary mesenchyme. *mp.* Primary madreporic pore. *musc.* Muscle-fibres. *n. c.* Nerve-cells of ectodermic origin. *n'. c'.* Nerve-cells of cœlomic origin. *n. f.* Nerve-fibres. *œs.* Œsophagus. *p. c.* Pore-canal. *pd.* Postero-dorsal arm of larva. *ph.* Perihæmal space. *ph. 1, 2.* Perihæmal rudiment originating between hydrocœle lobes 1 and 2. *ph. 2, 3, ph. 3, 4, ph. 4, 5, ph. 5, 1.* Rudiments originating between lobes 2 and 3, 3 and 4, 4 and 5, and 5 and 1 respectively. *pl.* Postero-lateral arm of larva. *p. o.* Post-oral arm of larva. *r. a. c.* Right anterior cœlom. *r. cœ.* Right cœlomic vesicle as yet undivided. *r. hy.* Right hydrocœle. *r. p. c.* Right posterior cœlom. *sk.* Larval skeletal rod. *sk'.* Rudiment of adult skeleton. *sp.* Buccal spines, or "teeth" of adult. *st.* Stomach. *st. c.* Stone-canal. *stom.* Stomodæum. *vac.* Vacuolated ectoderm at posterior end of larva.

PLATE 31.

FIG. 1.—Optical section of blastula, seven hours old, reared from natural fertilisation. $\times 260$.

FIG. 2.—Optical section of blastula, seven hours old, reared from natural fertilisation, but more advanced in development than that shown in fig. 1. *mes.* Primary mesenchyme. $\times 260$.

FIG. 3.—Optical section of gastrula, twenty-one hours old, reared from natural fertilisation. The vacuolated crest is already strongly developed. $\times 260$.

FIG. 4.—Ventral view of larva, thirty hours old, reared from natural fertilisation. *cil.* longitudinal ciliated band. $\times 260$.

FIG. 5.—Ventral view of larva of same age and origin as that shown in fig. 4, but of more advanced development. $\times 260$.

FIG. 6.—Ventral view of larva, fifty-five hours old, reared from natural fertilisation. *r. cœ., l. cœ.* Right and left undivided cœlomic vesicles. *p. l.* Postero-lateral larval arms. *sk.* Calcified rods supporting the larval arms. $\times 260$.

FIG. 7.—Ventral view of larva, three days old, reared from natural fertilisation. *a. l.* Antero-lateral larval arms. *p. o.* Post-oral larval arms. $\times 260$.

FIG. 8.—Dorsal view of rather older larva picked out of Plankton. The larval skeleton has been dissolved out by the preservative. *l. a. c., l. p. c.* Anterior and posterior halves of the left cœlom. *r. a. c., r. p. c.* Anterior and posterior halves of the right cœlom, still connected. $\times 260$.

FIG. 9.—Dorsal view of a larva, five days old, reared from natural fertilisation. The cœlomic vesicle is completely divided on both sides into anterior and posterior halves. *m.p.* Primary madreporic pore. $\times 260$.

FIG. 10.—Dorsal view of a larva, six days old, reared from natural fertilisation. *p.d.* Rudiments of postero-dorsal arms. *r.hy., l.hy.* Rudiments of right and left hydrocœles respectively. $\times 260$.

FIG. 11.—Dorsal view of a larva, eight days old, reared from natural fertilisation. The postero-dorsal arms have acquired a fair length. $\times 150$.

PLATE 32.

FIG. 12.—Ventral view of a larva, sixteen days old, reared from artificial fertilisation. All the arms have attained their full length. 1—5. Lobes of the left hydrocœle, which has grown forward beyond the left anterior cœlom. $\times 150$.

FIGS. 13 *a* and *b*.—Dorsal and ventral views respectively of a larva picked out of Plankton, in which the metamorphosis is just beginning. The preservative has dissolved out the larval skeleton. 1—5. Lobes of the left hydrocœle, which is beginning to extend ventrally under the œsophagus; I—IV. Rudiments of three of the arms of the adult brittle-star. *int.* Intestine. *st.* Stomach. *stom.* Stomodæum. \times about 100.

FIG. 14.—Ventral view of a larva picked out of Plankton, in which the metamorphosis is more advanced than in the preceding figures. The preparation was somewhat opaque, and the larval skeleton has been dissolved by the preservative. Observe that the left antero-lateral arms and lobes 1 and 2 of the left hydrocœle have passed over the œsophagus. $\times 100$.

FIG. 15.—Ventral view of a larva twenty-three days old, reared from artificial fertilisation, in which the metamorphosis is very far advanced. All the larval arms except the postero-lateral are reduced in size. *az. t.* The terminal tentacle of one of the radial water-vascular canals. *b. t.* Buccal tentacle. $\times 100$.

FIG. 16.—Ventral view of a larva of the same age as foregoing, but of more advanced development, reared from artificial fertilisation. All the larval arms except the postero-lateral arms have disappeared; the developing adult arms are bent over the disc. The spines of the mouth-angles (*sp.*) have made their appearance. $\times 100$.

FIG. 17.—Ventral view of a young brittle-star which has just completed its metamorphosis. Artificial fertilisation, twenty-six days. *pl.* The calcareous rod of one of the two postero-lateral arms which have now disappeared. The hooks characteristic of the brephic stage of *Ophiothrix fragilis* are seen attached to the sides of the arms. $\times 100$.

FIG. 18.—Section of a segmenting egg. Artificial fertilisation. $\times 500$.

FIG. 19.—Longitudinal section of a blastula. Artificial fertilisation. *mes*. Primary mesenchyme filling the interior. $\times 500$.

FIG. 20.—Longitudinal section of a blastula in which gastrulation is about to commence. Artificial fertilisation. *mes*¹. Primary mesenchyme filling the interior. *mes*². Secondary mesenchyme budded from the endodermic area. $\times 500$.

FIG. 21.—Longitudinal sagittal section of a gastrula. Artificial fertilisation. Notice the tongue of endodermic cells projecting into the archenteron. $\times 500$.

PLATE 33.

FIG. 22.—Longitudinal frontal section of a larva two days old. Artificial fertilisation. *p. l.* First rudiment of one of the postero-lateral arms. $\times 500$.

FIG. 23.—Longitudinal frontal section of a larva two and a half days old. Artificial fertilisation. *cæ*. Cœlomic rudiment still opening into the archenteron. $\times 500$.

FIG. 24.—Longitudinal section of blastula seven hours old. Natural fertilisation. *mes*. Beginning mesenchyme. $\times 500$.

FIG. 25.—Longitudinal frontal section of incipient gastrula, one day old. Natural fertilisation. $\times 500$.

FIG. 26.—Longitudinal frontal section of more advanced gastrula, one day old. Natural fertilisation. $\times 500$.

FIG. 27.—Longitudinal frontal section of gastrula of one and a half days. Natural fertilisation. $\times 500$.

FIG. 28.—Longitudinal frontal section of larva, two days old. Natural fertilisation. Notice the origin of the cœlom. *cæ*. Cœlomic rudiment. *p. l.* Rudiment of the postero-lateral arm. $\times 500$.

FIG. 29.—Longitudinal frontal section of larva, two days old. Natural fertilisation. Notice that the cœlom is separated from the archenteron as a single vesicle. $\times 500$.

FIG. 30.—Longitudinal frontal section of a larva two and a half days old. Natural fertilisation. The stomodæum is in the act of opening into the œsophagus. *œs*. Œsophagus. *stom*. Stomodæum. $\times 500$.

FIG. 31.—Longitudinal frontal section of a larva picked out of Plankton. Estimated age three and a half days. *musc*. Muscular fibres encircling œsophagus developed from cœlomic sacs. $\times 260$.

PLATE 34.

FIG. 32.—Longitudinal frontal section of a larva picked out of Plankton. Estimated age four and a half days. *l.p.c.* Outgrowth from the left cœlomic sac, which will be cut off as the left posterior cœlom. *al.* Antero-lateral arm. $\times 260$.

FIG. 33.—Longitudinal frontal section of a larva four and a half days old. Natural fertilisation. *l.p.c.* The rudiment of the left posterior cœlom. It has now assumed a rounded shape. $\times 260$.

FIG. 34.—Longitudinal frontal section of a larva picked out of Plankton. Estimated age five days. *r.p.c.* Outgrowth from right cœlomic sac, which will be cut off as the right posterior cœlom. $\times 260$.

FIG. 35.—Transverse section of a larva three days old. Natural fertilisation. *mp.* Ectodermic invagination to form the madreporic pore. $\times 500$.

FIG. 36.—Transverse section of a larva three days old, more advanced in development than that shown in the preceding figure. Natural fertilisation. *mp.* Ectodermic invagination to form madreporic pore; this has now fused with an outgrowth from the left cœlomic sac. *st.* Stomach. $\times 500$.

FIGS. 37 *a* and *b*.—Two longitudinal frontal sections through a larva picked out of Plankton of estimated age fourteen to sixteen days. Fig. 37*a* is the more dorsal section passing through the œsophagus. Fig. 37*b* is a more ventral section passing through the stomach alone. *r.hy.*, *l.hy.* Rudiments of the right and left hydrocœles respectively. *pd.* Postero-dorsal arms: the last to appear. $\times 260$.

FIGS. 38 *a* and *b*.—Two longitudinal frontal sections through a larva somewhat older than that represented in fig. 37, picked out of Plankton. Fig. 38*a* passes through the left hydrocœle, fig. 38*b* through the right hydrocœle. The latter is the more dorsal section. $\times 260$.

FIG. 39.—Longitudinal frontal section through a larva picked out of Plankton, still older than that shown in fig. 38. The left hydrocœle is just beginning to be lobed; 1–5. Its lobes. $\times 260$.

FIG. 40.—Longitudinal frontal section through a larva picked out of Plankton older than that shown in fig. 39. The lobes of the left hydrocœle are finger-like. *r.a.c.*, *l.a.c.* Right and left anterior cœloms, just grazed. *cil.* Longitudinal ciliated band. *cil.ad.* Adoral ciliated band. $\times 260$.

FIG. 41.—Longitudinal median sagittal section through a larva picked out of Plankton, about the same age as that shown in fig. 40. $\times 260$.

PLATE 35.

FIGS. 42 *a* and *b*.—Two longitudinal sagittal sections through a larva picked out of Plankton, of same age as that shown in the preceding figure. Fig. 42 *a* is more to the left of the middle line than fig. 42 *b*. Fig. 42 *a* shows

the opening of the stone-canal into the left hydrocœle between lobes 1 and 2. Fig. 42 *b* shows the opening of the pore-canal to the exterior. *m.p.* Madreporic pore. *st. c.* Stone-canal. $\times 260$.

FIGS. 43 *a* and *b*.—Two longitudinal frontal sections through a larva picked out of Plankton, in which there is the first discoverable trace of metamorphosis. Fig. 43 *a* is the more dorsal section, and passes through lobes 1 and 5 of the left hydrocœle, whilst fig. 43 *b* passes through lobes 2, 3, and 4, thus showing that the hydrocœle is commencing to encircle the œsophagus. In fig. 43 *a* the right hydrocœle (*r. hy.*) is grazed, and the thickening of the outer wall of the left posterior cœlom, which is the first rudiment of an adult arm, has made its appearance. The prolongation of the lumen of the cœlom into the thickening is the origin of the dorsal cœlomic canal of the arm. *Pari passu* with the extension of the left hydrocœle goes the extension of the left half of the adoral ciliated band (*vil. ad.*). $\times 260$.

FIGS. 44 *a*, *b*, and *c*.—Three longitudinal frontal sections through a larva in the first stage of metamorphosis. Fig. 44 *a* is the most dorsal section, fig. 44 *c* the most ventral. Fig. 44 *a* shows the pore-canal (*p. c.*) opening into the left anterior cœlom (*l. a. c.*) from which is given off the perihæmal rudiment (*ph.* 1, 2). Both left dorsal (*l''. p''. c''.*) and right ventral (*l'. p'. c'.*) horns of the left posterior cœlom are seen. From the former is given off the perihæmal rudiment (*ph.* 2, 3), from the latter the perihæmal rudiment (*ph.* 3, 4). The right hydrocœle is seen, as are also the adult arms I and II, which now show as external protuberances. In fig. 44 *b* all five lobes of the left hydrocœle are shown. The stomodæum, or thin-walled portion of the œsophagus, is pushed over to the right, owing to the enormous growth of the adoral ciliated band. *p. d.* Left postero-dorsal larval arm carried forward by the preponderant growth of the left side. In fig. 44 *c* the tips of the lobes of the left hydrocœle are seen projecting into the stomodæum. Each lobe is covered with an ectodermal thickening. The left antero-lateral arm has passed over towards the right, so as to assume a mid-dorsal position. The opening of the stomach into the intestine is shown, the different histological characters of these two portions of the alimentary canal being strongly marked. $\times 250$.

PLATE 36.

FIG. 45.—Longitudinal frontal section through a larva of about the same age as that shown in fig. 44. Picked out of Plankton. The rotation of the left antero-lateral larval arm has not proceeded so far as in fig. 44. All five lobes of the left hydrocœle are shown. Three perihæmal rudiments are seen; one of them (*ph.* 3, 4) appears as a hollow outgrowth of the left posterior cœlom. The stomach (*st.*) is seen opening into the intestine. External to the right posterior cœlom a mass of mesenchyme cells is seen—probably the rudiment of one of the adult plates. $\times 250$.

FIG. 46.—Longitudinal frontal section through a larva of about the same age as that shown in figs. 44 and 45. Picked out of Plankton. The epineural folds (*ep.*) are shown; these roof over the ambulaeral grooves (i. e. the surfaces of the primary lobes of the hydrocœle) and give rise to the epineural canals. $\times 250$.

FIG. 47.—Longitudinal frontal section of a larva picked out of Plankton in the second stage of metamorphosis. The opening of the stone-canal into the water-vascular ring is shown; the pore-canal is indicated by dotted lines, as it does not lie in the plane of the section. The evagination of the left posterior cœlom to form the dorsal canal of the fourth arm is shown (arm IV). $\times 250$.

FIG. 48.—Longitudinal frontal section of a larva picked out of Plankton in the third and final stage of metamorphosis. *a. l.* Disappearing remnants of the antero-lateral arms. *p. d.* Remnant of right postero-dorsal arms. *peri.* Peri-oral cœlom originating as an outgrowth from the left posterior cœlom. *musc.* Muscles originating from dorsal canal (arm-cœlom) of arms IV and V. $\times 250$.

FIG. 49.—Portion of a longitudinal frontal section of a larva picked out of Plankton in the first stage of metamorphosis. *ph.* 4. 5. A perihæmal rudiment showing its origin as an evagination of the left posterior cœlom. *st.* Stomach. $\times 466$.

FIG. 50.—Portion of a longitudinal frontal section of a larva picked out of Plankton in the second stage of metamorphosis. *ep. s.* Epineural canals. *musc.* Muscular cells originating from the walls of the perihæmal space (*ph.* 3. 4.). *n. c.* Ganglion cells originating from the ectodermic wall of the epineural canal. *n. c.!* Ganglion cells originating from the wall of the perihæmal space. *n. f.* First nerve-fibres. *p. l.* Postero-lateral arm cut obliquely. $\times 466$.

FIG. 51.—Sagittal longitudinal section of a larva picked out of Plankton in the third stage of metamorphosis. *gen.* Primitive germ-cells originating from the wall of the left posterior cœlom. $\times 233$.

FIG. 52.—Sagittal section through a young *Amphiura squamata* 25 mm. in diameter taken from the maternal brood-pouch. *musc.* Muscular fibres developing from the cells lining a tentacle. *gen.* Primitive germ-cells forming a swelling, which is the beginning of the genital rachis. $\times 233$.

FIG. 53.—Longitudinal frontal section through an abnormal larva of *Ophiothrix fragilis* in the second stage of metamorphosis picked out of Plankton. 1', 2', 3', 4', 5'. Lobes of abnormally developed right hydrocœle. $\times 173$.

On the Segmentation of the Head of Diplopoda.

By

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With Plate 37 and 6 Text-figures.

INTRODUCTION.

THROUGH the kindness of Professor Minchin and Mr. Pocock I have been able to examine embryos and larvæ of a South African species of *Archispirostreptus* from Port Elizabeth. The adult specimens were obtained by Mr. Pocock for the Zoological Society, and the eggs were laid in the insect house at the Society's gardens.

The work was undertaken with a view to finding out, from embryological evidence, the number of segments and appendages concerned in the formation of the Diplopod gnathochilarium. Unfortunately none of the larvæ from the two batches of eggs that were laid lived long enough to allow the development of the gnathochilarium to be observed. The examination of some of the embryos has, however, led to one or two interesting results, and incidentally to the solution of the gnathochilarium problem itself.

Many thanks are due to Mr. Pocock, who spent much of his valuable time in preserving the earlier stages daily.

HISTORICAL SKETCH.

In an account of the developmental stages of several Chilognatha, Metschnikoff (1874) states that in them the

mouth parts are only two in number ; namely, mandibles and one pair of maxillæ. Heathcote's (1888) observations confirm this statement as also do those of vom Rath (1886), whose dissertation unfortunately I have been unable to see. While Heathcote merely mentions the fact of there being but one pair of maxillæ, Metschnikoff insists upon it as being important, and includes it among other points of resemblance between the Poduridæ and the Chilognatha.

More lately, zoologists have been quite awake to the significance of there being only two pairs of mouth parts in the Chilognatha, though but few observations have been made on the development of these myriapods. Folsom (1900) quotes Packard (1883) as saying that there can only be two pairs of oral appendages ; but adds that he (Folsom) would suggest that further embryological studies on Diplopods might show more. In his summary he homologises the Diplopod gnathochilarium with three Hexapod somites, namely, those of the superlinguæ, maxillæ, and labium.

Korschelt and Heider (1898) also, in their text-book quote Metschnikoff and vom Rath, and allude to the necessity for further investigation into this matter.

Heymons (1897), in an account of the embryo of *Glomeris*, states very decidedly that its gnathochilarium is formed of only one pair of appendages and the hypopharynx (hypostome), but ventures no surmises as to the origin of the hypostome.

Silvestri (1898), in describing the larva of *Pachyulus communis* says that though there is only one pair of appendages in the gnathochilarium yet two sterna are also concerned in its formation ; namely, the sternum of the maxillary segment and that of the post-maxillary segment, and that it is this latter sternum which corresponds with the hypostome of Latzel. This implies the existence of two maxillary segments, though one of them is without appendages.

The fact that only two jaw-bearing segments were known in Diplopods was one of several reasons given by Professor Lankester (1903), for placing the Diplopods (*Dignatha*

monoprosthomera) far away from the Chilopods, Hexapods, and Crustacea (*Pantagnatha triprosthomera*), in his recent classification of the Arthropoda.

The latest writer on the subject, Carpenter (1905), has found what he interprets as two pairs of maxillæ in the gnathochilarium of *Polyxenus* (adult). He also expresses the confident anticipation that the median parts of the gnathochilarium will be found to belong to the post-maxillary segment. This would imply the existence of three maxillary segments in *Diplopoda*.

Among the recent writings on the segmentation of the *Diplopod* head it seems only necessary to mention those of Professor Lankester (1903), Heymons (1901), and Carpenter (1905).

Professor Lankester, considering the head as monoprosthomeros, places the *Diplopoda* far away from the *Chilopoda*, *Hexapoda*, and *Crustacea*.

Heymons considers the differences between the *Diplopod* head and those of the other groups to be not radical, for he admits the existence of a tritocerebrum in the adult *Diplopod* (St. Rémy, 1890), and also because he would include the post-maxillary segment in the head, considering it as a rudimentary maxillary segment, thus giving the head six segments as in the other tracheates.

Carpenter too is unable to consider the *Diplopod* head as monoprosthomeros, partly because he believes in the existence of an ocular segment, and also because he is in hopes of a tritocerebral segment being demonstrated in an embryo millipede.

METHODS.

The eggs were fixed for the most part in Perenyi's fluid, but for some corrosive sublimate and acetic acid were used. Perenyi is excellent for hardening the yolk, but the second method gives far better histological results.

I can fully endorse Folsom's (1900) statement that the

best and easiest way of observing the minute embryonic appendages is to study the embryos whole with simply their shells removed. When this has been done sections can be cut, and they are of great use in giving complementary and explanatory evidence. The whole embryos were stained with Delafield's hæmatoxylin, the sections with Kleinenberg's hæmatoxylin and orange.

DESCRIPTION OF THE STAGES.

My observations were made chiefly on the two embryonic stages which are described below.

Stage A (fig. 1).

At this stage one can see on the ventral surface of the blastoderm:

(1) The mouth which has the appearance of a semicircular pore.

(2) The clypeus lying in front of the mouth, and already showing division into two lobes.

(3) A rudimentary brain consisting of three lobes on either side.

(4) An incomplete nerve ring on which lies one pair of post-oral ganglia.

(5) Two other pairs of post-oral ganglia.

(6) A large pair of procephalic lobes.

(7) Four pairs of rudimentary post-oral appendages.

(8) Markings or grooves showing divisions between the segments in which the appendages lie.

The nerve-ring, with its thickenings, is here very minute. I have represented it (fig. 1) and them as being slightly raised above the rest of the blastoderm. This is done to emphasise their appearance.

Heymons (1901), in his account of the development of

Scolopendra, describes the primitive brain as consisting of three lobes on either side. In his drawing, which, like mine, is somewhat diagrammatic, the lobes lie more in series than do those shown here (Pl. 37, fig. 1). Nevertheless, there is a great similarity between the two brains.

It seems that we have in the *Archispirostreptus* embryo an archicerebrum, in Professor Lankester's (1881) sense of the word (Heymons uses the words "archicerebrum" and "syncerebrum" with a different meaning), and one cannot help being reminded of the fact that in the *Chaetopod* brain, also an archicerebrum, there are three areas (Pruvot, 1885, and Racovitza, 1896).

The first post-oral ganglion, which is here very small, consisting of a just visible expansion of the thickening, is that belonging to the first post-oral segment which bears the antenna. I can find no trace of a pre-antennary segment in this embryo.

The segment which lies immediately behind the imperfectly closed nerve-ring is apparently the tritocerebral segment (intercalary segment of Heymons and others). It has no appendages, but shows distinct rudiments of a pair of ganglia, and is definitely cut off by grooves from the antennary segment in front and the mandibular segment behind.

The "intersegmental furrows" (Heymons) seem here to appear first in the median region of the blastoderm, while in *Scolopendra*, according to Heymons (1901) they make their first appearance laterally.

The rudimentary nervous system is here extremely small and difficult to make out. From the deep way in which it stains with Delafield's hæmatoxylin, I believe it to be as yet merely a thickening of the ectoderm.

Behind the mouth, a very little way in front of the first "intersegmental furrow," there is a very slight expansion of the thickening on both sides of the nerve-ring. This expansion I take to be the first indication of the antennary ganglion. The ring is not completely closed, but the two cords lie very near each other behind the imperfect closure. These cords

have a rather wavy outline all through their length, but after much careful observation I was able to make out in each cord a dip or incurving, surrounded by a semicircular thickening, of the cord. This incurving lies about half-way between the imperfect closing of the nerve-ring and the second "inter-segmental furrow"—i. e., in the middle of the second post-oral segment. This appearance is repeated in the mandibular segment. These semicircular, thickened incurvings of the cords must be the rudimentary tritocerebral and mandibular ganglia.

One cannot help being struck by the well-marked doubleness of the nervous system shown here. In *Scolopendra*, according to Heymons, the median unpaired ectodermal thickening (which he calls the archicerebrum) is the part of the nervous system which appears first. Later on it forms a junction between the two halves of his syncerebrum, ultimately becoming fused with them. These two halves, therefore, are never separated from each other by non-nervous tissue. But Heathcote (1886), if I understand him rightly, records a double origin for the nervous system of *Julus*, and Metschnikoff (1874), with more clearness, speaks of the first rudiment of the brain in *Strongylosoma* as consisting of two "Scheitelplatten."

I do not believe that the tritocerebral segment has, up till now, been observed in a *Diplopod* embryo. It is, however, not surprising to find it, since St. Rémy (1890) has described a well-marked tritocerebrum in the brains of *Julus* and *Glomeris* (adult).

Before leaving the future head, a word or two must be said about the procephalic lobes. They resemble those found in embryonic insects, arachnids, etc., and those figured by Heymons (1897) in the embryo of *Glomeris*. Their size would seem to me to almost preclude the possibility of finding a separate præ-antennary segment here.

Behind the tritocerebral segment lies that of the mandibles, and following that two segments bearing maxillæ. I have not been able to demonstrate the ganglia belonging to these last

two segments. They have probably not yet made their appearance.

The antennæ and mandibles are slightly raised above the rest of the blastoderm, and each of these appendages shows a slight depression in its centre. The anterior pair of maxillæ are less rounded and smaller than the second pair, and they also lie rather nearer to the middle line. They are most likely homologous with the maxillulæ or superlinguæ described by Hansen (1893) and Folsom (1900) in some of the *Thysanura* and *Orthoptera*. They are also the homologues of the first maxillæ in *Chilopoda* and the first maxillæ in *Crustacea*.

I am guided to the making of this homology mainly by Hansen's (1893) observation that the structure of the first maxillæ in *Machilis* agrees with that of the second maxilla in the *Eumalacostraca*, though I am well aware that serial position is of more importance than structure in such a case as this.

Folsom's (1900) account of the development of the maxillulæ or superlinguæ in *Anurida* is somewhat unsatisfactory, for he says that they do not make their appearance until some little time after the maxillæ have become evident. This statement may, however, be due to an error in observation, and the ultimate position of these small appendages is between the mandibles and the first maxillæ.

Another piece of evidence which, though structural, seems to me to count for a good deal is this:—My observations on the developing *Archispirostreptus* have enabled me to give ample confirmation to Heathcote's (1888) statement as to the mesodermal origin of the salivary gland in *Diplopoda*. This gland, then, must be a cœlomoduct, and is most probably homologous not only with the salivary gland in *Peripatus*, but also with maxillary gland (in the second maxilla) in *Crustacea*.

Hansen has recently (1903) found maxillulæ in *Scolopendrella*, and this gives another reason in favour of the above-mentioned homology; for if the *Symphyla* be not true *Diplopods* they are, at any rate, very nearly related to them, as they are also to the *Thysanura*.

I would, therefore, arrange the mouth parts in the four groups thus :

Hexapoda.	Crustacea.	Diplopoda.	Chilopoda.
Mandibles.	Mandibles.	Mandibles.	Mandibles.
Maxillulæ (Hansen and Folsom).	First maxillæ.	First maxillæ.	First maxillæ.
First maxillæ.	Second maxillæ.	Gnathochilarium.	Second maxillæ.
Labium.	First maxillipede.	Post-maxillary seg- ment.	Maxillipede.

In stage B the size of the first maxillæ as compared with that of the other appendages is much reduced, and no trace of them can be found in the mouth parts of older larvæ. My material was very scanty, and only three series of longitudinal sections through stage B were cut. In each of these series, however, one could see the first maxillæ apparently in process of fusion with the mandible. Perhaps it would be better to say that one could see it being absorbed by the mandible (fig. 4). And just as one could see the apparent absorption of this maxillula by the mandible, so also could one see the absorption of its ganglion by the mandibular ganglion (fig. 5). Provided that any fusion of ganglia or appendages takes place the above is the manner in which one would expect it to do so. For the tritocerebral rudiments undoubtedly join the deuterocephalon which lies in front of them, and not the mandibular ganglion which lies behind them, and the general tendency of fusion in the Arthropoda is from behind forwards.

