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EDITED BY

SIR RAY LANKESTER, K.C.B., M.A., D.Sc., LL.D., F.R.S.,

HONORARY FELLOW OF EXETER COLLEGE AND HONORARY STUDENT OF CHRIST CHURCH, OXFORD;
MEMBER OF THE INSTITUTE OF FRANCE (ASSOCIÉ ÉTRANGER DE L'ACADEMIE DES SCIENCES);
CORRESPONDENT 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
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FOREIGN ASSOCIATE OF THE NATIONAL ACADEMY OF SCIENCES, U.S., AND MEMBER OF THE
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HONORARY FELLOW OF THE ROYAL SOCIETY OF EDINBURGH;
LATE 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;
EVERETTUS PROFESSOR OF ZOOLOGY AND COMPARATIVE ANATOMY IN UNIVERSITY COLLEGE, UNIVERSITY OF LONDON

WITH THE CO-OPERATION OF

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

BRYER PROFESSOR OF ZOOLOGY IN THE UNIVERSITY OF MANCHESTER;

GILBERT C. BOURNE, M.A., D.Sc., F.R.S.,

LINACRE PROFESSOR OF COMPARATIVE ANATOMY, AND FELLOW OF MERTON COLLEGE, OXFORD;

J. GRAHAM KERR, M.A., F.R.S.,

REGIUS PROFESSOR OF ZOOLOGY IN THE UNIVERSITY OF GLASGOW;

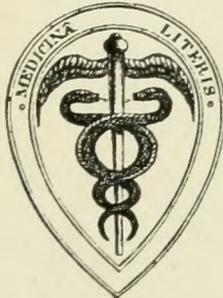
AND

E. W. MACBRIDE, M.A., D.Sc., LL.D., F.R.S.,

PROFESSOR OF ZOOLOGY AT THE IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY.

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CONTENTS.

CONTENTS OF No. 241, N.S., MAY, 1915.

MEMOIRS:	PAGE
A Further Study of the Mitotic Spindle in the Spermatocytes of <i>Forficula auricularia</i> . By C. F. U. MEEK. (With Plates 1 and 2)	1
The Embryonic Area and so-called "Primitive Knot" in the Early Monotreme Egg. By Profs. J. T. WILSON, F.R.S., and J. P. HILL, F.R.S. (With Plate 3 and one Text-figure.)	15
The Larva of the Starfish <i>Porania pulvillus</i> (O.F.M.). By JAMES F. GEMMILL, M.A., D.Sc., Lecturer in Embryology at Glasgow University, and in Zoology at Glasgow Provincial Training College. (With Plates 4 and 5).	27
Double Hydrocoele in the Development and Metamorphosis of the Larva of <i>Asterias rubens</i> , L. By JAMES F. GEMMILL, M.A., D.Sc. (With Plates 6 and 7)	51
On the Life-History of the Sporozoa of Spatangoids, with Observations on some Allied Forms. By HELEN L. M. PIXELL-GOODRICH, B.Sc., Beit Memorial Research Fellow. (With Plate 8)	81
Studies on Parasitic Protozoa. III.—(a) Notes on the Flagellate <i>Embadomonas</i> . (b) The Multiplication Cysts of a <i>Trichomastix</i> . By DORIS L. MACKINNON, D.Sc., Assistant in the Zoology Department, University College, Dundee. (With Plate 9)	105
Note on the Relation of Spermatozoa to Electrolytes and its bearing on the Problem of Fertilization. By JAMES GRAY, B.A., Fellow of King's College, Cambridge	119
<i>Klossiella muris</i> . By A. C. STEVENSON, M.B., D.P.H., University College Hospital Medical School. (With Plate 10)	127

CONTENTS OF No. 242, N.S., JULY, 1915.

MEMOIRS:	PAGE
The Chorda Tympani and Middle Ear in Reptiles, Birds, and Mammals. By EDWIN S. GOODRICH, F.R.S., Fellow of Merton College, Oxford. (With Plates 11, 12, and 13, and 5 Text-figures.)	137
Studies on the Turbellaria. Part III.— <i>Didymorchis</i> . By W. A. HASWELL, M.A., D.Sc., F.R.S., Challis Professor of Zoology, University of Sydney. (With Plate 14, and 1 Text-figure.)	161

	PAGE
The Placenta of a Lemur. By J. W. JENKINSON, M.A., D.Sc., University Lecturer in Embryology, Oxford; late Fellow of Exeter College. (With Plates 15, 16, and 17, and 7 Text-figures.)	171
On a New Species of Pentastomid from a N. African Snake (<i>Zamenis ravigieri</i>). By MARY L. HETT, B.Sc., Demonstrator of Zoology, Bedford College, University of London. (With 5 Text-figures.)	185
REVIEW:	
'Text-Book of Embryology,' Vol. I, Invertebrata. By E. W. MACBRIDE, M.A., D.Sc., LL.D.; F.R.S., Professor of Zoology at the Imperial College of Science and Technology, South Kensington	201

CONTENTS OF No. 243, N.S., MARCH, 1916.

MEMOIRS:

The Gregarines of <i>Glycera siphonostoma</i> . By HELEN L. M. PIXELL-GOODRICH, B.Sc., Beit Memorial Research Fellow. (With Plate 18.)	205
Observations on the Insect Parasites of some Coccidæ. By A. D. IMMS, M.A., D.Sc., Reader in Agricultural Entomology in the Victoria University of Manchester. I.—On <i>Aphelinus mytilaspidis</i> Le Baron, a Chalcid Parasite of the Mussel Scale (<i>Lepidosaphes ulmi</i> L.). (With Plates 19 and 20, and 5 Text-figures.)	217
The Transition of Peritoneal Epithelial Cells into Germ Cells in some Amphibia Anura, especially in <i>Rana temporaria</i> . By J. BRONTÉ GATENBY, Exhibitioner of Jesus College, Oxford. (With Plates 21 and 22 and 5 Text-figures.)	275
On the Embryology of <i>Stratiodrillus</i> (Histriobdellidæ). By W. A. HASWELL, M.A., D.Sc., F.R.S., Challis Professor of Zoology, University of Sydney. (With Plate 23 and 4 Text-figures.)	301
Note on Intra-uterine Eggs of <i>Heterodontus</i> (<i>Cestracion</i>) <i>Phillipi</i> . By Professor W. A. HASWELL, M.A., D.Sc., F.R.S., Sydney. (With 2 Text-figures.)	313
The Development of the Sperm Duct, Oviduct, and Spermatheca in <i>Tubifex rivulorum</i> . By J. BRONTÉ GATENBY, Exhibitioner of Jesus College, Oxford. (With Plate 24 and 1 Text-figure.)	317
<i>Dendrocometes paradoxus</i> (Stein). Part II.—Reproduction (Bud-formation). By GEOFFREY LAPAGE, M.Sc., Late Assistant Lecturer in Zoology, Victoria University of Manchester, and J. T. WADSWORTH, Research Assistant in Zoology, Victoria University of Manchester. (With Plates 25 and 26 and 16 Text-figures.)	337

CONTENTS.

v

PAGE

CONTENTS OF No. 244, N.S., JULY, 1916.

MEMOIRS:

- On the Development and Morphology of the Pharyngeal, Laryngeal, and Hypobranchial Muscles of Mammals. By F. H. EDGEWORTH, M.D., Professor of Medicine, University of Bristol. (With Plates 27—39) 383
- On the Corpora lutea and Interstitial Tissue of the Ovary in the Marsupialia. By CHAS. H. O'DONOGHUE, D.Sc., F.Z.S., Senior Assistant in the Zoological Department, University College, London. (With Plate 40) 433

TITLE, INDEX, AND CONTENTS.

A Further Study of the Mitotic Spindle in the Spermatocytes of *Forficula auricularia*.

By

C. F. U. Meek.

With Plates 1-2.

INTRODUCTION.

At the beginning of 1913 I stated that the lengths of the mitotic spindle at the conclusion of the primary and secondary spermatocyte metaphases of *Forficula auricularia* seemed to be constants, and that the ratio between these lengths was almost identical with the ratio between the radii of two spheres of which the volume of one is equal to twice that of the other. The primary spermatocyte cell divides to form two secondary spermatocytes; and, since no period of growth seems to separate their mitoses, the volume of the primary spermatocyte cell in the metaphase is presumably equal to twice that of the daughter secondary spermatocyte: connection was therefore suggested between the spindle-length and cell-volume at this stage.

I then measured spindle-lengths at the conclusion of the spermatocyte metaphases of *Helix pomatia*; the lengths again seemed to be constants, and the ratio between them was found to be identical with the second ratio mentioned above. Moreover, independent investigations made at this time by von Winiwarter showed the same phenomenon in the spermatocyte mitoses of man.

I have since published photo-micrographs verifying my earlier camera lucida drawings of the spindles of *Forficula*

auricularia and *Helix pomatia*; but, in new preparations of the former, have observed primary spermatocyte spindle-lengths that are excessive, and that consequently do not accord with the ratio previously found. Four explanations were put forward to account for these lengths. "Firstly, the volume of these cells in the metaphase may vary, and our proposition may still be valid. In this case, however, various lengths will presumably be found at the conclusion of the secondary spermatocyte metaphase; and I have not observed such lengths. Secondly, the daughter-chromosomes may remain apposed to one another in the equatorial plane for a considerable time after constriction is complete; if centrosome divergence continues during this period, the various and excessive lengths may be explained. This, however, cannot always occur; for, in this organism, I have found and drawn primary spermatocyte cells in which the daughter-chromosomes have begun to move towards the poles when the spindle-length is only slightly greater than that estimated for the conclusion of the metaphase. Thirdly, our proposition may require modification in that the length of the spindle may be affected by the shape of the cell. My original measurements in *Forficula* and *Helix* were made from cells that were approximately spherical, and this may explain the constant lengths observed. When, however, cells are closely packed together in a cyst, the spherical form disappears, and if our modification is valid, the spindle-length will vary with the shape assumed. Lastly, the length of the spindle at this stage may be connected with neither the volume nor shape of the cell; and in this case our proposition is entirely disproved. If, however, this is so, why has the ratio in question been observed in *Helix pomatia* and man?"

We know little concerning the nature of the mitotic spindle, although it has been a subject of investigation for many years. We have found that it cannot be regarded as a figure formed entirely by the action of forces at its poles. I have since shown that its length at the conclusion of certain spermatogenetic metaphases cannot be correlated with the

volume of chromatin present; and, if we can prove that the length at this stage is or is not determined by the volume of the cell, we shall have succeeded in establishing another proposition.

In the circumstances, I now intend to investigate further the spermatocyte spindles of *Forficula auricularia*. That the length at the conclusion of the primary spermatocyte metaphase is not a constant has already been proved; but the results of this study may constitute new data affecting the proposition that I have put forward.

MATERIAL AND METHODS.

The material, which was obtained in July and August, was preserved in Flemming's strong chromo-aceto-osmic acid fluid. The testes remained in the fixative for twenty-four hours, and, after being thoroughly washed in running water and passed through successive strengths of alcohol, were cleared in xylol and embedded in paraffin. Sections were cut 8μ thick with a Cambridge rocking microtome.

All sections were stained on the slide. The slides were placed for four to six hours in an aqueous solution of ferric alum, and were then stained for twelve to fifteen hours in Heidenhain's iron-hæmatoxylin. In certain cases they were first stained for ten minutes in eosin.

The preparations were studied with a Zeiss apochromatic oil-immersion objective of 2 mm. focus and N.A. 1.30, and the various compensating oculars. The light was obtained from an inverted incandescent gas-burner, and was passed through a Watson holoscopic oil-immersion substage condenser. All photo-micrographs shown were made with a Zeiss camera, the apochromatic objective mentioned above, and compensating ocular No. 4. The camera extension was 50 cm. in the case of all photographs of individual cells, and 25 cm. in the case of photographs of cysts. The magnification was estimated with a stage micrometer graduated to read one hundredth part of a millimetre, and photographs of this scale are in-

cluded in the plates. Moreover, in order to minimize error, all measurements have been independently checked by my assistant, Mr. T. R. Goddard.

THE LENGTH OF THE SPINDLE IN THE PRIMARY SPERMATOCYTE METAPHASE AND EARLY ANAPHASE.

Fig. 1, Pl. 1, shows a section of two cysts containing primary spermatocyte cells, stained with iron-hæmatoxylin. Two polar views are seen, in which the reduced number of chromosomes can be counted; and, since both metaphases and telophases are observed, the cells are in various stages of mitosis. Fig. 2 shows a section of a cyst of these cells, taken from the testes of another specimen and stained with iron-hæmatoxylin and eosin; the cells in this section are seen to be in the metaphase. Fig. 46, Pl. 2, shows the magnification of these two photo-micrographs.

Figs. 7 and 8, Pl. 2, are polar views of primary spermatocyte cells in the metaphase, and show twelve chromosomes; the latter figure has been taken from the section of the cysts shown in fig. 1. These and all following photographs of cells have been made at a magnification approximately equal to twice that of figs. 1 to 6, Pl. 1, and a photograph of the divisions of the stage micrometer is given in fig. 47, Pl. 2.

Figs. 9 to 26 inclusive are lateral views of primary spermatocyte cells in the metaphase, and the chromosomes are seen to be constricting in the equatorial plane. Figs. 10 and 17 also show polar views, which are similar to those of figs. 7 and 8. Figs. 15, 18, and 23 have been taken from the section of cysts shown in fig. 1, in which photograph the first two figures are seen in focus. Fig. 25 belongs to the section and cyst shown in fig. 2. The lengths of these eighteen spindles, estimated from the magnification, are given in the following table¹:

¹ In this paper I am not attempting to express spindle-lengths in terms smaller than one quarter of a micromillimetre.

Fig. 9	11.25 μ	Fig. 18	14.0 μ
„ 10	11.25 μ	„ 19	14.5 μ
„ 11	11.5 μ	„ 20	14.75 μ
„ 12	12.0 μ	„ 21	16.0 μ
„ 13	12.25 μ	„ 22	16.25 μ
„ 14	12.5 μ	„ 23	16.5 μ
„ 15	12.75 μ	„ 24	16.5 μ
„ 16	12.75 μ	„ 25	16.75 μ
„ 17	13.25 μ	„ 26	17.5 μ

Now these figures prove that the length of the spindle may vary considerably at the stage immediately preceding the conclusion of the metaphase. The spindles shown in the photographs cannot be regarded as abnormal, and their lengths, which constitute an approximately graded series, exceed in every case that originally observed by me at the slightly later stage, when constriction of the chromosome is completed.

I said in my last paper that I had found various and excessive spindle-lengths at the latter stage; and in the course of the present research I have again observed such lengths, e. g. 12.0, 13.5, 15.25, 15.75, 16.25, 16.5, 17.0 and 18.0 μ . In the circumstances, we must realise that the length of the spindle in this mitosis is not a constant at either of the stages mentioned. Moreover, I have now found these excessive lengths in certain cysts of my older material; we have, therefore, no reason for supposing that individuals of the species differ in this respect.

We will now consider the early anaphase. Figs. 27 and 28 are lateral views of primary spermatocyte cells at this stage, and the spindle-lengths, estimated from the magnification, are respectively 11.25 and 11.5 μ . These lengths are smaller than those seen in the cells represented by figs. 12 to 26 inclusive, and the figures consequently prove that the length of the spindle when the daughter-chromosomes have begun to move towards the two poles may be smaller—and sometimes considerably smaller—than the length before constriction of the chromosomes is completed. Furthermore, I have found

cells in which the distance between the daughter-chromosomes is approximately the same as that in figs. 27 and 28, whereas the spindle-length greatly exceeds 11.5μ . The amount of divergence of the daughter-chromosomes is therefore not invariably proportional to the length of the spindle in the anaphase of this mitosis.

THE LENGTH OF THE SPINDLE IN THE SECONDARY SPERMATOCYTE METAPHASE AND EARLY ANAPHASE.

Figs. 3 and 4, Pl. 1, show sections of cysts of secondary spermatocyte cells in various stages of mitosis; these sections were stained with iron-hæmatoxylin. Figs. 5 and 6 show sections of similar cysts, taken from the testes of another specimen and stained with iron-hæmatoxylin and eosin; the cells in these figures are seen to be in the metaphase. These four photographs have been made at the same magnification as figs. 1 and 2.

Figs. 29, 30 and 31, Pl. 2, are polar views of secondary spermatocyte cells in the metaphase, and twelve chromosomes can be counted in each. Fig. 29 was taken from the section of the cyst shown in fig. 3, Pl. 1, and the figure is seen to be in focus in the latter photograph.

Figs. 32 to 42 inclusive are lateral views of these cells in the metaphase, before constriction of the chromosomes is completed. Figs. 36 and 37 belong to the section of the cyst shown in fig. 3; figs. 41 and 42 belong to that shown in fig. 5. Fig. 40 was taken from the section and cyst shown in fig. 6, and fig. 32 belongs to the same section and cyst as fig. 30. The lengths of the spindle in these eleven cells, estimated from the magnification, are given in the table below:

Fig. 32	6.75μ	Fig. 38	8.75μ
„ 33	7.0μ	„ 39	9.0μ
„ 34	7.0μ	„ 40	10.25μ
„ 35	7.5μ	„ 41	10.25μ
„ 36	7.5μ	„ 42	11.25μ
„ 37	7.5μ		

Now, it is evident from these measurements that the length of the spindle is not a constant at the stage immediately preceding the conclusion of the secondary spermatocyte metaphase; the lengths that I originally observed at this stage and at the conclusion of the metaphase were respectively 7.8 and 8.1 μ , and the lengths shown in the table are in every case greater or smaller than these. This presupposes various lengths at the conclusion of the metaphase, and I have now found such lengths both in my new material and in certain cysts of my older preparations. The discovery of these various lengths is important; for I stated in my last paper that I had not found them in this cell generation.

Figs. 43, 44 and 45 are lateral views of these cells in the anaphase. Fig. 43 was taken from the section of the cyst shown in fig. 3; figs. 44 and 45 were taken from that shown in fig. 4. The lengths of the spindle in these cells are respectively 8.25, 8.75, and 10.75 μ ; and, since we have observed greater lengths in cells in which constriction of the chromosomes is not completed, the results are similar to those already obtained from the study of the primary spermatocyte mitosis. Moreover, I have found cells in which the distance between the daughter-chromosomes is approximately the same as that in figs. 43 and 44, whereas the spindle-lengths considerably exceed 8.75 μ ; the length of the spindle cannot therefore be proportional to the amount of chromosome divergence in the early anaphase of the secondary spermatocyte cells.

THE FOUR EXPLANATIONS PREVIOUSLY PUT FORWARD.

Having proved that the spindle-length in these two mitoses is not a constant at either the conclusion of the metaphase or the stages immediately preceding and following the conclusion, we can consider the explanations that were put forward when I discovered various and excessive lengths in the primary spermatocyte cells. Of these explanations, three imply the

validity of my original proposition, or of a modification of that proposition; the fourth implies its entire refutation.

Firstly, I suggested that the volumes of the primary spermatocyte cells vary in the metaphase. Secondly, I suggested that the daughter-chromosomes remain opposed to one another in the equatorial plane for a considerable time after constriction is completed; if centrosome divergence continues during this period, the various and excessive lengths may be explained. Both explanations assume that the length of the spindle is proportional to the volume of the cell at the moment when constriction of the chromosomes is completed. We will deal first with the former.

We know that a long period of growth separates the last spermatogonial mitosis and that of the primary spermatocytes, but we do not know that the consequent increase of volume of the latter cells is the same in all cases. Furthermore, we have no proof that the volumes of the parent spermatogonia are constant, and therefore cannot say that all primary spermatocytes are identical in volume at the moment of their formation. The objection to this explanation was that the spindle-length must presumably vary at the conclusion of the secondary spermatocyte metaphase, whereas it seemed to be constant. This objection, however, has now been removed; for the further study of these cells has revealed the existence of various lengths at this stage.

Now, the photographs of individual cells given in Pl. 1 show marked differences between the areas enclosed by the cell outlines. We cannot, however, infer from this that the volumes vary; for the cells are seen in only two dimensions. If we wish to prove that the volumes vary, we must study the cells in various horizontal planes; and, since we cannot hope to form more than a rough estimate of the volume in any individual case, it will be advisable to compare cells in which the volumes in corresponding planes differ considerably. Moreover, in dealing with this explanation we must be careful to compare cells to which the second explanation cannot apply; otherwise no trustworthy conclusion can

be drawn from our results. The conditions mentioned above are fulfilled in the cells shown in figs. 21 and 25 of the plate. We will first consider the former.