If, as I believe, these maxillulæ fuse with or become part of the mandibles, they can hardly be homologous with the maxillulæ found by Carpenter (1905) in the adult *Polyxenus*, for the inferences which he draws from his observations on the mouth parts of that Diplopod would lead one to suppose that the maxillulæ there had fused with the maxillæ during the growth of the gnathochilarium.

This matter certainly requires further investigation, though it seems, for reasons which are given below, extremely

improbable that more than one pair of appendages are concerned in the formation of the gnathochilarium.

Stage B (fig. 2).

This is the stage which immediately precedes the breaking and final casting off of the egg-shell. In an external view of it there can be seen :

- (1) The three-lobed archicerebrum.
- (2) The antennary ganglia, to which the tritocerebral rudiments are now joined, and which still lie behind the mouth.
- (3) The ganglia of the mandibles.
- (4) The ganglia of the maxillulæ, much reduced in comparative size.
- (5) The ganglia of the maxillary segment.
- (6) The ganglia of a post-maxillary segment which is without appendages.
- (7) The ganglia of the three segments bearing rudimentary legs.

There are also to be observed the mouth and the clypeus, as well as a pair of appendages for each pair of ganglia, excepting that in the post-maxillary segment.

In each of the three lobes of the archicerebrum and in every other ganglion there can now be seen a distinct depression, a little pit in fact, the opening of which lies in about the middle of the ventral surface of the ganglion. Heathcote (1888) mentions these depressions as occurring in the ganglia of the embryonic *Julus*, but states that they do not appear until the ganglia have left the ectoderm. He also says that he does not consider them to have anything to do with the "cerebral grooves." These "cerebral grooves" are similar cavities which he found in the ganglia of the embryonic brain.

In my specimens I can find no difference between the depressions in the ganglia forming the archicerebrum and those in the ganglia of the cords; and further, these depressions seem to me to appear before the ganglia have com-

pletely left the ectoderm (see fig. 5), which represents a longitudinal section through part of one of the nerve cords in stage B). In this section there can also be seen the small tritocerebral rudiment as well as the rudimentary ganglion of the maxillulæ. This last is being absorbed by the mandibular ganglion.

The tritocerebral rudiment here shows no depression. It is simply a mass of nervous tissue, lying between the antennary ganglion and that of the mandible. The ganglion of the maxillula on the other hand, does show traces of a depression, although it is fusing with, or rather becoming part of, the mandibular ganglion.

It can be noticed that all the appendages (first maxillæ or maxillulæ excepted) have grown in size, as also has the clypeus. The mouth has moved a little further back, but still lies in front of the antenna and its ganglion. The first maxillæ appear very much flattened between the mandibles and the second maxillæ.

Behind the second maxillary segment there is a segment definitely marked off from those in front of and behind it, and showing distinctly a pair of ganglia. This is the post-maxillary segment. Behind it there follow three segments, each of which bears a pair of rudimentary legs. These appendages spring from the hindmost region of the segments in which they occur, so that they have the appearance of belonging to the segment behind their own. Heymons (1897) has observed a similar appearance in the embryo of *Glomeris*, and, what is far more important, he has noticed in the same embryo a post-maxillary segment which bears no appendages. While being firmly of the opinion that there go to the making of the gnathochilarium only one pair of appendages and the hypopharynx, he homologises the post-maxillary segment with the labium-bearing segment in Hexapods, and the second maxillary segments in Chilopods. The knowledge of the existence of a pair of maxillæ in front of the gnathochilarium compels one now to homologise these segments differently. (See Table given above.)

The Gnathochilarium.

Silvestri (1898) found a post-maxillary, legless segment in the larva of *Pachyulus communis*. He expresses the opinion that the sternum of this (post-maxillary) segment corresponds with the hypostome of Latzel, and that therefore the gnathochilarium consists of the sternum and appendages of the maxillary segment together with the sternum of the labial (post-maxillary) segment.

Further, he says that the dorsal part of this labial (post-maxillary) segment does not join the head at all, but forms a neck joined to the first segment of the body. This last statement is fully confirmed by my observations in *Spirostreptus*. My reasons for thinking that the sternum of this segment does not form the hypostome are given further on.

Unfortunately none of the larvæ hatched in the Zoological Gardens lived long enough to enable the actual formation of the gnathochilarium to be worked out. But that is now a matter of comparatively small importance since the real question at issue was not the formation of the lower lip, but the number of maxillary segments present in the Diplopoda.

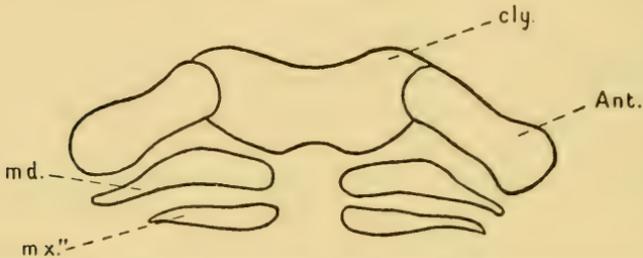
When the larval *Archispirostreptus* leaves the egg the mandibles and the second pair of maxillæ are clearly laid down, as indeed can be seen from Pl. 37, fig. 2, which represents an embryo still within the egg-shell. The first maxillæ have disappeared, being by this time completely absorbed by the mandibles. But the gnathochilarium is far from being fully formed, and in this my observations do not agree with those of vom Rath (1891), who found that the young larvæ of the Julidæ and Glomeridæ left the egg with mouth parts like those of the adult.

As the larvæ grew in age and size the maxillæ gradually approached each other, and in that which lived longest they touched each other; but as yet they were not fused together, nor could I see that they had fused with any other part of the head, or body (see text-figures 1—4).

There were no adult *Archispirostreptus* at my disposal, I have therefore made use of some adult *Spirostreptus* collected by Professor Minchin in Uganda.

In the adult *Spirostreptus* there is behind the hypo-

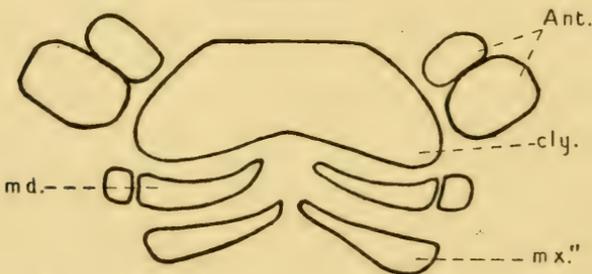
TEXT-FIG. 1.



Diagrammatic plan of the mouth-parts of the larva of *Archispirostreptus* five days after it leaves the egg-shell.

stome a piece of chitin, which, though larger than the hypostome, resembles it in shape (text-fig. 5). The simplest way of accounting for this would be to consider it as representing the sternum of the post-maxillary segment. The tergum of

TEXT-FIG. 2.



Mouth-parts of larva ten days after leaving the shell.

this segment is exceptionally large, and seems to represent two terga, namely, that of the post-maxillary segment and that of the first leg-bearing segment. The segment following that which bears the third pair of legs (fifth body segment) is apodous in *Spirostreptus*.

Heathcote (1888) in his account of the development of

Julus says that he believes the first body segment, i. e. the post-maxillary segment, to be the apodous one. He states that he believes it to be equivalent to the second maxillary

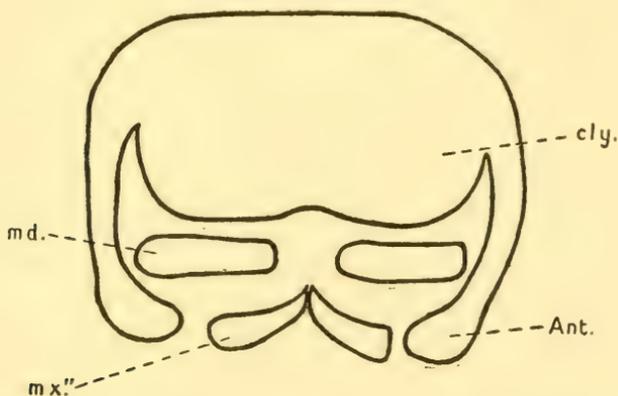
TEXT-FIG. 3.



Mouth-parts of larva fifteen days after leaving the shell.

segment in the Insecta, and also asserts that its ganglion fuses early with that of the segment in front of it. I can find no trace of such a fusion in either the embryos or larvae of *Archispirostreptus*. Moreover, I have cut longitudinal

TEXT-FIG. 4.



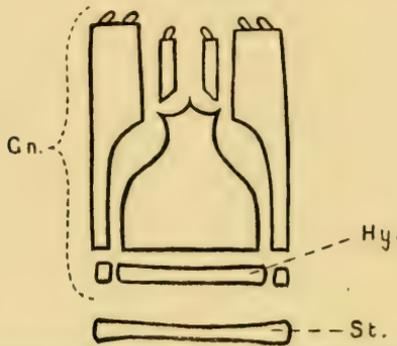
Mouth-parts of larva twenty days after leaving the shell. *Ant.* Antenna. *Cly.* Clypeus. *Md.* Mandible. *Mx.''* Second maxilla.

sections through the subœsophageal ganglion in several adult *Spirostreptus*, and have found it to consist of only two pairs of ganglia (Pl. 37, fig. 6), namely, those of the mandibles and maxillæ.

I am well aware that the evidence is incomplete and that

what is really needed is an examination of larvæ intermediate in age between the oldest which I have had and the adult. But such evidence as we have at present seems to show that

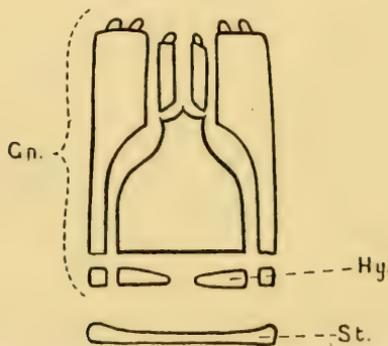
TEXT-FIG. 5.



Gnathochilarium from adult *Spirostreptus*, with hypostome as usually present.

only one pair of appendages (the second pair of maxillæ) are concerned in the making of the gnathochilarium, though the sternum of the segment which bears them most probably

TEXT-FIG. 6.



Gnathochilarium from adult *Spirostreptus*, showing double hypostome.
Gn. Gnathochilarium. *Hy.* Hypostome. *St.* Sternum of post-maxillary segment.

also takes part in it. The fact that the subœsophageal ganglion consists of only two pairs of ganglia seems to point strongly to this conclusion.

In taking out the subœsophageal ganglia from several adult *Spirostreptus* I naturally looked at their gnathochilaria. In two of these I found the hypostome to be distinctly double (text-fig. 6). This would seem to show that it had its origin rather in two appendages than in one sternum.

If, as above suggested, the gnathochilarium consists of but one pair of appendages we have another reason in favour of the homology proposed in the first part of this paper.

Of making many homologies for the segments in different arthropods there is no end, but most authorities are agreed in assigning six segments to the head in Crustaceans, Hexapods and Chilopods. Now the head of the adult Diplopod is very distinctly marked off from the body, being separated from it by a narrow neck, which forms, as it were, a joint. This is more apparent in the recently hatched larva before the chitin has developed than in the adult (fig. 3). Even in so young an embryo as that shown in fig. 2 the ganglia of the head have a different appearance from those of the body. The Diplopod head can now be shown to consist of six segments thus :

(1) A segment which, together with the acron (Heymons), forms the procephalic lobes.

(2) The antennary segment.

(3) The tritocerebral segment, representing the second antennary segment of Crustacea, and tritocerebral rudiments in Hexapoda and Chilopoda.

(4) Mandibular.

(5) First maxillary (rudimentary).

(6) Second maxillary segment, the appendages of which are fused to form the gnathochilarium.

As the head of *Spirostreptus* is distinct from the body so also, notwithstanding the forward movement of the ganglia, is the subœsophageal ganglion distinct from that of the post-maxillary segment. I mention this as further evidence to show that the post-maxillary segment belongs to the body and not to the head.

SUMMARY OF RESULTS.

There are, in the embryo of *Archispirostreptus*, two segments, the possession of which would seem to give the Diplopoda a place in the Arthropod system nearer to the Chilopoda and Hexapoda than that which has of late been assigned to them.

These additional segments are :

(1) A tritocerebral segment representing the tritocerebral rudiments found by Wheeler (1893) and others in Hexapoda, and by Heymons in *Scolopendra*, and also the tritocerebral segment in Crustacea.

(2) A pair of maxillæ (rudimentary) lying in front of the pair which forms the gnathochilarium in the adult. These are most likely homologous with the first maxillæ in Chilopoda and Crustacea, and with the superlinguæ (Folsom) of Hexapoda.

With regard to the development of the gnathochilarium I have unfortunately not been able to add much to our previous knowledge. But the importance of this matter has now become comparatively small, since the existence of a first pair of maxillæ has been demonstrated.

The evidence that I have is incomplete, but it certainly goes to show that the gnathochilarium is part of the head, being formed by the second pair of maxillæ which are the only appendages in it; and that the post-maxillary segment of Heymons and Silvestri takes no part in the formation of this gnathochilarium, but is purely a body segment.

May 27th, 1907.

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EXPLANATION OF PLATE 37,

Illustrating Miss M. Robinson's paper "On the Segmentation of the Head of Diplopoda."

REFERENCE LETTERS.

Ag. Antennary ganglion. *Ant.* Antenna. *Arc.* Archicerebrum. *Cly.* Clypeus. *Ec.* Ectoderm. *M.* Mouth. *Md.* Mandible. *Mdg.* Ganglion of mandibular segment. *Mes.* Mesoderm. *Mx'.* First maxilla. *Mx'g.* Ganglion of first maxilla segment. *Mx''.* Second maxilla. *Mx''g.* Ganglion of second maxilla segment. *Pmx.* Post-maxillary segment. *Pmxg.* Ganglion of post-maxillary segment. *Pmxl.* Post-maxillary tergum. *Proc.* Procephalic lobe. *Isf.* Intersegmental furrow. *Trc.* Trito-cerebral segment. *Treg.* Trito-cerebral ganglion.

FIG. 1.—Embryo of *Archispirostreptus* (sixteen days old) about four days before leaving the shell.

FIG. 2.—Embryo of *Archispirostreptus* one day before leaving the shell

FIG. 3.—Larva of *Archispirostreptus* five days after hatching.

FIG. 4.—Longitudinal section through appendages of embryo shown in fig. 2.

FIG. 5.—Longitudinal section through ganglia of embryo shown in fig. 2.

FIG. 6.—Longitudinal section through the subœsophageal and post-maxillary ganglia in the adult *Spirostreptus*.

The Fixation of the Cypris Larva of *Sacculina*
carcini (Thompson) upon its Host,
Carcinus mænas.

By

Geoffrey Smith, M.A.,
New College, Oxford.

With 6 Text-figures.

THE fixation of the Cypris larva of *Sacculina* has hitherto been successfully observed only by Delage (1), and since a considerable degree of doubt has been thrown on his account it may be of interest to record that I have been able this autumn, at Plymouth, to rear the larvæ and observe all the stages of fixation upon their host. My inability to obtain the Cypris larvæ of any of the Rhizocephala at Naples had left a gap in my observations, which I am now in a position to fill, and thus I am able to present a complete account of the life-history of *Sacculina* from my own observations (2).

It is extremely easy to obtain large batches of the Nauplii of *Sacculina* or *Peltogaster* by selecting a crab whose parasite is nearly ready to emit its Nauplii from the brood pouch, and keeping it for a few days in a vessel of sea water. The readiness of the parasite to emit the Nauplii can be judged in *Sacculina* by the purple colour of the mantle, and in *Peltogaster* by the mantle becoming a paler pink than usual.

The Nauplii of *Peltogaster* are not actively heliotropic, but they are negatively geotropic, and in a short time all of them reach the surface film of the water and perish, and I have been unable to obviate this difficulty in rearing the larvæ. The Nauplii of *Sacculina* are actively heliotropic, but there is no marked negative geotropism; nevertheless, with the

Neapolitan races of *Sacculina* which infest *Inachus mauritanicus*¹ (Lucas) and *Pachygrapsus marmoratus* (Fabr.), I did not succeed in keeping them alive for more than four days, or in observing the transformation into the Cypris stage.

The Nauplii of the *Sacculina* on *P. marmoratus* appeared to be hardier than those of the parasites on *I. mauritanicus*, and I suggested (2, p. 44) that this might be due to the former crab being a distinctively littoral species, and thus subjected to more varying conditions than the Spider-crabs; this suggestion is probably correct since the Nauplii of the *Sacculina* of *Carcinus mænus* are evidently the hardiest of all, and this crab is more decidedly littoral in habit than *P. marmoratus*.

The successful rearing of the Nauplii of *Sacculina carcini* was effected by selecting "purple" *Sacculinæ* and obtaining a batch of healthy Nauplii; they were then transferred to sea water which had been purified by mixing with powdered charcoal and filtering in the manner used by the Director of the Plymouth Laboratory, Dr. E. J. Allen, in his preparations of pure cultures.

It is of extreme importance that water of great purity should be used, since the larvæ are to live in this water for nearly a fortnight, and the multiplication of Infusoria and Algæ in the water is highly injurious to them. The larvæ, of course, require no food, since they possess no gut and subsist entirely on the yolk reserves which they contain.

The Nauplii, actively swimming the whole time, undergo four successive moults in four days; they are then transformed by a single moult into the Cypris larvæ. Before these larvæ fix upon their host, Delage observed that they had to spend at least two more days in a free-swimming state; fixation also only takes place in the dark. These two points I am able to confirm from my own observations.

The fixation of the Cypris larvæ, as followed by myself,

¹ By an error in nomenclature *I. mauritanicus* (Lucas) was called *I. scorpio* (Fabr.) throughout in my monograph (2).

takes place exactly in the manner described and figured by Delage (1). In every case the larva was fixed by one of its antennulæ to the base of a hair, most frequently on the legs of the crab, and preference was shown for the sparsely scattered, plumose hairs upon the flat surfaces of the proximal joints of the legs. The larvæ appeared to avoid fixing on the dense hairs which fringe the edges of the appendages. This fact is fortunate, since the Cypris when fixed upon an isolated hair on the smooth surface of an appendage is a most conspicuous and unmistakable object.

The crabs which I placed with the Cypris to be infected were about 7—15 mm. in breadth, and I selected specimens which had recently undergone a moult, since the skin of these individuals is clean and easy to manipulate.

Soon after fixation the Cypris larva casts away bodily all its thoracic appendages with their attached muscles; I was fortunate to obtain this stage, the particular specimen exactly resembling Delage's Plate 23, fig. 21 (2). During the next two days a process goes on inside the Cypris shell which leads to the formation of the Kentrogon larva. According to Delage, the ectoderm of the larva draws away from the Cypris shell and comes to surround a mass of mesodermal cells lying in the anterior region of the body; the ectoderm secretes a layer of chitin externally to the whole, the Cypris shell falls off, and with it the degenerated remains of the larval muscles, pigment, sense-organs, and broken-down food material, and the so-called Kentrogon larva, a little oblong sac encased in chitin, is left attached to the hair of the crab by means of the antennule of the Cypris.

Delage's figures representing these changes were so completely reproduced in the larvæ observed that I have nothing to add to his description.

With regard to the contents of the Kentrogon larva a word is necessary. Delage considered that a layer of ectoderm is present surrounding a mass of cells which he calls the ovary. This conception I disputed (2, p. 43) after finding the earliest internal stages of the parasite in which no visible differentia-

tion of the cells into distinct layers or organs was present. I therefore suggested the term "embryonic cells" to designate the cellular contents of the Kentrogon. After observing in several instances the formation of the Kentrogon larva, it appears to me that the ectoderm of the Cypris is undoubtedly included in the Kentrogon together with the mesodermal cells, as Delage originally maintained. At the same time, his designation of the mesodermal mass of cells as the ovary is obviously a misnomer, since this mesodermal mass gives rise to all the mesodermal organs and tissues of the adult, and, at the early stages of the endoparasitic development, is quite undifferentiated (see 2, pp. 47 and 55).

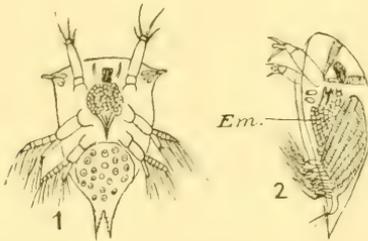
We may, therefore, retain the term "embryonic cells" for the cellular contents of the Kentrogon, with the admission that these embryonic cells are composed of embryonic ectoderm and embryonic mesoderm. It may be remarked that, in this manner, the Kentrogon of the Rhizocephala almost exactly corresponds to the post-nauplius stage of *Monstrilla*, as described by Malaquin (3), which, on penetrating the ectoderm of its host, loses all the larval organs, and consists of a little chitinous sac containing embryonic ectoderm and meso-endoderm, from which the adult organs are regenerated. In *Monstrilla*, of course, the endoderm is not entirely suppressed, as is the case in the Rhizocephala, though it is on the way to becoming so.

This interesting parallelism between the Rhizocephala and the *Monstrillidæ* has not been sufficiently insisted on.

In the extreme anterior region of the Kentrogon, where it is attached to the base of the crab's hair, I have been able to observe the formation of the dart which gives the name to this larval stage (*κέντρον*, dart; *γόνοσ*, larva). This hollow dart, as Delage described, elongates and pierces the base of the hair, thus opening a passage through which the embryonic cells of the Kentrogon can pass into the hæmocœl of the host. The dart is continuous with the inner cuticle of the Kentrogon larva, and is presumably a product of the ectoderm.

After the formation of the Kentrogon larva with its dart, a process obviously preparatory to the infection of the host, the next stage in the life-history which is known is that which I have described (2, p. 47) under the name *Sacculina interna migrans*.

The parasite at this stage consists of a mass of embryonic cells undergoing rapid division by mitosis; it has an irregular shape, a few small roots beginning to grow out from a central tumour, and the whole is enclosed in a thin chitinous cuticle. There is no visible differentiation of any of the adult organs. It is clear that the gap between this stage and the Kentrogon larva is very slight, since the morpho-



TEXT-FIGS. 1 and 2.—1. Nauplius of *Sacculina*.
2. Cypris of *Sacculina*.

logical structure is practically identical. These small migrant *Sacculinae* were found in the hæmocœl of the crabs applied to the upper part of the intestine just below the stomach, that is to say, far away from the point where the body of the adult *Sacculina externa* is situated. This position of the earliest known internal stage of *Sacculina* is completely in accord with the indefinite position of the fixation of the Cypris larvæ upon the crab, and is irreconcilable with the theory that the Cypris fixes upon the under surface of the crab's abdomen, and is transformed into the adult *Sacculina in situ*.

We are still confronted with the problem how the cells of the Kentrogon that enter the hæmocœl of the crab at any

point are transported to the position of the *Sacculina interna migrans*. Delage, who did not observe the latter stage, believed that the cells of the Kentrogon, on entering the crab, at once began to send out roots, and to grow always in a determinate direction until the central tumour reached the point where the adult *Sacculina* was to be evaginated. But, since the youngest *Sacculinae* of the migrant stages which I observed were not provided with elongated roots stretching to the skin at any point where the *Cypris* might have fixed, I incline to the opinion that the embryonic cells of the Kentrogon, after entering the hæmocœl, are carried passively about in the blood stream until they, sooner or later, reach the large blood spaces surrounding the intestine, and that, arrived there, they begin to throw out the root system, while the central tumour grows down toward the junction of thorax and abdomen where the body of the *Sacculina externa* is differentiated. The method of this differentiation, the formation of the adult organs, and the evagination of the *Sacculina* to the exterior have been fully described by Delage (1) and myself (2).

The life-history of *Peltogaster*, the only other of the *Rhizocephala* that has been at all worked out (2), is very similar to that of *Sacculina*, save that it seems probable that the *Cypris* fixes upon the abdomen of the hermit-crab, and that the migrant phase of the internal development is not so marked.

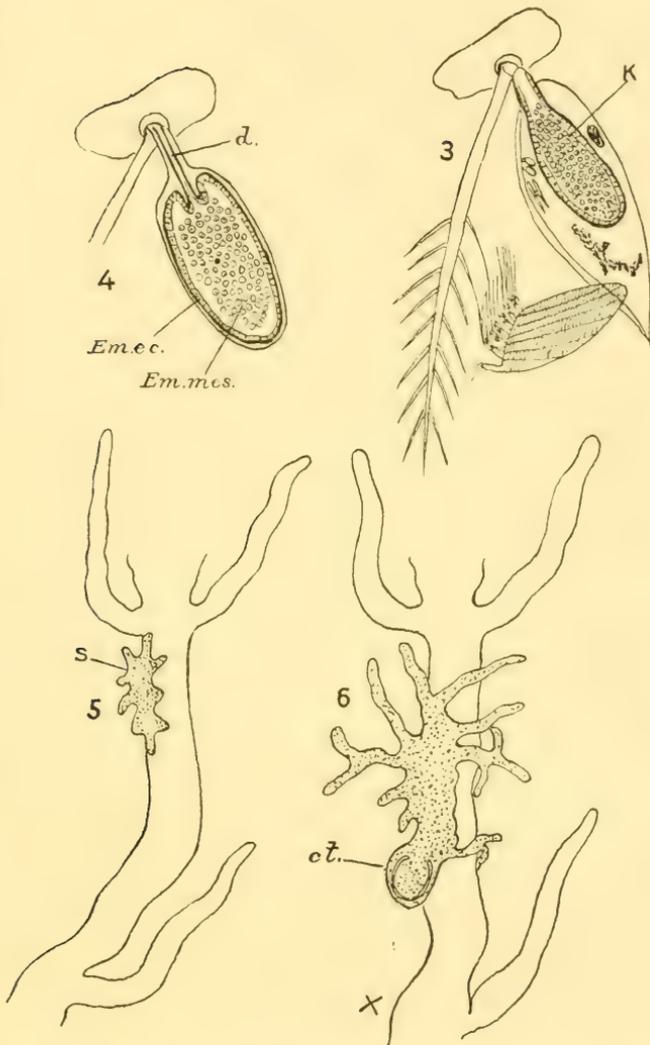
The life-history of *Sacculina* may be shortly summarised as follows:

The eggs undergo maturation in the brood pouch, and are self-fertilised.

Development up to the *Nauplius* stage proceeds in the brood pouch.

The *Nauplii* are expelled to the exterior, and lead a free-swimming existence for four days, undergoing four moults.

The *Cypris* stage is attained at the fifth day, and, after two or three days of free existence, the *Cypris* larvæ attach



TEXT-FIGS. 3-6.—Diagrams illustrating the life cycle of Sacculina. 3. Cypris fixed on hair; degeneration of larval organs, and formation of Kentrogon (*K*). 4. Kentrogon, formation of dart (*d.*); *Em.ec.* Embryonic ectoderm; *Em.mes.* Embryonic mesoderm. 5. *Sacculina interna migrans* (*S.*) on gut of host. 6. *Sacculina interna*, later stage, with central tumour (*ct.*) passing to position of evagination (*X*).

themselves by their antennulæ to a hair upon any portion of a young individual of the host, preferably upon the appendages.

The Cypris casts off its thoracic appendages, the ectoderm draws away from the shell, and comes to surround a mass of mesodermal cells; it secretes a chitinous coat, and in this manner the Kentrogon larva is formed. The Cypris shell, together with all the larval organs, are thrown off.

The ectoderm of the Kentrogon secretes a hollow dart in the anterior region which projects up into the antennule by which the larva is fixed to the base of the hair on the crab, and gradually penetrates the base of the hair so as to open a means for the cells contained in the Kentrogon to enter the hæmocœl of the crab.

The embryonic cells of the Kentrogon, consisting of ectoderm and mesoderm, enter the hæmocœl of the crab, and are carried about in the blood-stream until they reach the large blood-spaces surrounding the intestine. They are enclosed in a thin chitinous cuticle. The *Sacculina interna migrans* now proceeds to grow rapidly, to throw out roots in all directions, while the central tumour grows down the intestine towards the junction of thorax and abdomen of the crab. As it grows in this manner, the adult organs are differentiated in the most posterior portion of the central tumour, which soon arrives at the position of evagination of the adult *Sacculina*. Here differentiation proceeds, and the pressure of the growing tumour upon the epithelium of the crab causes it to degenerate, and thus, when the crab next moults, a hole is left in the new chitin, through which the *Sacculina* protrudes, and so gains the exterior.

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Physiological Degeneration in Opalina.

By

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With Plate 38 and 2 Text-figures.

INTRODUCTION.

In many respects *Opalina ranarum*, Purk. and Val., is one of the most interesting of the ciliated Protozoa. Although its adult appearance has been familiar to all zoologists for a long time, it is only in the course of the last year that its interesting life-cycle has become fully known. We owe this knowledge to Neresheimer, who has, however, only given us a preliminary account of his important researches. His full paper will be awaited with much interest.

It will be unnecessary for me to give a detailed description of this very common species. But I would remind the reader that it is a large, multinucleate, holotrichous form found in the large intestine of our common frog and toad (*Rana temporaria*, L., and *Bufo vulgaris*, L.). The nuclei may be regarded as consisting of meganucleus and micronucleus fused to form a synkaryon. For an account of the life-history the reader is referred to accounts already published, but more especially to the memoirs of Zeller and Neresheimer. It will be necessary for me to give a brief account of the earlier part of the life-cycle, however; that is to say, of the part prior to encystment, which takes place in the adult host.

Neresheimer has accurately, though briefly, described this phase, and I have been able to confirm fully his observations.

In the spring, at the frog's breeding season, *Opalina* encysts, and is cast out into the water in the excreta. The changes which take place before encystation are as follows:—The adult animal divides in an oblique manner, giving rise to two daughter individuals. Each of these then divides into two, and these again divide until small *Opalinæ* containing several nuclei are formed. During these divisions important changes occur in the nuclear apparatus. The nuclei are seen to become less distinct, this being due to the fact that the chromatin is cast out into the cytoplasm in the form of small strands and particles, or chromidia. Sometimes I have observed these united to form a network through the creature. Finally, the original nuclei vanish, and we are left with only distributed chromatic material. The chromidia soon become aggregated at certain centres, and thus synthesise new nuclei, which are from two to ten in number. These nuclei are seen to be composed of large chromatin granules, arranged irregularly. They change, however, with the approaching encystment of the animal, which soon takes place. The chromatin travels to the periphery of the nucleus where it becomes arranged in a thin layer with two to four large cap-like thickenings.¹ In optical section these nuclei appear as rings. The cap-like projections are soon cast off into the cytoplasm, where they degenerate, this constituting the first nuclear reduction. Encystment follows, and in the normal course of events the cyst is cast into the water, where a second reduction of chromatin occurs. I will leave the description of the life-cycle at this point, merely noting that each nucleus is now a reduced gamete nucleus, and takes part in the formation of a single ciliated gamete whose destiny is to conjugate in the tadpole's gut. The essential points in the process just described are (1) formation of chromidia, (2) synthesis of fresh nuclei from these chromidia, (3) reduction of chromatin, and (4) encystment.

¹ First described by Loewenthal as "micronucleus-like structures,"

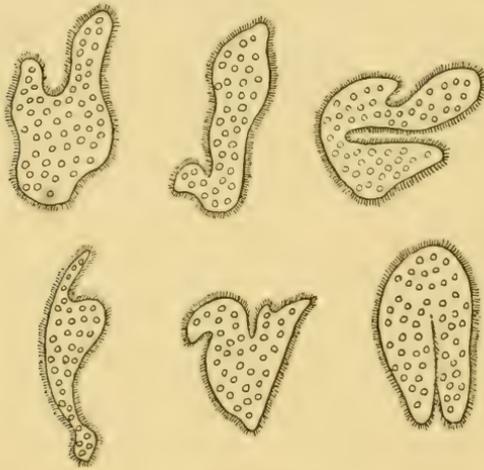
DESCRIPTION OF DEGENERATION IN OPALINA.

Having cleared the ground by describing the ordinary course of events preceding gamete formation, I will now pass on to a description of the physiological degeneration of the forms whose usual destiny is to encyst.

Two different kinds of degenerative changes may be distinguished—the one caused by removal from the host, the other within the host. The former is much the more rapid, and is easily induced at any time. Degeneration results from drying, from increase in the number of bacteria, and also doubtless from lack of food, and increase of metabolites. The entire organism simply decomposes, often after first throwing out its nuclei in fragments. It is the second kind of degeneration—that within the host—with which I am here concerned.

In nature, as we have seen already, the encystation of *Opalina* is contemporary with the sexual activity of its host. The set of degenerative changes which I am about to describe took place when the ordinary activities of the host animals were modified by captivity and starvation. Frogs, as is well known, can endure starvation for many weeks. But the contained *Opalinæ*, apparently, cannot do so—at all events at their encystation period. Starvation is, I believe, the determining factor in their degeneration. Other causes, which materially influence degeneration in the organisms when removed from their host—such as change in reaction of the medium, drying, increase in the number of bacteria, etc.—do not appear to come into play. For after lengthy starvation the rectal contents of the frogs and toads examined consisted of only a small quantity of a clear, mucous fluid, alkaline in reaction, and containing but few bacteria. It was in cases such as this—where starvation of the host had sometimes lasted for at least two months—that the most advanced stages in the degeneration of *Opalina* were encountered. My observations extend over a period from the middle of

January to the beginning of April, and are based upon the careful examination of the rectal contents of over fifty frogs and toads. The original object of the research was to study the life-histories of the small Protozoa (flagellates and amœbæ) which occur in this situation. My attention was attracted by the curious degeneration forms, although their true nature was not, for some time, understood. However, after a careful study of fresh material, the complete series of degeneration changes here described became apparent.



TEXT-FIGURE 1.

One of the earliest changes which the Opalinæ undergo is a change of shape. Instead of remaining of a flattened, ovate form, they become modified into all sorts of indefinite shapes. Some of these are shown in text-fig. 1, but a great number of other forms may be seen. The drawings are schematic, and merely indicate the kind of thing which one encounters. These forms do not divide in the normal manner, but simply constrict off pieces, apparently at random, of all shapes and sizes. These again divide until small, irregularly discoidal, or ovoid forms are produced which have a length of about 10μ to 30μ . These usually contain from one to

four nuclei, but much larger individuals—up to 50μ , with nine or ten nuclei—are also found in the same condition. Such forms undergo two remarkable changes—(1) they completely lose all cilia, and (2) they give rise to globules of a substance of high refractivity in their cytoplasm. The nature of these globules I am unable to determine. I may mention, however, that they have the following properties:—

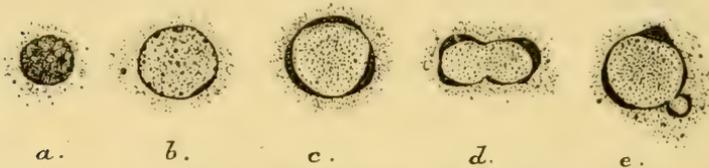
In the fresh state they are somewhat greenish, and very highly refringent. They are coloured a bright pink with eosin, and a bright greenish-yellow with picric acid. With iodine they appear to become slightly more greenish, but the reaction is not well marked. They are insoluble in water, alcohol, and weak acids and alkalies. Heidenhain's iron hæmatoxylin colours them a dark greyish- or brownish-black—not so dark, however, as the chromatin. Delafield's hæmatoxylin does not colour them—neither does borax-carminé.

From their remarkably vivid coloration with eosin I have termed these globules "eosinophile" bodies, in ignorance of their chemical constitution. Although they first appear as separate globules of small size, they ultimately run together, forming large masses lying in the cells. They do not appear to have any connection with the nucleus.

If these degenerate forms be obtained at the right stage, the loss of cilia may be observed in the living animals. It takes some days for all to be completely lost, and all do not seem to disappear in the same way. Apparently some of them actually dissolve, for they become gradually fainter and fainter, and finally disappear. Others are thrown off entirely, and after moving spontaneously for a short time after detachment, they become motionless and fade away. Still others undergo fusion with one another, and ultimately with the cytoplasm. In this manner many individuals arise which are completely divested of ciliary covering, and contain refringent eosinophile bodies. The nucleus also undergoes remarkable modifications. I term these forms the atrichous forms.

The nuclear changes which the atrichous forms undergo are

as follows, and may be easily seen in the living animal:—The chromatin which was at first evenly distributed through the nucleus becomes massed in granules at the periphery, whilst the nucleus itself increases in size until it becomes sometimes double its original diameter (text-fig. 2, *a*, *b*).



TEXT-FIGURE 2 (from permanent preparations, stained with Heidenhain's iron-hæmatoxylin).

The chromatin becomes evenly disposed in a single layer, so that in optical section the nucleus has a very characteristic annular appearance (*c*), the ring being thickened at various points (see also Plate, figs. 8, 12). A typical atrichous form is therefore distinguished by having no cilia, by possessing "eosinophile globules" and a large ring-like nucleus (or nuclei). When first seen they have a remarkable appearance, and their connection with the ordinary Opalina would hardly be suspected. These forms are quite motionless.

In many of the larger atrichous forms division of the nucleus takes place, followed frequently by division of the cytoplasm. Division may be equal, by a constriction appearing in the middle (text-fig. 2, *d*), or unequal. In this latter process a blister-like elevation of the chromatin appears, and is finally constricted off. This is shown in text-fig. 2, *e*. Above is a cap-like outgrowth of the chromatin, whilst below a later stage is seen in optical section (cf. also Plate, figs. 10, 11).

When cytoplasmic division follows the result is either equal bipartition or budding (cf. Plate, figs. 9, 10). The buds so produced are sometimes very small, not reaching a greater diameter than 4—5 μ . Occasionally buds are produced in which no nuclear material whatsoever can be

detected, and very commonly small buds are given off which contain an eosinophile globule, but no nucleus. All these enucleate buds appear to die and disintegrate. Finally, a number of uninucleate atrichous forms result, which are of an average diameter of about $20\ \mu$. At this stage they show a marked tendency to attach themselves to one another, thus forming small colonies (cf. Plate, fig. 12). No fusion, as a rule, appears to take place.

The chromatin of the nucleus, which is of a very variable size, but on an average about $8\text{--}10\ \mu$ in diameter, is seen to be arranged in lumps peripherally. It soon leaves the nucleus, however, and fills the cytoplasm, where it takes the form of irregular granules and masses of different shapes and sizes. These chromidia, as they may be called, appear to be sometimes in the form of minute hollow spheres, ring-like in optical section. By their formation the original nucleus dwindles away, and finally disappears (see Plate, figs. 1, 2, 7). As a rule most of this chromatin is cast out of the organism, which then dies and breaks up. But occasionally a remarkable thing happens. Only a part of the chromatin is cast out and perishes. The remaining granules run together again, very much as drops of oil might run together in a watery medium. All the irregular chromidial masses may become aggregated at a single centre, but at other times two such centres are formed, so that finally two nuclei, consisting of solid chromatin, are synthesised. These two solid lumps then approach one another and fuse (see Plate, figs. 3—6). During the chromidial stages a soft cyst-wall is sometimes formed.

I have been unable to obtain any further stages after this, except such as are disintegrative. Kept under a waxed coverslip or in a hanging drop they always perish by discharging their nuclei in fragments and then breaking up. This also appears to happen in the frog's gut. It would be exceedingly interesting to know whether a recovery could be made under suitable conditions or not. I have endeavoured to restore some of these degenerate fragments by transfer-

ring them into the boiled rectal contents of a normal frog, but without success. I have not been able to obtain sufficient material for more extended experiments in this direction.

The final result then is death; and with this I finish my description of degenerative changes in *Opalina ranarum* so far as I have observed them. Before leaving the subject, however, I must draw attention to the extraordinary parallel which exists between these changes and certain so-called "sexual" processes. In many Protozoa gamete nuclei are formed from the original compound nuclei by resynthesis from chromidia, very much in the same way as the solid chromatin nuclei which I have just described in *Opalina*. In certain autogamic processes the nuclei are formed and fuse in the same cell. Compare, for example, the autogamy of *Bodo lacertæ*, Grassi, as described by Prowazek. The animal encysts, and inside the cyst the nucleus gives off chromidia into the cytoplasm. From these chromidia a new nucleus is built up, and this divides into two. Each daughter nucleus forms two "polar bodies," and the reduced nuclei (the gamete nuclei) approach one another and fuse. In *Bodo lacertæ* heterogamy also occurs, but in *Trichomastix lacertæ*, Blochmann, and in *Entamoeba coli*, Lösch, only autogamy is known—no other "sexual" act.

It is possible that the process which I have just described in *Opalina* is a kind of autogamic attempt on the part of the organism to reconstitute itself. But I believe that the sole explanation of this curious set of changes is to be sought in the alteration in chemical and physical properties which living protoplasm undergoes in dying.

In conclusion, mention may be made of certain other observations which have been made on degeneration in other Protozoa. But little attention has been bestowed upon the matter, although in a few species degenerative changes have been studied in considerable detail. Among these I may name *Actinosphærium* (Hertwig), *Amœba* (Prandtl), *Paramœcium* (Maupas, Calkins, etc.), *Trichosphærium* (Schaudinn), and the sporont of *Cyclospora caryolytica*

(Schaudinn). In the first three of these increase in the size of the nucleus has frequently been observed as a preliminary occurrence. In *Actinosphærium* giant nuclei are formed when the animal degenerates owing to overfeeding. Breaking up and discharge of the nucleus usually follows—that is to say, chromidia play a part in the degenerative phenomena. In *Trichosphærium*, when degeneration is induced by starving the organism, the nuclei clump themselves together at certain points. This agglomeration is not followed by fusion. Stole has observed a similar condition in starved *Pelomyxa*. And I may here recall the observation of Maupas on *Paramœcium*, that the fragments of the old meganucleus sometimes fuse with the new meganucleus of an exconjugant if it be starved.

In the degenerating sporont of the coccidian *Cyclospora* the polar bodies divide until eight are formed, and microgametes then attempt to fertilise each of these. In later stages of degeneration pigment is formed; and Prandtl has shown that pigment appears in a degenerating *Amœba proteus*, and is formed from the chromatin of the nucleus. In this particular form there is also a curious tendency for the nucleus to surround food masses in the cytoplasm.

Nuclear fusion sometimes takes place—independently of any sexual process—in multinucleate Protozoa during, or following, encystment: e. g. in *Dileptus* (Prowazek).

Hyper-regeneration occurs in *Stylonychia* if mutilated when in a degenerate condition (Prowazek). Loss of appendages has been frequently observed in many different Protozoa undergoing degenerative changes. It is unnecessary to give a number of examples, but *Trichosphærium* and *Paramœcium* may be cited as good instances.

Chromidia of the type I have described in *Opalina* (i. e. bladder-like, or bläschenförmig) have only been noticed, so far as I am aware, in one other Protozoon, *Bodo lacertæ*, Grassi. And here they are formed as a preliminary to gamete formation and autogamy (Prowazek).

Very curious in many other ways is the parallel which

exists between degenerative and "sexual" processes. Besides the fact that chromidia are formed in both, we have the observation that an amœboid condition may occur in degenerating Protozoa, and also sometimes just before conjugation. Fusion also occurs in degenerating forms of various kinds. I have observed it especially in flagellates, e.g. *Trichomastix*, *Trichomonas*, etc. Senile *Paramœcia* enter upon what Calkins calls the "miscible state," when they tend to adhere to one another. Similarly, Roux has observed that isolated, living blastomeres of frog's eggs become amœboid and run together; though, according to Driesch, this is merely due to the capillary forces between the cells. These facts, and many others of a similar nature, are not without interest, both from a pathological and from a zoological point of view. I may mention merely their possible bearing upon the remarkable fusion which appears to take place between leucocytes and cancer cells, and its unknown significance. And since the work of Calkins seems to indicate that chemical change in protoplasmic composition is the chief beneficial effect of conjugation, and there is some proof that Protozoon individuals have chemical compositions differing from one another (cf. Jensen), is it not at least possible that the physico-chemical changes which cause fusion in degenerating cells are of a similar nature to those which gave rise to the first cell-couplings, and which still determine the fusion of one gamete with another?

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ADDENDUM.

Neresheimer's full account of the life-cycle of *Opalina* has appeared since this paper was submitted for publication. It is a very complete description, with full references to the literature, and is to be found in 'Arch. f. Protistenk.' Supplement i (Festband für R. Hertwig), 1907, p. 1.

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EXPLANATION OF PLATE 38,

Illustrating Mr. C. Clifford Dobell's paper on “Physiological Degeneration in *Opalina*.”

[Figs. 7 and 8 are drawn from living specimens, under a 2·5 mm. (apert. 1·25) apochromatic water immersion objective by Zeiss; compensating ocular 12. The remainder are from permanent preparations: fixed sublimate-alcohol (2:1). Figs. 1—6, 9 and 12 from specimens stained with Heidenhain's iron-hæmatoxylin. Fig. 10, Heidenhain and eosin. Fig. 11, Weigert's iron-hæmatoxylin and eosin. Drawn under a 2 mm. (apert. 1·40) apochromatic oil immersion (Zeiss), with compensating ocular 12.

All figures are drawn in monochrome for sake of uniformity. In all cases the darker masses are chromatin; the paler masses surrounded by a light area are the eosinophile bodies.]

FIG. 1.—Atrichous form in which the nucleus has largely broken up into chromidia. The remains of the nucleus are seen near the middle, with a single large cap-like mass of chromatin. Beneath is seen one large eosinophile body. Note that many of the chromidia are in the form of hollow spheres—annular in optical section.

FIG. 2.—Smaller specimen, completely filled with chromidia.

FIG. 3.—Specimen in which the chromidia are running together to form two new nuclei. Some indication of the formation of a cyst can be seen.

FIG. 4.—The chromidia have fused to form two new nuclei, which are approaching one another.

FIG. 5.—A cyst-wall has been formed, and the two solid chromatin nuclei are applied to one another.

FIG. 6.—Specimen showing a still later stage in the fusion of the nuclei. A thick, soft cyst has been formed.

FIG. 7.—Fresh preparation in which a cyst has been formed and the nucleus has broken up into chromidia.

FIG. 8.—Atrichous form in fresh condition, showing nucleus with peripherally placed chromatin masses (annular in optical section).

FIG. 9.—Large multinucleate atrichous form breaking up to form smaller ones. At least one nucleus is dividing.

FIG. 10.—An individual in the act of forming buds. Small blister-like nuclei are budded off, and the cytoplasm has become constricted round one of these, forming a complete bud.

FIG. 11.—Similar form, in which nucleus is seen in optical section. One nucleus has been completely separated off, and another is almost so.

FIG. 12.—Association of eleven typical atrichous forms. None of these have as yet formed chromidia, though one appears to be enucleate.

Some Facts in the Later Development of the Frog, *Rana temporaria*.

Part I.—The Segments of the Occipital Region of the Skull.

By

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With Plates 39 and 40.

THE question of the segmentation of the vertebrate head is one which for a long time has attracted the attention of many investigators. With regard to the segmentation of the anterior and middle parts of the head the facts are conflicting, and opinions differ; but, with regard to the post-otic portion, there is a converging mass of evidence, drawn from the neural, muscular, and skeletal tissues, that a varying number of segments serially homologous with the trunk segments have been drawn into the head. Besides this there is also evidence that in this region, in some groups at all events, a certain number of segments have altogether dropped out, and are not now represented by any rudiments, even in the embryo.

In the Cyclostoma the cranium terminates with the labyrinth region—there is no occipital portion of the skull.

The post-otic region of the Selachian head has been investigated by Neal, Fürbringer, Van Wijhe, Rabl, Beard, Sedgwick, C. K. Hoffman, Sewertzoff, Braus, Fropiep, and Dohrn. The chief results of their inquiries about this region may be summarised as follows.

There is present an occipital region of the cartilaginous skull, lying behind the labyrinth region. This is penetrated by a number of nerves, serially homologous with the spinal nerves, and known as the spino-occipital nerves. These are often three in number in the adult, but may vary from none

(Torpedo) to five (Hexanchus), and only retain their ventral roots; but in the embryo as many as seven may be indicated, and all provided with the rudiment of a dorsal ganglion (embryo Torpedo). More usually, however, there are only five or six (Spinax) of these nerves in the embryo, and only the last two or three show dorsal ganglia. But even in these cases we may still find seven post-otic somites (Spinax).

The skull itself is not usually segmented, but the myocomata are attached to the parachordal masses in segmental fashion. Rosenberg has shown in some sharks (Carcharias) distinct signs of extra vertebræ joined to the post-otic region of the skull, but leaves it uncertain whether these represent the spino-occipital segments of other sharks or whether they are additional trunk vertebræ which are being drawn into the skull.

In the Selachians we may therefore conclude that at least seven trunk segments have been fused with the head in the post-otic region, though in some forms (Scyllium and Pristiurus) traces of no more than five are still visible during development.

In other fishes there has been a further pushing up of trunk segments into the head. In Accipenser as many as six more segments have been fused, and in the Teleostei probably only three more.

In Amphibia the line of division between head and trunk is supposed to lie in the same place as in Selachians, but if so a number of the occipital segments have entirely disappeared, as at most three spino-occipital segments can be demonstrated in the embryo, and in the adult there are no spino-occipital nerves. In the Anura, indeed, the first spinal nerve and its ganglion also disappear.

In the Urodeles, Miss Platt has shown for Necturus that there are three post-otic segments in the head of the embryo, the first not giving rise to any muscles, the second partly uniting with the third, and the muscles from the second and third segments being hard to distinguish in the adult. She has also shown that there are two rudimentary dorsal arches

connected with these segments: a pre-occipital, which fuses with the otic capsule, and an occipital, which forms the occipital region of the skull.

Dr. Gadow suggested to me that, in view of the absence of the first spinal nerve in the Anura, I should cut a series of sections in the neck region of the developing frog to see what is the embryonic condition in this region—whether the first spinal nerve is present in the embryo, and what other traces there are of the disappearance of segments in this region.

Fig. 1 is a horizontal section of this region in a 9-millimetre tadpole.

The vagus is seen arising by a number of roots—five in this section with traces of a sixth—of which the first is larger than the rest and ganglionated, and there is a distinct gap between the first and the succeeding roots.

The latter have no ganglion-cells, and this fact, together with their more posterior position, suggests that they are not homologous with the first, and that they may be ventral roots of spinal nerves drawn into the head, and running forward to join the original cranial vagus.

In another section there are as many as seven roots to the vagus.

The third myotome is the first visible in this section, and in front of it lies a string of mesodermic tissue with elongated nuclei running from the anterior myotome to the otic sac.

If we follow the series of sections we can trace two myotomes in front of this one. In fig. 2 the second is shown, and in fig. 3 we have the complete series. The first is smaller than those posterior to it, and extends forward almost to the otic sac. There is, however, some mesoderm anterior to the first myotome, but it does not give rise to a muscle plate, though in a similar section of an animal of about the same age I saw signs of a muscle fibre in this region. We may, I think, fairly look on this mesoderm as representing at least one or more somites in front of that from which the first myotome is developed.

The second myotome has a trace of a rudimentary ganglion

(see fig. 4) associated with it, and the third myotome is accompanied by a well-developed ganglion.

In fig. 2 the distinction between the first ganglionated root of the vagus and the succeeding group of roots is well marked. The more posterior roots have formed a common trunk which is running forward to join the first root. There is a rudimentary ganglion to the second myotome, but it is not cut in either fig. 2 or fig. 3, and the ganglion of the third myotome is only just shaved in the section shown in fig. 3. Neither in this nor in any other section is there any trace of a ganglion in connection with the first myotome, nor with the mesoblast lying in front of the first myotome. If the segments are counted back to the cloaca (the only approximately fixed point, as the limbs have not begun to develop) they are found to be eleven in number.

Fig. 4 is a horizontal section through another tadpole of about the same age, showing all the anterior myotomes and associated ganglia. The first myotome is small, and has no trace of a ganglion, the second has on the left side a rudimentary ganglion associated with it; each of the other myotomes corresponds with a well-developed ganglion. The vagus roots on the right side are seen crossing outwards over the first myotome. In fig. 4*a* we have the rudimentary ganglion of myotome 2 enlarged. In fig. 4*b* we have a typical spinal ganglion from the same tadpole enlarged to show the contrast.

Fig. 5 is a horizontal section through a much older tadpole, about 13 millimetres long, in which the cartilaginous dorsal neural arches are developed. The first neural arch lies at *a*, on the myoseptum between the first and second myotomes now present. In front of this, however, are two small cartilages *b* and *c*, which look serially homologous with the neural arches, and lie just behind the ganglion of the vagus. In more ventral sections they may be seen to fuse with cartilage continuous with the otic capsule. These, I believe, represent two neural arches—are, in fact, homologous with the pre-occipital and occipital arches which Miss Platt found in *Necturus*. The first myotome now present has a distinct

though degenerating ganglion, and the myotome is small. This ganglion is lost in the adult, and belongs to the missing first spinal nerve. This is obvious from its position just in front of the first neural arch. The number of myotomes now present as far as the cloaca is only 9.

I therefore conclude that the first two myotomes and the rudimentary ganglion associated with the second, present in a 9-millimetre tadpole, have now disappeared—that these segments are represented by the two small cartilages *b* and *c*—and that the third myotome and its ganglion are much reduced in size, and are disappearing.

In fig. 6 we have a figure drawn combining two sagittal sections of a somewhat larger tadpole, about 20 millimetres long, in which the tissues of the hinder limb are beginning to show differentiation. The limb is drawn in from a section some distance farther on in the series, but the myotomes have been counted so as to correspond. The section shows the neural cord, cut a little on one side, the myotomes of that side, and the neural arches and ganglia almost all the way from the anterior end to the cloaca. The letter *a* points to the first myotome still remaining at this stage, and is, as in fig. 5, the third of the complete series. Anterior to it we see a blood-vessel *b*, and just to the right of this blood-vessel, at *f*, there are some cells which are evidently degenerated muscle-cells of the second myotome. Two small nodules of cartilage, *c* and *d*, are united to the parachordal bar by connective tissue, which already shows signs of chondrification. These are the same as the cartilages *b* and *c* in fig. 5. The first myotome present in this section—*a*—has a well-developed nerve and ganglion, and as this ganglion is in the space between the posterior end of the skull and the first neural arch, it is the first spinal nerve, which disappears in the adult.

Behind this we see ganglia, neural arches, and myotomes in orderly series, and counting myotome *a* as the first, we find that the ninth myotome gives off muscles to the cloaca.

The limb which is drawn from another section of the same animal is provided with three nerves, which we can see are

probably those of the eighth, ninth, and tenth myotomes—the connection of the middle one with the ninth myotome is quite clear. It is now possible to make a table showing the connection of the segments found in the tadpole with the nerves and vertebræ of the adult frog.