The area enclosed by the outline of this cell in the horizontal plane through the centrosomes is approximately 430 sq. μ , and camera lucida drawings of the cell-outline at various vertical distances from this plane prove that the area enclosed does not vary appreciably throughout the section. The greatest vertical distance between any two planes studied is 8 μ , i. e. the thickness of the section; and, since the mean area may be said to be 430 sq. μ , the volume of the portion measured must be approximately 3440 c. μ . Unfortunately, I have not been able to follow the contour further; for I have failed to identify the cell in the sections immediately preceding and following that in which the spindle lies. We have reason for supposing that a large portion lies outside this section; but, whether this is or is not so, the minimum volume of the cell cannot be less than 3440 c. μ .

We will now consider the cell shown in fig. 25. I have made numerous photo-micrographs at various vertical intervals of the cyst in which this cell lies; the horizontal planes represented extend through three consecutive sections, and cover a vertical distance of 20 μ . A comparison of these photographs shows that the maximum area enclosed by the cell-outline is found in the horizontal plane that passes through the centrosomes, and that the cell cannot extend for a distance greater than 8 μ above and below this plane. The maximum area enclosed is approximately 160 sq. μ ; and, since the depth of the cell cannot exceed 16 μ , the volume must be less than 2560 c. μ . But we have already proved that the volume of the cell shown in fig. 21 is at least 3440 c. μ : it is therefore evident that the volumes of the primary spermatocyte cells vary in the metaphase.

We must now compare the lengths of the spindle in these two cells. A glance at the table on page 5 shows that the smaller spindle-length is found in the larger cell; consequently, the length of the spindle at the stage shown cannot

be proportional to the volume of the cell. A study of the equatorial plates of these two figures at a high magnification shows respectively only one and two chromosomes that have not completed constriction. For our present purpose, therefore, the stage shown may justifiably be identified with the conclusion of the metaphase; and, since the second explanation cannot be applied in this case, we must realise that the length of the spindle at the conclusion of the primary spermatocyte metaphase is not proportional to the volume of the cell. But the first and second explanations were put forward to support the proposition that the spindle-length at this stage is proportional to the volume of the cell: neither explanation need therefore be discussed further.

Two explanations remain to be considered. One implies a modification of my original proposition in that the length of the spindle at the conclusion of the metaphase is said to depend upon both the volume and the shape of the cell; the other denies that it is controlled by this combination. The explanations are accordingly antithetical, and proof of one must constitute disproof of the other.

The only satisfactory method of dealing with this question is to compare cells in which both factors are identical, or cells in which one factor differs; if the suggested connection is not to be disproved, we must find similar spindle-lengths in the former, and different spindle-lengths in the latter respectively. And, in either case, the cells compared must be spherical: a rough estimate may suffice to prove that cells of various shapes differ in volume; but we cannot hope for accuracy sufficient to prove that in certain cases the volumes of such cells are identical. Unfortunately, my material is unsuited to this investigation; for the cells are closely packed together in the cysts, and are consequently distorted. We must therefore defer consideration of the two remaining explanations until suitable material is available.

CONCLUSION.

The present research is now finished, and we have seen that the results obtained contradict the proposition put forward in my earlier paper upon this organism. I have carefully checked the measurements that led me to put forward this proposition, and have found them to be accurate; but the new evidence before us shows that it cannot be universal. In the circumstances, another negative proposition seems to have been established concerning the spindle. We have found that it is not a figure formed entirely by the action of forces at its poles; we have found that its length at the conclusion of the metaphase is not proportional to the volume of the chromatin; and we have now found that the length at this stage is not proportional to the volume of the cell. We must, however, remember that these negative propositions have been established for individual cases, and are therefore generalisations only in that their antitheses cannot be put forward as being invariably valid.

Furthermore, we have seen that the volumes of the primary spermatocyte cells of *Forficula* vary in the metaphase; and we have no reason for supposing that such variation is confined to this organism. In the circumstances, a comparison of the volumes of spermatocyte cells in different organisms cannot prove specific similarities or dissimilarities until we have satisfied ourselves that the volumes do not vary, or until we have ascertained the limits of variation in each case.

I hope to deal further with these questions in a subsequent paper. The spindle is a phenomenon of mitosis; and, if we can discover the factors that determine its length, we shall be one step nearer an understanding of its nature and the phenomena that are inseparably connected with it.

SUMMARY.

(1) The length of the spindle at the stage immediately preceding the conclusion of the primary spermatocyte metaphase is not a constant.

(2) The length of the spindle at the conclusion of the primary spermatocyte metaphase is not a constant, and is sometimes smaller than that observed at the stage immediately preceding the conclusion.

(3) The length of the spindle in the early primary spermatocyte anaphase is not proportional to the amount of chromosome divergence, and is sometimes smaller than the lengths observed at the stages mentioned in (1) and (2).

(4) The volumes of the primary spermatocyte cells vary in the metaphase.

(5) The length of the spindle at the conclusion of the primary spermatocyte metaphase is not proportional to the volume of the cell.

(6) The length of the spindle at the stage immediately preceding the conclusion of the secondary spermatocyte metaphase is not a constant.

(7) The length of the spindle at the conclusion of the secondary spermatocyte metaphase is not a constant, and is sometimes smaller than that observed at the stage immediately preceding the conclusion.

(8) The length of the spindle in the early secondary spermatocyte anaphase is not proportional to the amount of chromosome divergence, and is sometimes smaller than the lengths observed at the stages mentioned in (6) and (7).

April, 1914.

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EXPLANATION OF PLATES 1 AND 2,

Illustrating Mr. C. F. U. Meek's paper on "A Further Study of the Mitotic Spindle in the Spermatocytes of *Forficula auricularia*."

PLATE 1.

Fig. 1.—Section of cysts containing primary spermatocyte cells in various stages of mitosis. Section stained with iron-hæmatoxylin.

Fig. 2.—Section of cyst containing primary spermatocyte cells undergoing mitosis. Section stained with iron-hæmatoxylin and eosin.

Figs. 3 and 4.—Sections of cysts containing secondary spermatocyte cells in various stages of mitosis. Sections stained with iron-hæmatoxylin.

Figs. 5 and 6.—Ditto. Sections stained with iron-hæmatoxylin and eosin.

PLATE 2.

Fig. 7.—Polar view of primary spermatocyte cell in the metaphase, showing twelve chromosomes.

Fig. 8.—Ditto. (Taken from section of cyst shown in fig. 1.)

Figs. 9 and 10.—Lateral view of primary spermatocyte cell in the metaphase; the chromosomes are constricting. Length of spindle 11.25μ .

Fig. 11.—Ditto. Length of spindle 11.5μ .

Fig. 12.—Ditto. Length of spindle 12.0μ .

Fig. 13.—Ditto. Length of spindle 12.25μ .

Fig. 14.—Ditto. Length of spindle 12.5μ .

Fig. 15.—Ditto. Length of spindle 12.75μ . (Taken from section of cyst shown in fig. 1.)

Fig. 16.—Ditto. Length of spindle 12.75μ .

Fig. 17.—Ditto. Length of spindle 13.25μ .

Fig. 18.—Ditto. Length of spindle 14.0μ . (Taken from section of cyst shown in fig. 1.)

Fig. 19.—Ditto. Length of spindle 14.5μ .

Fig. 20.—Ditto. Length of spindle 14.75μ .

Fig. 21.—Ditto. Length of spindle 16.0μ .

Fig. 22.—Ditto. Length of spindle 16.25μ .

Fig. 23.—Ditto. Length of spindle $16.5\ \mu$. (Taken from section of cyst shown in fig. 1.)

Fig. 24.—Ditto. Length of spindle $16.5\ \mu$.

Fig. 25.—Ditto. Length of spindle $16.75\ \mu$. (Taken from section of cyst shown in fig. 2.)

Fig. 26.—Ditto. Length of spindle $17.5\ \mu$.

Fig. 27.—Lateral view of primary spermatocyte cell in the early anaphase, showing divergence of daughter-chromosomes. Length of spindle $11.25\ \mu$.

Fig. 28.—Ditto. Length of spindle $11.5\ \mu$.

Fig. 29.—Polar view of secondary spermatocyte cell in the metaphase, showing twelve chromosomes. (Taken from section of cyst shown in fig. 3.)

Figs. 30 and 31.—Ditto.

Fig. 32.—Lateral view of secondary spermatocyte cell in the metaphase; the chromosomes have not completed constriction. Length of spindle $6.75\ \mu$.

Figs. 33 and 34.—Ditto. Length of spindle $7.0\ \mu$.

Fig. 35.—Ditto. Length of spindle $7.5\ \mu$.

Figs. 36 and 37.—Ditto. Length of spindle $7.5\ \mu$. (Taken from section of cyst shown in fig. 3.)

Fig. 38.—Ditto. Length of spindle $8.75\ \mu$.

Fig. 39.—Ditto. Length of spindle $9.0\ \mu$.

Fig. 40.—Ditto. Length of spindle $10.25\ \mu$. (Taken from section of cyst shown in fig. 6.)

Fig. 41.—Ditto. Length of spindle $10.25\ \mu$. (Taken from section of cyst shown in fig. 5.)

Fig. 42.—Ditto. Length of spindle $11.25\ \mu$. (Taken from section of cyst shown in fig. 5.)

Fig. 43.—Lateral view of secondary spermatocyte cell in the early anaphase, showing divergence of daughter-chromosomes. Length of spindle $8.25\ \mu$. (Taken from section of cyst shown in fig. 3.)

Fig. 44.—Ditto. Length of spindle $8.75\ \mu$. (Taken from section of cyst shown in fig. 4.)

Fig. 45.—Ditto, at a slightly later stage. Length of spindle $10.75\ \mu$. (Taken from section of cyst shown in fig. 4.)

Fig. 46.—Divisions of stage micrometer, $10\ \mu$ apart, showing magnification of figs. 1 to 6 inclusive.

Fig. 47.—Ditto, showing magnification of figs. 7 to 45 inclusive.

The Embryonic Area and so-called "Primitive Knot" in the Early Monotreme Egg.

By

Professors J. T. Wilson, F.R.S., and J. P. Hill, F.R.S.

With Plate 3, and 1 Text-figure.

SINCE the appearance, in 1906, of our "Observations on the Development of *Ornithorhynchus*" (1) there have appeared for the most part only isolated comments upon the facts there set forth and on our interpretation of them. This is doubtless owing to the absence or the extreme rarity of such material as might serve as a reliable basis for criticism of our results.

Such commentary as has been forthcoming has mainly had reference to a feature on which we laid considerable stress, namely, the apparent co-existence of a primitive knot, which, in consequence of its general similarity to that of reptiles, we interpreted as an archenteric knot, with a quite independent primitive streak. Having arrived at this conclusion, we were forced to interpret later phenomena in its terms. This involved an identification of the obvious Hensen's knot of a subsequent stage (our "post-gastrular") with the earlier primitive knot. We therefore sought to explain how the latter structure might come to be included in the later embryonic area by a process of forward extension of that proliferative area which in the earlier phase extends from, and is traversed by, the primitive streak.

Our interpretation of the primitive knot of *Ornithorhynchus* has been challenged both by Dr. Assheton and Prof. Keibel, who have, independently of one another, suggested a different explanation.

In a reference to our paper in a footnote to his account of the mammalian germ-layers, Keibel (2) makes the following observation: "In their [Wilson and Hill's] opinion the primitive node, which marks the position of the blastopore, comes into relation with the primitive streak only secondarily; originally it lies outside the embryonic shield. Yet it seems to me questionable if the structure which the authors regard as the primitive node in early stages is the same structure which they so designate in later stages. I have wondered whether the primitive node of younger stages may not be the yolk-navel."

Substantially the same position was taken up by Assheton in the course of his criticism of Hubrecht's views on the early ontogenetic phenomena in mammals. In this contribution (3) Assheton has gone beyond mere suggestion of an alternative interpretation, and has endeavoured to establish it both by an independent critical examination of our own facts and findings and by further evidence of what he regards as similar occurrences in the blastoderm of Sauropsida. He has also endeavoured to bring the facts as interpreted by him into line with the Entherian condition as manifested, e. g. in the rabbit.

Still more recently, Assheton has returned to this subject in a paper on "Tropidonotus and the Archenteric Knot of Ornithorhynchus" (4), in which he exhibits a striking parallel in respect of a knot-structure between the reptilian and prototherian blastoderms. "If," he says, in this latest contribution, "my comparison is a correct one, the archenteric knot of Ornithorhynchus with its anterior and posterior lips of the blastopore, and its 'commencement of true archenteric invagination' may be dismissed, and another stumbling-block will be removed from the path of the student of mammalian embryology" (p. 634).

In view of these important criticisms of our previously expressed views, we feel it incumbent upon us to indicate our opinion of their validity. We should, indeed, have done so at an earlier date had it not been for the difficulties in

the way of collaboration at the Antipodes and the absence of any specific occasion for a further publication. Such an occasion has now, however, offered itself in the form of an opportunity of together investigating another egg of *Ornithorhynchus*, of which some account will be given further on in this paper.

We now desire to say at the outset that even prior to the appearance of Assheton's second paper (4) we had both, independently of one another, become convinced of the justice of the main contentions of Keibel and Assheton and of the general adequacy of their re-interpretation of the condition we recorded as existing in the *Ornithorhynchus* blastoderm. Our "primitive" or "archenteric" knot in the early egg of *Ornithorhynchus*, we are now prepared to regard as a yolk-knot or yolk-navel, as one might term it, a structure to be explained on the general lines suggested independently by Keibel and Assheton.

The interesting parallel which Assheton (4) has recently traced between the "primitive knot" of *Ornithorhynchus* and a similar structure in *Tropidonotus* seems to afford a further convincing proof of the homology he had previously established, and the question that remains for us with regard to the knot itself merely concerns the detailed interpretation of the various parts of the structure and the manner of its production.

Whilst it can never be wholly palatable to have to confess to an error of interpretation, it is no small mitigation in this case to recognise that the developmental processes described by us in *Ornithorhynchus* now assume a less complicated aspect. We feel, with Assheton, that "another stumbling block has been removed from the path of the student of mammalian embryology."

When we come to consider the modifications in our former work necessitated by the newer point of view, we are surprised to note how circumscribed the error really is, and how little it affects the major part of our investigation. It is true that the mistaken interpretation occupies a very prominent

position in our work. But this was due rather to its apparent intrinsic interest and importance than to any really fundamental significance. Its withdrawal does not after all seriously violate our general conception of monotreme development outside the area of the error itself.

It is, of course, our interpretation of the so-called gastrular stage and of its supposed relationship to the stages immediately preceding and succeeding that is practically alone affected. Withdraw that interpretation and our descriptive account of these other stages remains valid and open to a less extraordinary and in some sense easier and more natural explanation.

The modifications of our former publication (1) which are demanded by our change of opinion may best be summarised by indicating the necessary amendments in the several published summaries on pp. 59-61, 90-91, and 116-17, of that work.

On p. 60, (*e*), the "primitive streak-area" here referred to must be regarded as an embryonic area, whilst the "axial thickening of the mesoderm" can only be a so-called "head-process." In (*f*), the "primitive knot" here referred to must be interpreted in terms of Assheton's and Keibel's suggestion.

Otherwise this summary holds good.

On pp. 90-91, the only amendment required is the deletion of proposition (*d*).

On pp. 116-117, the propositions expressed under the letters (*a*) to (*h*) can no longer be maintained. The remainder of this summary, in our opinion, still holds good. The conception implied in the term archenteron may be open to discussion, but our employment of the term is not now—and was not formerly—dependent merely on the view now discarded.

We have again examined the sections of the blastoderm of our former specimen "Q" in the region of the axial thickening of the mesoderm in front of the anterior end of the primitive streak. We now agree with Assheton that the embryonic area of our Text-fig. 7 expands into that of our

Text-fig. 8, as corresponding areas do in other mammals and in birds; whilst, as above noted, we now regard the axially thickened forward extension of the mesoderm in front of the primitive streak as a "head-process," and therefore as being directly related, as an early phase, to the long "archenteric" process of Text-fig. 8. Figs. 14-16 on Pl. 5 of our previous memoir represent cross-sections through the axial mesoderm in question. In our Text-fig. 7 the anterior limit, represented as that of the primitive streak, was fixed by, and actually indicates, the separation of the ectoderm from the mesodermal cell-thickening beneath. It thus really corresponds to the anterior limit of Hensen's knot, and any axial thickening of mesoderm in front of this must thus be "head-process." In our specimen "Q," such an axial thickening can be traced forwards in the sectional series through 42 sections in front of Hensen's knot. Thus the "head-process" should be plotted in, in Text-fig. 7, as extending 2.7 mm. (on the paper) in front of what is shown there as primitive streak, but whose anterior end actually represents Hensen's knot. It is to be noted, however, that neither the lateral nor the anterior limits of this head-process are sharp, but merge gradually into the thinner mesodermal sheet.

The absence from our collection of material of any stage which we could look upon as the immediate forerunner of our "gastrular" stage has been throughout a matter for regret. The specimen "a" of our paper was the only one at our disposal which at all approximated towards the gastrular. As appears from our paper, the examination of this egg showed completion of the bilaminar blastoderm, i. e. complete establishment of a bilaminar blastodermic vesicle in the mammalian sense, and one area of proliferative activity over the white yolk pole. This area showed a thickish cell-plate (our Pl. 2, text-fig. 5), forming a patch of about .5 mm. in its greatest diameter, which we took to be "the initial stage in the formation of the primitive knot." This opinion can no longer be maintained, and we are compelled to regard this area as simply the embryonic region of the blastoderm in an

early phase of its differentiation. The "accumulation of cells of irregular shapes and sizes, which appear to be actively proliferating," and which we formerly noted as existent "beneath the surface layer of the embryonic patch," and in proliferative continuity with the same, may very well represent the earliest product of that proliferative activity which gives origin to the primitive streak.

The strangest feature of the specimen as now interpreted is the entire absence of any trace of a yolk-navel or any equivalent structure. We have re-examined our material most carefully, and have found no trace whatever of any area which we could interpret as the site of coalescence of the margins of the blastoderm. In particular we can positively state that there is no such trace over the lower hemisphere of the egg. In the examination of a relatively large spheroidal structure, which necessarily has to be divided up for examination, it is impossible to be absolutely certain that nowhere near the lines of division could there have been some such trace. But at least none has been discoverable after the closest search; and we are, therefore, no nearer the solution of the problem of the yolk-knot after than before the examination of specimen "a."

The opportunity of examining another egg intermediate between our former specimens "a" and "Q" was, therefore, a very welcome one.

This egg, which appears in our list under the letters GW., was placed at our disposal by Prof. Gregg Wilson of Belfast, whose generous courtesy we desire here to acknowledge.

The egg was obtained at Gayndah, Queensland, in 1898, and was fixed in corrosive sublimate. As received by us it was somewhat collapsed and showed a rupture on one side. In this condition it measured 8.5×7.5 mm.

After cutting through the shell from the ruptured area, the blastocyst was separated in a more or less shrunken and collapsed state, and in this condition occupied a space of about 5.5×4 mm. It contained disseminated yolk material as well

as a more coherent yolk-mass, ovoidal and flattened in shape and about 4×2 mm. in diameter.

On examination in alcohol, an embryonic area traversed by a primitive streak was recognisable. It exhibited a primitive groove over about the anterior half of its extent. The length of the streak—neglecting the curvature of the blastoderm—was approximately 2.75 mm. and its breadth 0.3 to 0.4 mm. Throughout the greater part of its extent the primitive streak region showed as a longitudinal prominence on the surface of the somewhat shrunken blastoderm. This prominence was sharply accentuated at the anterior end of the streak, which here appeared to terminate in a knob-like thickening, representing a definite "Hensen's knot." The latter was readily recognisable by transmitted as well as by reflected light. Under transmitted light it appeared as a more opaque circular patch at the anterior extremity of the streak. It appeared as if definitely limited in front and no trace of a "head process" was perceptible on examination *in toto*. About 0.5 mm. anterior to Hensen's knot, a small local accumulation of yolk-spheres, about 0.5 mm. in diameter, was adherent to the deep surface of the blastoderm. Subsequent examination of the sections has not led us to attach any special significance to this patch.