Skel.	No. seg.	Myotome.	Gang.	Nerve.	Distribution in adult.	Remarks.	
HEAD	Parietochordal cartilage	x	None	None	{ Post. rootlet of vagus }	Mesoblast in front of 1st myotome.	
	Pre-occip. cart.	1	Pres.	None	{ Post. rootlet of vagus }	Disappears by time limbs appear.	
	Occip. cart.	2	Pres.	Rudy.		Disappears by time cartilaginous arches appear. May be degenerating cells. (See Fig. 6.)	
Vert. 1		3	Pres.	Pres.		Nerve and ganglion disappear in adult—still present when cart. arches are formed. First persistent spinal nerve. Really Sp. II.	
Vert. 2		4	Pres.	Pres.	{ Hypoglossal II }		Muscles, tongue
Vert. 3		5	Pres.	Pres.	III		Brachial plexus
Vert. 4		6	Pres.	Pres.	IV		Brachial plexus
Vert. 5		7	Pres.	Pres.	V		Body wall
Vert. 6		8	Pres.	Pres.	VI		Body wall
Vert. 7		9	Pres.	Pres.	VII		Body wall
Vert. 8		10	Pres.	Pres.	VIII		Sciatic plexus
Vert. 9		11	Pres.	Pres.	IX		Sciatic plexus
Vert. 9		12	Pres.	Pres.	X		Sciatic plexus
Dorsal arch fuses urostyle		13	Pres.	Pres.	XI		Pelvis

Rana therefore entirely agrees with *Necturus* in the segmentation of the head. There are two post-otic segments giving rise to the first and second myotomes, and in front of this a mass of mesoblast, which may represent one or an indefinite number of disappearing segments. There is no ganglion connected with the first myotome, and an extremely rudimentary one with the second.

The neural arches between the first and second and second and third somites persist, and form the occipital region of the skull. They are homologous with the pre-occipital and occipital arches which Miss Platt found in *Necturus*.

In the 9-millimetre tadpole the third myotome is of full size, and so is the corresponding ganglion. But they have already begun to show signs of diminution in the tadpole, in which the dorsal cartilaginous arches are present (see fig. 5). The nerve of this segment is the missing first spinal nerve of the adult.

If the division between head and trunk is homologous in Selachians and Amphibia, as is generally assumed, a number of post-otic somites must have entirely vanished in the Amphibia, leaving no obvious trace at any stage of development. For in the Selachians Braus has demonstrated seven spino-occipital segments, and in the Amphibia we have traces at most of three, as shown for both *Urodela* and *Anura*.

The curious origin of the vagus may point to a possible reminiscence during development of these otherwise totally missing segments. The difference between the first and the succeeding roots, the presence of ganglion cells on the former and their absence on the latter, and the union of all the posterior roots into a common trunk, which passes forward to join the first root, are, I think, indications of a difference of origin of the posterior group. The position of the first myotome opposite to these roots, together with the absence of any trace of nerve connected with it, suggests at once that its nerve may have run forward to join this group; and it is easy to carry the suggestion further, and see in the more anterior roots of the posterior set a rudiment of the

nerves of segments now lost; segments whose myotomes are represented by the undifferentiated mesoblast in front of the first existing myotome.

To sum up the results obtained—

1. The first spinal nerve with its ganglion and associated myotome is well developed in the young tadpole, and still present, though somewhat reduced, in the tadpole which has developed dorsal cartilaginous arches, and whose limbs have begun to develop.

2. Two myotomes are present in the 9-millimetre tadpole in front of this segment. Cartilaginous arches appear in connection with these, and fuse with the parachordals, from which they are still distinct in a 20-millimetre tadpole. There is no ganglion in the segment of the first myotome, but there is a rudimentary one in the segment of the second myotome. Both these myotomes and the rudimentary ganglion disappear by the time that the cartilaginous dorsal arches are present, while the cartilaginous arches corresponding to them form the occipital region of the skull.

3. The vagus arises by numerous roots. The first root has a ganglion, and lies well forward and distinctly separated from the remaining roots. The latter may possibly represent ventral roots of the nerves of the missing post-otic segments, and also of the segment in which the first myotome is developed.

4. There is a string of true mesoblast lying between the otic sac and the first myotome. This may also be the remains of the tissue of segments anterior to that of the first myotome.

5. The segmentation of the post-otic region of the skull in *Rana* agrees completely with that in *Necturus*.

My sincere thanks are due to Dr. Gadow, both for his suggestion of the subject of this investigation, and for his kind interest and advice during its progress.

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EXPLANATION OF PLATES 39 AND 40,

Illustrating Miss Agnes I. M. Elliot’s paper on “Some Facts in the later Development of the Frog, *Rana temporaria*.”

ABBREVIATIONS.

Br. Brain. *C.c.sp.c.* Central canal of spinal cord. *C.E.C.* Cartilaginous ear capsule. *Cl.* Cloaca. *Cl.mus.* Cloacal muscles. *C.N.S.* Central nervous system. *Cl.* Connective tissue. *Deg.cs.* Degenerating cells. *E.* Eye. *Gan.V.* Ganglion of V cranial nerve. *Gan.VIII.* Ganglion of VIII cranial nerve. *Gan.X.* Ganglion of X cranial nerve. *Gr.m.sp.c.* Grey matter of spinal cord. *H.K.* Head kidney. *H.L.* Hind limb. *L.S.* Lymph space. *Lg.* Lung. *Mes.Ant.* Mesoblast cells stretching forward from anterior myotomes to otic capsule. *Mes^{me}.* Mesenchyme. *My.* 1—13. Myotomes numbered from anterior end. In figures 5 and 6 myotome 1 is the first myotome still remaining, and corresponds to myotome 3 in figures 1, 2, 3, and 4. *N.c.* Nerve cell. *Nch.* Notochord. *Pch.* Parachordal cartilage. *Pr.O.A.* Preoccipital arch. *R.sp.gan.* Rudimentary spinal ganglion. *Sp.cd.* Spinal cord. *Sp.gan.* 1—11. Spinal ganglia 1—11. *V.as.br.* Nerve trunk running forward, formed from posterior roots of vagus. *Vagus R. 1.* First root of vagus. *Vagus R. 6.* Sixth root of vagus. *V. 1—7.* Dorsal cartilaginous arches corresponding to vertebræ 1—7. *Wh.m.sp.c.* White matter of spinal cord.

PLATE 39.

FIG. 1.—Horizontal section of 9-millimetre tadpole, showing roots of vagus. Magnification 260.

FIG. 2.—Horizontal section of same tadpole, more ventral; showing second myotome and ascending roots of vagus. Magnification 100.

FIG. 3.—Horizontal section of same tadpole, still more ventral; showing first and succeeding myotomes and spinal ganglia corresponding to myotomes 3, 4, 5, 6, and 7. Magnification 83.

FIG. 4.—Horizontal section of 10-millimetre tadpole; showing myotomes and spinal ganglia, including the rudimentary spinal ganglion corresponding to the second myotome. Magnification 50.

FIG. 4a.—Rudimentary spinal ganglion corresponding to second myotome, enlarged from Fig. 4. Magnification 800.

FIG. 4b.—Spinal ganglion corresponding to fourth myotome, enlarged from Fig. 4. Magnification 800.

PLATE 40.

FIG. 5.—Horizontal section of 13-millimetre tadpole; showing cartilaginous arches, myotomes, and spinal ganglia at anterior end. (Combined from three sections.) For explanation of *a*, *b* and *c* see page 650. Magnification 100.

FIG. 6.—Sagittal section of 20-millimetre tadpole; showing cartilaginous arches, myotomes, and spinal ganglia of anterior region, and developing hind limb. (Combined from two sections.) For explanation of *a*, *b*, *c*, *d* and *f* see page 651. Magnification 36.

INDEX TO VOL. 51,

NEW SERIES.

- Ænigma ænigmatica*, by G. C. Bourne, 253
- Anatomy of *Notoryctes*, by G. Sweet, 325
- Anomiacea, structure of *Ænigma*, by G. C. Bourne, 253
- Antedon and *Synapta*, spicules of, by W. Woodland, 31
- Ascidians, spicules of, by W. Woodland, 45
- Auricularia*, wheel-spicules of, by W. Woodland, 483
- Babesia bovis* (see *Piroplasma bigeminum*, 297)
- Bourne, G. C., structure of *Ænigma ænigmatica*, 253
- Buds of *Cephalodiscus*, plumes of, by W. G. Ridewood, 221
- Carcinus*, fixation of *Sacculina* upon, by G. Smith, 625
- Cattle-fever, parasite of Texas, by H. B. Fantham, 297
- Cephalodiscus*, development of plumes in, by W. G. Ridewood, 221
- Cephalodiscus*, *Neurosporidium*, a sporozoön in, by W. G. Ridewood and H. B. Fantham, 81
- Chaetognatha*, or primitive Mollusca, by R. T. Günther, 357
- Chemical composition of *Radula*, by I. B. J. Sollas, 115
- Chromatin masses of *Piroplasma bigeminum*, by H. B. Fantham, 297
- Convoluta*, green cells of, by F. Keeble and F. W. Gamble, 167
- Cypris larva of *Sacculina*, fixation of, by G. Smith, 625
- Degeneration in *Opalina*, by C. C. Dobell, 633
- Dendy, A., parietal sense-organs of *Geotria*, 1
- Development of Frog, occipital region, by A. I. M. Elliot, 647
- Development of head-muscles in *Gallus*, by F. H. Edgeworth, 511
- Development of *Ophiothrix fragilis*, by E. W. MacBride, 557
- Development of plumes in *Cephalodiscus*, by W. G. Ridewood, 221
- Development of *Radula*, by I. B. J. Sollas, 115
- Development of teeth in *Ornithorhynchus*, by J. T. Wilson and J. P. Hill, 137

- Diplopoda, segmentation of head of, by M. Robinson, 607
- Dobell, C. C., physiological degeneration in *Opalina*, 633
- Dobell, C. C., *Trichomastix serpentis*, 449
- Doncaster, L., gametogenesis in *Nematus ribesii*, 101
- Edgeworth, F. H., head-muscles of *Gallus* and other *Sauropsida*, 511
- Elliot, A. I. M., later development of *Frog*, 647
- Factors in production of various forms of spicules, by W. Woodland, 55
- Fantham, H. B., chromatin masses of *Piroplasma bigeminum*, 297
- Fantham, H. B., and W. G. Ridewood, on *Neurosporidium cephalodisci*, 81
- Fertilisation in *Nematus ribesii*, by L. Doncaster, 101
- Fixation of *Cypris* larva of *Sacculina*, by G. Smith, 625
- Fleure, H. J., and M. M. Gettings, notes on *Trochus*, 459
- Formation of skeleton in *Madreporaria*, by M. M. O. Gordon, 473
- Forms of spicules, factors concerned, by W. Woodland, 55
- Frog*, later development of, by A. I. M. Elliot, 647
- Gallus*, development of head-muscles, by F. H. Edgeworth, 511
- Gamble, F. W., and F. Keeble, green cells of *Convoluta*, 167
- Gametogenesis in *Nematus ribesii*, by L. Doncaster, 101
- Geotria*, parietal sense-organs of, by A. Dendy, 1
- Gettings, M. M., and H. J. Fleure, notes on *Trochus*, 459
- Gordon, M. M. O., formation of skeleton in *Madreporaria*, 473
- Green cells of *Convoluta*, by F. Keeble and F. W. Gamble, 167
- Günther, R. T., the *Chaetognatha*, 357
- Hair, skin, and reproductive organs of *Notoryctes*, by G. Sweet, 325
- Head-muscles in *Gallus* and other *Sauropsida*, by F. H. Edgeworth, 511
- Head of Diplopoda, segmentation of, by M. Robinson, 607
- Hewitt, C. G., structure of House-fly, 395
- Hill, J. P., and J. T. Wilson, tooth-development in *Ornithorhynchus*, 137
- House-fly, structure of, by C. G. Hewitt, 395
- Keeble, F., and F. W. Gamble, green cells of *Convoluta*, 167
- Lamprey, New Zealand, (*Geotria*), parietal sense-organs of, by A. Dendy, 1
- Later development of *Frog*, by A. I. M. Elliot, 647
- MacBride, E. W., development of *Ophiothrix*, 557
- Madreporaria*, formation of skeleton in, by M. M. O. Gordon, 473
- Mollusca, *Chaetognatha* or primitive, by R. T. Günther, 357
- Mollusca, spicules of, by W. Woodland, 45
- Molluscan *Radula*, by I. B. J. Sollas, 115
- Musca domestica*, structure of, by C. G. Hewitt, 395
- Nematus ribesii*, gametogenesis in, by L. Doncaster, 101

- Neurosporidium cephalodisci*, by W. G. Ridewood and H. B. Fantham, 81
 New Zealand Lamprey, parietal sense-organs of, by A. Dendy, 1
 Nicoll, W., *Parorchis acanthus*, a new Trematode, 345
 Notes on skeleton of *Madreporaria*, by M. M. O. Gordon, 473
 Notes on *Trochus*, by H. J. Fleure and M. M. Gettings, 459
 Notoryctes, skin, hair, and reproductive organs of, by G. Sweet, 325
 Observations on tooth-development in *Ornithorhynchus*, by J. T. Wilson and J. P. Hill, 137
 Occipital region of Frog, development, by A. I. M. Elliot, 647
 Ogilvie Gordon, M. M., formation of skeleton in *Madreporaria*, 473
 Opalina, degeneration in, by C. C. Dobell, 633
Ophiothrix fragilis, development of, by E. W. MacBride, 557
 Ophiuroidea and Echinoidea, spicules of, by W. Woodland, 31
 Origin and nature of green cells in *Convoluta*, by F. Keeble and F. W. Gamble, 167
Ornithorhynchus, tooth-development in, by J. T. Wilson and J. P. Hill, 137
 Parasite of Texas cattle-fever, by H. B. Fantham, 297
 Parietal sense-organs of *Geotria*, by A. Dendy, 1
Parorchis acanthus, a new Trematode, by W. Nicoll, 345
 Physiological degeneration in *Opalina*, by C. C. Dobell, 633
Piroplasma bigeminum, chromatin masses of, by H. B. Fantham, 297
 Plate-and-anchor spicules of *Synapta*, by W. Woodland, 483
 Plumes of buds of *Cephalodiscus*, by W. G. Ridewood, 221
 Preliminary consideration, forms of spicules, by W. Woodland, 55
 Primitive Mollusca, the *Chaetognatha* or, by R. T. Günther, 357
 Production of various forms of spicules, by W. Woodland, 55

Radula, composition and development, by I. B. J. Sollas, 115
 Reproductive organs, skin, and hair of *Notoryctes*, by G. Sweet, 325
 Ridewood, W. G., development of plumes in *Cephalodiscus*, 221
 Ridewood, W. G., and H. B. Fantham, on *Neurosporidium cephalodisci*, 81
 Robinson, M., segmentation of head of *Diplopoda*, 607

Sacculina, fixation of *Cypris* larva of, by G. Smith, 625
Sauropsida, head-muscles in, by F. H. Edgeworth, 511
 Scleroblastic development of spicules in Mollusca and *Ascidians*, by W. Woodland, 45
 Scleroblastic development of spicules in *Ophiuroidea*, etc., by W. Woodland, 31
 Segmentation of head of *Diplopoda*, by M. Robinson, 607
 Segments of occipital region of Frog, by A. I. M. Elliot, 647
 Sense-organs, parietal, in *Geotria*, by A. Dendy, 1
 Skeleton of *Madreporaria*, by M. M. O. Gordon, 473
 Skin, hair, and reproductive organs of *Notoryctes*, by G. Sweet, 325

- Smith, G., fixation of Cypris larva of Sacculina, 625
- Sollas, I. B. J., the Molluscan Radula, 115
- Spicule formation, studies in, Part V, by W. Woodland, 31; Part VI, 45; Part VII, 483
- Spicules, various forms of, by W. Woodland, 55
- Sporozoön from Cephalodiscus, by W. G. Ridewood and H. B. Fantham, 81
- Structure of *Ænigma ænigmatica*, by G. C. Bourne, 253
- Structure of House-fly, by C. G. Hewitt, 395
- Studies in spicule formation, Part V, by W. Woodland, 31; Part VI, 45; Part VII, 483
- Sweet, G., skin, hair, and reproductive organs of *Notoryctes*, 325
- Synapta, plate-and-anchor spicules, by W. Woodland, 483
- Texas cattle-fever, parasite of, by H. B. Fantham, 297
- Tooth-development in *Ornithorhynchus*, by J. T. Wilson and J. P. Hill, 137
- Trematodes, new genus, *Parorchis*, by W. Nicoll, 345
- Trichomastix serpentis*, by C. C. Dobell, 449
- Trochus, notes on, by H. J. Fleure and M. M. Gettings, 459
- Various forms of spicules, factors concerned, by W. Woodland, 55
- Wheel-spicules of *Auricularia*, by W. Woodland, 483
- Wilson, J. T., and J. P. Hill, tooth-development in *Ornithorhynchus*, 137
- Woodland, W., forms of spicules, factors concerned, 55
- Woodland, W., studies in Spicule Formation, Part V, 31; Part VI, 45; Part VII, 483

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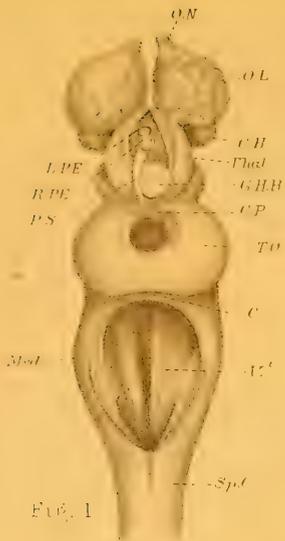


Fig. 1.



Fig. 3.

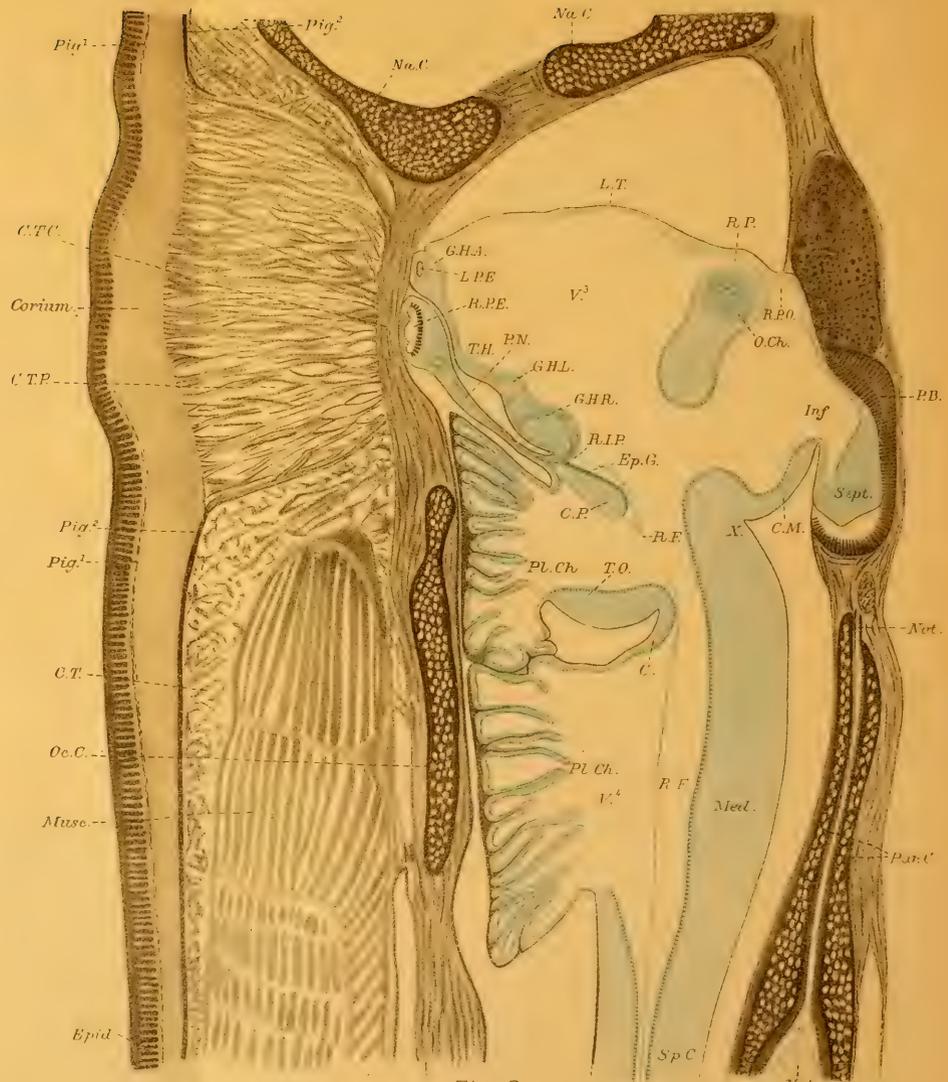


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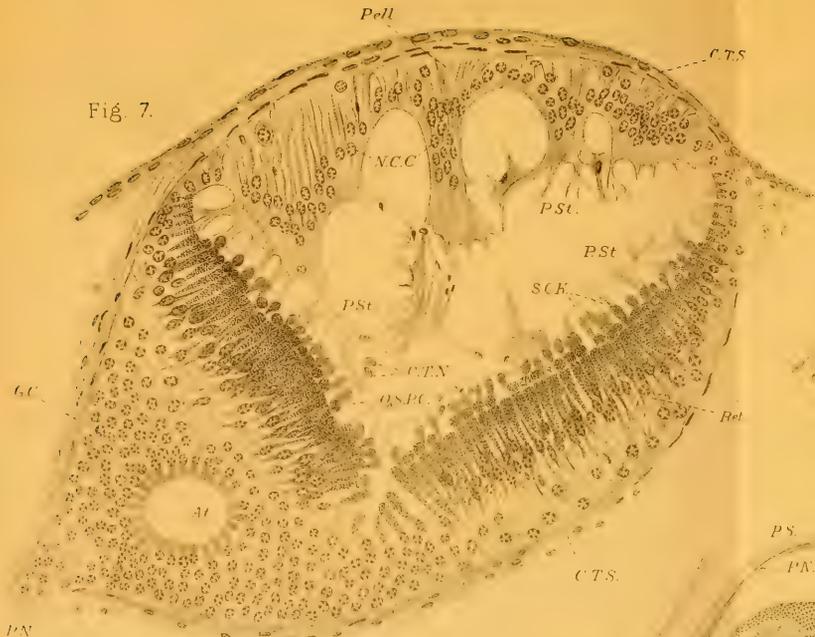


Fig. 7.

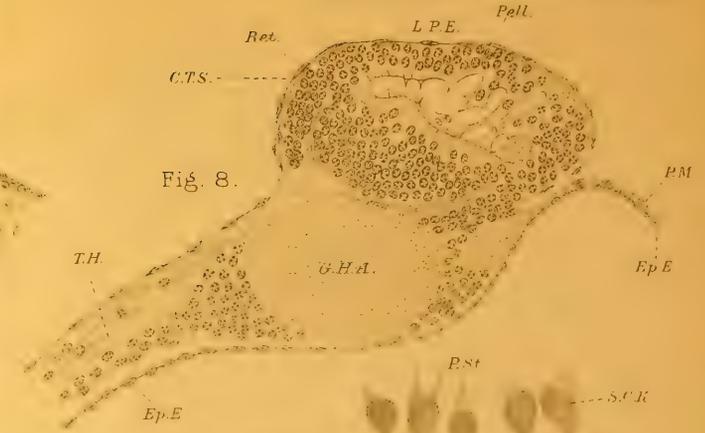


Fig. 8.

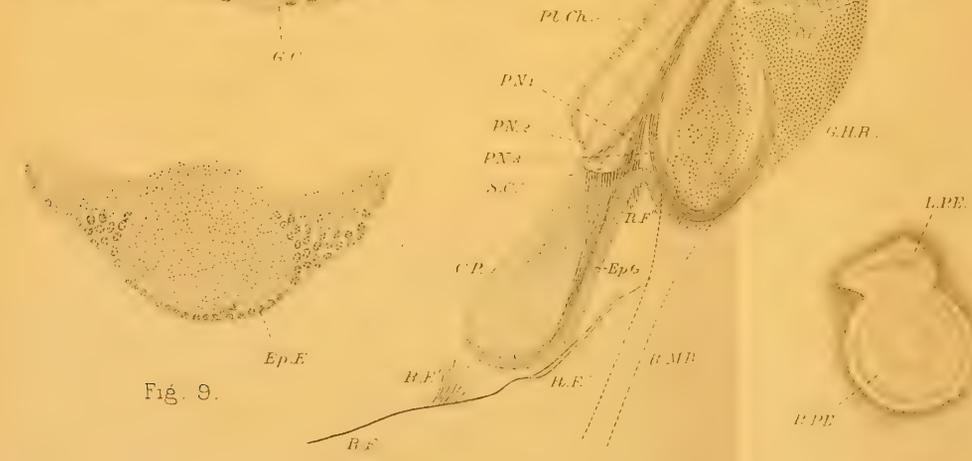


Fig. 9.

Fig. 6.



Fig. 4.

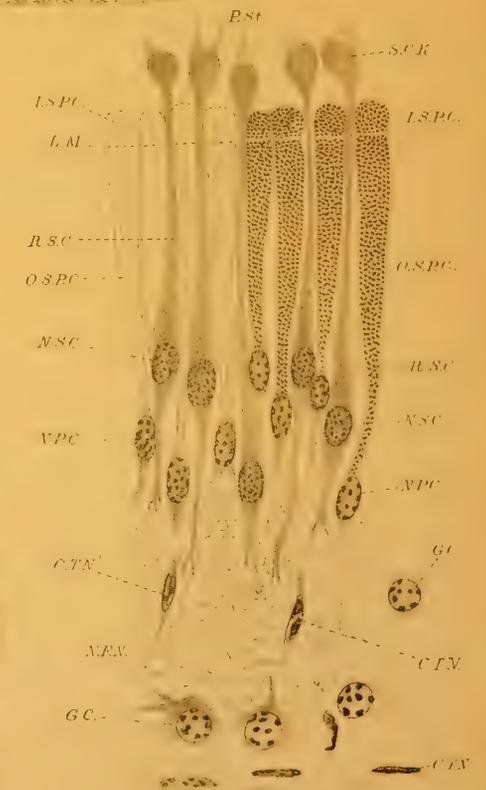


Fig. 5.