After removal of the lower (antembryonic) polar area of the blastodermic vesicle, we sought very carefully for a yolk-knot or some equivalent appearance, but entirely without success. And we may at once state that the subsequent examination of the sections equally failed to afford any evidence of the existence of such a structure. As in the case of our former specimen "*a*," we are quite satisfied that no differentiated area of the kind was present over the lower polar area. For the same reasons as in the case of "*a*," there must remain a shade of uncertainty as regards the immediate vicinity of the lines of division of the blastoderm. At all events, we could detect nothing which even remotely suggested a yolk-knot, or indeed any differentiation other than the embryonic area itself. It is certainly most remarkable

that this should be the case when it is remembered in all four specimens of the only slightly later stage which we termed "gastrular" a relatively conspicuous yolk-knot (our "primitive knot") was found.

After preliminary infiltration in a 0.25 % solution of cedar oil-phoxoilyn, the upper hemisphere of the blastocyst was divided transversely into anterior and posterior segments and both portions as well as the lower hemisphere were embedded in paraffin and cut into serial sections of 8 μ . The total antero-posterior extent of the two portions of the upper hemisphere was represented by about 798 sections or just under 6.5 mm.

Examination of the sectional series showed that the primitive streak together with Hensen's knot extended through about 416 sections, or practically 3.3 mm. The anterior limit of Hensen's knot was easily defined by the abrupt cessation of its prominent convexity. But contrary to the suggestion of the surface examination an axial thickening of mesoderm extended forwards from the knot, forming a "head-process" of quite similar character to that we now recognise as existent in specimen "Q" of our former paper (cf. supra, p. 19). As in the latter case, so also here, this axial thickening of mesoderm is continuous, both bilaterally and in a forward direction, with the thinning out mesodermal sheet. As a "head-process" or recognisable axial thickening, it may be traced forwards for just one-third of a millimetre in front of the plane of its continuity with Hensen's knot.

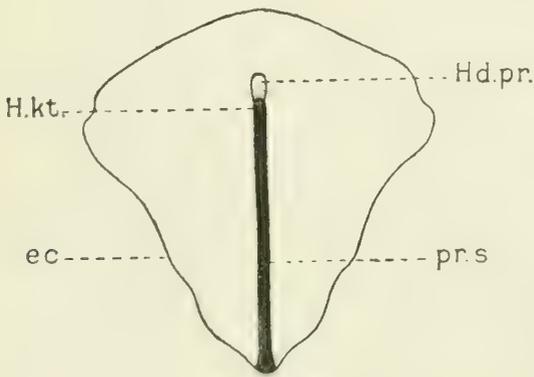
It is impossible in this specimen to determine definitely the hinder limit of Hensen's knot. As we formerly showed, such a limit does, at least at a later period, become distinguishable, probably as a result of inequalities in the growth rates of the different regions of the axial differentiation. In the present instance, a markedly convex prominence of the axial region of the embryonic area continues backwards from Hensen's knot, and the latter appears to merge gradually in the primitive streak tissue. It may be noted, however, that

examination of the blastoderm by transmitted light did indicate a posterior mass-limitation of Hensen's knot.

The sections show the entodermal sheet as everywhere complete. It continues across the embryonic region, underlying both the primitive streak and Hensen's knot. It is yolk-laden throughout, although, axially, where it underlies the latter structures, its cells appear as if more delicate in texture, suggesting a more active yolk-sphere digestion.

The yolk-entoderm of the general interior of the vesicle is of a character quite similar to that formerly described in

TEXT-FIG. 1.



Scheme of embryonic region of *Ornithorhynchus* Egg, GW, plotted to scale from the serial transverse sections. ($\times 625$)
ec. Outer limit of area of thick ectoderm. *H. kt.* Region of Hensen's knot. *Hd.-pr.* "Head-process." *pr. s.* Primitive streak.

nearly related stages. For purposes of comparison with the Text-figs. 7 and 8 of our previous paper, we have plotted the embryonic region of this specimen at the same magnification as that formerly employed (Text-fig. 1).

The embryonic area here shown is that definable by a thickening of the ectoderm, but here, as in the case of the other specimens, there is no abrupt line of demarcation, since the transition to the uniform thin ectoderm of the rest of the vesicle is a gradual one. The definite periphery shown must be accepted with this qualification. We have not thought it necessary to plot the limits of extension of the mesodermal

sheet. As might be expected, the extent of this sheet is considerably less than in our specimen "Q" (Text-fig. 7 of our previous paper). It is also distinctly weaker than in the latter case.

We have, however, plotted in, in front of the anterior limit of Hensen's knot, the extent of the axial mesodermal thickening or "head-process." Our former Text-fig. 7 (of specimen "Q") ought now to be amended, as we have already stated, by the insertion of a similar outline of the extent of the corresponding axial mesodermal thickening or "head-process," which we illustrated in the sectional Pl. 5, figs. 14-16, of our paper. If Text-fig. 7 were thus amplified, the "head-process" would appear as a projection in front of the anterior end of the primitive streak (really, here, Hensen's knot), extending for a distance in the figure of 2.7 mm. The breadth of the extension thus plotted would be about 4 mm. It is to be noted, however, that neither the anterior nor the lateral limits of the "head-process" so represented are sharply defined, but merge gradually in the thinner mesodermal sheet as seen, e. g. in our former Pl. 5, fig. 15.

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2. Keibel, F., and F. P. Mall.—"Manual of Human Embryology," Lippincott Co., 1910, vol. i, pp. 46-47, footnote.
3. Assheton, R.—"Professor Hubrecht's paper on the Early Ontogenetic Phenomena in Mammals," '*Quart. Journ. Micr. Sci.*,' vol. 54, 1909-10.
4. ———. "Tropidonotus and the 'Archenteric Knot' of *Ornithorhynchus*," '*Quart. Journ. Micr. Sci.*,' vol. 54, 1909-10.

EXPLANATION OF PLATE 3,

Illustrating paper of Professors J. T. Wilson, F.R.S., and
J. P. Hill, F.R.S., on "The Embryonic Area and so-called
'Primitive Knot' in the Early Monotreme Egg."

Photomicrograph of embryonic area of Ornithorhynchus egg,
(G. W.). $\times 25$ diam.

The Larva of the Starfish *Porania pulvillus*
(O.F.M.).

By

James F. Gemmill, M.A., D.Sc.,

Lecturer in Embryology at Glasgow University, and in Zoology
at Glasgow Provincial Training College.

With Plates 4 and 5.

CONTENTS.

	PAGE
EARLY STAGES	28
Spawning, etc.	28
Origin of Enterocœles	31
Double Hydropore	31
The Ciliated Bands	32
Posterior Enterocœlic Outgrowth	32
THE BIPINNARIA AND BRACHIOLARIA	35
External Characters	35
Comparison with other identified Larvæ	36
Feeding	37
Locomotion	37
Growth of the Enterocœles	38
The Hydrocœle	39
Pharyngeal Cœlom	40
The Dorsal Sac	40
Aboral Arm-rudiments	40
Neuro-muscular Fibres	41
Fixation	42
Probable Course of Metamorphosis	42
Double Hydrocœle	43
CONCLUSIONS	44
REFERENCES	45
EXPLANATION OF PLATES	47

IN its general outlines the larval history of *Porania* resembles that of *Asterias rubens*, L., recently described by me in a paper (7), to which reference may be made throughout the following, for data regarding corresponding stages and structures in the development of other Asterids. The methods employed in order to obtain the larvæ were the same as in the case of *A. rubens* (7, p. 224), the fertilisations being made at the Millport Marine Biological Station, and the rearing carried out in the Embryological Laboratory at Glasgow University.

EARLY STAGES (Pl. 4, figs. 1-12, 17-19).

The **gonads** are bunches of short, simply-branched tubes, reddish-yellow in the female, whitish in the male, confined to the interradial, i. e. not extending outwards into the arms. As many as 150 of these tubes may be present in each gonad in large specimens. At the root of the gonad a single duct, after receiving the openings of four or five short side ducts, passes obliquely outwards through the greater part of the thickness of the body-wall and then expands to form a small dilatation, which opens on the surface by six or seven tiny apertures.

The eggs are very slightly larger than those of *A. rubens*, and contain rather more food yolk. They are brownish-yellow in transmitted and also in reflected light, the depth of the tint varying in ova from different individuals. **Spawning** has not been observed, but the presence of a few fertilised eggs in an isolated tank containing several adult *Porania* was noted at the Millport Marine Station in the beginning of June, 1913. One practically never finds ripe ova in the gonads of starfishes, and *Porania* is no exception to the rule, but during a period extending from the end of April till the middle of June, the ovaries contain abundance of eggs, which, if shredded out into sea-water, will undergo the characteristic maturation changes and be capable of fertilisation and subsequent normal development. We may call this

period one of facultative ripeness. Probably in nature, spawning occurs twice or thrice during the period named, some physiological stimulus coming in from time to time to induce ripening and extrusion of a batch of eggs. In *Solaster endeca* (6, p. 9), *Asterias rubens* (7, p. 220), and *A. glacialis* (7, p. 221) the eggs undergo maturation while spawning is actually in progress.

The **maturation changes** are practically the same as in *A. glacialis* (9), and *A. rubens*, and experimentally we find that they are completed in from three to four hours after the eggs have been teased out into sea-water. If sperm is now added, the membrane of fertilisation begins to form almost immediately (within two minutes), and is completely separated within five to seven minutes, while one or both of the polar bodies lie external to the membrane of fertilisation. If the sperm be introduced immediately after the ova have been shredded out, impregnation occurs prior to the extrusion of the first polar body, and accordingly both of these bodies are found inside the membrane of fertilisation. When much spermatic fluid is added, the eggs are rotated, oscillated, or otherwise moved about under the action of the crowding spermatozoa, as in the case of *A. rubens* (7, p. 203), *A. glacialis*, and *A. vulgaris* (1, p. 6).

The first and second **divisions** are equal, the former occurring, in the case of ova already ripe, about two and a half hours after the addition of sperm, and the second about two hours thereafter. The four cells lie in the same horizontal plane. The third division is usually not perfectly equal, but gives rise to four slightly smaller and clearer cells lying above four others which are very slightly larger and less transparent. During the succeeding segmentation stages there is little or no trace of an internal cavity, the **morula** (eighteen hours) being practically solid (Pl. 4, fig. 1). At the end of about thirty hours the formation of the hollow **blastula** is in active progress. This process is marked (Pl. 4, fig. 2) by egression of the central cells along lines, which, appearing at first as superficial grooves, afterwards become smoothed out,

leaving the blastocœle empty of cells. The process resembles that which occurs on a much larger scale in *Cribrella* (Masterman, 13, p. 378), and *Solaster* (Gemmill, 6, p. 12). Mortensen (15) has also described it as characteristic of the *Luidia* blastula, which is similar in size to that of *Porania*. Meantime, the membrane of fertilisation which hitherto surrounded the developing egg has become lost, and cilia having appeared on the surface, the blastula begin to move about (thirty-six hours). The posterior or blastoporic end of the larva has also become evident through flattening, opacity, and slightly greater thickness of the part which will become invaginated to form the archenteron (Pl. 4, fig. 3). **Gastrulation** (third and fourth days) is accompanied by rapid lengthening of the larva. This characteristic feature is early present in *Porania* and becomes still better marked in the immediately succeeding stages (Pl. 4, figs. 5, 6), although the late bipinnaria and the brachiolaria (Pl. 5, fig. 21) are, if anything, rather shorter than those of *A. rubens*. The wall of the archenteron is somewhat thick and opaque (Pl. 4, figs. 4-6), no doubt owing to the fact that, to begin with, the egg possessed an appreciable quantity of darkly-tinted yolk.

The blind end of the archenteron now dilates slightly; its walls become thinner and more transparent than the rest of the archenteron, and bud off stellate **mesenchymal cells**, which wander in all directions through the blastocœle. There is no apical thickening which could be interpreted as sensory or nervous at this stage.

We next find (fifth and sixth days) that the blastopore migrates ventralwards (Pl. 4, fig. 7) causing a dorsally convex curvature in the archenteric canal, while on the ventral aspect of the larva, near the junction of its second and third fifths, there appears a slight ridge, indicating the transverse portion of the **preoral ciliated band**. A similar ridge soon becomes evident about a fifth of the length of the larva forwards from the posterior end, and marks the transverse portion of the **post-oral ciliated band** (Pl. 4, fig. 7). Between these ridges is the circumoral field or depression, within which

the **stomodæal pit** has appeared in the mid-ventral line just behind the preoral ridge. Meanwhile the dorsal portion of the blind end of the archenteron, which we may call the **enterocœlic portion**, is being constricted off, and at the same time is growing backwards on either side, while the bottom of the stomodæal pit becomes connected by mesenchymal cells with the ventral aspect of the blind end of the archenteron in the middle line. The walls of the stomodæal pit and of the archenteron are next laid against one another, and shortly afterwards break down, establishing the opening into the entodermic œsophagus. This occurs at much the same time as the separation of the enterocœlic sacs from the archenteron. Probably in the majority, and certainly in at least 50 per cent., of the larvæ the two enterocœles were seen to be in open communication with one another for a brief period after their constriction from the archenteron was complete (cf. Pl. 4, fig. 10). The connecting isthmus between the two enterocœles next divides and the sacs shift backwards, partly owing to migration and partly to greater relative growth of the anterior portion of the larva. As a rule the left enterocœle is slightly the larger, and it practically always acquires a **hydropore** (seventh day). This opening is formed by the enterocœle sending up a hollow process tipped by a small clump of cells to reach the epiblast, two or three cells of which at the place of contact become elongated. A lumen appears in the middle of these cells, as well as in the clump before-mentioned, and thus the hydroporic opening and canal are formed. In many cases the right sac acquires an entirely similar opening (Pl. 4, fig. 9), and in many others there is formation of the hollow process with its cap of cells in contact with thickened epiblast, but the lumen which should appear in the two structures last named is either absent or of minute size. The proportion of **double hydropores** of the first kind was approximately 30 to 40 per cent., while the second or modified form occurred in addition in almost equally large numbers, so that only about 25 per cent. of the larvæ showed no trace

of the formation of a right hydropore. A single instance was observed in which the hydropore occurred on the right side alone.

As regards the **archenteron**, we have now to note its differentiation into gullet, stomach, and intestine. As in *A. rubens* the stomodæum provides the buccal cavity, and the anterior fifth or so of the larval œsophagus:

Meantime the larval ciliated bands, of which the first indication was noted in the form of transverse ridges across the ventral aspect of the larva, have extended obliquely forwards on each side, and merge into an antero-dorsal area near the apex of the larva (Pl. 4, fig. 9). This area retains meantime its richer ciliation, and is provided with somewhat numerous gland cells. Probably it is in some degree sensory or nervous in character. The early elongation of the larva gives this area from the first a more anterior and seemingly apical position than it has in the case of *A. rubens*, where it is more dorsally placed, and might as well be compared with a supra-œsophageal as with an apical nerve centre (7, p. 232).

The whole of the blastula wall is richly covered by long cilia. In later development these cilia are not lost, though they become reduced in number and to some extent in activity over the general surface of the body, while by contrast they are powerful and closely massed together along the ciliated tracts.

The larva has now reached the stage shown in figs. 9 and 10. A most important point to notice is the formation of a small body which I take to be a rudimentary **posterior enterocœlic outgrowth** arising (seventh to eighth day) dorsally by proliferation of the stomach endoderm, usually on both sides of the middle line, but sometimes in the middle line, or on one side only (Pl. 4, figs. 9-11, 17-19). The tiny masses thus formed, which at first are somewhat dark-coloured like the endoderm, project into the blastocœlic space, but soon separate off from the stomach-wall and break up into mesenchyme-like cells, some of which join the posterior wall

of the enterocœle, while others do not. Very rarely the mass forms a connection with the enterocœle while still joined on to the stomach. I cannot find that it develops a central cavity of its own, which is afterwards added on to the cavity of the enterocœle, as occurs in the case of *A. glacialis* (7, p. 233). When only one body is present it occurs rather oftener on the right than on the left side, a contrast to the condition in *Asterias rubens*, where the corresponding body (a still smaller one) appears most commonly on the left side (7, p. 233).

Striking differences are recognisable in the mode of origin of the cœloms throughout Echinoderms and in Enteropneusts. To illustrate these we need only refer to the fact that in *Echinus esculentus* (11), and in *Ophiothrix* (12), the various enterocœlic cavities on each side are derived from an anterior outgrowth, while in *Cribrella* (13), and *Solaster* (6), the cavity which becomes the hypogastric cœlom has a posterior origin from the archenterom. Similarly in the New England *Tornaria* according to Morgan (14) and in *Balanoglossus* Kow. according to Bateson (2), there are separate origins for the proboscis cœlom, and the collar and trunk cœloms, while in *Dolichoglossus*, according to Davis (3), these three cavities take origin from a single anterior outgrowth. MacBride in 1896 (10, p. 397) put forward the conception of a group ancestral to the Echinodermata, Enteropneusta and Chordata, which he named the "Protocœlomata," the characteristic feature of the group being the presence of three cœlomic cavities, viz. an anterior (probably unpaired), and middle and posterior (paired) cavities. It seems to me that we must extend this conception so far as to postulate **potential independence** of origin of the middle and posterior cœloms in the descendants of the group. We recognise among Echinoderms, on the one hand, a tendency for the separate origins of the middle and posterior cavities to be replaced by backward extension of the anterior one. This has been effective in *Echinus*, *Ophiothrix*, *Asterina gibbosa*, *A. rubens*, *A. glacialis* and *Porania*, although rudiments of the posterior outgrowth still remain

in the three species last named. On the other hand, in *Cribrella* and *Solaster*, as I believe, the posterior outgrowth has appeared only on the left side, giving rise to the hypogastric coelom, of which the morphological equivalent on the right side, namely, the epigastric coelom, is derived from the anterior vesicle. In *Antedon* the appearances described can be interpreted by assuming a posterior origin for the right and left posterior coeloms. In *Ophiura brevispina*, if Grave's account be correct (8), we have an example in which the hydrocele arises by forward extension from the posterior vesicle on the left side. It need hardly be added that the same principle, i. e. primitive independence of origin of the three primary coelomic cavities in the ancestral form, also throws light on certain peculiarities in the mode of origin of the coeloms among the chordate descendants of the Proto-coelomata, for example, the separate origin of the anterior coelomic cavity in *Amphions*.

The following variations in the development of the enterocoelic vesicles are of interest: (1) a united condition of the two vesicles across the mid-dorsal line, not within the preoral lobe, but opposite the middle of the oesophagus, and at a period earlier than that at which union of the anterior ends of the two enterocoeloms should take place. This condition was noted in several larvæ during the second and third weeks, and although the earlier history of these individual larvæ had not received particular attention, it is in every way probable that the united condition was due to a failure on the part of the vesicles to become separated from one another at their time of origin. These larvæ survived for a considerable time, but did not differentiate further. (2) Presence of a small median coelomic cavity in the preoral lobe above the buccal cavity at a time when the two anterior ends of the right and left enterocoeloms are still far from the stage at which they unite with one another in the preoral lobe. In this case the transverse isthmus between the right and left enterocoeloms present at their time of origin seems to have persisted and become an independent cavity. (3) Failure on the part of

the stomodaal pit to effect union with the anterior end of the archenteron (Pl. 4, fig. 11). The growth of the frontal region became arrested while the rest of the larva continued to develop for a time. In all such instances coming under my notice, the right and left enterocœles were connected across the middle line by an isthmus situated to the ventral side of the blind end of the entodermic œsophagus. Larvæ of *A. rubens* with the same initial malformation showed the two enterocœlic vesicles separated from one another.

We have, in these different *Porania* variations, evidence of a single origin for the enterocœles which if not constant in the species was at any rate present in the individuals afterwards showing the abnormalities described.

THE BIPINNARIA AND BRACHIOLARIA. (Pl. 4, figs. 13-16, and Pl. 5, figs. 20, 23.)