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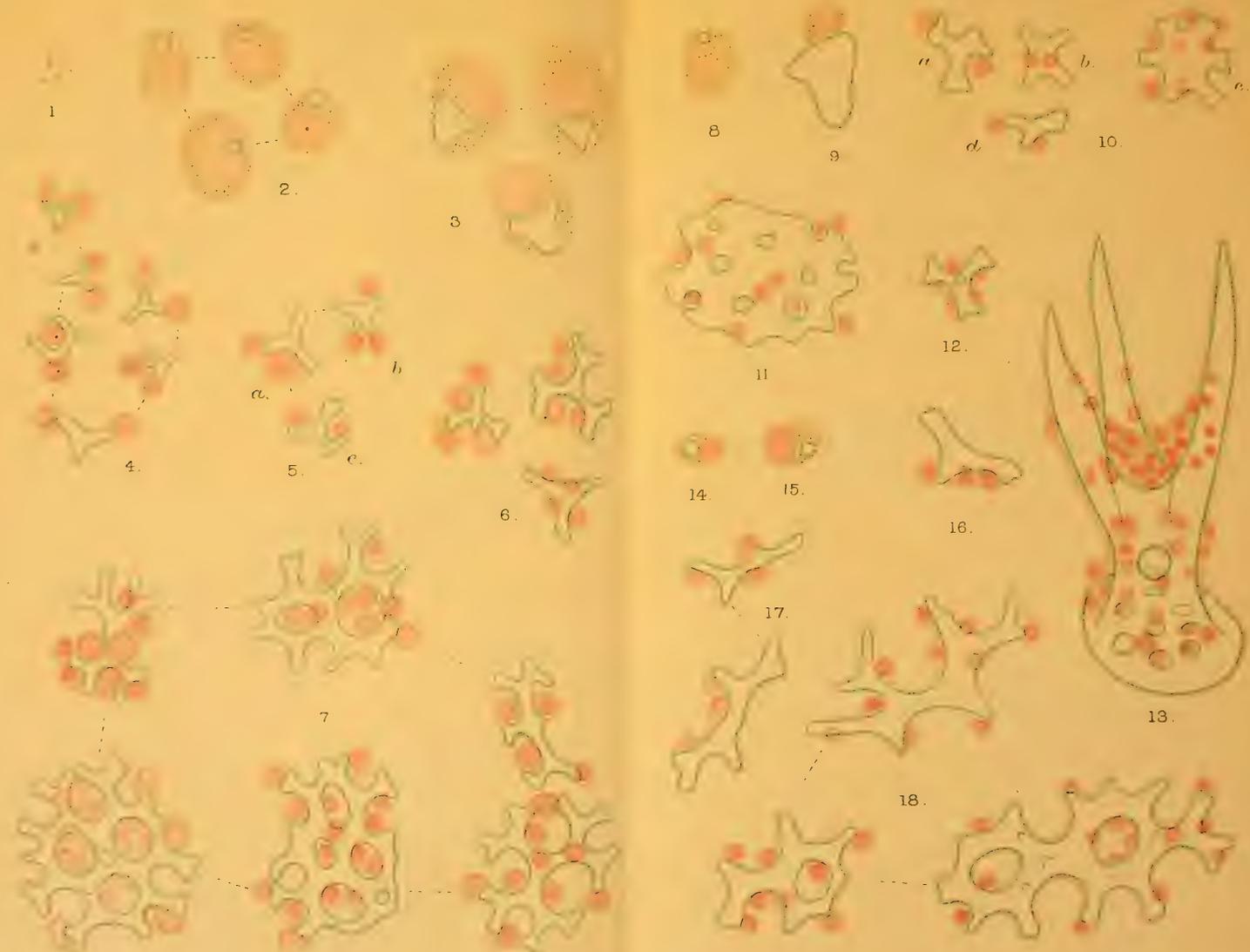
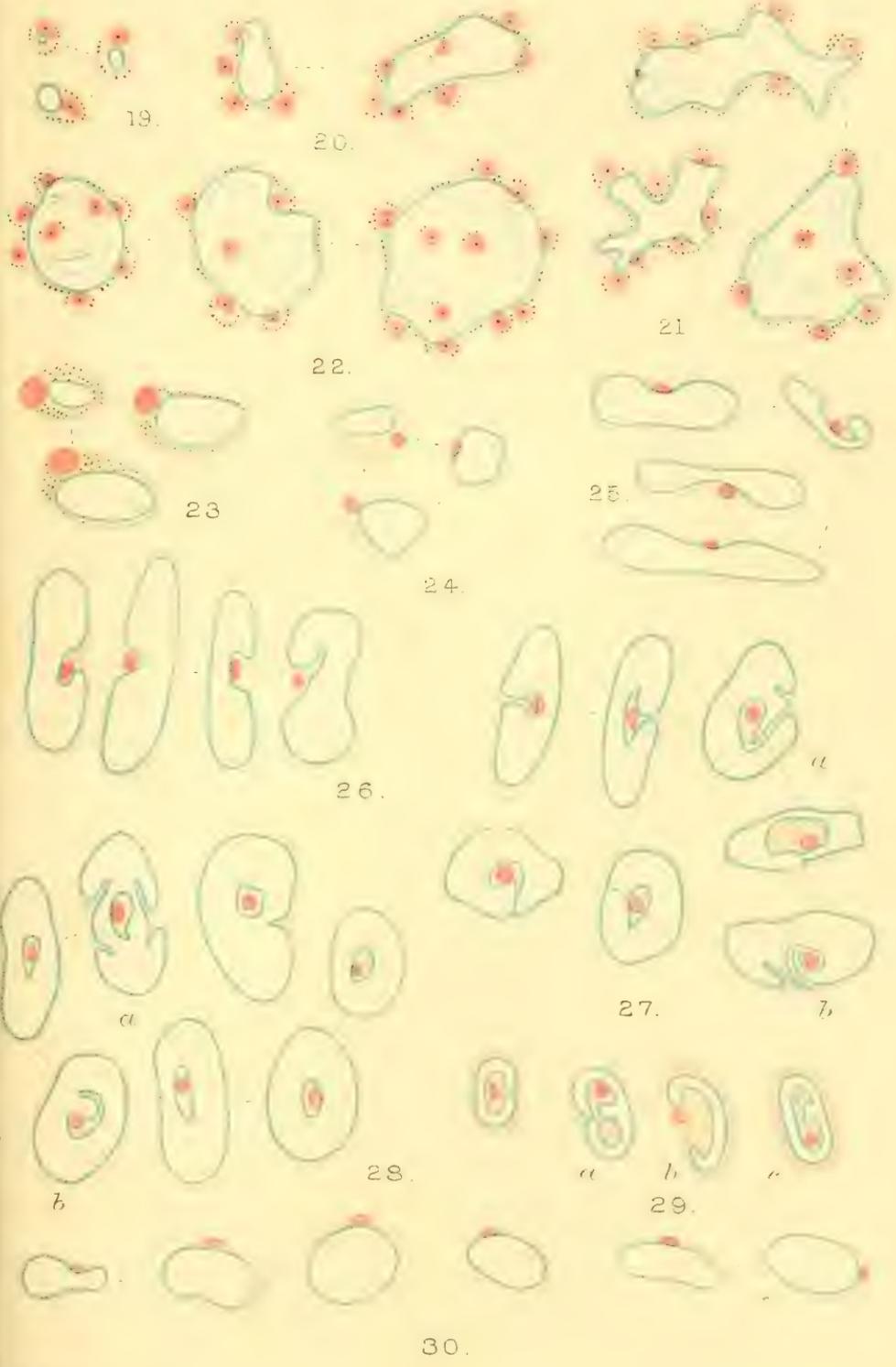
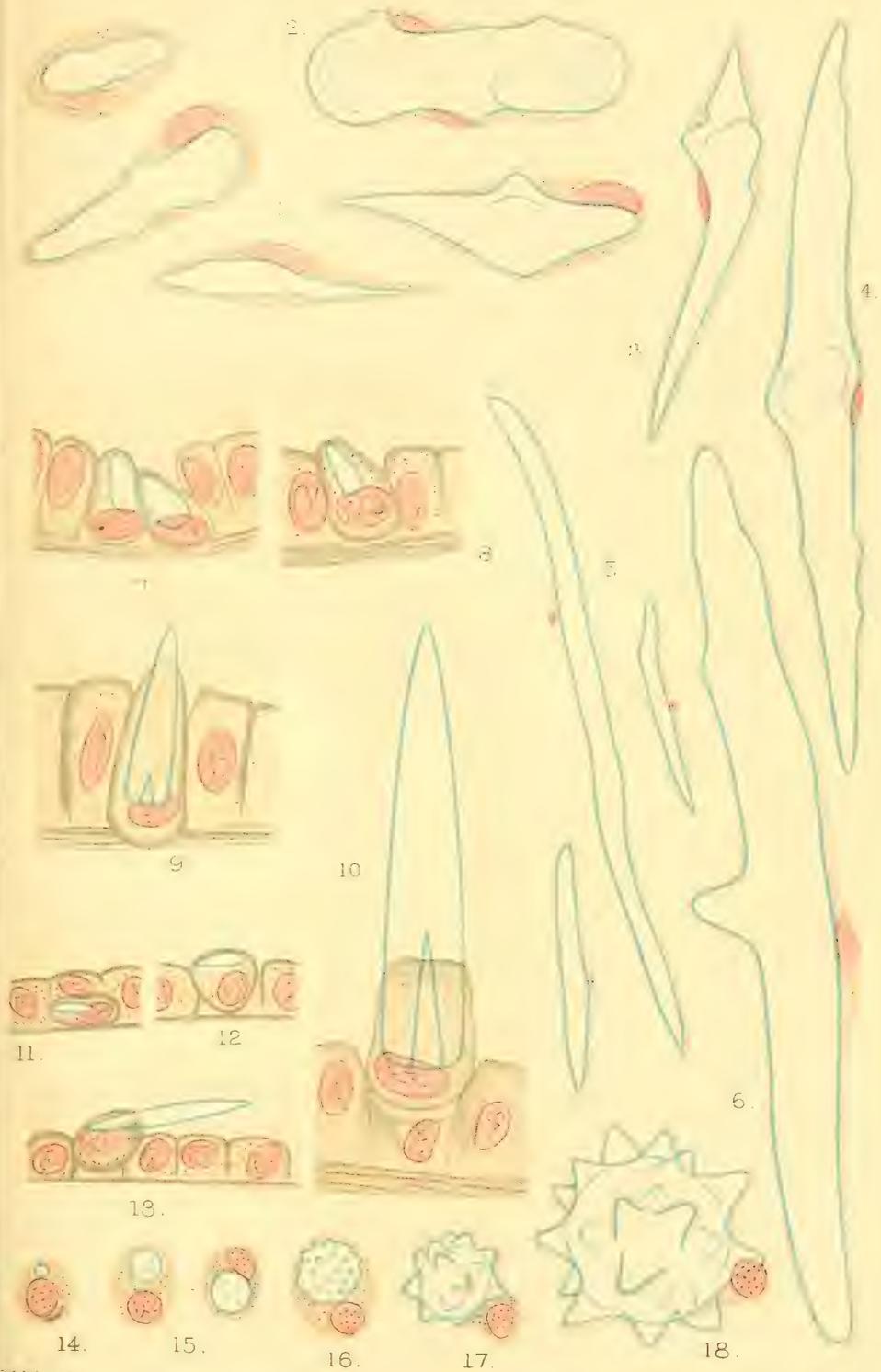


Fig. 18.

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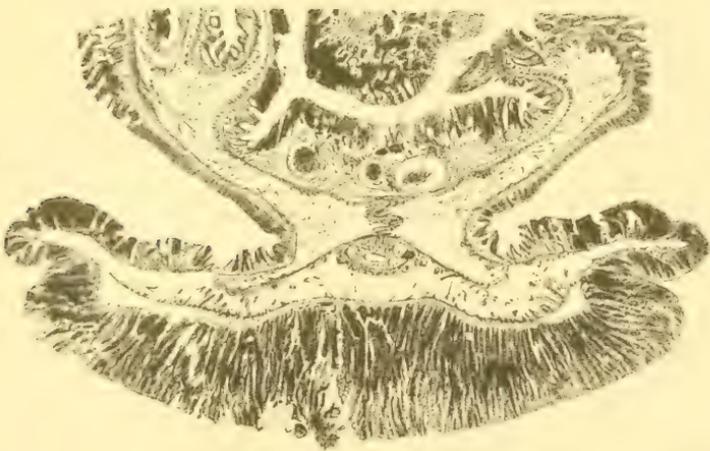
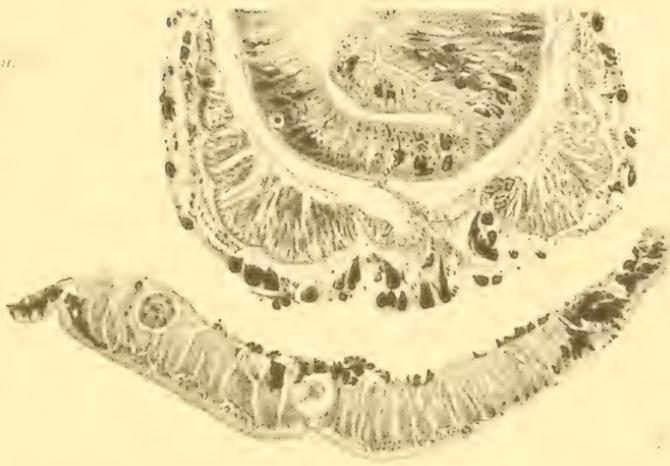


FIG. 1

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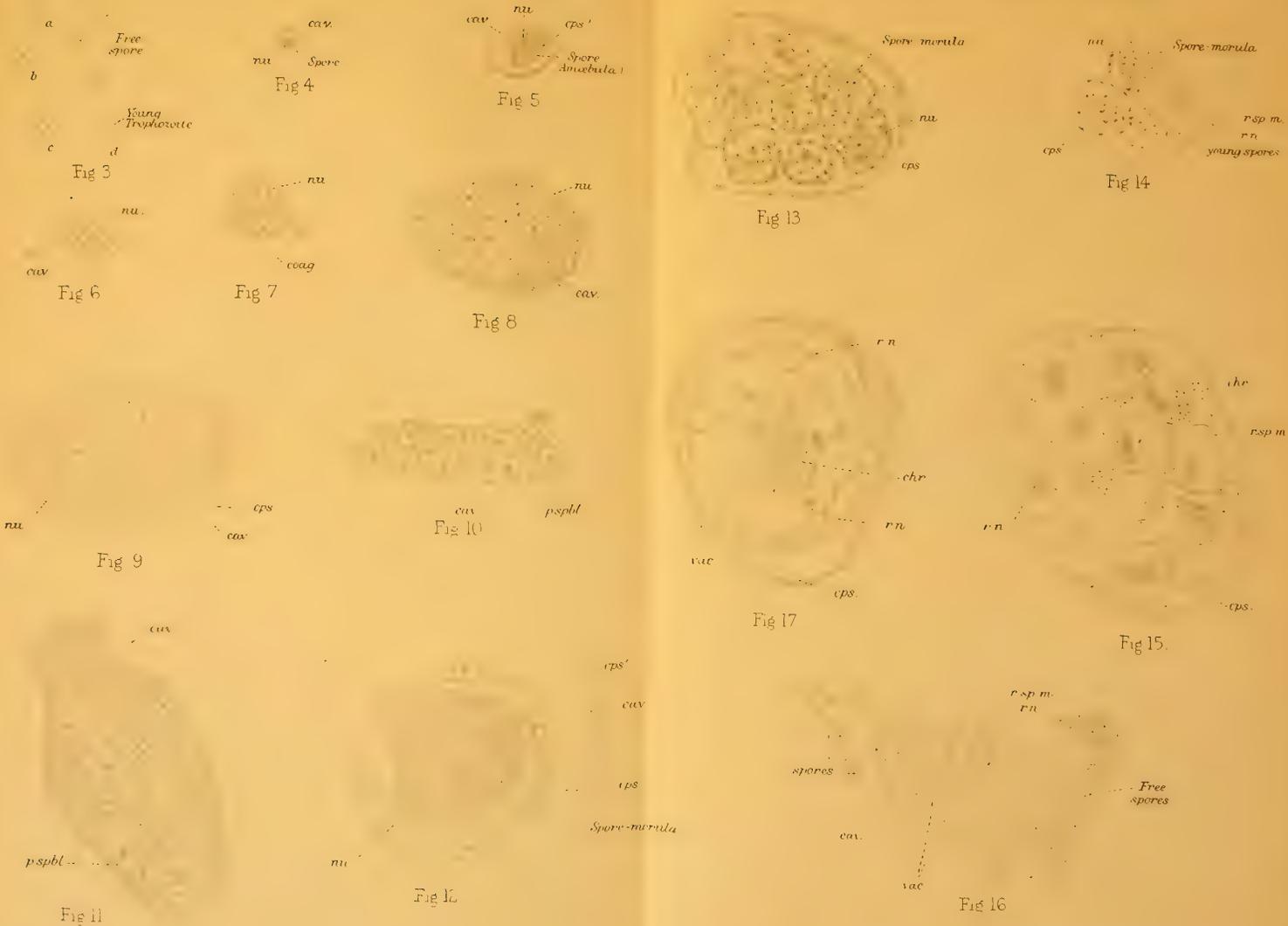
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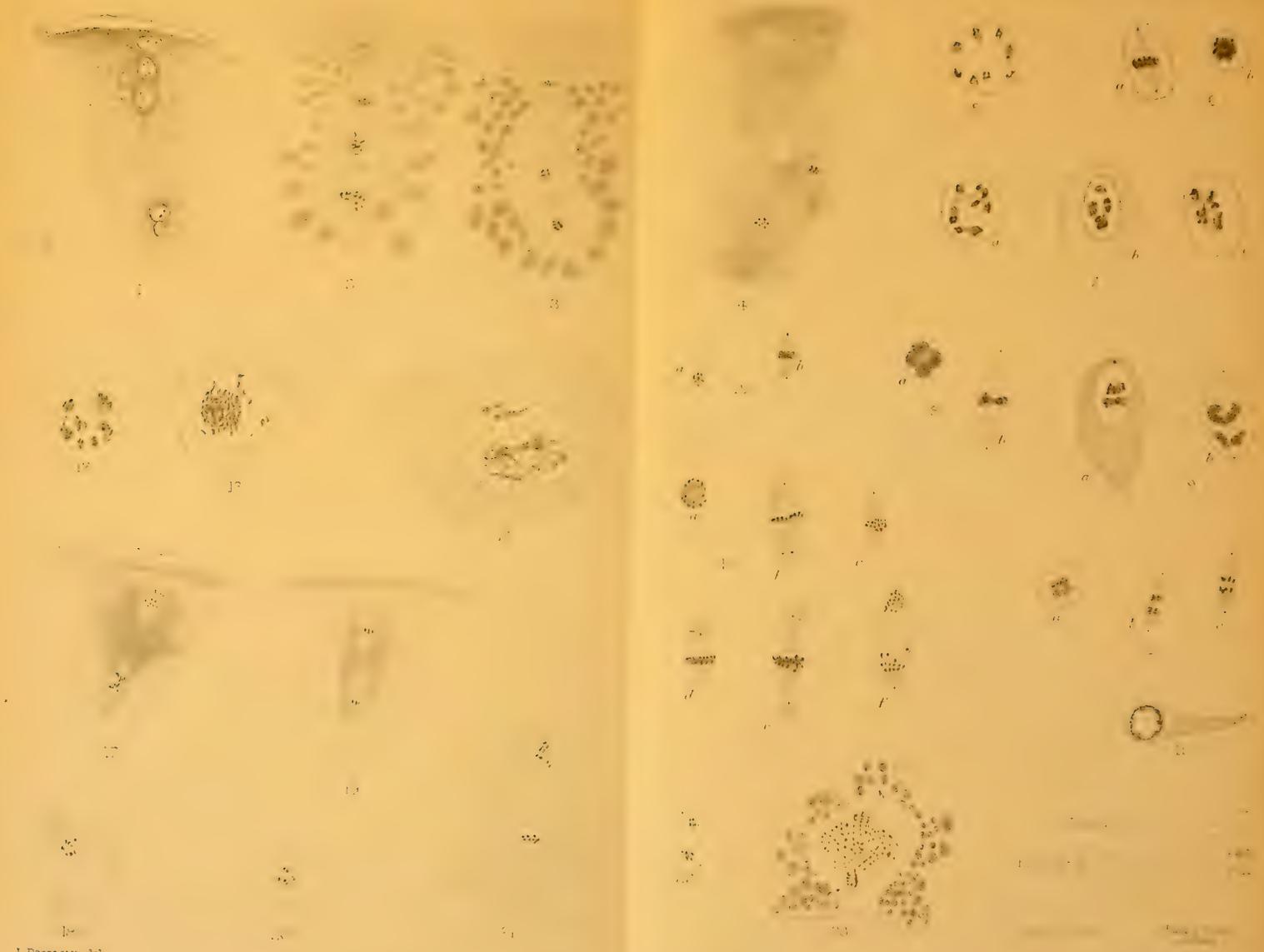




H.B. Fantham del.

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 in the nerve tracts of *Cephalodiscus nigrescens*



L. Doncaster, del.

GAMETOGENESIS AND FERTILISATION IN NEMATUS RIBESII



1.



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7.



e



d



c



11.



c



b



b



a

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13.



a

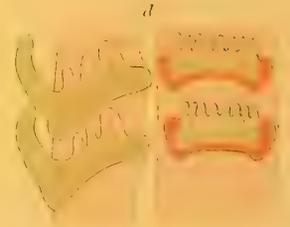
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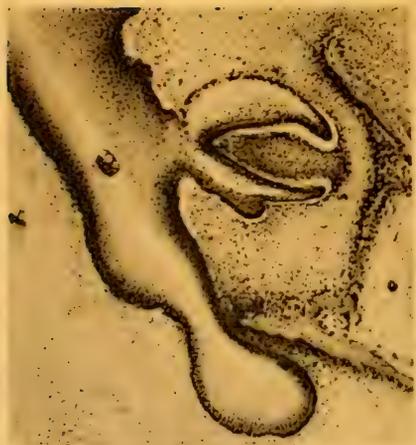
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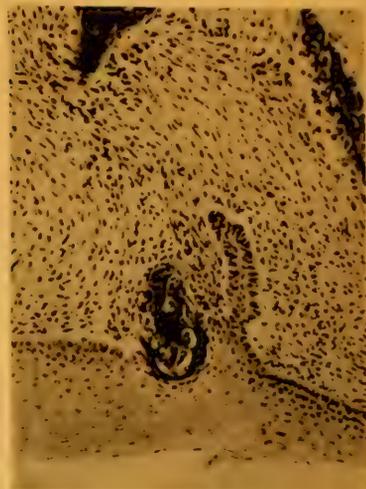
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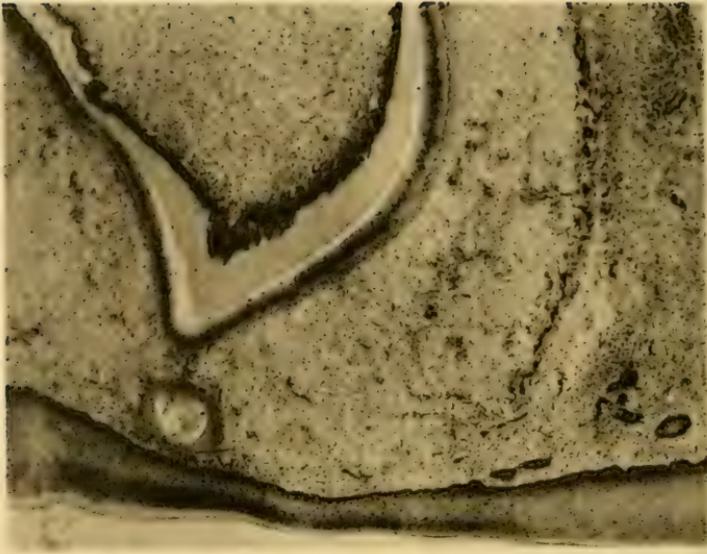
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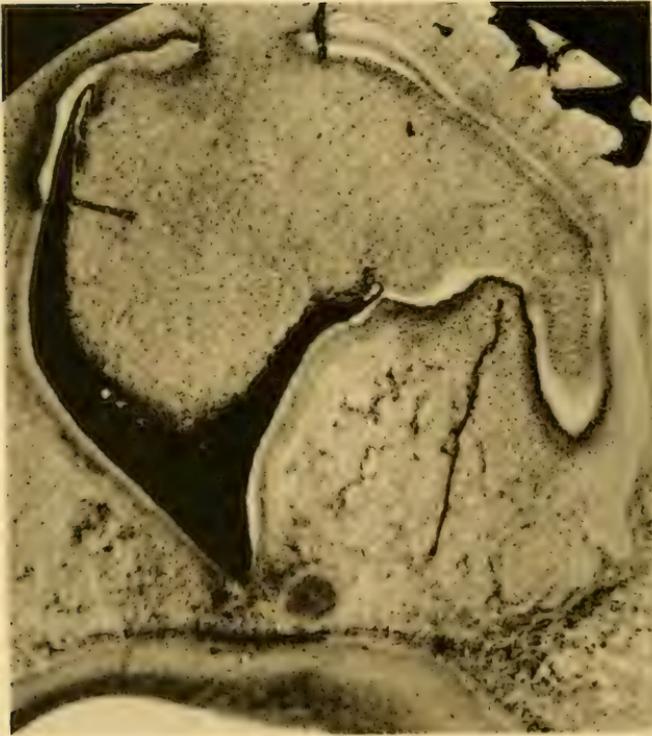
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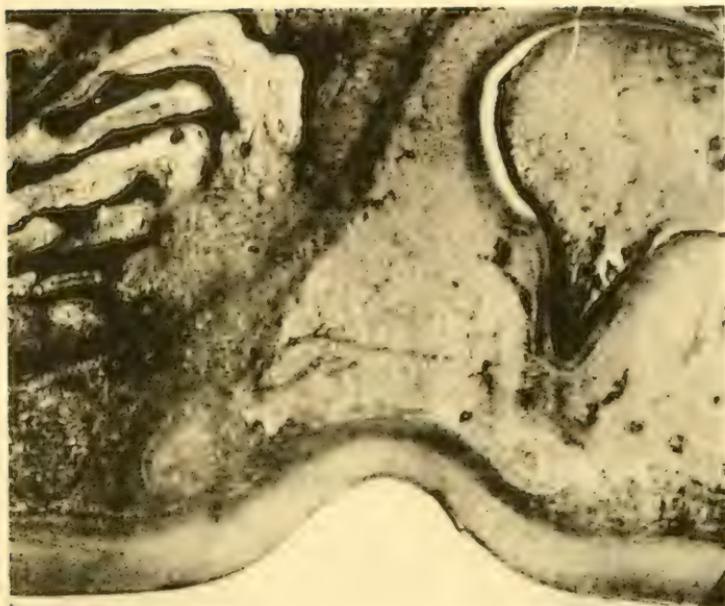
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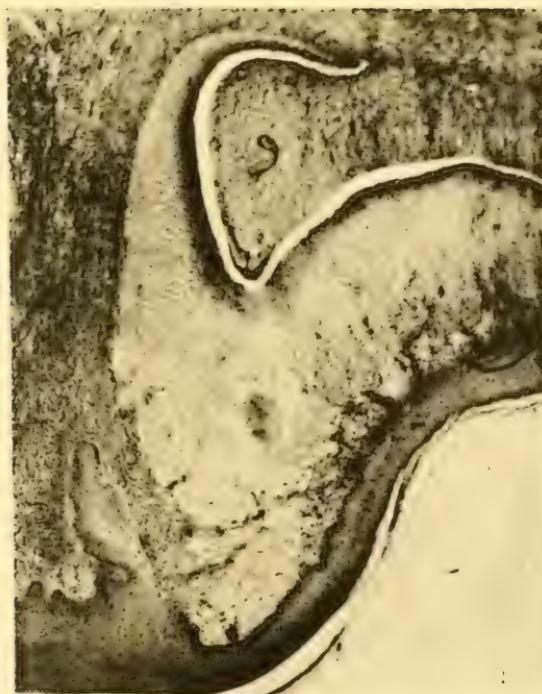
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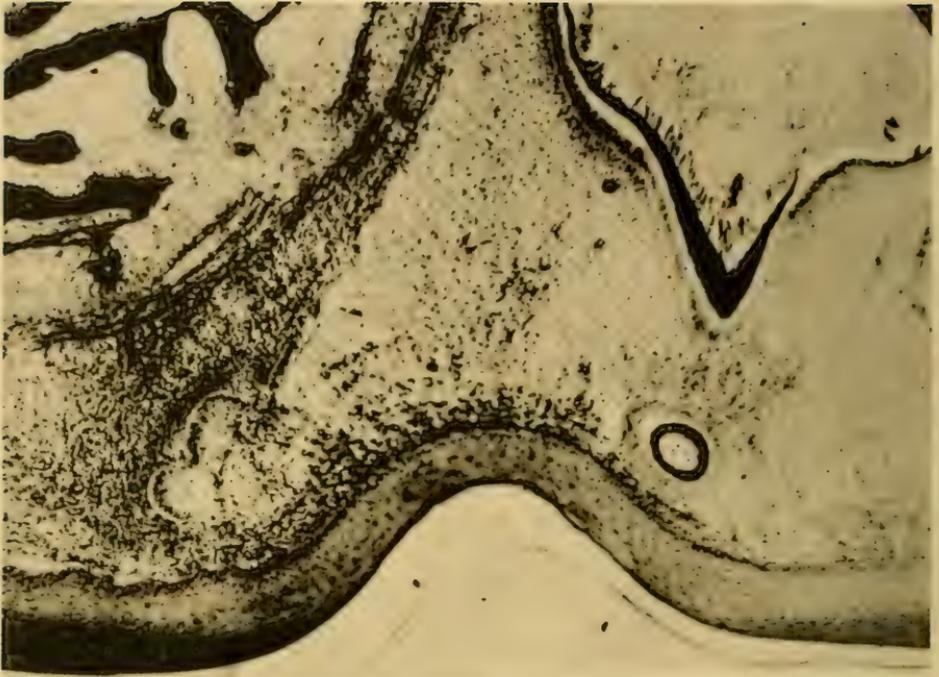


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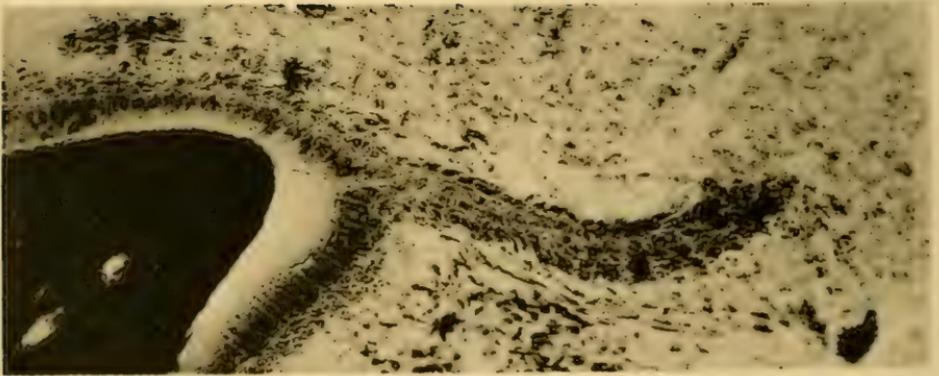


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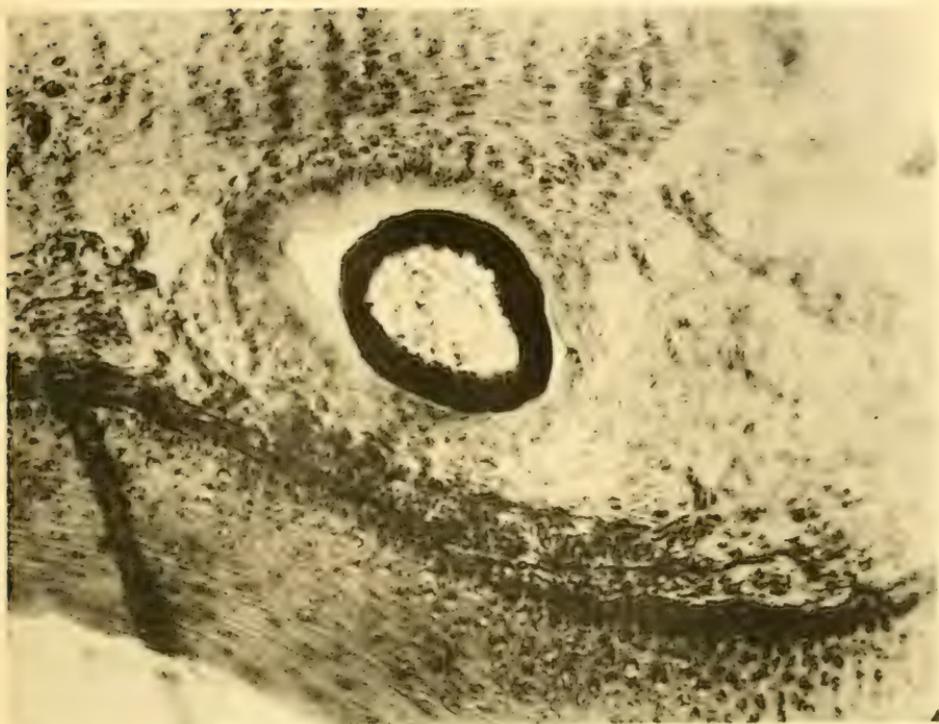
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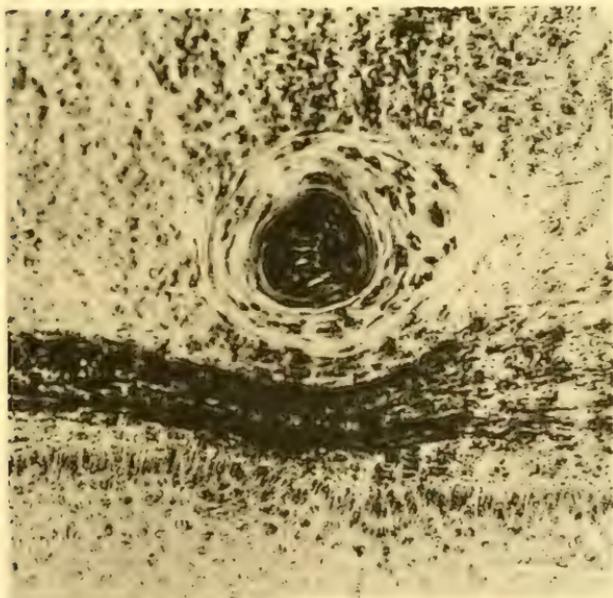
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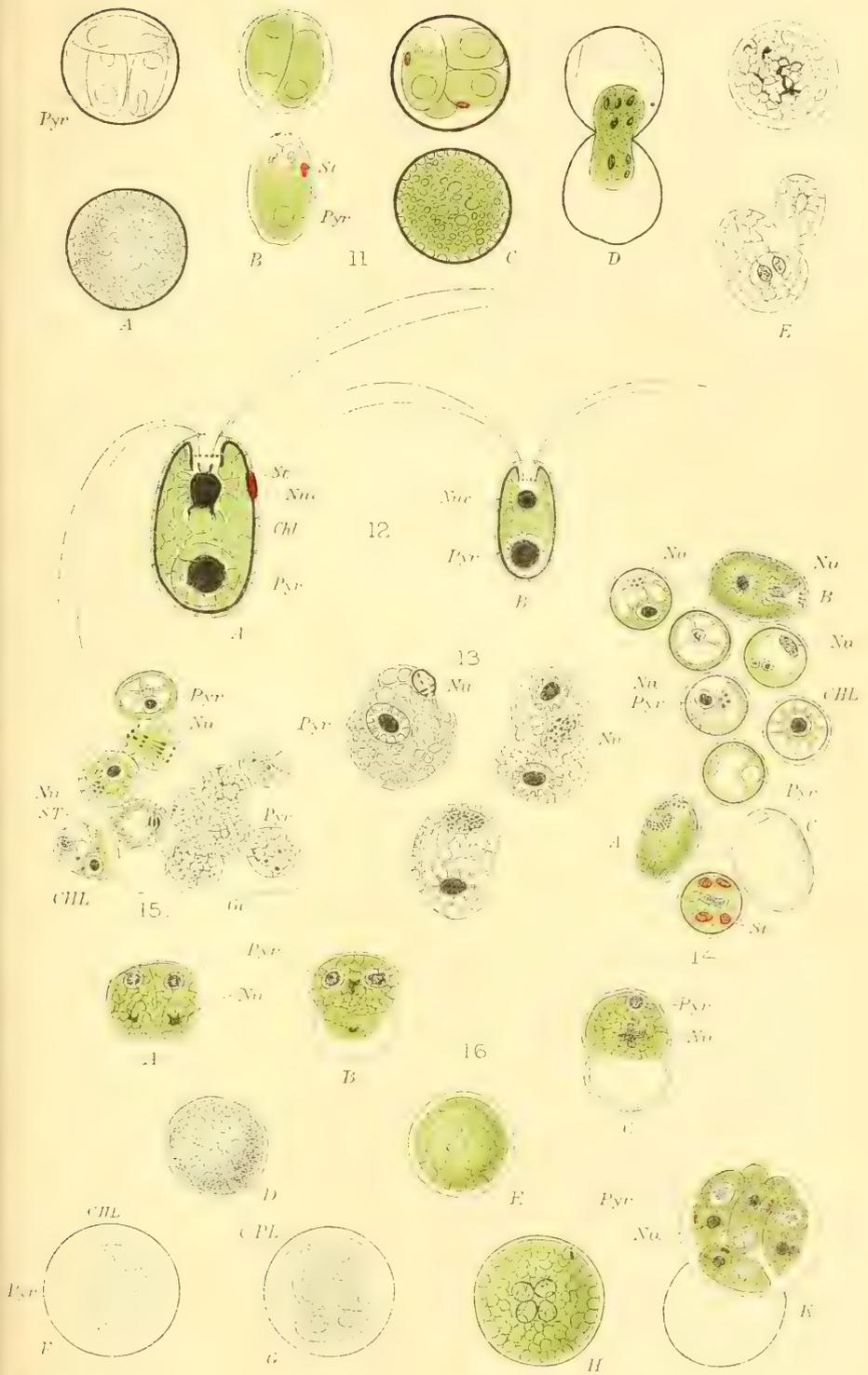
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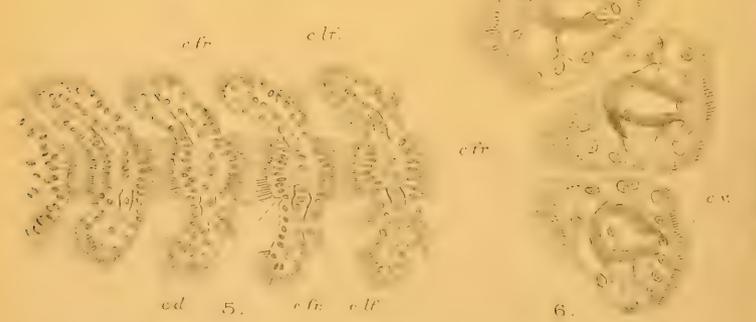
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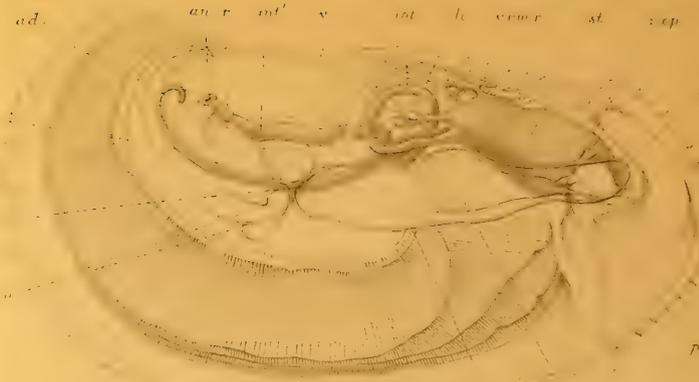


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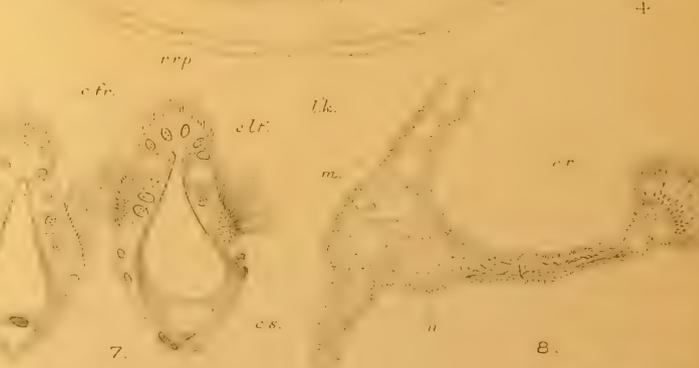
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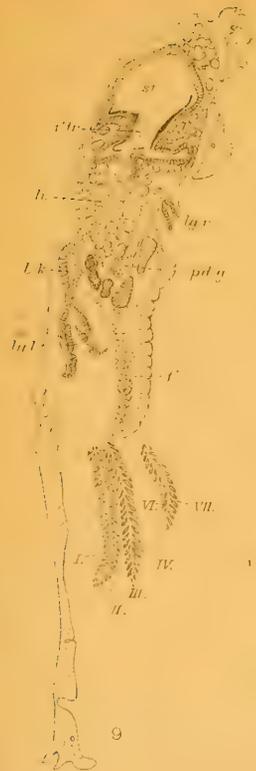
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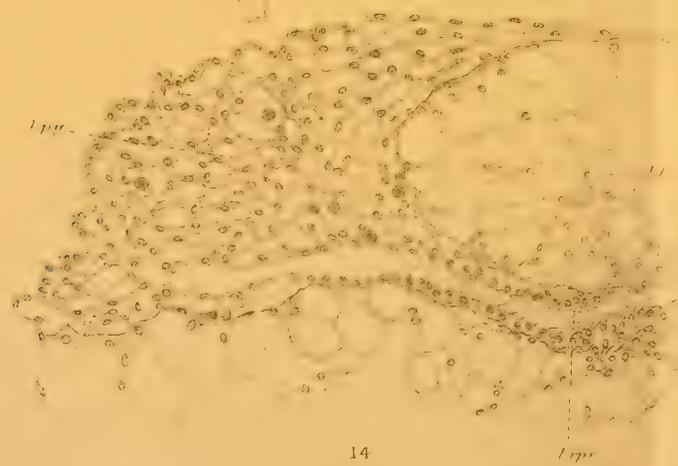
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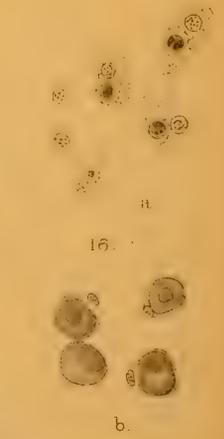
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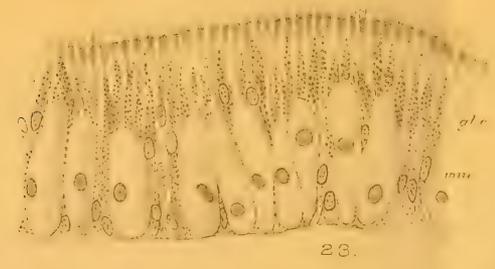
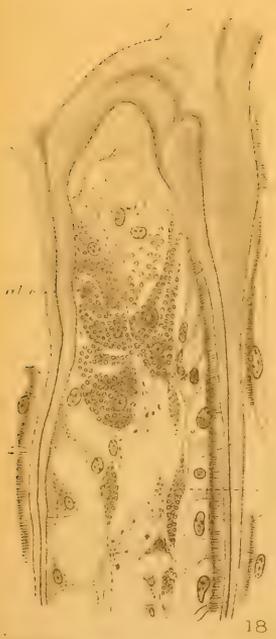
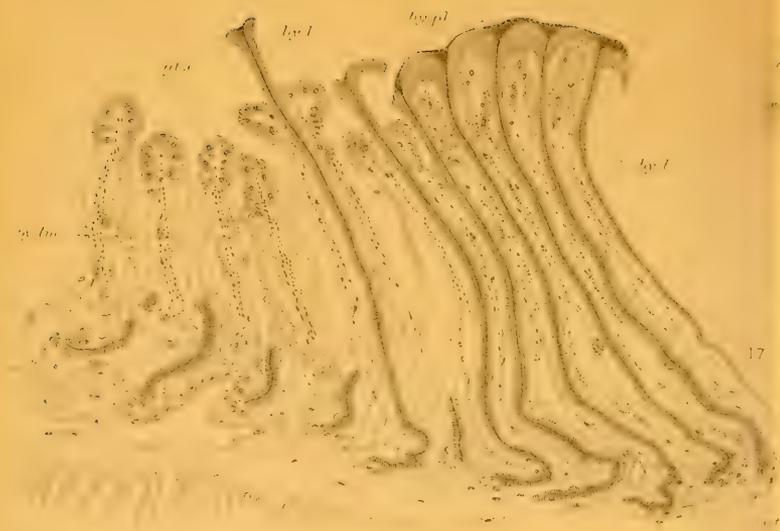




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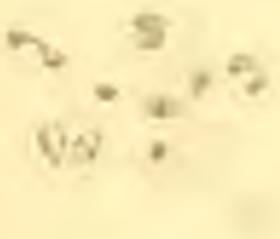


Fig. 3.



Fig. 5.

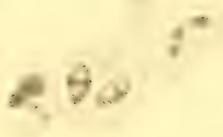


Fig. 4.

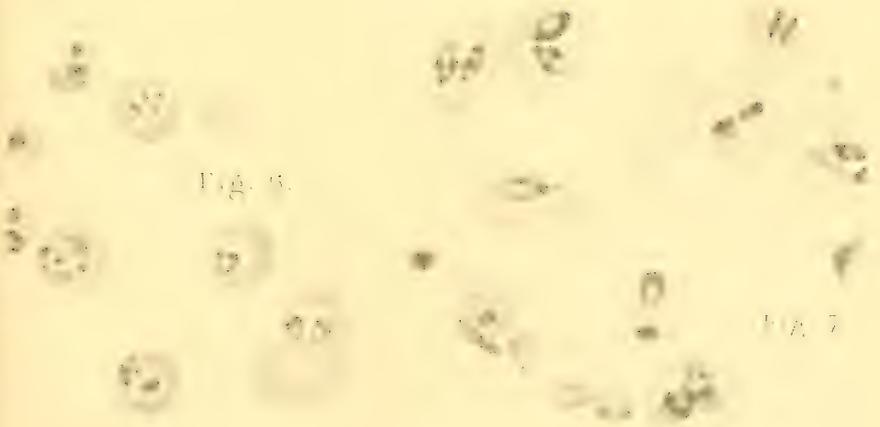


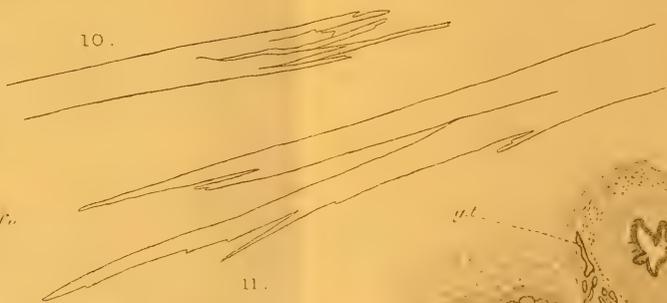
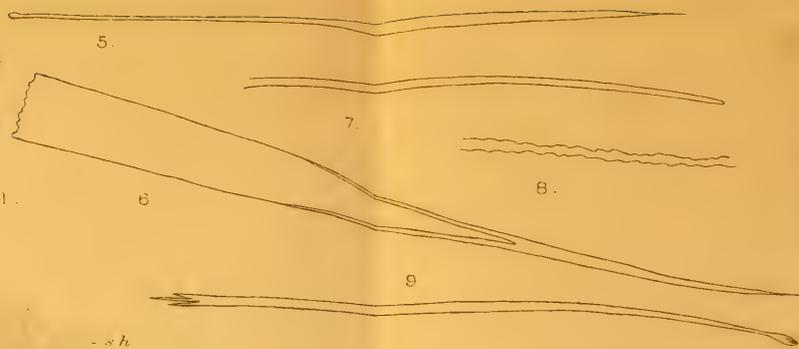
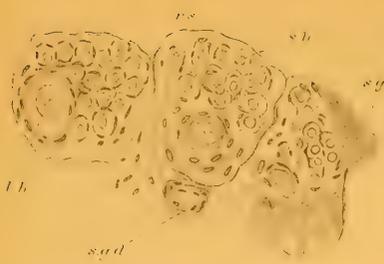
Fig. 6.

Fig. 7.

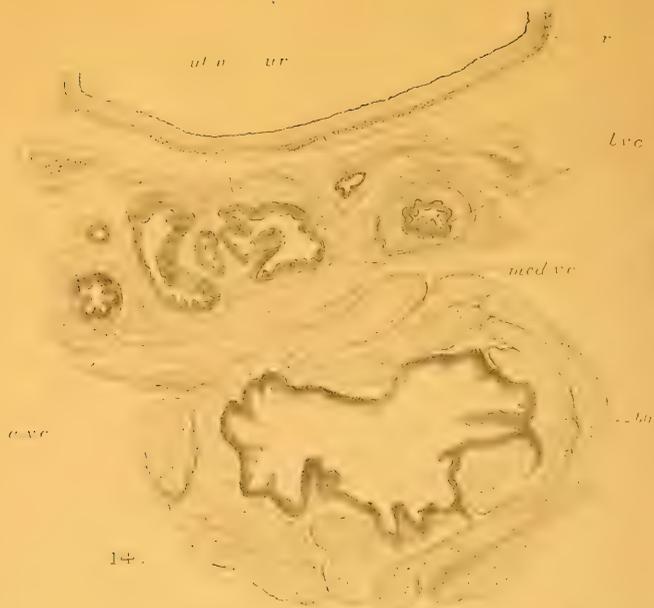
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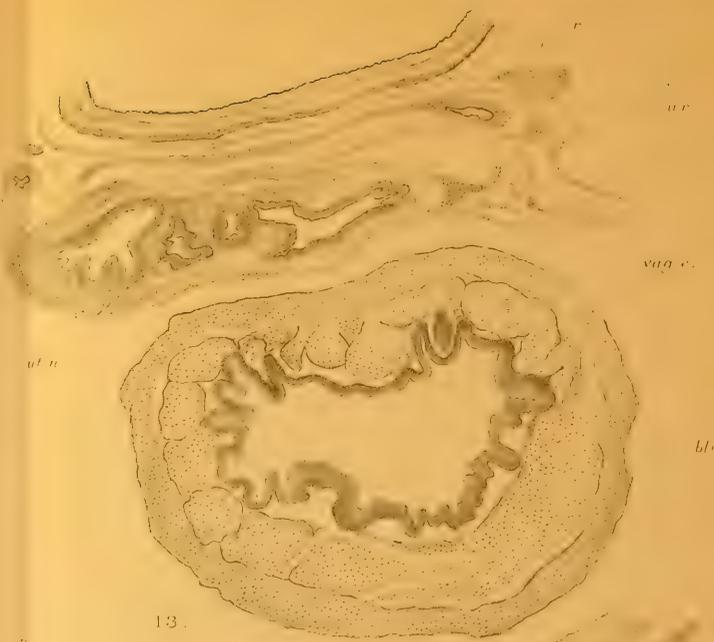
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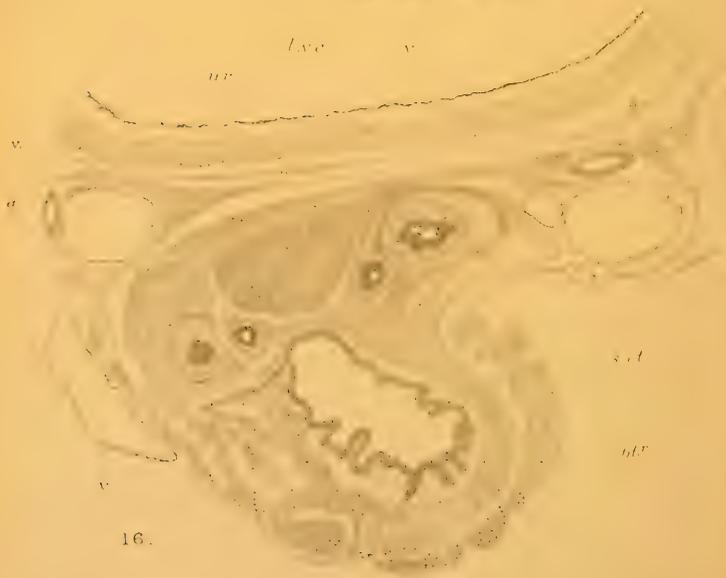
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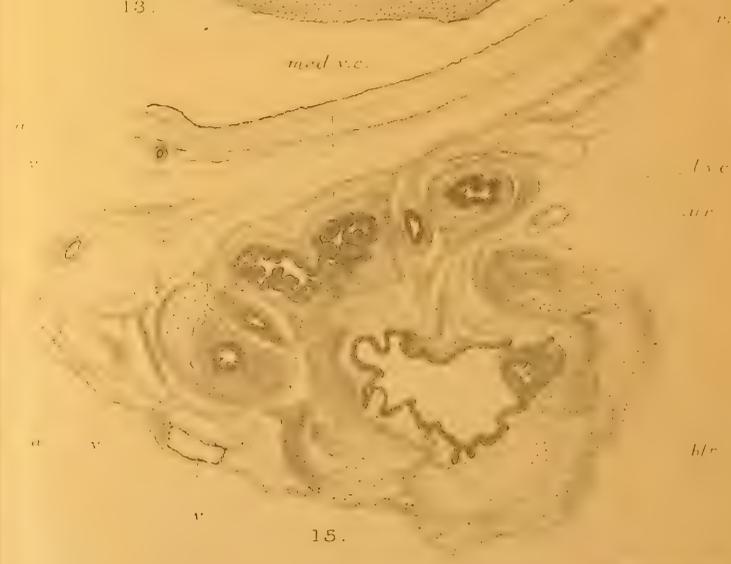
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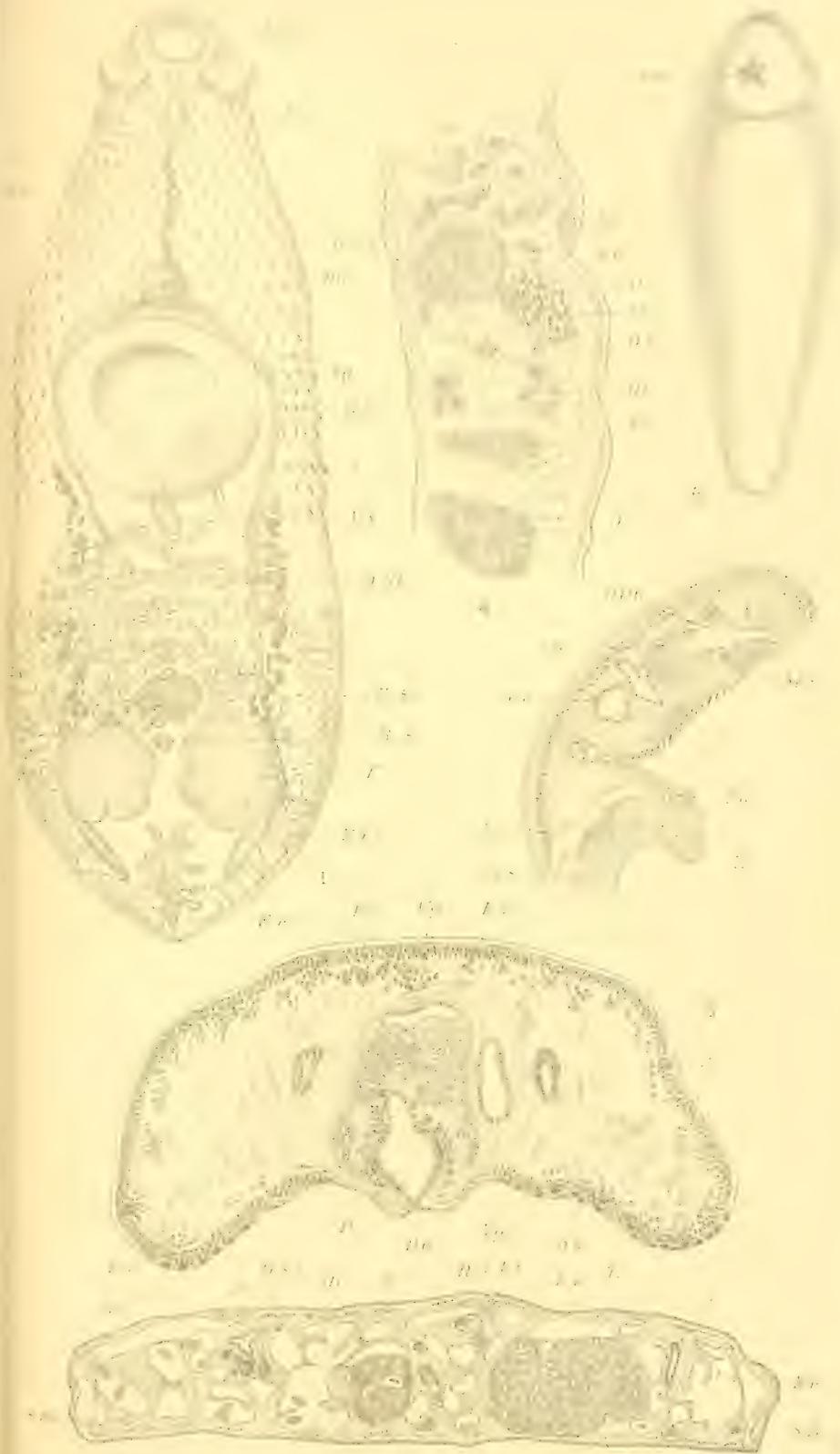
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Wilson del