The general appearance of the early and advanced bipinnaria, and of the brachiolaria is shown in the figs. referred to above. The late period (about the seventeenth day) at which the anterior ends of the preoral and postoral ciliated bands are finally differentiated and become separated from one another is a characteristic feature when comparison is made with *A. rubens*. It is also characteristic that until the median brachium has attained a considerable size, the anterior portions of the preoral and postoral ciliated bands should reach forwards for approximately the same distance. Accordingly we do not have at any stage a markedly projecting median dorsal lobe or process, as in the larva of *A. rubens*. The various ciliated processes characteristic of starfish larvæ are all present, but do not reach such full development as in the case of *A. rubens* and *A. vulgaris*. In particular the postero-lateral processes remain small. The preoral and the anterior dorsal processes are relatively the largest. The median dorsal process is small from the first, and in late brachiolaria becomes reduced almost out of existence, forming simply a ridge on the back of the anterior brachium (Pl. 4, fig. 16).

There is a distinct middle dorsal lobe or lappet on the post-oral ciliated band opposite the mouth on either side. This lappet, although not provided with an outgrowing ciliated process, corresponds, I believe, with the middle dorsal lobe of the auricularian larva, and is seen also in the brachiolaria of *A. rubens* (7, p. 235) and *A. glacialis* (7, p. 235).

The brachia develop in the usual manner, beginning to appear about the fortieth day. The anterior one carries over it, as it grows out, the apex of the preoral ciliated band, while the lateral ones show a similar relation to the band further back. The brachia have an arrangement of papillæ, which at once marks out the *Porania* larva from that of *A. rubens* (Pl. 4, fig. 16, and Pl. 5, fig. 21). The extremities of the brachia are not truncated, but rounded, and are provided with ten or twelve papillæ, while about the same number of papillæ occur on each side in a row down the anterior brachium past the sucker and up the lateral brachia. It was during the seventh and eighth weeks that the brachiolaria reached their full development, the length attained being about 2.4 mm. The period of growth was thus longer and the ultimate size less than in the case of *A. rubens* (six to seven weeks and 3 to 4 mm. respectively).

The only starfish larvæ known to possess an arrangement of papillæ at all resembling that of *Porania* are the brachiolaria of *Asterias glacialis*, and a brachiolaria from Messina figured and described by Joh. Müller (17), to which Mortensen has given the name *Bipinnaria papillata* (15, p. 44). In the former the papillæ appear to form a circle round the sucker and not to be continued up the sides of the arms. In the latter the brachia are flattened on the sides which look towards the sucker, while there is a very long dorsal median ciliated process. In *A. rubens* (7) and *A. vulgaris* (1), the brachia are truncate and their extremities carry six to eight papillæ; as a rule only two papillæ occur to each side of the sucker and there are none on the sides of the brachia.

The long cilia with which the preoral and post-oral bands are provided lash in a backward direction, as also do the cilia over the general surface of the preoral and post-oral fields. The larvæ, therefore, **progress** with the anterior end in front. The dextral rotation characteristic of the gastrula is retained, but becomes much slower. There is also associated with the rotation and progression a characteristic backward swerving movement of the anterior end of the body, which varies in amount in different larvæ and in general is more marked throughout the earlier than the later stages. On the whole, my larvæ of *Porania* showed a greater tendency all through to sink to the bottom and remain there than did those of *A. rubens*.

In general the **adoral ciliation** resembles that of *A. rubens* (7, p. 239). There is a peristomal ciliated ring, the dorsal part of which comes in contact with the transverse portion of the pre-oral ciliated band, while its mid-ventral portion forms the true lower lip, and from its sides there pass backwards two ridges which converge in the floor of the first part of the œsophagus. About halfway through larval life the ends of these ridges unite in the middle line, thus forming the loop, which, in the case of *A. rubens*, I distinguished as the œsophageal loop of the adoral ciliation (7). The interior of the buccal cavity and of the œsophagus retains its ciliated character, and the cilia act everywhere towards the stomach except on the lower lip, where, at times, if not always, their action seems to be in the outward direction. The space (circumoral field) between the preoral and the post-oral ciliated bands serves for the **gathering of food**, and the cilia on this field, though relatively few in number, are vigorous in action, and bring particles from all parts of the field to the corners of the mouth, directing them inwards under the overhanging upper lip formed by the transverse portion of the preoral ciliated band. This structure drives the particles into the buccal cavity, being aided by the peristomal ring except on the ventral segment of the latter. The activity of the cilia on the circumoral field varies very greatly

at different times according as the larva is accepting food or not. The cilia on the œsophageal loop also act towards the stomach, and their particular function appears to me to be that of striking back towards the bottom of the œsophagus particles which would otherwise be swept away by the currents of waste water flowing up the middle of the œsophagus and escaping over the lower lip, the cilia of which, as was noted above, can lash outwards. As compared with *A. rubens*, the œsophagus is somewhat capacious, especially in transverse measurement. There is variation of activity on the part of the cilia on the œsophageal loop in correspondence with the variation previously noted in the activity of the cilia on the circumoral area. Emptying of buccal cavity and first part of œsophagus can be produced, as in *A. rubens*, by quick backward flexion of the preoral lobe on the rest of the body.

We now take up the growth of the enterocœlic sacs and of the cavities, etc., to which they give rise. It was seen previously (p. 31) that a **right hydropore** is present in a large number of the early larvæ. Almost invariably the right hydropore becomes lost. Atrophy, constriction, and separation of the canal occur at its junction with the epiblast, a small thickened patch of the latter being left for a time at the point where the opening was originally present. A tiny clump of cells may also be observed for a time on the apex of that part of the right enterocœle which led towards the hydropore.

The two enterocœlic vesicles grow at a practically equal rate forwards into the preoral lobe, where they unite with one another, and also backwards, covering the sides of the stomach. A dorsal constriction appears on each side behind the level of the hydropore, and gradually passes ventrally, cutting off the **posterior cœloms**. Besides these we may now distinguish on each side an **anterior and a middle cœlomic region**,¹ which, however, are not separated off from

¹ The terms anterior and middle cœlomic regions are used here for descriptive convenience, and not as indicating separate morphological

one another (figs. 14, 21). It is the left middle cœlomic region which receives the persisting left hydroporic opening. Meantime the left posterior cœlom has sent round a **ventral horn** to the right side between stomach and rectum. This horn lays itself against and finally opens into the right middle cœlomic region, exactly as in *A. rubens*, except that the expansion of the horn to the right of the rectum is smaller than in the species named. The **hydrocœle pouches** appear as outward pockets of the wall of the l. m. c. (Pl. 5, fig. 21). We number² them according to the same system as the arms or rays of which they ultimately form a principal part. Pouches III and IV are the first to become evident, pouches II and V next, while pouch I is slightly later than the rest. In very advanced larvæ the rudiment of the **stone canal** groove may be seen passing from near the inner end of the hydroporic canal to the interspace between pouches I and II. Before this time a secondary opening has formed, as in *A. rubens*, between the dorsal horn of the l. p. c. and the left middle cœlom, the opening lying deeply between pouches I and II of the hydrocœle. The **rectum** becomes involved in a sheath derived from the l. p. c. by the growth from the ventral horn of hollow folds which meet and unite exactly as in the case of *A. rubens* (see 7 Pl. 18, fig. 7).

or ontogenetic units. Combined they represent the proboscis and collar cœloms of *Balanoglossus*, but not singly and respectively (7, p. 278). For example, that part of our left middle cœlomic region into which the hydropore opens (aboral part of axial sinus) is obviously homologous with a portion of the proboscis cœlom, as the hydropore is with the proboscis pore.

² The madreporite is counted as lying between rays I and II, the former being to its dextral and the latter to its sinistral side. Rays III, IV and V continue the series sinistrally from ray II, and inter-radius V/I is the anal inter-radius. The system is the same as that used by MacBride (10) and exactly the converse of that employed by Ludwig in Bronn's *Klassen*, and Masterman (13). The reasons for adopting it are given in detail elsewhere (7, p. 276). Throughout this paper dextral and sinistral indicate respectively the sides to which, or away from which, the hands of a watch would seem to move if looked at on the starfish disc from the aboral side.

It seemed, however, that in *Porania* the length of rectum surrounded in this manner was less than in the case of *A. rubens*.

Towards the end of the sixth week an outgrowth from the l. p. c. for the **pharyngeal cœlom** arises near the tip of the dorsal horn of the l. p. c. The outgrowth pushes first to the right then ventralwards within the mesentery separating the l. m. c. from the l. p. c., following the curvature of the hydrocœle rudiment. This corresponds with the mode of formation of the same outgrowth in *Asterina gibbosa* (10) and in *A. rubens* (7, p. 259).

Although I had not the opportunity of cutting and examining as full a series of stages as in the case of *A. rubens* (7, p. 246), it seems probable that in *Porania* the **dorsal sac** or madreporic vesicle arises from mesenchymal cells situated immediately to the right of the hydroporic opening, and that these cells are not derived from the wall of the pore canal or from the wall of the enterocœles, but date back to the origin of the general mesenchyme from the archenteron. As in *A. rubens*, the sac is sometimes connected in its early stages by a strand of cells with the wall of the pore canal. In *Porania*, **pulsation** of the floor of the sac was observed towards the beginning of the sixth week, the rate being much the same as in the case of *Asterias rubens*, viz. once every six or seven seconds to begin with, but slowing down to once in eighteen seconds or thereby prior to metamorphosis. During the resting intervals the floor of the sac becomes swollen irregularly upwards by fluid which gathers in the underlying tissue spaces. In each contraction the floor of the sac descends, displacing the contained fluid, and no doubt serving, however imperfectly, as a circulatory mechanism. On the whole, the sac is slightly larger and more definite than in *A. rubens*, and its pulsations can more readily be observed (see further on p. 44).

The aboral arm rudiments (Pl. 5, figs. 20, 21), arise in typical fashion around the posterior end of the larvæ, there being as in most other starfish larvæ a wide gap—the aboral

brachiolarian notch—between rudiments II and I. The dermal tissues of the arm rudiments and of the disc early become somewhat more opaque than in the case of *A. rubens*, making it less easy in living specimens to observe the nature of the calcifications for the first formed ossicles. But so far as I could make out, these arise in typical fashion and include the usual elements, namely, a single terminal for each arm rudiment, a single basal in each inter-radius and one dorso-central. While the dorso-central and four of the basals overlie the r. p. c. (epigastric cœlom), the basal belonging to inter-radius I to II is superficial to the dorsal sac. Numerous small calcifications for the spines appear in the superficial mesodermal tissues of the aboral body-wall, each calcification being embedded in a rather thicker cushion of tissue and forming a rather larger papilla on the surface than in the case of *A. rubens*. It is interesting to note that in the adult, spines are absent, except along the margin of the body, the ambulacral grooves and around the anus. However, if one clears the body-wall in the adult with oil of cloves or some such agent, minute calcifications may be found in the superficial layer all over the aboral surface, and these may be the remains of spines. A tiny dredged specimen 16 mm. across disc and arms showed quite definite spines projecting from the aboral surface.

In most of my late brachiolariaë, there could be made out a "skeleton" set of fine fibres, which looked like ordinary muscular fibrils in contraction, but did not, like the latter, disappear from view during relaxation. They showed the same arrangement in different individuals, and in particular they sent branches to the brachia and to all parts of the ciliated tracts. One could distinguish two nodes on each side, an anterior beside the base of the median dorsal lobe, and a posterior dorsally opposite the middle of the œsophagus. The anterior nodes were connected across the mid-dorsal line by a single fibre. The posterior nodes were less definite. Careful observation showed that the fibres shortened somewhat when the general muscular tissue of the larva was in

contraction. At the same time they were too few to effect movements by themselves, and the conclusion seems probable that we have here a set of neuro-muscular or neuro-epithelial fibres which are specialised for conducting impulses. They seem to have the same origin as the muscular fibrils, but there is always the chance that they may have arisen from epiblastic cells which migrated inwards. Possibly the occurrence of these fibres is the earliest manifestation of, or at any rate is related to, the presence of an entoneural nervous system in the adult.

Several brachiolariae entered on the stage of fixation, becoming attached first by the brachia and afterwards by the sucker as in *Asterias rubens* (7, p. 250). However, none had the vitality required to complete metamorphosis. In a short time they all went back as regards shape and texture, those which survived longest becoming little more than masses of round cells without structural differentiation. Although metamorphosis did not supervene, there is every reason to expect that it will conform with the procedure in *A. rubens*, certain features of which may be detailed here. The preoral lobe, etc., are retracted into the left side and this side gives rise to the oral surface of the starfish. The r. p. c. becomes the epigastric and the l. p. c. with the help of the pharyngeal outgrowth becomes the hypogastric cœlom; the right middle cœlomic (hydrocœlic) region disappears. The left middle cœlomic region gives rise to hydrocœle, axial sinus, and internal oral circular sinus, the anterior cœlom with its right and left limbs disappearing completely. The larval mouth closes, but a large part of the larval œsophagus is retained and becomes incorporated with the stomach probably forming the pharyngeal region of the adult gastric cavity. The larval anus and the terminal part of the larval rectum are lost, but the intestine and rectal sac of the starfish are derivatives of the intestinal tube of the larva. The adult rectum is a new formation. It is probable also that as in *A. rubens*, at the end of metamorphosis the sucker and stalk do not become absorbed,

but are "walked away from" and left adherent to the surface of attachment (7, p. 253).

Several typical examples of **double hydrocœle** were noted and one of these is illustrated in Pl. 5, fig. 23. As in similar larvæ of *A. rubens* there was, to begin with, failure of the ventral horn of the l. p. c. to unite with the right middle cœlomic cavity. The latter cavity, thus left isolated like the l. m. c., proceeded to develop into a hydrocœle with a set of hydrocœle pouches exactly like those of the left side. Later, it also showed a pharyngeal outgrowth, as well as rudiments of a stone canal and axial organ. A right hydro-pore was not present in any of my specimens. The r. p. c. and l. p. c. became united between stomach and rectum and also sent round hollow folds to enclose the rectum. Secondary openings were formed between the dorsal horns of the right and left posterior cœloms and the right and left middle cœloms respectively, exactly as occurs normally on the left side alone. This shows that in normal development the l. p. c. (hypogastric cœlom) is homologous with the r. p. c. (epigastric cœlom). Masterman's view (13) that the hydrocœle is the equivalent of the epigastric cœlom cannot be reconciled with the data from double hydrocœle in *Porania* and *A. rubens* (7). Very frequently also the dorsal horns of the r. p. c. and l. p. c. communicated with each other across the middle line by means of an opening which has no direct homologue in normal development, but is no doubt an expression of the normal tendency of the dorsal horn of the l. p. c. to extend to the right and unite with the ventral horn of the same cavity behind axial sinus and dorsal sac, in order to complete the hypogastric cœlomic ring. As regards the arrangement of the aboral arm rudiments, naturally a set of these tended to be formed on the right as well as on the left side, and thus the two sets were brought back to back against one another forming a strap-like "disc" round the posterior end of the larva. The full series was not present in any instance, rudiments I being always wanting, while rudiments V were

usually much smaller than normal and fused with one another. Separate terminal ossicles appeared on each side in rudiments II, III and IV, while the small composite rudiments V showed a single terminal. No elements corresponding with basals were observed in any of my *Porania* double-hydrocœles, although occasionally in the case of *A. rubens* small basal calcifications occurred near the mid-dorsal line of the disc.

A dorsal sac was present in the mid-dorsal line to the left of the hydroporic opening. In a particular double hydrocœle larva which I had the opportunity of observing repeatedly the sac was somewhat elongated in shape, being compressed between the hydrocœles. The contractions of the floor of the sac were observed to progress from behind forwards. This seems to indicate a forward passage of fluid absorbed from the stomach, and is one of a number of circumstances which justify a close comparison between the hæmal system of *Balanoglossus* and of Asterids. In this view dorsal sac and head-process of axial organ correspond respectively with the pericardium and heart of *Balanoglossus*; the gastric hæmal tufts are dorsal afferent vessels; the axial organ, oral hæmal ring, and radial hæmal strands correspond with the left (efferent) pharyngeal vessel and its continuations; while the pharyngeal cœlom is the equivalent of the left pharyngeal cavity (7, p. 278).

CONCLUSIONS.

The more important results brought out in this paper are as follows:—

(1) The fact that the general course of larval development is similar to that of *A. rubens*, L. and *A. vulgaris*. Sladen (18) has divided Asterids into the two great families, **Phanerozonia** and **Cryptozonia**, according as their marginal plates are well developed or not. He believed the former to be the more primitive. *Porania* is the first Phanerozonate Asterid which has been shown to exhibit a feeding brachio-

larian stage in its life history. It is evident that the division of Asterids into Phanerozonia and Cryptozonia is not necessarily associated with fundamental differences of development. That the division in question is not an entirely natural one has been pointed out by Jeffrey Bell (2*a*), MacBride (10*a*), and others. Curiously enough I have recently found, among the material dredged at the Millport Marine Station, two specimens of *Porania* with a number of actinal gills. It will be remembered that in Phanerozonates the gills are usually confined to the abactinal surface bounded by the supero-marginal plates.

(2) The fact that blastula formation is by egression of central cells in lines appearing externally as surface furrows (p. 29).

(3) The practically constant presence of what seems to be a rudimentary posterior enterocœlic outgrowth, and its important bearing on the general question of the origins of the cœloms (p. 32).

(4) The fact that the main enterocœles arise often, if not always, by a single outgrowth (p. 31).

(5) The great frequency of double hydropore in the early larvæ (p. 31).

(6) The determination of the special characters of the brachiolaria (p. 36).

(7) The fact that the late larva is provided with a system of neuro-muscular or neuro-epithelial fibres (p. 41).

(8) The occurrence of larvæ with double hydrocœle, and the light they throw on the homologies of certain parts (p. 43).

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EXPLANATION OF PLATES 4 AND 5,

Illustrating Mr. James F. Gemmill's paper on "The Larva of the Starfish *Porania pulvillus*."

1, 2, 3, 4, 5, 6.—The larval ciliated processes in order as follows: 1. Median dorsal; 2. anterior dorsal; 3. posterior dorsal; 4. posterolateral; 5. post-oral; 6. preoral.

I, II, III, IV, V.—These numerals lead by continuous lines to hydrocœle pouches I, II, etc., and by dotted lines to the corresponding aboral arm-rudiments; (*r*) and (*l*) after the numerals indicate respectively that the structures in question belong to the right or to the left side.

LETTERING AND ABBREVIATIONS EMPLOYED.

a. c. Anterior cœlom. *ant. br.* Anterior brachium. *anus.* Anus. *ap.* Apex. *arch.* Archenteron. *bl.* Blastopore. *bl.c.* Blastocœle. *co. a.* Circumoral area. *ect.* Stomodæal pit. *egr.* Egression grooves of blastula. *entc.* Enterocœle. *hy. can.* Hydroporic canal. *inf. lab.* Inferior labial loop of adoral ciliation. *l. a. c.* Left anterior cœlom. *lat. br.* Lateral brachium. *l. entc.* Left enterocœle. *l. mid. c.* Left middle cœlom. *l. post. c.* Left posterior cœlom. *l. p. entc.* The left posterior rudimentary enterocœlic body. *mes.* Mesenchyme. *med. lob.* Middle dorsal lobe or lappet of the post-oral ciliated band. *node.* Neural node. *œs.* Œsophagus. *œs. lp.* Œsophageal loop of the adoral ciliation. *pap.* Papillæ. *p. entc.* The posterior rudimentary enterocœlic bodies. *per. bd.* Peristomal band of the adoral ciliation. *po. cil. bd.* Post-oral ciliated band. *pr. cil. bd.* Preoral ciliated band. *r. a. c.* Right anterior cœlom. *r. br.* Right brachium. *rect.* Rectum. *r. mid. c.* Right middle cœlom. *r. p. entc.* The right posterior rudimentary enterocœlic body. *r. post. c.* Right posterior cœlom. *s.* Sucker. *st.* Stomach.

PLATE 4.

Fig. 1.—Late stage in segmentation, surface view. At this stage there is practically no segmentation cavity.

Fig. 2.—Optical section of stage in blastula formation by egression of centrally placed cells.

Fig. 3.—Commencement of gastrulation. The surface irregularities are all smoothed out and invagination of the archenteron is beginning.

Fig. 4.—Gastrula stage. The elongated shape of the gastrula will be noted, as also the fact that the greater part of the archenteric invagination is thick-walled and opaque, while the bottom of the invagination is expanded, has thinner, clearer walls, and is budding off mesenchymal cells.

Fig. 5.—Slightly later gastrula stage showing further elongation with expansion and thinning of the bottom of the archenteron. The general mesenchyme has now been budded off and is found everywhere throughout the archicœle.

Fig. 6.—Stage slightly later, at which the orientation can be made out. The anterior end of the larva is somewhat pointed. The blastopore is beginning to migrate ventrally and the archenteron to show a dorsally convex curvature. The slight prominence on the ventral side is an indication of the transverse portion of the preoral band, and the concavity behind it an indication of the circumoral field.

Fig. 7.—Slightly older larva showing commencement of preoral and post-oral bands, which, however, are still incomplete dorsally. The anterior portion of the archenteron is now horse-shoe shaped as seen from above, but is here shown in side view, and is becoming constricted off. The stomodæal pit is beginning to deepen. The future stomach is becoming slightly dilated, and the blastopore has migrated distinctly towards the ventral side.

Fig. 8.—View from dorsal side of larva at much the same stage as in Fig. 7. For description see under Fig. 7.

Fig. 9.—View from right side of young bipinnaria, nine days old. The enterocœlic sacs are nearly separated off from the archenteron and from one another (c.f. Fig. 10). The different regions of the alimentary canal can now be made out. The rudimentary posterior enterocœlic outgrowth is also indicated. The preoral and post-oral ciliated bands lack definiteness toward their anterior ends, and in that region the ectoderm as a whole is somewhat elongated. This figure and the next bring out the characteristic shape of the larva at the present stage.

Fig. 10.—Ventral view of larva of same age as in Fig. 9, but from a specimen in which the two enterocœles were still united together by a transverse canal after their separation from the archenteron.

Fig. 11.—View from left side of abnormal specimen described on p. 35, in which the bottom of the stomodæal pit has failed to unite with the anterior end of the archenteron (œsophagus). The larva is about ten days old and still shows the two posterior enterocœlic outgrowths, but its characteristic feature is that the main enterocœlic sacs are in wide communication with each other across the middle line

ventral to the anterior end of the œsophagus. Two hydropores are present.

Fig. 12.—Larva twenty-five days old seen from left side. The extension anteriorly and posteriorly of the enterocœlic vesicles will be made out. The growth of the larva is relatively slower than in the case of *A. rubens*.

Fig. 13.—Dorsal view of larva at the same stage as in last fig.

Fig. 14.—Ventral view of larva at same stage as in Fig. 11.

Fig. 15.—Lateral view of anterior part of larva about forty-two days old. The enterocœlic vesicles have united in front and the united portion is sending out hollow processes for the brachia. The relatively short median dorsal process will be made out.

Fig. 16.—Anterior end of larva about fifty-six days old showing the brachia well developed and provided with papillæ on and around their extremities which are somewhat rounded. Papillæ are also present on the sides of the brachia (see Fig. 21). The median lobe or lappet of the post-oral ciliated band opposite the mouth will also be seen.

Fig. 17.—Transverse section through wall of stomach showing formation by solid cell proliferation of the posterior enterocœlic body on the left side of an eight days old larva.

Fig. 18.—Transverse section through stomach region of larva about ten days old showing the posterior body almost separated off from the stomach-wall on the right side and considerably larger than that on the left side which has now become mesenchymal.

Fig. 19.—Transverse section through stomach region of larva about thirteen days old showing the posterior bodies now broken up into mesenchyme. On the left side the main enterocœle is shown as having extended back into the region which the section cuts and one of the mesenchyme cells above referred to is joining the wall of the sac.

PLATE 5.

Fig. 20.—View from right side of specimen about fifty days old showing the posterior part of the body and the arm rudiments. The enterocœlic cavities are not outlined in this illustration.

Fig. 21.—View from ventral aspect of late branchiolaria. The hydrocœle lobes have appeared in the *l. m. c.* The *l. p. c.* has extended to the right side ventrally and has formed a communication with the *r. m. c.* Aboral arm rudiment I is developing over the expanded ventral horn of the *l. p. c.* This horn has also encircled the rectum. The relative shortness of the ciliated processes, and the large size of the anterior part of the larva will be made out, this last feature being

here present in a slightly exaggerated degree. The arrangement of the papillæ on the brachia is a characteristic feature.

Fig. 22.—Brachiolaria in dorsal view showing the set of fibrils interpreted as neuro-muscular or neuro-epithelial in character. Something of the nature of nodes or centres in this system appears to exist at the points marked on either side. The characteristic single transverse fibre between A and A' will be made out.

Fig. 23.—Ventral view of advanced double hydrocele brachiolaria. The *r. m. c.* has developed hydrocele outgrowths like the *l. m. c.* The *r. p. c.* is symmetrical with the *l. p. c.* and takes its share in the formation of a coelomic ring round the rectum. The aboral arm-rudiments lie posteriorly and form a double set except that rudiments I are entirely wanting. In this specimen the only ossicles to appear were the terminals of II, III, IV, and V, those of the three first-named being separate and those of the last being small and united. The rest of the larva is perfectly normal.

Double Hydrocœle in the Development and
Metamorphosis of the Larva of *Asterias*
rubens, L.

By

James F. Gemmill, M.A., D.Sc.

With Plates 6 and 7.

CONTENTS.

	PAGE
NORMAL DEVELOPMENT PRIOR TO METAMORPHOSIS	52
DOUBLE HYDROCÆLE PRIOR TO METAMORPHOSIS	53
NORMAL METAMORPHOSIS	57
DOUBLE HYDROCÆLE METAMORPHOSIS	59
UNSYMMETRICAL DOUBLE HYDROCÆLE	66
FREQUENCY; OTHER INSTANCES	67
DOUBLE HYDROPORE AND DOUBLE HYDROCÆLE	69
CAUSATION	70
CONCLUSIONS	71
EXPLANATION OF PLATES	76

As is well known, the hydrocœle or water vascular system of Echinoderms arises from a portion of the left cœlom. The early larvæ being bilaterally symmetrical, a great deal of discussion has taken place as to whether any definite portion of the right cœlomic cavity corresponds with the hydrocœle, and if so, whether this portion gives rise to any adult structure which can be identified. There has also been much speculation regarding the bilateral ancestor. On both of these problems a study of double hydrocœle—that is, the condition in which a right as well as a left hydrocœle appears—is calculated to throw light. While this is true for any kind of Echinoderm, it applies particularly in the case of *A. rubens*,

since there is evidence that the ontogenetic history of our species belongs to the least modified type of larval development we yet know of within the phylum (4, p. 279). Again, various points in normal development are difficult to determine with certainty, on account of the great and rapid change of symmetry which occurs at metamorphosis. Welcome additional light on a number of these points (p. 72) has accrued from a study of double hydrocœle.

NORMAL DEVELOPMENT UP TILL METAMORPHOSIS.

(c f. Pl. 6, fig. 1).

It is advisable at this stage to give a brief account of the formation of the hydrocœle in normal larvae, and also of the subsequent course of development till the commencement of metamorphosis, mentioning only those points which are of importance in connection with double hydrocœle. The complete account appears elsewhere (4).

The two enterocœles arise separately, one from each side of the anterior end of the archenteron, and, as a rule, the left one alone acquires a hydroporic opening. During the early growth of the larva the enterocœles extend forwards into the preoral lobe, and backwards over the lateral aspects of the stomach. At about the twenty-first day the enterocœles unite across the middle line in the preoral lobe. The front portion of each enterocœle may now be called the anterior cœlomic region¹ (*A. a. c.* or *a. a. c.*). Shortly thereafter each enterocœle begins to show a constriction just behind the level of the hydropore. By gradual extension of these constrictions, from the dorsal to the ventral side, the right and left posterior cœloms (*r. p. c.* and *l. p. c.*) are cut off from the corresponding middle cœlomic regions¹ (*r. m. c.* and *l. m. c.*). Before this process is complete the left posterior cœlom has begun to grow more rapidly than the right one, and in particular has sent out a ventral and a dorsal horn. The former pushes its way to the right, across the middle line between stomach and rectum, and its extremity undergoes expansion, and finally comes to lie against the ventral corner of the right middle cœlomic region. Shortly afterwards the intervening septum breaks down, so that now the right middle and the left posterior cœlomic regions are in communication with one another. The right middle cœlomic region ceases to undergo further differentiation or to increase markedly in size. On the other hand, the left middle cœlomic region,

¹ The terms anterior and middle cœloms or cœlomic regions are used here for descriptive convenience and not as indicating separate structural or morphological units (see 4, p. 254).

into which the hydropore opens, being at this stage completely isolated from the two posterior cœloms, begins to undergo expansion and to exhibit the five hydrocœle pouches which are the rudiments of the rays of the water vascular system. Still later, the dorsal horn of the *l. p. c.* acquires a secondary opening into the left middle cœlomic region on the deep aspect of the interval between pouches I and II,¹ Meantime the ventral horn of the *l. p. c.* has extended so as to enclose the rectum (cf. 4, Pl. 18, fig. 7), while the rudiment of the calcareous plate for ray I has appeared superficial to the cavity derived from the expanded extremity of this horn, to the right of the midventral line. The other four arm-rudiments with their terminal ossicles, all overlying the margin of the *l. p. c.*, have also appeared, the series beginning with arm-rudiment II, behind and to the left of the hydropore. Between arm-rudiments II and I is the aboral brachiolarian notch, the last-named rudiment being formed on the opposite side of the notch from hydrocœle pouch I, although the rudiment in question afterwards becomes associated with hydrocœle pouch I to form ray I of the adult starfish. Interradial ossicle I-II develops over the dorsal sac, but the other interradials and the dorso-central are superficial to the (larval) right posterior, or epigastric, cœlom.

The dorsal sac or madreporic vesicle develops from mesenchymal cells at or near the mid-dorsal line, becoming evident as a vesicle about the twenty-fifth day, and showing rhythmic contractility eight or ten days later. It is the floor of the sac which pulsates, and the effect is to displace the fluid in the underlying spongy tissue, the tissue becoming turgid and bulging up the floor of the sac in the intervals between the contractions. Owing to the absence of vessels we cannot say that there is a definite circulation in the larva, while the adult hæmal system is still a subject of discussion. However, there is reason for thinking that the dorsal sac and underlying spongy tissue serve as a heart-complex (4, p. 249), and that the general result even in the larva is to distribute nutritive fluid absorbed from the stomach (see further on, p. 58).

DOUBLE HYDROCÆLE UP TILL METAMORPHOSIS.

(Pl. 6, figs. 1-5, and Pl. 7, figs. 10-12.)

It is impossible to tell what larvæ will develop double hydrocœle until a little after the time when the opening of the

¹ The numbering of the rays adopted in this paper is as follows: On the aboral side passing sinistrally (i. e. counter-clockwise) from the madreporite we come in series to rays II, III, IV, etc., while ray I is immediately dextral to the madreporite (see 4, p. 276).

ventral horn of the *l. p. c.* into the right middle cœlomic region ought to be effected, and the earliest sign of double hydrocœle I could recognise was that in certain cases, without there being any permanent failure of health, the ventral horn of the *l. p. c.* did not succeed in forming the communication in question. Two different environmental factors may be concerned in evoking this condition (see p. 70). The first, less common and of less importance, is a disturbance of growth due to temporary malnutrition, in virtue of which the ventral horn of the *l. p. c.* does not exhibit sufficient preponderance to carry it over to the right side of the rectum. Accordingly, the horn in question has not the opportunity of meeting and uniting with the ventral corner of the right middle cœlomic region, which thus remains isolated from the cœloms behind it, exactly like the left middle cœlomic region. However, sooner or later, if there is further growth, the ventral horn of the *l. p. c.* meets and unites with the ventral angle (ventral horn) of the *r. p. c.*, which has meantime advanced towards it, and from the common cavity thus formed a hollow sheath begins to extend around the rectum. We have now an absence of that polarity between the right and left sides which should be present at this critical stage in the development of the larva. Two courses are open: (*a*) There may be failure of further differentiation, including failure of formation, or a rudimentary condition, of the hydrocœle pouches even on the left side; (*b*) the right and left middle and posterior cœlomic regions may proceed to differentiate more or less symmetrically, the left side imposing its course of development on the right one and causing, through secondary homœosis (p. 71), the formation of a right as well as of a left hydrocœle.

The second, and much the more important of the environmental factors concerned in evoking double hydrocœle, appears to be excessive nutrition. The larvæ in one or two of my cultures grew very rapidly, their stomachs becoming widely expanded and globular. The ventral horn of the *l. p. c.* thus had too great a distance to travel before it could

unite with the right middle cœlomic region, and accordingly the union in question did not take place, the last-named cavity being left isolated from the posterior cœloms, as in the different mode of causation already described. The effect, however, is the same if differentiation proceeds. The left cavities impose their developmental symmetry on the right ones, one of the results being the formation of double hydrocœle.

In all such cases the chief points in aberrant development which have to be noted prior to metamorphosis, have reference to the following structures: hydrocœle pouches, stone canal grooves, posterior and pharyngeal cœloms, the aboral arm-rudiments, and the skeletal ossicles. The preoral structures develop along normal lines.

The **hydrocœle pouches** appear on the right side either at the same time as on the left, or very slightly later. The sequence in their formation is the same on both sides, that is to say, III and IV are the first to become evident, then II and V, and I very slightly later. Towards the end of larval life, a secondary opening forms between the dorsal horn of the *r. p. c.* and the *r. m. c.* exactly like that between the dorsal horn of the *l. p. c.* and the *l. m. c.* The **stone canal groove** appears on the right side as definitely as on the left, and in a corresponding position. There does not, however, appear to be any tendency to the formation of a right pore canal.

It was noted above (p. 52) that the right and left **posterior cœloms** unite with one another around the rectum through fusion of their ventral horns. A communication,¹ which has no homologue in the normal development of *A. rubens*, is next established between the two cœloms dorsally, owing to the formation of a larger or smaller perforation in the

¹ The formation of this opening in double hydrocœle is no doubt traceable to the normal tendency of the dorsal horn of the *l. p. c.* to meet and unite with the ventral horn of the same cavity during metamorphosis in order to complete the circle of the hypogastric cœlom. Curiously enough Delage (see 4, p. 244) describes the presence of a dorsal opening between the *l. p. c.* and the *r. p. c.* as being normal for the late larva of *A. glacialis*.

dorsal mesentery. Elsewhere, the posterior cœloms are separated by the mesentery in question, the layers of which in some instances are closely apposed and in others are separated by a wide intervening space. The last-named difference depends on whether the disc-rudiments are broad or not, and this, in turn, depends chiefly on the size of the stomach. The space between the mesenteries, when well-marked, takes on a sparsely cellular character, the cells being round rather than branched, and the space itself becoming pseudo-cœlomic. A true lining membrane is of course absent, but interrarial ossicles may develop over the space as they do normally over the right larval (epigastric) cœlom. It was noted earlier, in connection with the middle cœloms, that an opening appears between each and the corresponding posterior cœlom, exactly like the opening normally present in late larvæ between the *l. m. c.* and the dorsal horn of the *l. p. c.*

The pharyngeal cœlom develops on the right side as well as on the left, in the form of an evagination from the dorsal horn of the posterior cœlom, which passes first inwards towards the middle line; then curves ventralwards and finally forwards within the root of the mesentery separating the posterior cœlom from the hydrocœle. At first the two pharyngeal cœloms are separate from one another, but in late larvæ and in specimens which are undergoing or have undergone metamorphosis they are found to be united across the middle line for a short portion of their length not far from their places of origin. This mesial portion may extend backwards for some distance on its own account, so as to appear an independent cavity in transverse sections which show the two pharyngeal cœloms separated laterally from one another (cf. Pl. 7, figs. 13, 16).

Aboral **arm-rudiments** I are not developed, while arm-rudiments II to V meet back to back, forming a paired series of (right and left) elements, which together give rise to a strap-like "disc" overlying the dorsally convex aspect of the larval body (cf. Pl. 6, figs. 4, 5). A **terminal ossicle** appears in each of the arm-rudiments. Of the **interrarial ossicles**,

that which overlies the dorsal sac, viz. interradial I-II, is practically always present and is always, so far as I have observed, a single piece. Interradial V-I is never found. The other interradials, viz.: II-III, III-IV and IV-V are absent as a rule, but if the stomach is unusually large and the "disc" broad, they may be present in pairs (see Pl. 6, fig. 4). When present they develop not over an epigastric cœlom, but over a pseudo-cœlomic space between the layers of the dorsal mesentery (pp. 56, 64). It may be of importance as regards the homologies of the skeleton in different echinoderms to recognise that the asterid interradial I-II is different from the other interradials, in respect that it arises over the dorsal sac and is therefore essentially unpaired and mesial in position, as we find it in double hydrocœle. When the spines appear, so far as space allows, they develop just in the same number and position as in normal larvæ. Thus in a specimen with broad "disc," at the commencement of metamorphosis, twelve spines may be counted over each of the terminal plates (cf. 4, p. 266).

It remains to be added that during all this time, the anterior and middle portions of the larva have been developing along perfectly normal lines. Even the same kinds of variation as are found in ordinary larvæ (4, p. 236), occur also in double hydrocœle, for example, the presence of three or more papillæ on each side of the sucker instead of only two, entire absence of the median dorsal process, variation in the length of ciliated processes, and in particular great shortening of the postero-lateral process on the right side.

NORMAL METAMORPHOSIS.

We must preface the description of metamorphosis in double hydrocœle with a short account of normal metamorphosis. At the commencement the brachia give temporary attachment, but definitive fixation takes place by means of the sucker, the cells of which secrete a strongly adhesive cement. The whole anterior part of the larva including the ciliated processes becomes retracted and is finally amalgamated with the oral (left larval) aspect of the disc, considerable histolysis,

both of epidermal and mesenchymal tissues taking place during the process. A stalk, which soon becomes solid, is left connecting the sucker with the oral aspect of disc. In the end this stalk, which has meantime grown slender and elongated, ruptures near the disc, sucker and stalk being left behind. The stalk-cavity before its disappearance, is seen to be encircled by the just completed hydrocœle ring, as in *Asterina* (MacBride 6, p. 356), and *Solaster* (Gemmill 3, p. 27). The larval mouth closes and the larval œsophagus becomes divided near the junction of its stomodæal and endodermal portions, the former joining the epiderm of the (starfish) oral surface, and the latter being taken into the stomach wall through gradual widening of the cardiac orifice. The anus and terminal part of the rectum in the larva degenerate, but the larval intestine and the rest of the rectum are retained to form the intestine and rectal sac of the adult. The paired radial cœca grow out from the aboral (pyloric sac) region of the stomach. The permanent mouth and anus are new formations, the latter appearing aborally in interradius I-V.

The right middle cœlomic (right hydrocœlic) region is early obliterated. The common mesial portion of the right and left anterior cœlomic regions at first become greatly expanded by taking the rest of these cavities into itself. For a time it reaches some distance into the stalk, but it becomes gradually smaller, and finally disappears. Hydrocœle, internal oral circular sinus (inner perihæmal ring), axial sinus, stone canal, and axial organ are products of the left middle cœlomic region. The larval *r. p. c.* and *l. p. c.* become respectively the epigastric and hypogastric cœloms. The pharyngeal cœlom forms a ring round the mouth on the oral aspect of the stomach, and is afterwards practically amalgamated with the hypogastric cœlom through formation of interradiial and radial perforations in the intervening mesentery, whereby the adradially placed oral gastric ligaments are left.

The dorsal sac of the larva persists as the dorsal sac of the adult, the reticular tissue underneath it being invaginated upwards and forming the so-called head process or glandular process of the axial organ. The axial organ appears as a fold-like thickening of the walls of the *l. m. c.*, running parallel with the stone canal. It consists at first of spongy hæmal tissue, in which, however, cells of parenchymatous nature soon appear. The origin of these cells is ascribed by MacBride to downward growth from the gonad rudiment (6), but I think they are derived simply from the lining of the *l. m. c.* (4, p. 261).