Hutch, Lith. London



1. *Musca domestica* ♀



2. *Anthomyia radicum* ♀

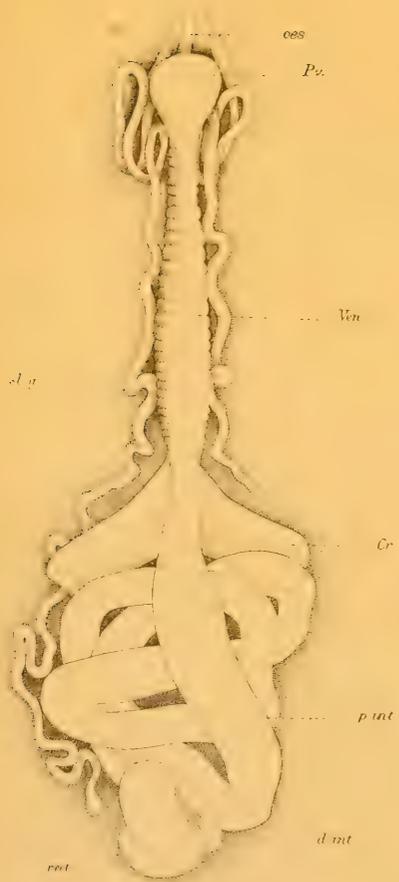


3. *Homalomyia canicularis* ♂

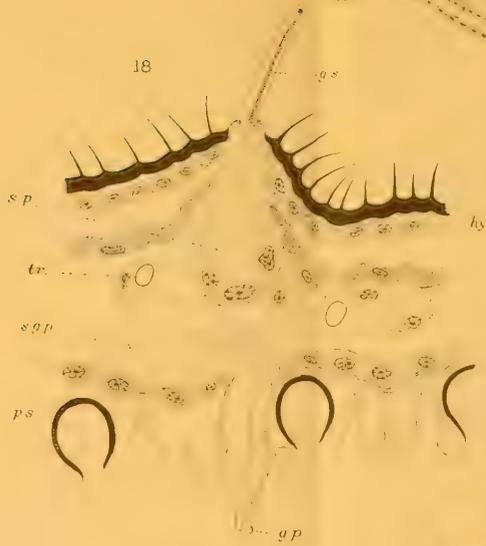


4. *Stomoxys calcitrans* ♀

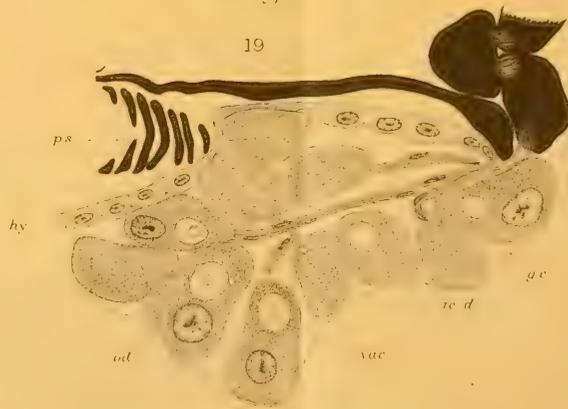
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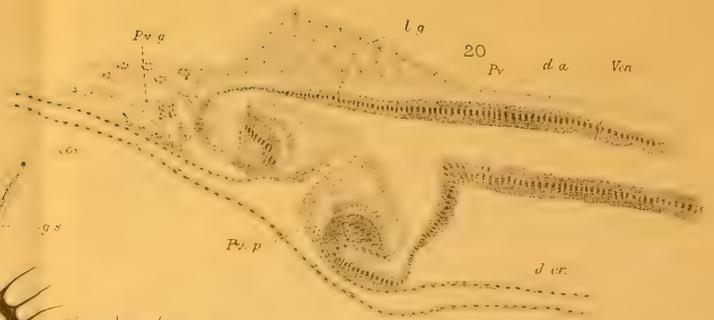
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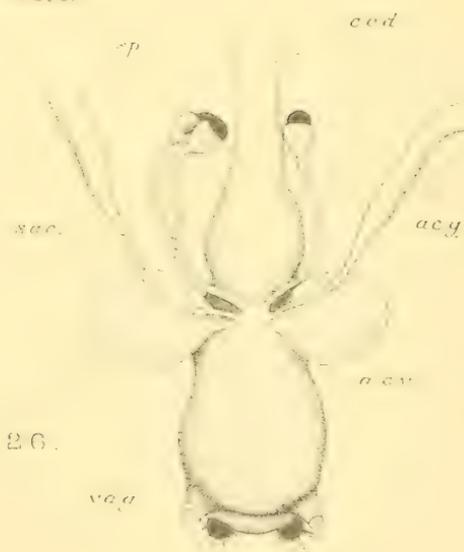
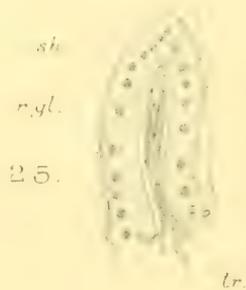
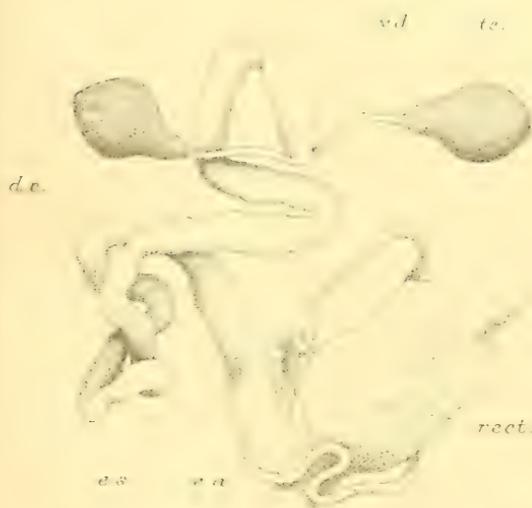
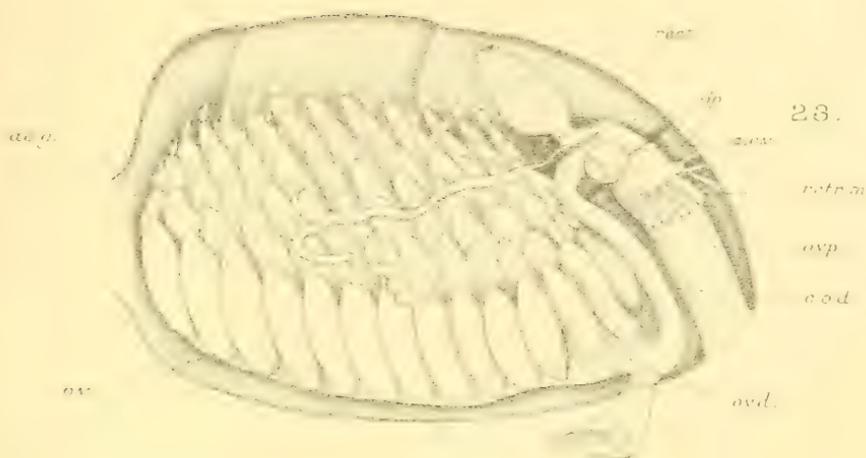


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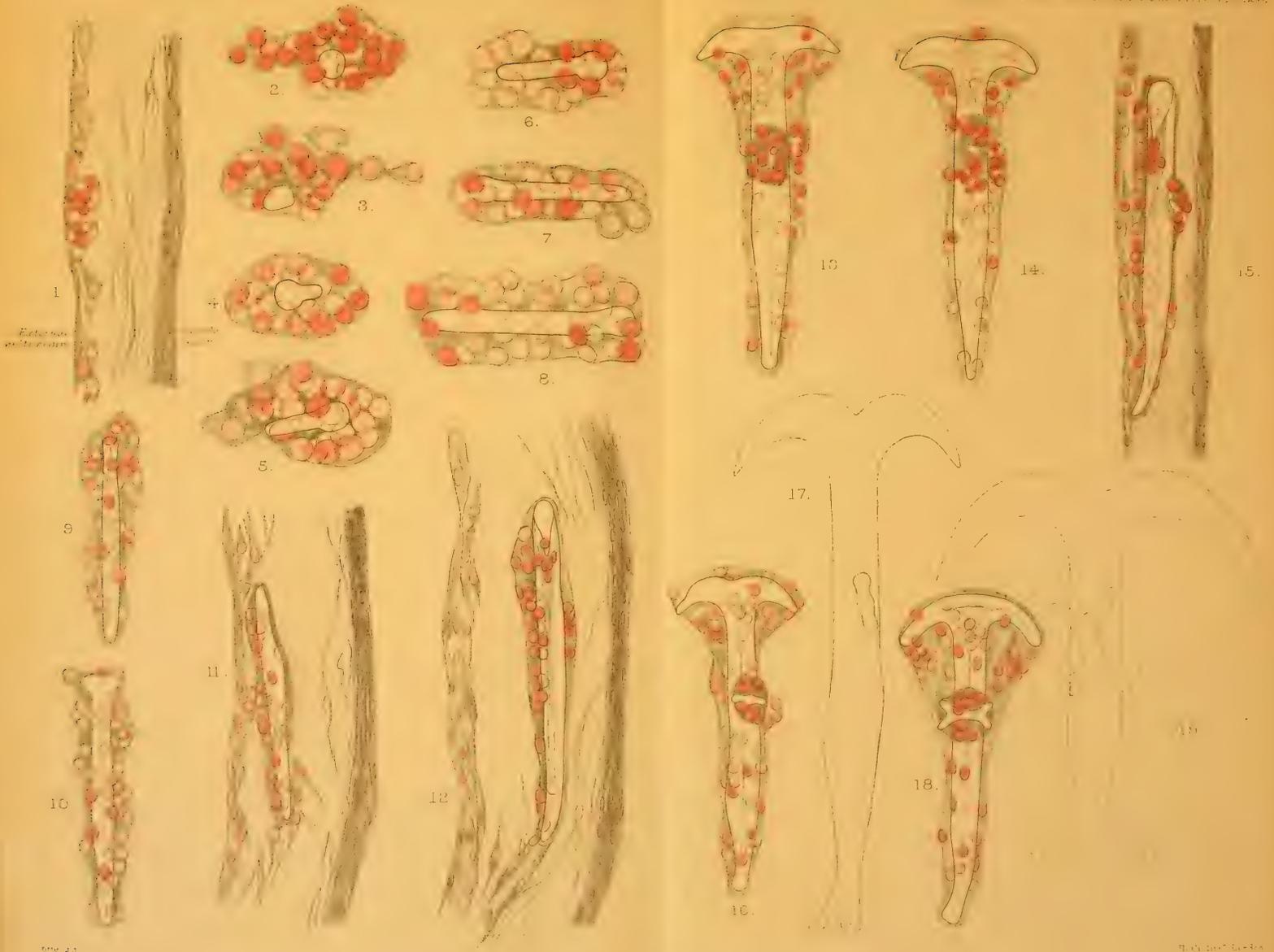




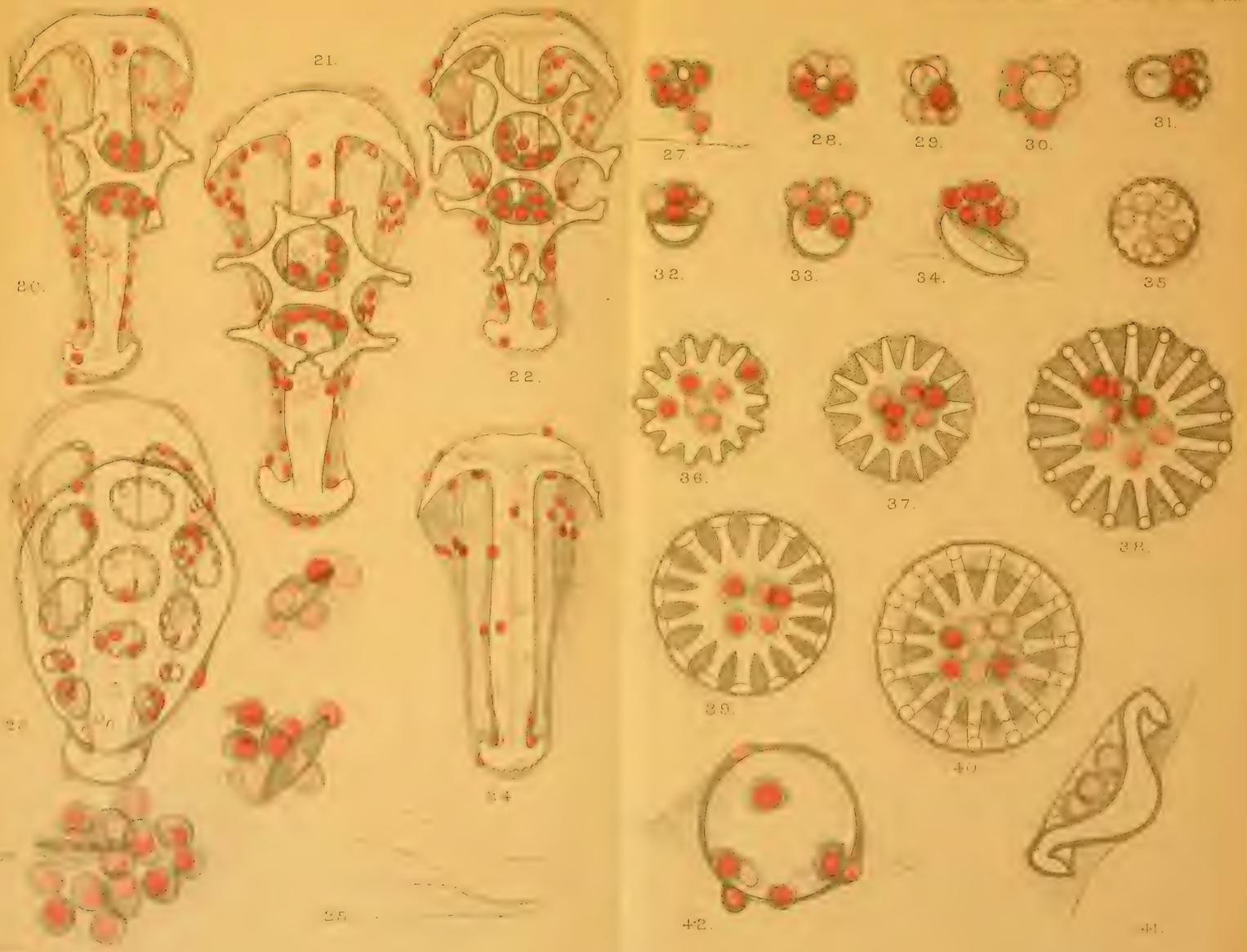


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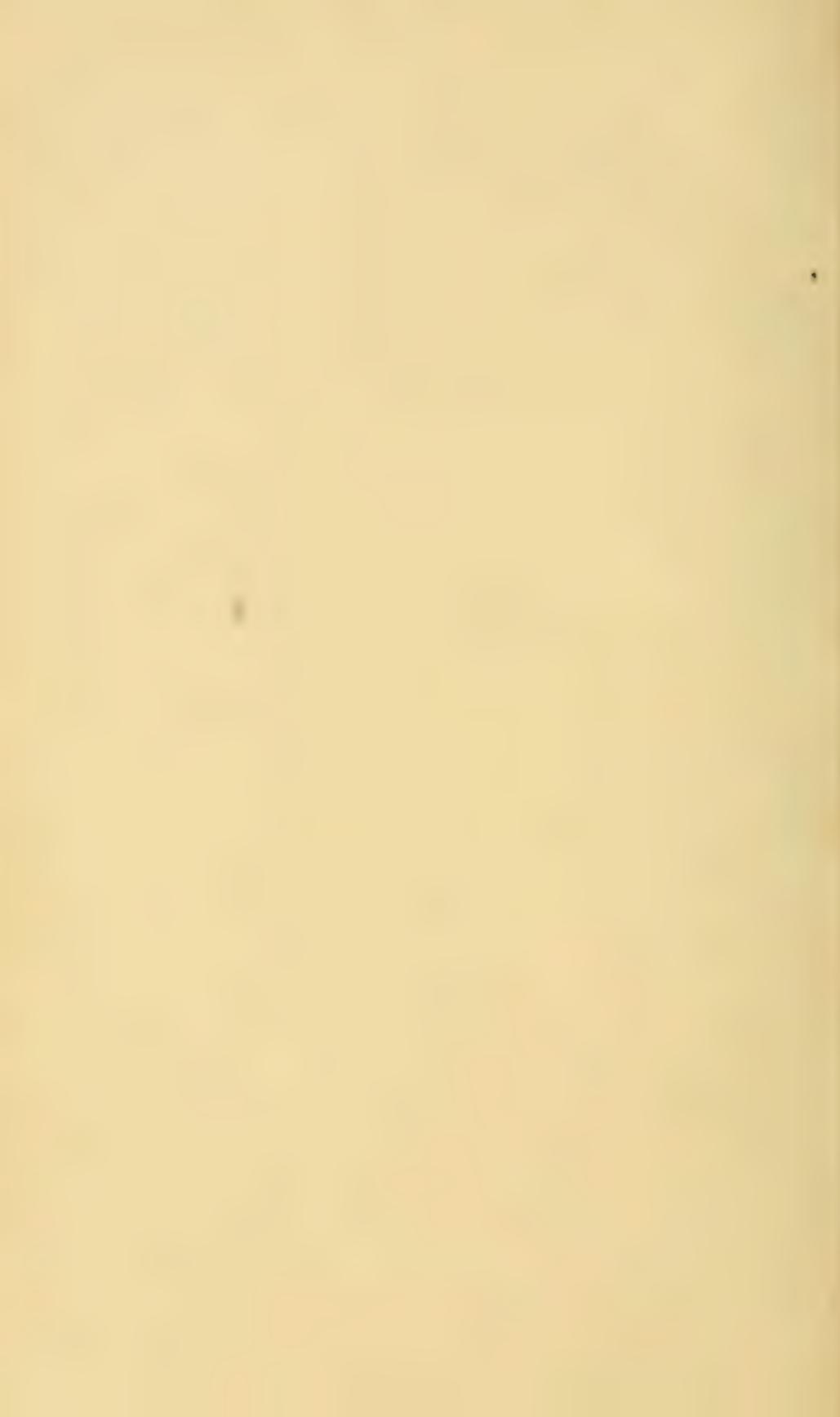
TRICHOMASTIX SERPENTIS.



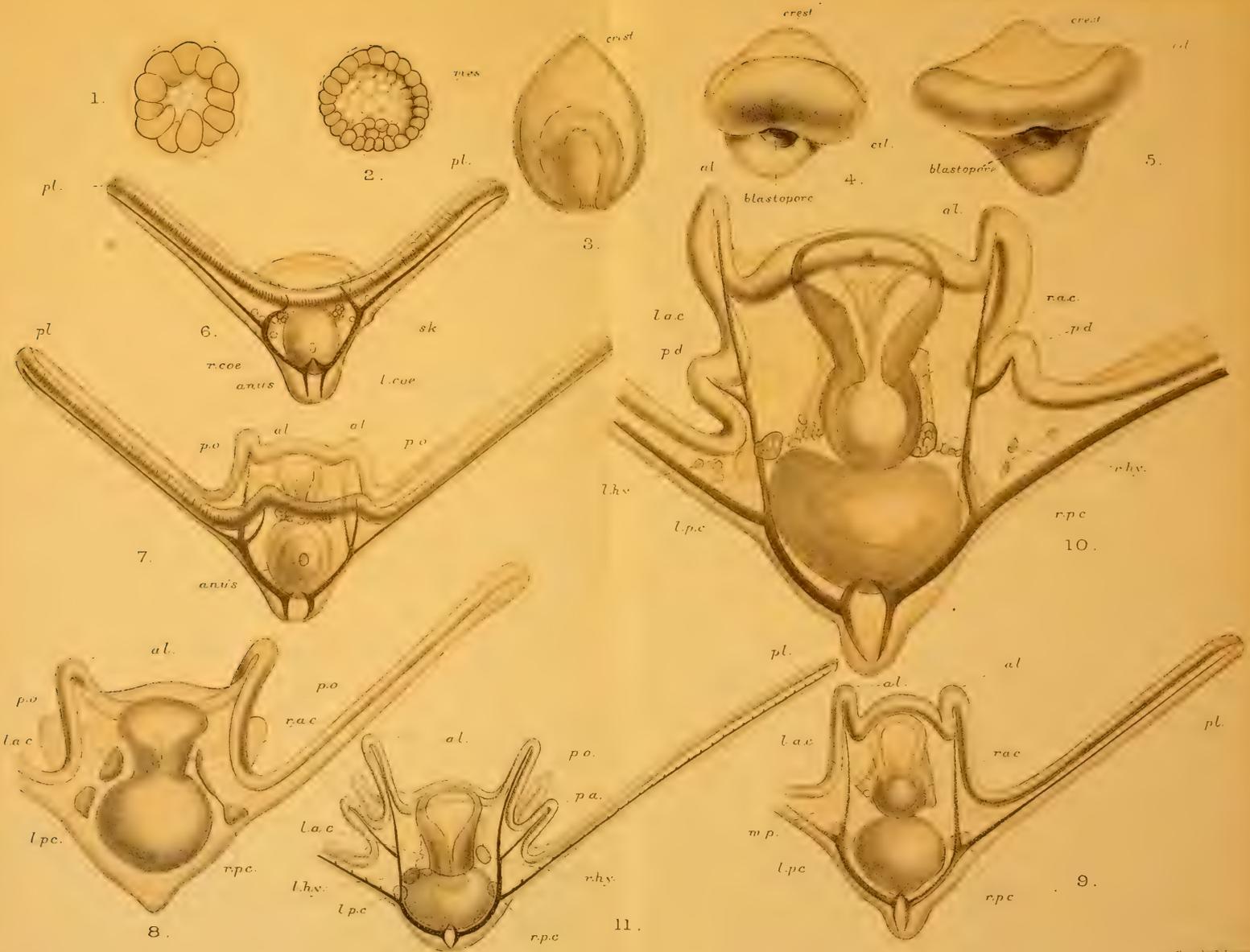
DEVELOPMENT OF PLATE- & ANCHOR SPICULE OF SYNAPTA.



DEVELOPMENT OF SPICULES OF SYNAPTA AND AURICULARIA LARVA.