It is probable that nutriment (absorbed from the stomach, the pyloric sac, and the pyloric cœca) passes by way of the gastric hæmal tufts to the axial organ, and then (*a*) through the hæmal tissue within the aboral perihæmal sinus to the genital organs; and (*b*) down the hæmal channels of the axial organ to the internal oral hæmal ring and the

radial hæmal strands, supplying nourishment in particular to the central nervous system, the sucker feet, and the muscles of the ambulacral ossicles. The head process of the axial organ, being invaginated into the dorsal sac, acts as a heart, or, at any rate, as a pulsating spongy ampulla at the nodal point in this circulation. The severance of the nutritive and the respiratory functions in Echinoderms is probably the key to the peculiarities of the hæmal system in this phylum.

METAMORPHOSIS IN DOUBLE HYDROCÆLE.

(Pl. 6, fig. 6, and Pl. 7, fig. 9, surface views ; Pl. 7, figs. 14-18, sections.)

We find the same signs of approaching metamorphosis here as in normal development. The brachia move actively in all directions and not infrequently effect temporary adhesion to smooth surfaces. At the same time, longitudinal contractions of the body-wall begin to take place, tending to dilate the median cavity in the preoral lobe, and to push out the sucker. A little later, attempts at sucker fixation occur, and in the end cement fixation takes place, not without several short periods of temporary attachment, which are effected in the manner I described for normal larvæ before the gland cells of the sucker shed their cement and thus produce final fixation (4, p. 250). In the intervals between the temporary attachments just referred to, the brachia are the adhering organs, and the starfish may be described as crawling by the combined action of brachia and sucker. The larva is now shrinking slightly in size, and as its tissues are becoming more condensed it is no longer able to swim freely up from the bottom by the help of its ciliated processes.

After fixation has taken place the longitudinal contractions of the body-wall become stronger and more regular, and under their influence the whole of the preoral lobe is gradually pulled towards the disc or body portion of the larva. Since in double hydrocæle the right and left sides of the larval body are symmetrical and the aboral arm rudiments, etc., so far as they are represented, overlie the dorsal aspect of the larval body, we find that as metamorphosis proceeds the

preoral lobe structures, including the sucker and the larval mouth, are retracted towards the mid-ventral line, and, as no torsion of preoral lobe on body of larva takes place, the remains of the larval mouth comes to lie approximately in the middle of the ventral surface while the sucker is in the mid-ventral line anterior to this part. An extremely early stage at the very commencement of metamorphosis is shown in lateral view in Pl. 6, fig. 5, and a later stage with retraction well advanced in Pl. 6, fig. 6. During these changes the mesenchyme throughout the anterior portion of the larva and within the ciliated processes breaks down, and becoming semi-fluid is transferred into the disc in the same manner as in normal development. In the end the preoral structures are reduced till only the sucker and a long slender stalk are left, the latter projecting from the mid-ventral line in the position previously indicated. The last remains of stalk and sucker are not absorbed, but are left to disintegrate, rupture taking place at the junction of stalk and body. This very interesting fact, which I found to occur normally in the metamorphosis of *A. rubens*, was actually first observed by me in the case of a double hydrocœle specimen, namely, that represented in Pl. 7, fig. 8. In this figure we are looking at the left aspect of the bilateral "starfish," there being five rays on the right side, not seen in the drawing, but entirely similar to the five rays on the left side. There is no question as to which are the anterior and posterior ends of the strap-like "starfish," reckoning by the hydrocœle lobes. The first of the hydrocœle lobes has not an aboral arm-rudiment of its own, since, as we saw above, aboral arm-rudiment I is not developed in double hydrocœle larvæ. The dorsal sac or madreporic vesicle lies in the middle line close under the dorsal surface at the anterior end. When in due course this young "starfish" became separated from its stalk and crawled away it used its sucker feet like an ordinary starfish, resting apparently with indifference at one time chiefly on its right, and at another chiefly on its left sets of sucker feet. In shape it was more convex dorsally than would appear from Pl. 7, fig. 8, in which,

for purposes of illustration, it is partly straightened out. A mouth was formed in the ordinary way, but the formation of an anus could not be made out. The "starfish" lived for five or six weeks after breaking away from its stalk, and seemed to be attracted by small portions of animal tissue (shredded ovary of sea urchin), which were placed in the vessel containing it. Unfortunately a satisfactory histological examination was rendered impossible owing to the fact that the specimen became unhealthy when I was away during part of the autumn vacation, and on my return had suffered such degeneration that, though still alive, it proved useless for the study of finer details when cut into serial sections. However, several other double hydrocœle specimens were obtained at various stages in metamorphosis, and an examination of serial sections of these specimens allowed the following facts to be made out.

The Stalk.—Since the adjacent ends of the two (half) rings of the hydrocœle do not actually unite (p. 63), we cannot speak of the stalk being encircled by the ring canals of the hydrocœle, although insertion of stalk into disc falls well within the area virtually enclosed by these canals. It would be a point of no little interest to determine whether the base of the stalk is surrounded by the circular oral nerve area. In the specimen previously referred to this seemed to be the case as far as one could judge by external observation, while examination of sections of another specimen at a relatively early stage in metamorphosis led, though not quite decisively, to the same conclusion. In normal development the base of the stalk is not surrounded by the circular oral nerve. Probably in double hydrocœle the inclusion is not real but apparent, resulting from apposition of the open ends of two nervous half rings as in the case of the hydrocœle half rings just mentioned.

Anterior Cœlomic Regions.—In the stages immediately succeeding fixation, as the preoral lobe is rapidly shortened and the œsophagus ruptured, we find that the anterior cœlomic regions give rise to a relatively large cavity surrounding

the œsophageal cone, entering the stalk and passing backwards into the right and left middle cœlomic regions (c f. Pl. 7, figs. 14, 17). Later, when the stalk lengthens, the contained cavity, as in normal development, keeps retreating towards the disc, so that except for a short funnel passing into its commencement, the stalk is now solid. We have to note that the main cavity just referred to, i. e. that derived from the anterior cœlomic regions, is a single space, not divided into right and left chambers. Later this cavity undergoes reduction and disappears after remaining for a time in widely open communication with the two **internal oral circular sinuses**.

Middle Cœlomic Regions.—Under this heading we have to notice hydropore, axial sinus, stone canal, axial organ, hydrocœle lobes, openings between posterior and middle cœloms, and internal oral circular sinuses (inner perihæmal rings). The **hydropore** is single and to the left in all my specimens, whether early and late, of double hydrocœle. The **axial sinuses** are symmetrical on the right and left sides except for the fact that the right sinus is not provided with a hydroporic canal. The tissue between the two sinuses becomes reduced at metamorphosis till it forms merely a thin septum between them. However, the sinuses in question do not communicate with each other at the end of metamorphosis, and accordingly we may be certain (as in the case of the hydrocœle ring canals and the internal oral circular sinuses) that they are derivatives of the middle cœlomic regions (c f. Pl. 7, fig. 16). We are justified in inferring that this also holds good in normal development on the left side for the structures named—a conclusion I had already reached (4, p. 276). The **stone canals** begin as grooves on either side, which become closed to form tubes opening at the ends into their respective hydrocœle half-ring canals and axial sinuses. It is noteworthy that on the right side the opening last referred to is still present, even though there is no pore canal related to it. The two stone canal grooves are illustrated in Pl. 7, figs. 10, 13, 16. The **axial organ** is present on

the right as well as the left side and arises as in normal development, in the form of a loosely cellular fold of the wall of the middle cœlomic region parallel to the stone canal. The two folds unite aborally to form a single upper portion and head process. The latter is derived as normally from the spongy tissue underneath the dorsal sac, which becomes invaginated into the sac at metamorphosis and it corresponds, as I believe, to the heart of *Balanoglossus*. At the end of metamorphosis the two axial organs lie almost back to back against each other on either side of the septum mentioned above, as separating the two axial sinuses.

The ring canals of the **hydrocœle** becomes separated from the right and left middle cœlomic regions as occurs in normal development on the left side. The hydrocœle pouches expand rapidly at metamorphosis, becoming trilobed and then five-lobed as in normal development, at the time when the preoral region of the larva is undergoing retraction. The trilobed and five-lobed conditions represent stages respectively at which the rudiments of the terminal tentacle and of the first, or of the first and second, pairs of sucker feet are present. By the time a third pair of podial rudiments has appeared, the earliest formed pair is showing movements and beginning to serve for attachment. Ray I of the hydrocœle does not have the opportunity of associating itself with an aboral arm-rudiment, since, as was stated above, only four arm-rudiments are formed, namely, those which belong to rays II to V. The two hydrocœle rays I accordingly are found close together at the front end of the larva partly under the shelter of arm-rudiments II. A point of very great interest to be noted is that the ring canals of the two hydrocœles simply formed half rings, the extremities of which were close to, but not united with each other.

At the end of metamorphosis the right and left **internal oral circular sinuses** (inner perihæmal rings), like the two half-ring canals of the hydrocœle formed separate cavities. This goes to show that the sinuses in question are derived normally

from the middle cœlomic regions alone, since, if the common anterior cœlomic region had shared in their formation, they ought not to have become separated from one another in double hydrocœle.

Right and Left Posterior Cœloms.—At metamorphosis the right and left posterior cœloms communicate with each other around the rectum and terminal part of intestine of the larva, as also dorsally across the middle line in front where their two anterior horns are in communication by a smaller or larger opening. During the course of metamorphosis these openings are not closed off, and accordingly the two hypogastric cœloms at the end of metamorphosis may be described as forming a single cavity all round. In cases where the disc is broad, the two layers of the longitudinal mesentery between the right and left posterior cœloms become widely separated, and the space left between them takes on a pseudo-cœlomic character, in the manner noted on p. 57. Accordingly what may be called a pseudo-epigastric cœlom (cf. Pl. 7, fig. 18), is produced underneath the middle area of the disc.

Pharyngeal Cœloms.—The origin of the two pharyngeal cœloms has already been referred to. They extend in the same manner as in normal development, and the mesentery between them and the posterior cœloms afterwards undergoes radial and interradial perforations, the strands which remain forming right and left sets of oral gastric ligaments. It was noted earlier that the two pharyngeal cœloms communicate with one another. This communication is on the anterior side of the œsophageal cone. At the end of metamorphosis the posterior extremities of the pharyngeal cœloms appear also to establish a communication between each other behind the point where the mouth develops, and thus in the end we have the pharyngeal as well as the hypogastric cœloms forming a circular cavity.

Perihæmal Pouches.—It was ascertained that the perihæmal pouches for interradii I–II arise from the posterior cœloms, as also do the pouches for II–III, III–IV, and

IV-V. The material available did not include stages giving conclusive evidence as regards I-V. Normally, in *A. rubens*, I had come to the conclusion (4, p. 260), that the perihæmal pouch I-II arises from the *l. p. c.* as in *Solaster* (3, p. 35), but the point was difficult to settle, owing to the proximity to the pouch of the opening from the *l. p. c.* into the *l. m. c.* In *Asterina* MacBride (6, p. 360), and Goto (5), and in *Cribrella* Masterman (11, p. 392), described the perihæmal pouch I-II as arising from the anterior cœlom. It is probable that the difference is connected with the presence of the communication above referred to, and that primitively all the perihæmal pouches belonged to the *l. p. c.*

Alimentary Canal.—It was established that in double hydrocœle, as in normal development, the œsophagus becomes divided near its junction with the buccal cavity and forms a cone projecting into the common expanded anterior cœlomic cavity (cf. Pl. 7, fig. 17). This cone is gradually incorporated with the stomach, and the new mouth forms in the neighbourhood of the former apex of the cone. As in normal development, the cardiac orifice of the stomach becomes gradually dilated. The anus and terminal part of the rectum degenerate leaving the intestine and first part of the rectum, the former being enclosed between the layers of the dorsal mesentery, and the latter projecting into the hypogastric cœlom posteriorly at the junction of the right and left components of this latter cavity. My material did not show the formation of a new anus, but there can be no doubt that, if formed, it would appear in the mid-ventral line behind the mouth outside the hydrocœle ring and under cover of the posterior edge of the "disc," i. e. the part formed by arm rudiments V. The pyloric sac region became elongated at metamorphosis following the shape of the disc, and sent out right and left sets of pyloric cœca into the right and left arm rudiments. Four pairs of pyloric cœca were recognisable on each side, namely, those belonging to arm rudiments II-V. What became of the cœcal rudiments for ray I could not be

made out with certainty. Probably they united together forming a single median anterior cavity seen, for example, in Pl. 7, fig. 18.

UNSYMMETRICAL DOUBLE HYDROCŒLE. (Pl. 7, figs. 9, 18.)

Several examples of this condition were observed in early and late larvæ, as also after the completion of metamorphosis (Pl. 7, fig. 9). In every instance the right hydrocœle was smaller than the left one. In certain cases the full set of hydrocœle pouches was not present on the right side, the number being reduced to four or three or even to two. When only two were present they were usually, but not always, pouches III and IV. In every instance the right middle cœlomic region was not so large and did not hang so far back as in symmetrical double hydrocœle. As a consequence the left posterior cœlom expanded to a greater size than the right one, and accordingly, when the structures of the disc (aboral arm-rudiments and skeletal plates) began to be formed, these structures were not in the mid-dorsal line but were pushed to the right so as to encroach on the right side of the larval body. During the course of metamorphosis the inequality became more marked, and in extreme cases (cf. Pl. 7, fig. 9) the result was an almost apparently normal starfish-disc, which was made up of the five rays belonging to the left component, but was notched more or less deeply between arm-growths I and IV, and had the remains of the rays belonging to the right hydrocœle crowded together in the notch, especially towards the oral aspect. Comparison of several such instances leads to the conclusion that in normal development the last remains of a right hydrocœlic element, if such an element persisted, would have to be looked for on the oral side of the disc in interradius V-I, superficial to the hydrocœlic and perihæmal rings.

The specimen of unequal double hydrocœle shown in Pl. 7, fig. 9, possessed rudiments of the full set of five right hydrocœle pouches. The mouth was somewhat elongated and the

stomach and pyloric cavity were single. An anus had not appeared. There was a well-developed dorsal sac to the dextral side of the single hydroporic canal as viewed aborally. There were two stone canals, each opening into its own axial sinus and ring canal. The axial sinuses did not communicate with one another, nor did the hydrocœles, nor did the internal oral circular sinuses (inner perihæmal rings). However, the oral nerve ring formed a complete circle around the mouth.

FREQUENCY; OTHER INSTANCES.

In 1912 I obtained only four double hydrocœle larvæ in all, but in 1913 the total number amounted to over sixty. Examples occurred in practically all my cultures, but were particularly numerous in two vessels, the larvæ in which happened to grow with great rapidity and to reach an unusual size. These larvæ were fed with *Nitschia* having a chance bacterial and flagellate infection. Six or seven of the larvæ went on to metamorphosis. Of ordinary plankton larvæ I have had the opportunity of examining a considerable number from the Firth of Clyde; several hundreds from St. Andrews Bay, through the kindness of Prof. W. C. McIntosh; and nearly a hundred sent me from the Little Belt by Dr. Th. Mortensen. Among all these not a single specimen of double hydrocœle was observed.

There seem to be no records of double hydrocœle from the **Crinoids** or **Holothurians**. Joh. Müller (1846, Taf. I, fig. 2d) referred to by Metschnikoff (13, p. 16) and MacBride (7, p. 578) figured an *Ophiopluteus* (obtained from plankton) which had a right as well as a left water vascular rosette, and to which he gave the name *Pluteus paradoxus*. In *Amphiura squamata*, the eggs of which develop within the parent, Metschnikoff (13) described the formation normally of a right as well as of a left hydrocœlic rudiment, and stated that the former sometimes went on to further differentiation. MacBride obtained examples of double hydrocœle larvæ in

his cultures of *Ophiothrix fragilis*, and one such larva from plankton (7, p. 578, fig. 53).

As regards the **Echinoidea**, Metschnikoff (14, p. 64) mentions that he obtained from plankton near Messina a Spatangoid larva with two perfectly equal urchin plates, each having ambulacral feet and spines. In *Echinus* MacBride (8) has figured and described two very interesting double hydrocœle plutei. The first (*E. miliaris*) was about six weeks old and had an early "echinus-rudiment" and amnion cavity on the right side precisely similar to those on the left side, the arms and other structures of the larva being perfectly normal. The second specimen (*E. esculentus*) was about fifty-five days old and had right and left echinus-rudiments, the former being rather smaller than the latter. Both rudiments were sufficiently advanced to show the oral discs with the unpaired and paired tube feet, the nerve ring, groups of interradial spines, and the five dental pockets, with the mouth and œsophagus between them. As MacBride pointed out, in this case a practically complete set of definitely echinid structures were induced to form in the layers of the right side of the larva through the influence of an underlying right hydrocœle.

As regards **Asterids** we have to note that in 1874 Metschnikoff (13, p. 75) figured and described a late larva (referred by him to *Asteracanthion*, i. e., *Asterias*, but probably rather resembling the *Astropecten* type), which was provided with perfectly symmetrical right and left hydropores and hydrocœles. He made the very interesting suggestion that such larvæ showed a kind of symmetry which might well be compared with that of Ctenophores (see p. 74). MacBride in 1896 (6) described several examples of the double hydrocœle condition in *Asterina*, a starfish which has yolky eggs and direct development. No further examples in any starfish seem to have been recorded until 1912, when several appeared in my cultures of *Asterias rubens* (L.), and 1913, when they occurred in much greater numbers. Several advanced double hydrocœle larvæ were also obtained by me

in 1913 from cultures of *Porania pulvillus* (O.F.M.), a species which, like *A. rubens*, has a feeding larva of the brachiolarian type.

DOUBLE HYDROPORE AND DOUBLE HYDROCÆLE.

Double hydropore is not infrequently found in very early *A. rubens* larvæ, but, except in the rarest instances, the right hydropore disappears long before the stage at which double hydrocœle becomes evident. As a matter of fact, it would seem that double hydropore has little or nothing to do with the production of double hydrocœle in *Asterias*, since out of a total of over sixty double hydrocœle larvæ which I was able to pass under review, not a single one possessed two hydropores.¹ If, as seems entirely probable (4, p. 278), the hydrocœlic region of an Echinoderm corresponds to the left collar cavity of *Balanoglossus*, and the hydroporic canal to the proboscis pore, we may perhaps see in the independence of double hydropore and double hydrocœle an indication of the primitive independence of the anterior and the middle primary cœloms.

The frequency of double hydropore varies within the widest limits among different starfish, as among other Echinoderms (see 4, p. 230). Thus in *A. rubens* the proportion in early larvæ is about one to ten as a maximum, but usually very much less. In *A. glacialis*, on the other hand, as also in *A. vulgaris* (Field, 2, p. 111), most of the early larvæ in some cultures may develop two hydropores. The same thing is true in the case of *Porania pulvillus*. Among the Echinoids the larvæ of the regular urchins have only a single hydropore, while those of the common heart-urchin *Echinocardium cordatum* (MacBride, 9) are very frequently, if not normally, provided with two. We cannot, therefore, ascribe the incidence of double hydropore directly to ancestral causes, but must set down its variations to the influence of **Homœosis** (see below under Causation).

¹ Two hydropores are, however, seen in the double hydrocœle larvæ figured by Metschnikoff (13, p. 74) and MacBride (6, p. 369).

CAUSATION.

This is uncertain ground, and the following conclusions are simply put forward as appearing probable in the light of the evidence at present available.

My cultures (which were obtained from artificial fertilisations and reared by feeding with *Nitschia*) (4, p. 224) showed far greater numbers of double hydrocœle larvæ than occur in nature (p. 67). It is clear that something must have been at work to produce (*a*) disturbance of the normal course of development, and (*b*) deviation towards double hydrocœle. Under (*a*), as possible disturbing factors, we may single out (1) hurried nuclear maturation and probably also cytoplasmic immaturity in the ova employed, these having been shredded out from the ovary into sea water, where they underwent the nuclear maturation changes, and (2) defective or excessive nutrition of the larvæ. These factors could not, however, supply guidance in the production of double hydrocœle, and accordingly they are not truly causal of this condition, but only of developmental instability. However, we note further the following considerations coming under (*b*) above and making it seem natural for some at least of a group of unstably developing starfish ova to deviate into double hydrocœle:

(1) In the bilateral ancestor of Echinoderms the middle cœloms (from the left one of which the hydrocœle was derived) were no doubt symmetrical. The tendency to double hydrocœle may, therefore, have a directly atavistic foundation. It is not unreasonable to suppose that tendencies or potencies of ancestral origin may be strongly marked in ova that have undergone premature nuclear or cytoplasmic ripening.

(2) Distinguishable from directly atavistic potencies is the homœotic tendency to bilateral symmetry in the development of organisms, or of structures, which are normally no longer bilaterally symmetrical. There is evidence that this homœotic tendency may react so profoundly as to impose a particular

symmetry on the development of a species, although the symmetry in question is typically absent from the development of the group to which the species belongs. Certain facts in the incidence of double hydropore can best be explained on these lines; for example, the fact that in larvæ of the heart-urchin double hydropore is frequently, if not normally, found (MacBride, 9), while larvæ of the common sea-urchin almost always show a single hydropore. We may use the term "primary homœosis" to indicate the tendency which produces the kind of symmetry manifested as regards the hydropore by the larvæ of *Echinocardium*.

(2) There is a somewhat different homœosis which may best be described as "secondary" or "casual," though it is probably of chief importance as a factor in the production of double hydrocœle. This kind of homœosis manifests itself later in development, getting the opportunity to do so through failure of a particular difference between the right and left sides to become established at the proper time, development thereafter proceeding on the same lines on both sides. The initial failure may be due to environmental causes, which in the case of double hydrocœle seem to be connected with nutrition (p. 54).

CONCLUSIONS.

(1) Homologies.—The structural details in double hydrocœle show that the dorsal sac is a mesial organ, always present in cases of double hydrocœle, and in no way corresponding with a right hydrocœle. Interradial ossicle I-II is also essentially a mesial structure and not in full morphological series with the remaining interradials. The larval right posterior (epigastric) cœlom is the equivalent of the larval left posterior (hypogastric) cœlom. The presence of two stone canals, pharyngeal cœloms and axial organ rudiments, and their relation to each other and to the dorsal sac afford confirmation of the view that the homology between *Balanoglossus* and Echinoderms extends to many details

of structure. According to this view, the dorsal sac is the equivalent of the pericardium in *Balanoglossus*, while the axial organ and pharyngeal cœlom are equivalent respectively to the left pharyngeal efferent vessel and the left pharyngeal cœlom of *Balanoglossus* (4, p. 278). Direct observation of the contractions of the floor of the dorsal sac in a double hydrocœle larva of *Porania* showed that they progressed from behind forwards.

(2) Light on Normal Development.—Confirmatory evidence has been obtained from the study of double hydrocœle regarding certain points that were somewhat difficult to make out in normal development. These points had reference to (1) the retention of the larval œsophagus (p. 66; 4, p. 262); (2) the retention of the larval intestine and part of the rectum (p. 65; 4, p. 263); (3) the formation of the hydrocœle and axial sinus and of the internal oral circular sinus entirely from the cœlom of the left side (*l. m. c.* as defined on p. 52); (4) the formation of perihæmal pouch I–II from the left posterior cœlom (p. 64; 4, p. 260); (5) the view that hydrocœle pouch I should be looked upon as the most anterior and V as the most posterior of the hydrocœle lobes (p. 61; 4, p. 276).

(3) Causation.—We may infer that (1) the ova employed were developmentally unstable from the cause stated (p. 70); (2) at a certain stage the *r. m. c.* was left isolated posteriorly like the *l. m. c.*, from a cause proximately connected with nutrition (p. 55); (3) thereafter the *r. m. c.* followed the same course of development as the *l. m. c.*, through secondary homœosis; (4) the great majority of double hydrocœles are to be accounted for in this manner, but we cannot exclude the possibility that, given initial instability, the influence of atavism and primary homœosis may sometimes be sufficiently strong to induce the formation of double hydrocœle apart from the action of environmental factors.

(4) The Bilateral Ancestor of Echinoderms.—The probable characters of the ancestor are discussed elsewhere in connection with normal development (4, p. 279). The

double hydrocœle series described in the present paper shows us, in the most primitive type of Echinoderm larva, bilaterality carried much further than it is normally in this or any other larval type within the phylum. The data from double hydrocœle accord with the view that there has been a fixed stage in the evolution of Echinoderms; that fixation took place in the middle line of the preoral lobe; and that, as in *Antedon*, freedom was obtained by loss of the attaching stalk. On the other hand, the *Pentactæa* theory of Semon (16) receives no kind of support. Although not all the structures which are found in double hydrocœle after metamorphosis can be put down as proto-echinodermal in phylogeny, still I would include among such structures the dorsal sac, the two axial organ rudiments, and the two pharyngeal cœloms. Probably the two sets of pyloric cœca and the dorsally-convex protecting shield with its calcareous plates (cf. Pl. 6, fig. 5) should also be included. It will be remembered that in *Antedon* the stomach is provided with out-pocketings which may be compared with the radial cœca. Information regarding double hydrocœle in crinoid larvæ, especially if metamorphosis supervened, would be of extreme interest. The fact that even now a double hydrocœle starfish larva can undergo complete metamorphosis and remain capable of life favours the possibility that free bilaterally symmetrical Proto-echinoderms with right and left sets of food-collecting grooves, and with the other structures just referred to, may have appeared in early geological times.

Some such form, perhaps at first creeping but afterwards becoming attached by the preoral lobe, might well prove a starting-point for the differentiation of the primitive Crinoids from the primitive Asteroids. Certain obvious objections, based on habit, against deriving starfish as we know them from the stock in question will be greatly lessened, if not removed altogether, should the first results of feeding experiments at present in progress be ultimately fully confirmed. These results show that certain starfish, e. g. *Porania*, are able to collect and ingest food particles by means of their

actinal ciliation, healthy survival for considerable periods of time without loss of weight being thereby permitted. Observation of the ciliary currents, etc., in these starfish show that the currents in question actually bring particles to the mouth.

The sharp *ventral* curvature of the alimentary canal in the late brachiolaria and at the end of metamorphosis in double hydrocoele, no doubt marks a contrast, which, appearing early between the primitive Echinoderms and the Enteropneusts and Pterobranchs, became extreme as regards the last-named stock when the equally sharp *dorsal* curvature of the gut in this stock was established.

The immediate ancestor of the phyla named was, of course, a truly coelomate animal, with primitive metameric segmentation and with separate mouth and anus. Probably, also, the different coelomic cavities arose independently from the archenteron (4, p. 234). If we try to go still further back, we can only follow the well-known view which assumes that the coeloms are gastrovascular spaces that have become separated off from the digestive cavity, and that the gastrula opening has given rise by constriction to the mouth and anus. Masterman (10) derived the primitive metameric (or archicoelomatous, to use his own term) type directly from a tetramerous cœlenterate symmetry, and showed schematically how the arrangement of the coeloms in the higher phyla could thus be accounted for. On the other hand, Metschnikoff (13, p. 73) in 1874 called attention to the remarkable parallel that can be drawn between a double hydrocoele Asterid larva with two hydropores and such a Ctenophore as *Cydippe*; the hydropores, hydrocoele rays, and posterior coeloms of the former finding equivalents in the excretory pores, meridional canals, and paragastric tubes of the latter, while, in both, the digestive and coelomic cavities take origin from similarly placed regions of the archenteron. MacBride has lately (7, p. 593), in the light of recent work on the relationships of Ctenophores, laid further emphasis on the possible Ctenophore affinities of the early ancestor of the Coelomata.

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EXPLANATION OF PLATES 6 AND 7,

Illustrating Mr. James F. Gemmill's paper on "Double Hydrocœle in the Development and Metamorphosis of the Larva of *Asterias rubens*, L.

LETTERING AND ABBREVIATIONS EMPLOYED.

1, 2, 3, 4, 5, 6.—The larval ciliated processes in order as follows: 1. Median dorsal; 2. anterior dorsal; 3. posterior dorsal; 4. postero-lateral; 5. post-oral; 6. preoral.

I, II, III, IV, V.—Lead by continuous lines to hydrocœle pouches I, II, etc., and by dotted lines to arm-rudiments I, II, etc.; (*r*) and (*l*) after these numerals indicate respectively that the structures referred to belong to the right or to the left side.

a. c. Common united portion of the right and left anterior cœloms. *ant. br.* Anterior brachium. *anus.* Anus. *ax. org.* Axial organ. *ax. s.* (*l.*) Left axial sinus. *ax. s. (r.)* Right axial sinus. *b.* I–II. Basal or interradial ossicle I–II. *b.* II–III, etc., (*r*) or (*l*) Right or left interradial ossicle II–III, etc. *b. c.* Buccal cavity. *br.* The three brachia. *d. arm. r.* Aboral or disc arm-rudiments. *d. c.* Opening between dorsal horn of posterior cœlom and left middle cœlom. *d. h. l. p. c.* Dorsal horn of left posterior cœlom. *d. h. p. c.* In fig. 3 is over place of fusion of dorsal horns of right and left posterior cœloms. *hy.* Cavity belonging to hydrocœle. *hy.* I, II, III, etc. (*r*) or (*l*) Right or left hydrocœle pouches, I, II, III, etc. *hy. can.* Hydroporic canal. *hy. circ.* Circular canal of hydrocœle. *hyp. c.* Hypogastric cœlom. *int.* Intestine. *int. or s.* Internal oral circular sinus (inner perihæmal ring). *l. a. c.* Left anterior cœlom. *lat. br.* Lateral brachium. *l. hy.* I, II, III, etc. Radial pouches I, II, III, etc., of the left hydrocœle. *l. m. c.*, or *l. m. c. (l. hy.)* Left middle cœlomic region. *l. p. c.* Left posterior cœlom. *m.* Mouth. *n. r.* Nerve ring. *œs.* Œsophagus. *pap.* Papillæ beside sucker. *perih.* Perihæmal pouches of outer perihæmal ring. *phar. c.* and *phar. c. (l)* or (*r*) Pharyngeal cœlom and left or right pharyngeal cœlom. *po. cil. bd.* Post-oral ciliated band. *pod. l. hy.* or *pod. r. hy.* Sucker feet of the left or right hydrocœle. *pr. cil. bd.* Preoral ciliated band. *pr. l.* Preoral lobe. *ps. epg. c.* Pseudo-epigastric cœlom (see p. 64). *pyl. s.* Pyloric sac. *r. a. c.* Right anterior cœlom. *rect.* Rectum. *r. hy.* Right hydrocœle. *r. hy.* I, II, etc., Radial pouches I–II, etc., of right hydrocœle. *r. m. c.* or *r. m. c. (r. hy.)* Right middle cœlomic region or right hydrocœlic region. *r. p. c.* Right posterior cœlom. *s.* Sucker. *st. c. (l)* or (*r*) Left or right stone canal. *stk.*

Attaching stalk. *stom.* Stomach. *t.* II, III, etc. (*l*) or (*r*) Left or right terminal ossicle of rays II, III, etc. *ves.* Dorsal sac or madreporic vesicle. *v. h. l. p. c.* Ventral horn of the left posterior cœlom. *v. h. r. p. c.* Ventral horn of the right posterior cœlom. *w.* Small piece of seaweed.

PLATE 6.

Fig. 1.—Ventral view of normal larva about thirty-five days old showing hydrocœle lobes on left side, isolation of the right posterior or epigastric cœlom, and union of ventral horn of *l. p. c.* with right middle cœlomic cavity. The general description of this stage is given on pp. 52, 53.

Fig. 2.—Ventral view of double hydrocœle larva in which the right and left set of hydrocœle pouches have appeared. The two posterior cœloms are completely separated from the middle cœlomic regions, and their ventral horns have met and united with one another across the mid-ventral line between stomach and rectum, and have also sent round folds to enclose the rectum. Posteriorly the thickening due to the double set of aboral arm-rudiments is only shown in optical section and not in surface view.

Fig. 3.—Double hydrocœle larva a little further advanced than in last fig., and from the dorsal aspect. It will be seen that the two posterior cœloms have united with one another in front across the mid-dorsal line, and that each has acquired a secondary opening into the corresponding hydrocœle. The dorsal sac is elongated, and lies almost exactly in the middle line. In this specimen all the ciliated processes on the right side are somewhat smaller than those on the left, a condition not infrequently found in *A. rubens* larvæ apart from double hydrocœle. As in fig. 2 the disc thickening is shown only in optical section.

Fig. 4.—View, from dorsal side, of late double hydrocœle brachiolaria in which the rudiment of the disc is particularly broad, and, instead of having only the terminal ossicles on each side, shows also the interradians. The latter are in pairs, except that a single one, overlying the dorsal sac, represents the two we might have expected for interradius I-II (see p. 57). The rest of the interradians appear in the epiderm over a pseudo-cœlomic space which lies between the two layers of the dorsal mesentery, and is of the same nature as that shown in fig. 18 (see p. 57 and p. 64). Each of the terminals carries the usual number of spines.

Fig. 5.—Lateral view of double hydrocœle larva at the very commencement of metamorphosis and before attachment has taken place.

The contractions of the body-wall, which produce swelling of the anterior coelomic cavity and shortening of the preoral lobe, have just begun. On the preoral lobe an unusual arrangement of papillæ is present such as also occurs by way of variation in otherwise normal larvæ. The edges of the four arm-rudiments belonging to the left side are seen, each already showing the characteristic mesial notch.

Fig. 6.—View from ventral and slightly from left side, of double hydrocœle larva which attached itself temporarily and crawled for some distance with the help of the brachia and sucker in the manner described on p. 59. The disc rudiments are here relatively narrow, forming a contrast with those shown in fig. 4.

PLATE 7.

Fig. 7.—View from left side of double hydrocœle larva about twenty hours after metamorphosis has begun. The preoral lobe is now greatly retracted, and the ciliated processes are gradually disappearing, while the sucker is still somewhat prominent. It will be seen that the remains of the posterior ciliated band are within the circle of the hydrocœle lobes and apparently very much in the position where the oral nerve tract may be expected to form. However, examination of serial sections of different larvæ has made it practically certain that this band has nothing to do with the origin of the nerve-tract in question. As in fig. 5 the edge of the disc-rudiments is seen from the left side, only four rudiments being present, namely rudiments II-V. It will be understood that the larva would show the same appearance if viewed from the right side. Note at the anterior end the cavity of the dorsal sac and the shading to indicate the pore canal.

Fig. 8.—Young double hydrocœle starfish near end of metamorphosis as seen from left side. The slender stalk connecting the sucker with the body is seen, the insertion of the stalk being in the mid-ventral line and somewhat in front of the middle of the larva. Rupture of the stalk took place near its junction with the body as in normal development. The five hydrocœle rays belonging to the left side of this bilateral starfish are seen in the drawing, a corresponding set being present on the right side. The young starfish was more convex dorsally than is shown in the illustration (p. 62).

Fig. 9.—View from ventral aspect of an asymmetrical double hydrocœle starfish, after metamorphosis. The remains of the stalk have disappeared and the mouth is formed. The latter is somewhat curious in shape since it almost divided into two unequal parts by a fold or constriction which leaves the major opening in connection with the original left set of disc and ray structures. The smaller (right) set of rays occupies a wedge-shaped interval between pouches I and V and

arm-rudiments II and V of the larger (left) series. The vesicular appendage seen to the left of arm-rudiment V may represent a rudimentary arm-rudiment I in the original left series. Had it been fully developed and unhindered by the right hydrocœle, it would have travelled round in order to become associated with hydrocœle pouch I and thereby complete a normal starfish disc.

Fig. 10.—Transverse section through hydroporic region of the larva shown in fig. 7. Note in particular the single hydroporic canal and dorsal sac, the two stone canals and pharyngeal cœloms, and also the two extensions of the posterior cœloms which almost meet on the dorsal aspect of the rectum.

Fig. 11.—Transverse section of same larva but further forward, and showing opening of œsophagus into stomach, hydrocœle pouches I and V, the two pharyngeal cœloms and the single dorsal sac.

Fig. 12.—Transverse section of same larva but considerably further back, and passing through the dorsal opening between the *l. p. c.* and the *r. p. c.* The hydrocœle pouches are II (dorsally) and III (ventrally) on either side.

Figs. 13, 14, and 15.—Three transverse sections through the larva in early metamorphosis shown in fig. 6. In fig. 13 the section passes through the anterior portion of the junction between stalk and body, and cuts symmetrically the right and left posterior cœloms, hydrocœles, stone canals and pharyngeal cœloms. The mesial portion of the pharyngeal cœlom is seen. Fig. 14 passes a little behind the middle of the stalk and cuts the œsophagus along its full length as well as the dome of the buccal cavity. The latter still opens freely to the surface, further back than the level of the section. Fig. 15 is still further back in the series and passes through the anal opening, which is here seen in process of occlusion. For a short distance up from this opening, the wall of the rectum will also degenerate leaving, however, a portion of the rectum as well as the intestine of the larva to give rise to the rectal sac and intestine after metamorphosis.

Figs. 16 and 17.—Two transverse sections through a double hydrocœle larva in the middle stage of metamorphosis, to be compared respectively with figs. 13 and 14. Note in particular the fact that perihæmal pouch I-II on either side is arising from the posterior cœlom, and also that the rudiments of the two axial organs are appearing in front of their respective stone canals. In fig. 17 the œsophagus has become divided and the circular canal of the water vascular system is beginning to be constricted off from the *l. m. c.* Figs. 13-17 may, with advantage, be compared with figs. 27, 28, in my account of the normal development of *Asterias rubens* (4).

Fig. 18.—Vertical section through disc of the asymmetrical double

hydrocoele specimen described on p. 66, and illustrated in fig. 9. The section looks almost a normal one (cf. 4, Pl. 24, Fig. 31), but the portions of hydrocoele seen on opposite sides belong to different systems, that on the right side of the figure (including axial organ, stone canal and axial sinus) being part of the smaller right hydrocoele complex, while on the opposite side we have the left hydrocoele, etc. Note on the aboral side of the stomach a large pseudo-epigastric coelomic space (see pp. 57, 64).

On the Life-History of the Sporozoa of Spatangoids, with Observations on some Allied Forms.

By

Helen L. M. Pixell-Goodrich, B.Sc.,
Beit Memorial Research Fellow.

With Plate 8.

CERTAIN Spatangoids harbour peculiarly interesting parasites which were named by Giard in 1876 (8) *Lithocystis*, owing to the fact that their cysts invariably contain crystals. He interpreted the tail with which the spores are provided as two filaments which approached one another, and consequently did not realise the close affinities of this genus with *Urospora*, established by Schneider in the preceding year (23).

These gregarines were further studied by Léger in 1896, 1897 (13, 14), and he also allowed the genus *Lithocystis* to stand distinct from *Urospora*, owing to their possessing "des productions cristallines caractéristiques" (14, p. 145).

After an extensive study of different stages of these parasites I am quite convinced that such crystals are in no way characteristic of any one genus of gregarines, but more probably common to any parasites living in the highly mineralised cœlomic fluid¹ of Spatangoids; at all events, they are present in *Urospora* under these conditions.

¹ Presumably this cœlomic fluid has much the same composition as that of the Echinoids, *Toxopneustes lividus* and *Strongylocentrotus lividus*, analysed by Mourson and Schlagden (19, p. 792). There may, however, be slight differences, for, as Léger points out, true

In fact, no satisfactory characteristic has so far been put forward for distinguishing *Lithocystis* from *Urospora*, and it seemed at first advisable to include all the Spatangoid gregarines, of which there are certainly several distinct species, in the latter genus. However, on investigating their early stages, it was discovered that certain forms have not only flagellated gametes (Pl. 8, fig. 19) now for the first time, as far as I am aware, recorded with certainty in monocystid Gregarines, but also flagellated and therefore actively moving zygotes (Pl. 8, figs. 24-26). This characteristic appears to be constant for true *Urospora* (those with filiform tails to the spores). It is therefore suggested that on this account the genus *Lithocystis*, established by Giard (8) in 1876, be retained for the present distinct from Schneider's genus *Urospora*, established in 1875 (23).

I am much indebted to the British Association for the use of their tables, both at Naples and Plymouth, where I have studied these parasites. The investigation of *Lithocystis* was undertaken at Prof. Minchin's kind suggestion in the hope of being able to clear up the question as to the occurrence of solitary encystment. My observations on this subject are recorded on page 87. Through the kindness of Prof. G. C. Bourne, much of the work has been done in the Department of Comparative Anatomy, Oxford. My husband, Mr. E. S. Goodrich, has drawn some of the living specimens and given me other valuable assistance, especially with the figures. I take this opportunity also for expressing my thanks to Mr. A. T. Watson for most generously sending me many living specimens of *Pectinaria* from Llanfairfechan and from his collections in Sheffield.