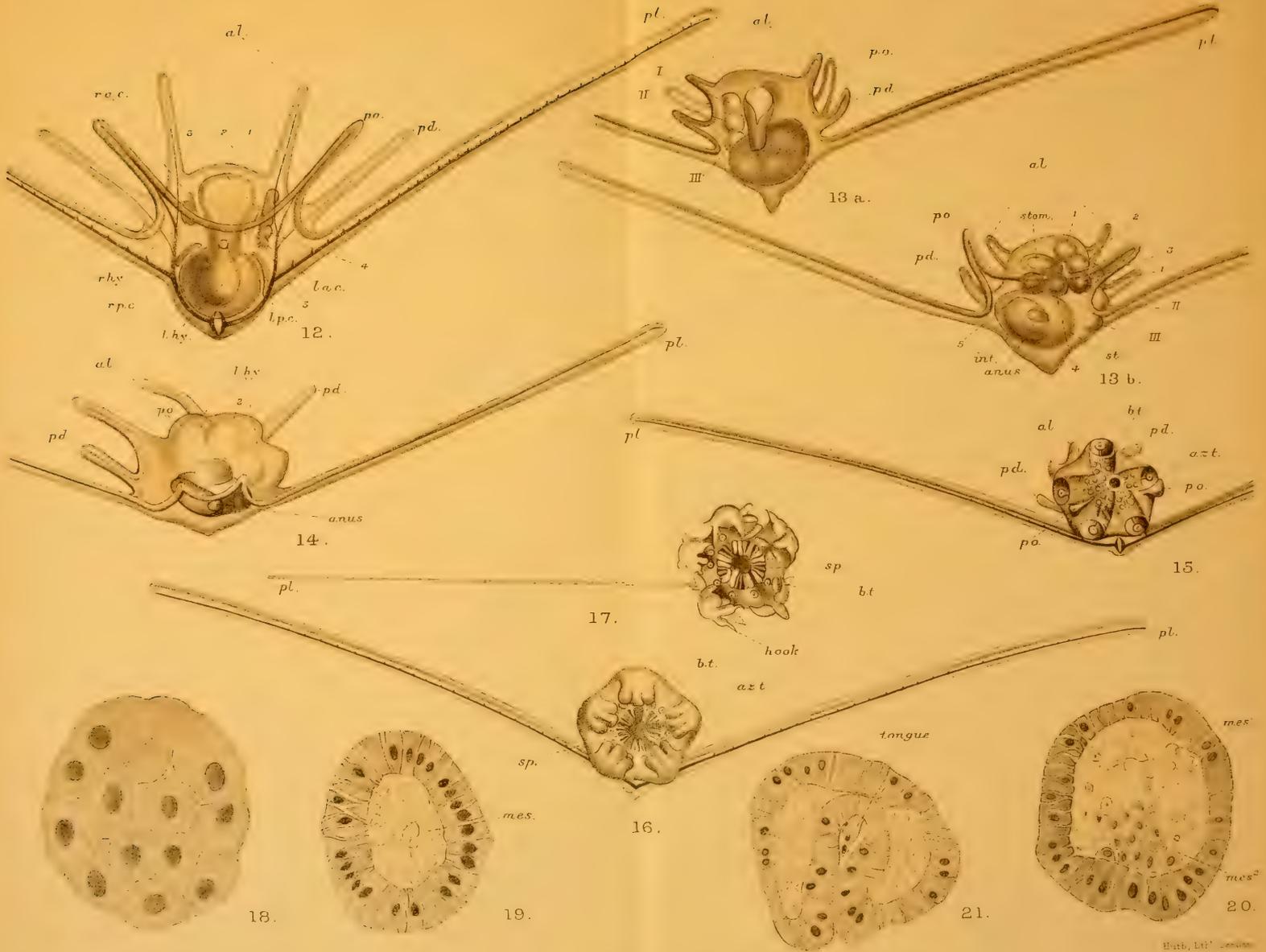






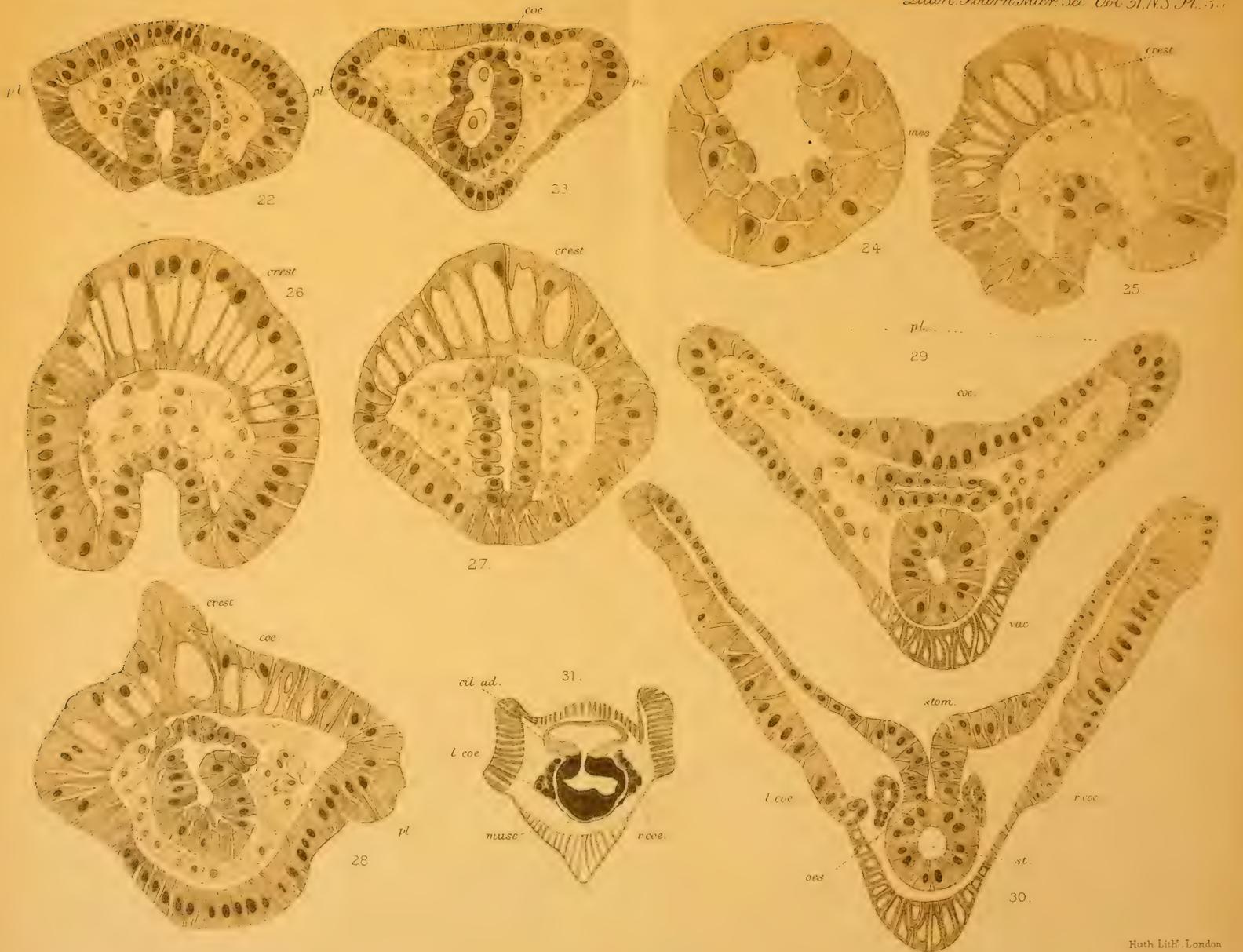
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Wm. Dyer, F.R.S.

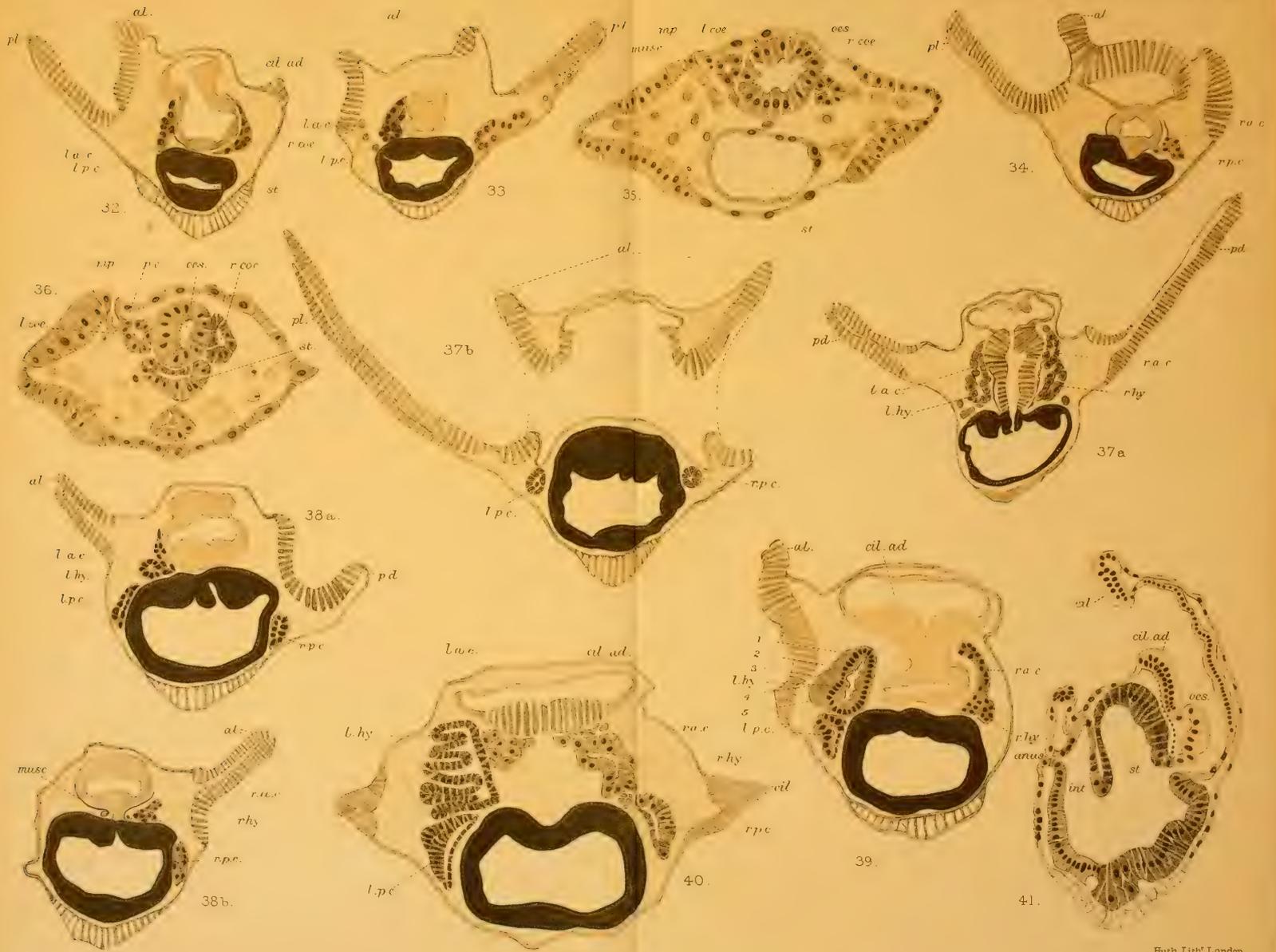


DEVELOPMENT OF OPHIOTHRIX FRACILIS.

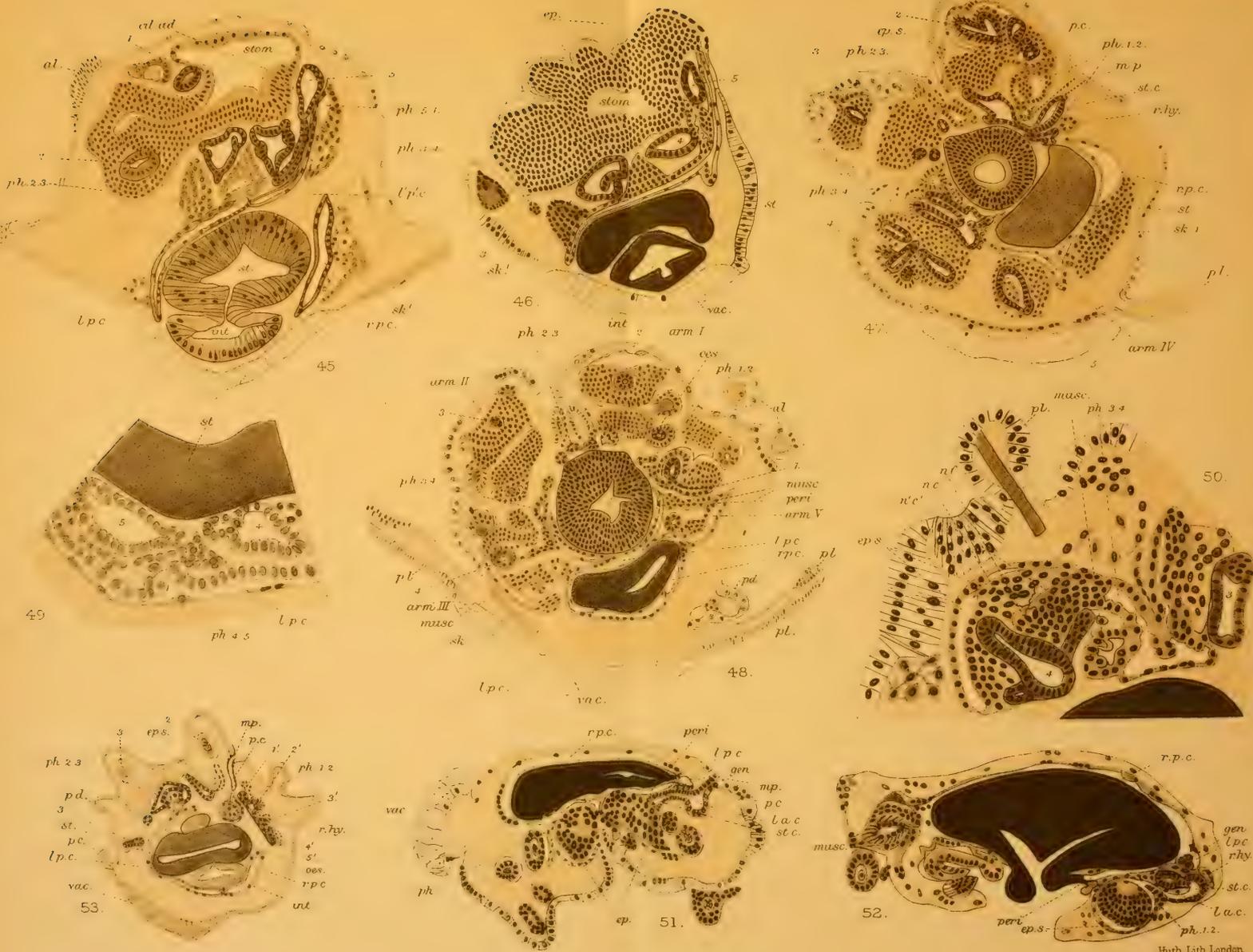
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DEVELOPMENT OF OPHIOTHRIX FRAGILIS.

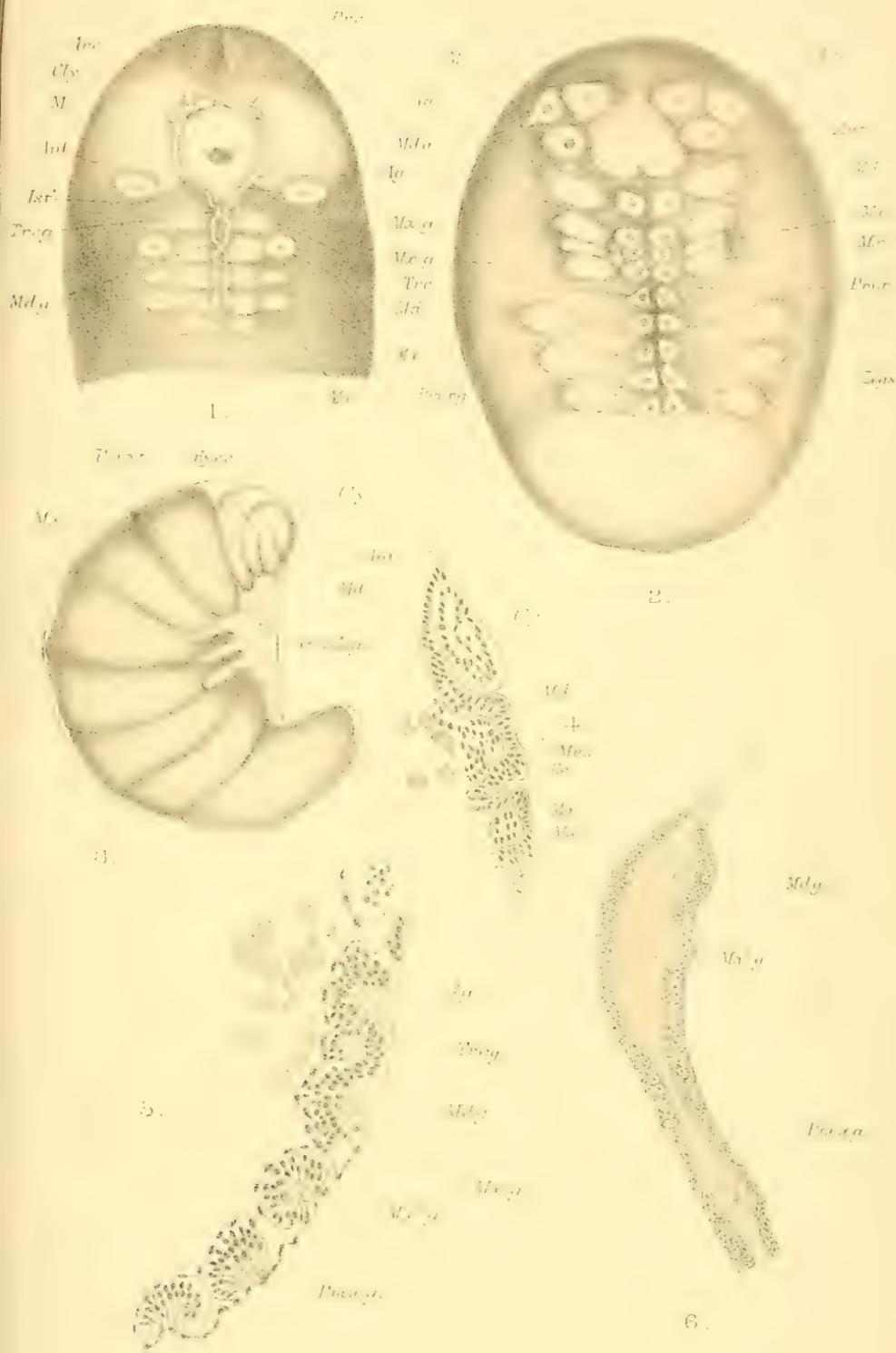


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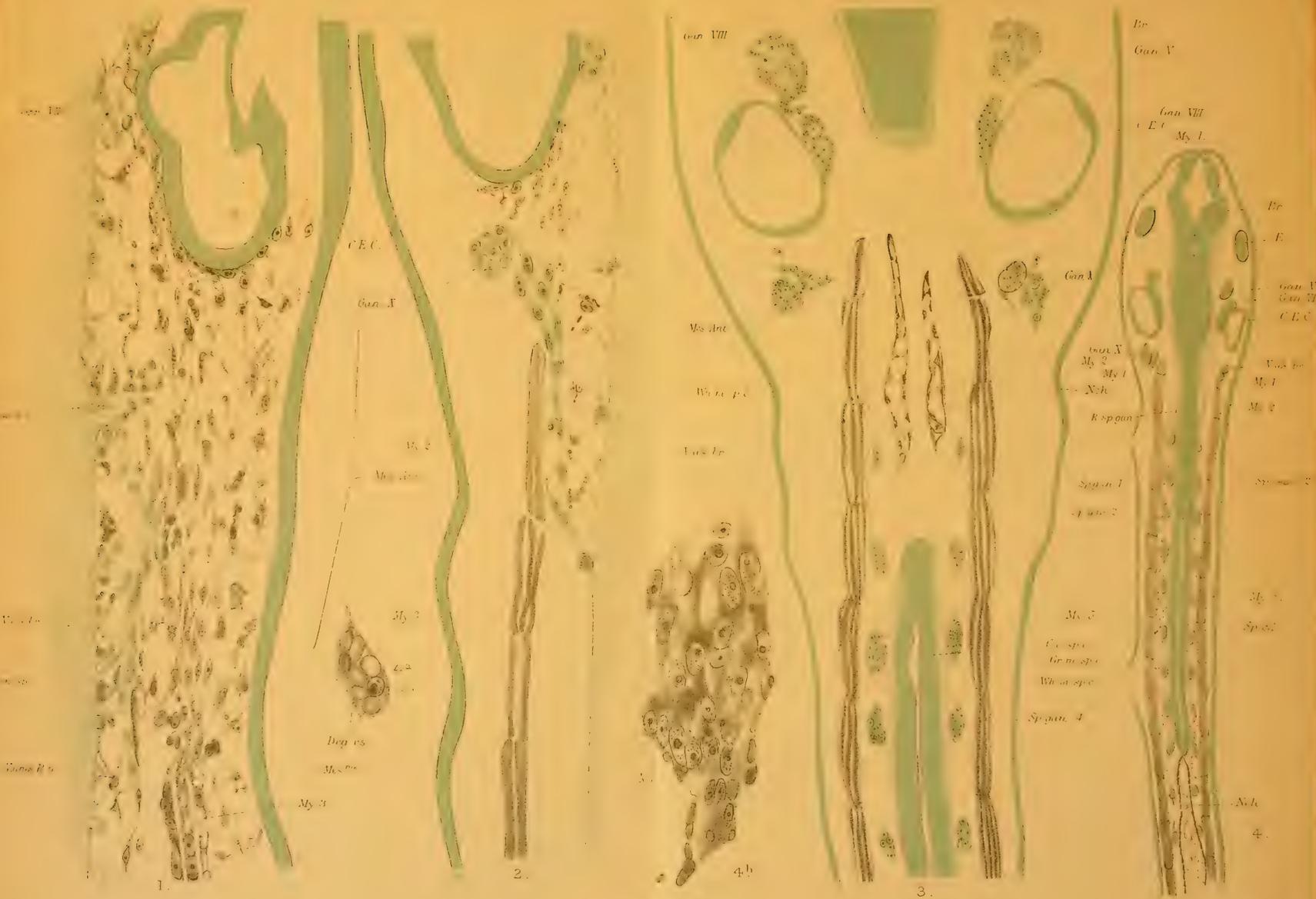
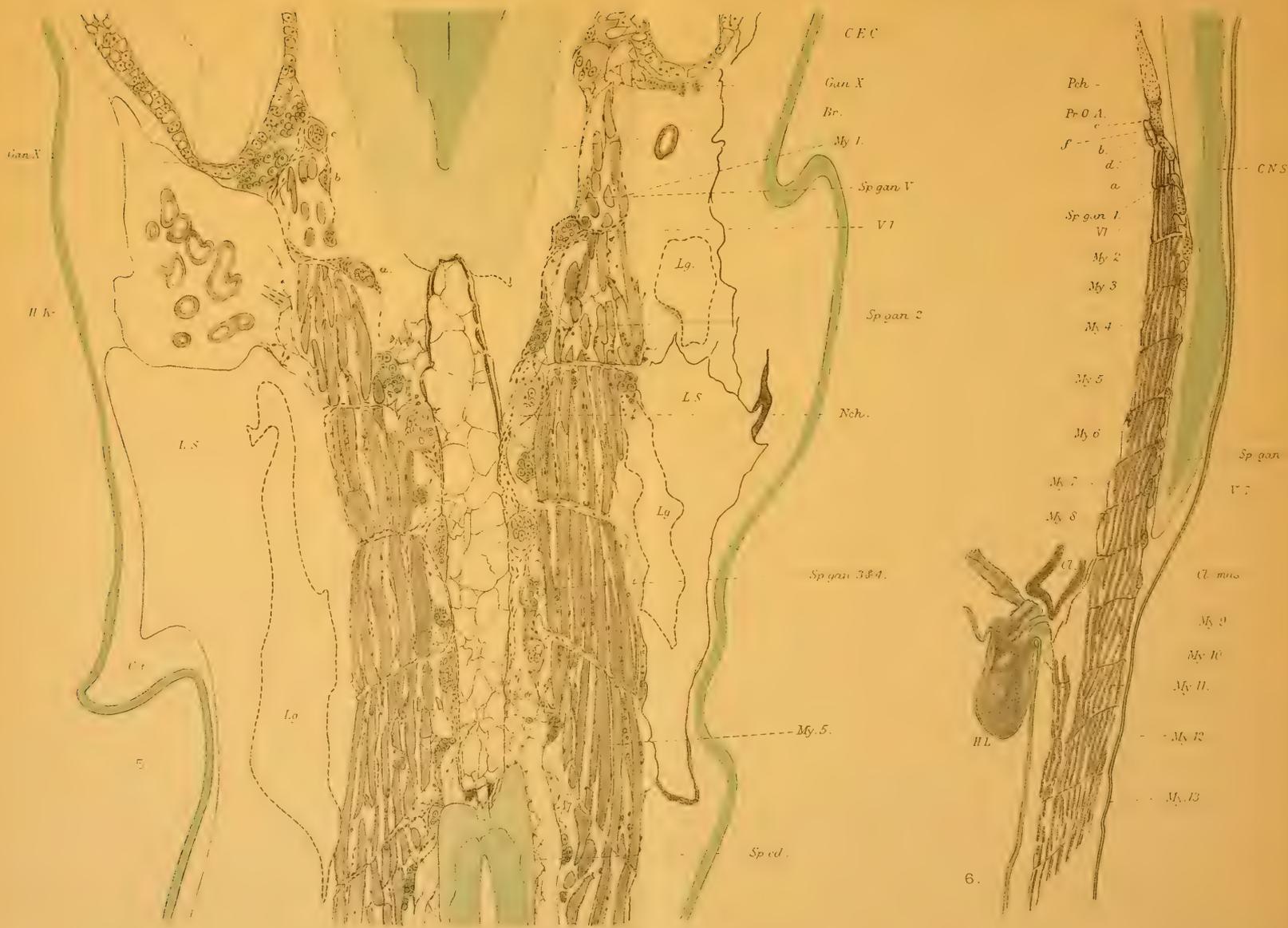


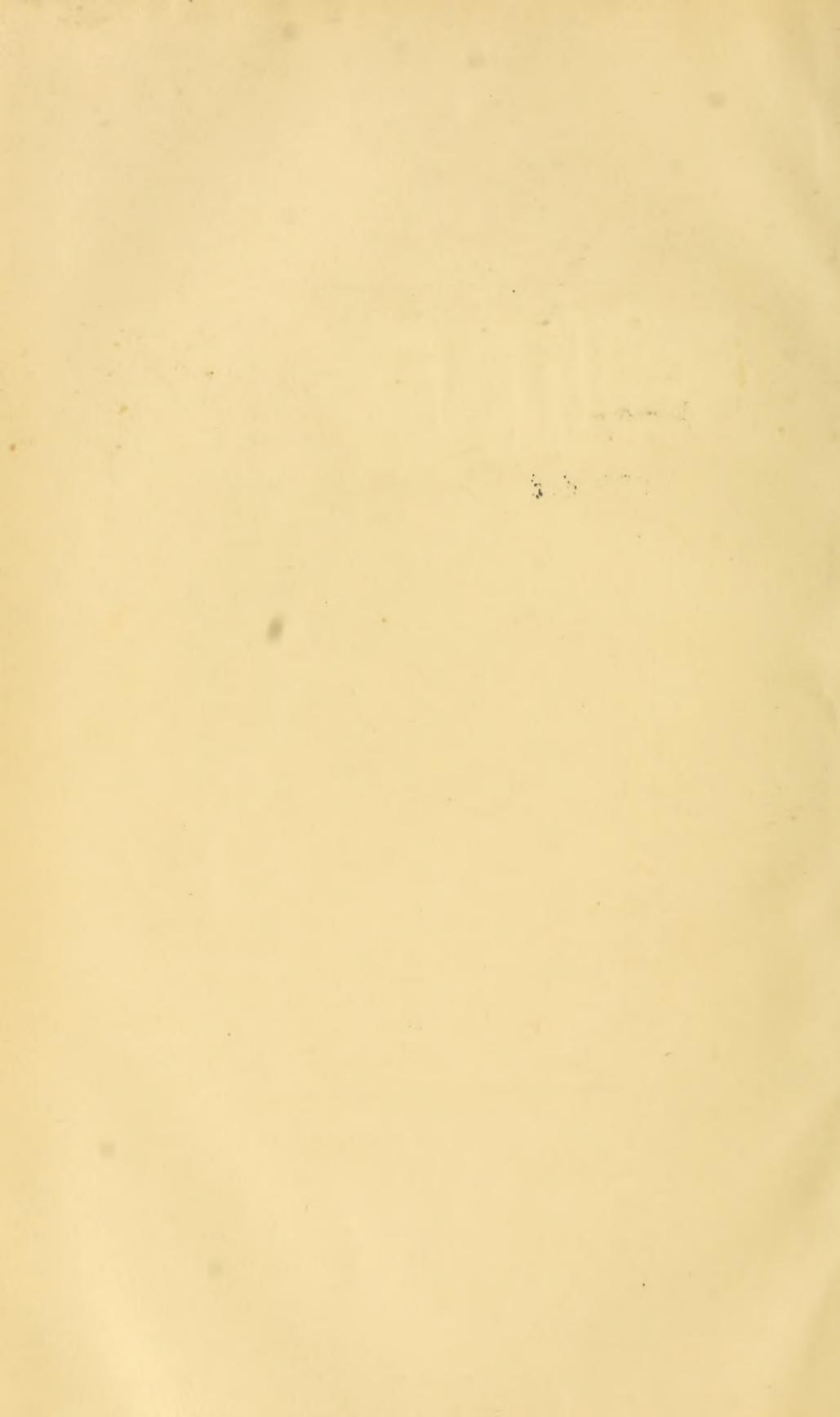
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