Echinoids in general are free from Gregarines. It may be well to mention here that, although some authors have included *Strongylocentrotus* as a possible host of *Lithocystis*, there seems to be no evidence in favour of such a procedure. Many specimens examined at Naples showed no Gregarines at all, but numerous Ciliates.

MATERIAL AND METHODS.

Echinocardium cordatum from Naples, Plymouth, and Port Erin have always been found to be well infected; also the deep-water species of *Echinocardium* and *Spatangus purpureus* from Plymouth.

In examining them it has been found best, after rubbing off the spines from a small area, to make a little hole in each side of the test, taking care, of course, to avoid the coils of the intestine. The cœlomic fluid, with the contents, can then be poured out into a suitable vessel and examined with a binocular microscope. Afterwards, the inside of the test is carefully washed out with sea-water introduced by a pipette through one of the holes and the washing collected and examined in a similar way. After this has been done it is rare, on cutting round the test, to find that any parasites remain except those ripe cysts generally fixed in masses to the oral surface of the host.

The cysts containing early stages are generally free in the cavity and readily distinguished by their opacity. The cyst walls of those with ripe spores, where not covered with amœbocytes, are so translucent that the spherical mass of crystals shows up with great clearness in the interior. Nearly all my work has been done on the living parasites, though films showing most of the stages have also been made, as well as sections where possible.

Hot corrosive sublimate and acetic acid mixture have been found satisfactory for fixing the sporozoite nuclei of the ripe spore. In studying differences in the shapes of the tails it has been found best to overstain with iron hæmatoxylin or hæmatein, which are fairly readily taken up by the epispor, but very readily lost again on differentiating with iron alum. Orange G and nigrosene also stain the epispor, but not very easily. Unless well stained the tails are of course practically invisible when mounted in Canada balsam. For rough comparison I have found ordinary ink (Stephens') very convenient for staining the tails of fresh spores.

GENUS LITHOCYSTIS.

Trophozoites and Associates.—The long narrow trophozoite of *Lithocystis* has already been drawn by Léger (14, Pl. 11) and some of his excellent figures are reproduced in most text-books of Protozoology (Minchin (17), Doflein (7)). Even at an early stage there is a tendency in the myocyte of opposite sides to contract alternately, thus forming right-angled bends in the middle of the parasite, where association is later effected; at times the more pointed end is used for temporary fixation. The extraordinary movements and alterations of shape that are undergone by specimens, either singly or in association, could only be portrayed by the cinematograph. It appears that the protoplasm streams at a great rate down the centre of the parasite and its direction is suddenly reversed at regular intervals. All the time there is an eddy at the sides flowing in the opposite direction to the main stream, so that when one end is just empty there is a thin stream of fine granules into it down each side, often producing a kind of dagger-like point before the main stream of protoplasm rushes back. Léger has described the structure of the trophozoite and its movements, so that there is little to add. He figures the nucleus with a single karyosome, whereas I have constantly found a large one at either side of the elongated nucleus; in a *Lithocystis* of 2 mm. in length the nucleus was 90 μ by 40 μ , and each karyosome 20 μ in diameter. The nuclear membrane is resistant and elastic, enabling the nucleus to be squeezed quite out of its normal shape in passing the bend, where the gregarine is at times very narrow. It is carried in the main stream of protoplasm, as are also the crystals, which begin to appear at an early stage. These large calcium oxalate crystals are presumably formed by the action of the soluble calcium salts absorbed from the host's cœlomic fluid on oxalic acid secreted by the parasite. They are not very noticeable at this stage when mixed up with the dense protoplasm. It is only after encystment that they collect into the

remarkable spherical mass, in which form they are no doubt less liable to interfere with the increased activities of the protoplasm.

Trophozoites are very frequently to be seen in association. Union, which is only slight for a long time, is effected at the bend, and for hours both associates keep up their extraordinary movements, having the appearance of a large writhing X (Léger (14), Pl. 11, Minchin (17), p. 195, fig. 29a). Selenidia have been seen to go through somewhat similar movements during association, but owing to the greater rigidity of their ectoplasmic layers, their movements are not accompanied, as in *Lithocystis*, with such marked changes in outline.

By keeping the associates in cœlomic fluid in a dark place it was hoped to induce them to continue their development, but sometimes, after twenty-four hours and more, they were found to have exactly the same appearance, though with slower movements.

Occasionally forms were to be seen which had become more or less rounded off, and in which movement had almost stopped, so that their surfaces were disturbed by a rhythmical wave-like movement only (fig. 1). In this condition the parasite is an easy prey to the numerous phagocytes of the host. These arrange themselves all over the surface, uniting by their pseudopodia to form a regular network. These stages have been beautifully figured by Léger (14, Pl. 12), so that it is unnecessary to draw them again. Especially long pseudopodia project outwards, giving the parasites a spiny appearance. The protoplasm also takes on a very characteristic vacuolar structure, with crystals lying in the vacuoles. In many all sign of movement has stopped, the nucleus has more or less broken down, and evidently general disintegration is about to follow. At any rate, no real cyst is formed, and no further developmental stages ever occur, so that there can be no doubt that such spiny forms are in an advanced pathological condition. Exactly the same fate may happen to a pair after association, as figured by Léger (14, Pl. 12, figs. 3 and 4),

and reproduced by Minchin (17, p. 195, fig. 39*b*). That this is no ordinary encystment, as is generally stated, is made quite clear by comparison with normal stages. Immediately after normal association is effected a definite cyst is formed, on the outside of which amœbocytes can accumulate without harm to the parasites.

These necrotic forms were specially plentiful in the Channel during October and the end of September of this year (1914). One specimen of *Echinocardium* contained as many as thirteen, and in only three out of about fifty infected *Spatangoids* examined at that time was an ordinary trophozoite of either *Lithocystis* or *Urospora* found.

One must conclude from this that there is a loss of vitality among the parasites at this time of year which enables the phagocytes of the host to attack them more easily. Whether the loss of vitality results only in loss of movement, or also in the cessation of some secretion, it is difficult to say. There seems good reason, however, to think that during the healthy life of trophozoites there may be a secretion to which the amœbocytes are negatively chemiotatic, as is maintained by Cuénot (6). An occasional specimen has been seen, for instance, which had nearly lost all movement, and rounded off without being submitted to attack (fig. 1.) The presence of such a secretion would account also for the fact that normal associates can keep themselves free from attack during loss of movement. However, this is a difficult problem, on which there has already been considerable discussion, Siedlecki (25, p. 437) taking the view that movement of the parasite alone is sufficient to prevent the attack by amœbocytes in the case of annelids.

Another observation of some interest in connection with these degenerate forms was provided in October by two associates which had managed to form a cyst round themselves for protection against phagocytes, but had evidently no vitality left to proceed further, for they were lying shrunken and obviously moribund at one side of their cyst. Now, in these the protoplasm had assumed that characteristic

vacuolar appearance with crystals in the vacuoles which, I maintain, is a sign of necrosis.

Sufficient proof has now been brought forward to show that the above-described forms, with amœbocytes forming a spiny covering, are undoubtedly in a pathological condition. These were, however, the forms referred to by Léger (14, p. 252) as cases of normal encystment and solitary encystment. Lithocystis has consequently been quoted in text-books as a Gregarine in which a single sporont can encyst by itself and proceed to form spores. It is clear, therefore, that there is now no reason in the case of Lithocystis at any rate for thinking that solitary encystment does, or can, take place as a normal stage of development.

As will be explained below, there are constant differences in the zygotes and spores, which make it quite clear that there is more than one species of Lithocystic parasitic in Spatangoids. To prevent confusion, therefore, it has been necessary to introduce new names by which to distinguish these species. These have been made as few as possible, and several forms have been mentioned as varieties, which may eventually have to be separated as distinct species. The trophozoites, as one would expect, show no important differences, and it is only by careful comparison that they can be distinguished. The original *Lithocystis schneideri*, described by Giard (8) from specimens of *Echinocardium cordatum* from Wimereux, is apparently the largest form. This is a species which I have found very commonly in *Echinocardium* from Plymouth and Naples. In the spore (Pl. 8, figs. 12 and 14) the two edges of the tail have somewhat the appearance, before being stained, of two flagella, and this, no doubt, accounts for Giard's interpretation referred to above. When full grown the trophozoites are 3 to 4 mm. long, and the cysts formed by an associated pair are the largest to be met with, being sometimes as much as 2 mm. in diameter. *L. foliacea*, n. sp., has a considerably smaller trophozoite, and its cyst seldom exceeds 6 mm. in diameter. This species is also found in *Echinocardium*, both in the Channel and Mediterranean.

The third form, *L. microspora*, n. sp., is found in *Spatangus* from the Channel. It is much smaller than the other species, having a trophozoite less than 1 mm. in length, and its cysts only .1 to .3 mm. in diameter.

With experience it becomes fairly easy to predict which species will be found in any one cyst by its size, but some of the *Urospora* cysts described below are exactly the same size as the small *Lithocystis* species (*L. microspora*). Generally the cysts containing adult spores of all species and both genera are massed together on the oral side of the host. The cysts are held in masses by, and more or less covered with, amoebocytes together with a quantity of dark purple or black pigment. Presumably these masses may sometimes represent an accumulation of years; at any rate, the number of cysts varies greatly in different individual hosts.

Gametes and Syngamy.

Parasites which had recently associated and safely encysted formed spheres which were generally found floating freely in the coelomic fluid. They could readily be distinguished from old cysts containing ripe spores by their greater opacity. By bursting cysts containing full grown gametes syngamy has been seen taking place on the slide. There is a slight sexual dimorphism, but apparently no constant difference in the size of the nuclei at this stage. The gamete which, as explained below on p. 94, must be considered as the female has its nucleus close to or extending into the small conical projection at the apex of which is the centrosome (Pl. 8, fig. 2). In the other gamete (fig. 3) a corresponding process has not been seen; it is probably lost at an early stage, the centrosome having probably travelled inwards with the nucleus, which tends to be central in position. Union between such a pair of gametes takes place along definite surfaces, producing a combination of very constant form in which the nuclei always occupy the relative positions shown in fig. 4. Exactly similar forms have been obtained directly, together with the slightly later

stages represented in fig. 5, by bursting other cysts. Consequently there seems to be no doubt that they are normal and that the gametes and zygotes do not possess at any time a flagellum such as is described below for *Urospora*.

Soon after this union fusion of the cytoplasm of the two gametes takes place, and the "male" nucleus approaches the "female," although they do not at once combine (fig. 5). This delay in the formation of the synkaryon is quite remarkable. The term zygote should, strictly speaking, only be used for the body formed by the complete fusion of the gametes—nuclei as well as cytoplasm. It therefore seems necessary to have another term to denote such stages as those represented in figs. 5, 11*a*, 26, etc., where the cytoplasm is completely fused but the gamete nuclei are still separate. I therefore venture to introduce the term prozygote to denote the body formed by fusion of the gametes prior to the fusion of their nuclei to form the the synkaryon (figs. 5 and 26). It will be observed that in the living (figs. 9, 10, 24, and 25) it is difficult, if not impossible, to distinguish whether the nuclei have fused or not.

The tail of the spore develops at the position of the cone and the centrosome of the female gamete, and is sometimes quite well developed before the synkaryon is formed.

Zygotes and Spores.

Even before this stage is reached it is quite easy to distinguish constant differences which make it quite clear that one is dealing with different species of *Lithocystis*. The adult spores are also very characteristically different as regards the shapes of the tails. Both Giard (8) and Léger (14) noticed the different sizes of the spores found in *Spatangoids* and referred to them as normal spores, microspores, etc. Léger also noticed the different shapes of the tails, but attributed them to stages in growth (14, p. 260). It seems, in the light of the present knowledge of spore-formation, very improbable that a passive organ like the tail of *L. schneideri* (fig. 14) corresponding to Léger's fig. 1, Pl. 13, could change its shape

to that of fig. 234 (Léger's fig. 2, Pl. 13) during a brief space of time. It is now proved that such is not the case by following the stages passed through from the zygote to the adult spore.

The tails in all *Lithocystis* are fundamentally hollow extensions of the epispore, but the walls of these tubular structures are thin and easily collapse, especially at the distal end. In *L. foliata* the whole tail becomes flattened at a very early stage, but specimens of *L. schneideri* are often to be seen in which the distal half or third of the tail is flattened while the rest remains tubular.

After the rounding off of the young spore into its final oval shape (Pl. 8, figs. 7 and 12) and the completion of the tail, the nucleus moves towards the centre of the spore and proceeds to divide into two, then into four. At this stage the epispore becomes considerably thickened. In order, therefore, to obtain good preparations of the spore with eight nuclei it has been found necessary to fix in hot corrosive acetic mixture. When the sporozoites become separated off there is a highly refringent granular residue left in the middle of the spore (fig. 13). With regard to the sporozoites themselves, there is little to say—the nucleus is about midway between the blunt and somewhat pointed extremities. Escape is effected through the funnel of the spore at the opposite end from the tail. If destined to develop, the spores, after being liberated by the rupture of their cysts in the sea water, are presumably taken in by fresh hosts with their food.

In *L. schneideri* the prozygote remains two-lobed for some time (Pl. 8, figs. 9–12), and the tail early assumes its normal tubular character. Apparently fusion of the nuclei does not take place until the prozygote has assumed the shape shown in (fig. 11*a*); the zygote (fig. 11*b*) then rounds itself off (fig. 12), but the synkaron remains near the tail until it begins to divide to form the sporozoite nuclei. The characteristic way in which young spores arrange themselves in groups inside the cysts with their tails towards the centre, and the later arrangement of the ripe spores in rosettes with their

funnels towards the centre and their tails projecting straight outwards, have been described by Léger (14). This characteristic rosette-formation is more marked in *L. schneideri* than in any other species. In some others there is often an arrangement in elongated groups.

In *Spatangus purpureus* from Plymouth the spore of a form, otherwise very similar to *L. schneideri*, often shows markings which give the epispore a somewhat papillated appearance. The tail of both spore and prozygote (fig. 10) is slightly longer than that of *Echinocardium* forms so that possibly it should constitute a distinct species.

In the case of *L. foliacea*, n. sp., the prozygote is top-shaped (Pl. 8, fig. 5) and soon passes into the true zygote (fig. 6); both of these forms have been obtained in the same cyst. Forms represented by figs. 4 and 5 have also been obtained in one and the same cyst, and as it is usual for all the contents of an individual cyst to reach any one stage almost simultaneously, it is concluded that in the case of this species, at any rate, the prozygote stage is very brief. After the formation of the synkaryon the characteristic tail grows apace; this, instead of remaining straight as in *L. schneideri*, expands near its distal end to form a flat leaf-like expansion considerably wider than the spore (Pl. 8, figs. 7 and 8). These tails are very thin and liable to twist, so that in unstained preparations it is difficult to make out their true shape.

In *Lithocystis microspora*, n. sp., both zygotes and spores are distinctly smaller than in the other two species of *Lithocystis* and are in consequence easily distinguished. The zygote has the form shown in Pl. 8, fig. 16 with a small triangular tail. The adult spore has a narrow tubular tail which is generally flattened and two to three times the length of the actual spore (Pl. 8, fig. 15).

GENUS UROSPORA.

Trophozoites and Associates.

The trophozoites of *Urospora* are smaller than *Lithocystis* rarely attaining a length of half a millimetre (Pl. 8, fig. 17).

They are elongated with coarsely granular protoplasm containing crystals as in *Lithocystis*. A clear epimerite may generally be distinguished at one end and by this temporary attachment may be effected. The parasite undergoes only sluggish sinuous movements with slight change of outline. The nucleus is nearly round and has often only a single karyosome. The trophozoites, either before or after association, may be attacked by amoebocytes in the same way as *Lithocystis*, and after studying many instances I feel confident that the same explanation should be given. After normal association encystment takes place as usual and the crystals collect into a spherical mass.

Gametes and Zygotes.

The cysts are, as a rule, considerably smaller than those of *Lithocystis* except *L. microspora*. They have fairly transparent walls, through which stages in nuclear division may be seen. At the stage represented in fig. 18 there is a distinct sexual difference in the nuclei—the upper associate with larger and less chromatic nuclei than the other, being presumably the female.

Any movement inside the cyst may be easily detected, and by breaking the wall syngamy has been detected taking place on the slide. One gamete, presumably the “male,” has a long flagellum (Pl. 8, fig. 19), by the lashing of which it moves actively and seeks out the “female,” in which no flagellum has been seen. Here, again, as in *Lithocystis*, union takes place along definite surfaces, so that a prozygote of characteristic shape is formed (figs. 20, 24–26). This retains its single flagellum, by the lashing of which it maintains a twirling movement about the rudimentary tail. This motion has been clearly seen through the cyst wall before rupture.

When Siedlecki (24) in 1889 described the true gametes of Gregarines and recorded for the first time the method of fertilisation, he quite realised that in the so-called “dance of the sporoblasts” the movements appeared to be due to flagella, although he could not distinguish them either in the

living or in fixed preparations. Other authors have also suggested their occurrence, but as far as I can make out this is the first genus of monocystid Gregarines in which they have been seen with certainty. Further, since here only the "male" gamete appears to be flagellated, the *Urospora* form a connecting link between those monocystid Gregarines in which isogamy is nearly perfect and such forms as the polycystid *Stylorhynchus*, in which, as shown by Léger (15), the "male" gamete is not only flagellated, but so very different from the "female."

In *Urospora*, as in *Lithocystis*, fusion of nuclei does not take place at once (fig. 26), so that there is a more or less prolonged prozygote stage during which the "female" nucleus is situated in the developing tail, towards which the "male" nucleus slowly moves from its original position near the flagellum.

The prozygote of the Mediterranean species *Urospora neapolitana*, n. sp., has a most striking appearance with its peculiar corkscrew-like tail (Pl. 8, fig. 20). This species has not been met with in the *Echinocardium* or *Spatangus* of the Channel.

The prozygote of *Urospora echinocardii*, n. sp., the form found commonly in the Channel *Spatangoids*, is somewhat larger and has a more or less straight and pointed tail (fig. 21). It only differs very slightly, if at all constantly, from the prozygote of the *Urospora* occurring in *Spatangus* (figs. 25 and 26). The spores into which they develop are also so similar that there seems to be no need at present, at all events, to establish another species for the *Spatangus* parasite.

After fusion of the nuclei, the oval shape of the adult spore is soon assumed (figs. 21, 22, and 28). The synkaryon divides by a simple process into two, four (fig. 29), then eight. Meanwhile, the epispore thickens considerably, and inside the spore fine protoplasm collects round the nuclei to form the eight sporozoites, leaving a coarsely granular and refringent residuum in the centre.

SPORES.

U. neapolitana is also easily distinguished from the Channel species by means of its spore. This is almost round, and it has a very long filamentous tail about twenty times the length of the spore itself. It remains very tightly coiled (fig. 23*a*) even during fixation, except in rare cases, when it unwinds (fig. 23*b*). In *U. echinocardii*, on the other hand, the loosely-coiled filamentous tail with which the spore emerges from the cyst easily unwinds (fig. 27). It is at most seven times the length of the spore itself and the proximal tubular region of the tail tapers much more gradually into the filament. It may be mentioned here that the filament is difficult to see in the living, and if unstained and mounted in Canada balsam it is practically invisible (fig. 29), so that spores appear under these conditions to have a short, tapering tail only.

HOMOLOGY OF THE GAMETES AND ZYGOTES.

In *Urospora* it seems clear that the flagellated gamete represents the "male" and the non-flagellated the "female." The tail therefore develops from that part of the prozygote which is derived from the "female." It is reasonable to suppose that the disposition of the gametes in these early zygotes of *Urospora* and *Lithocystis* is the same. Consequently, in *Lithocystis* the "female" gamete gives rise to that region of the prozygote from which the tail develops. In fact, the tail appears to be produced by the further growth of the conical projection bearing the centrosome of the original "female" gamete. It may even be suggested that the characteristic tail of these *Lithocystis* and *Urospora* spores has been phylogenetically derived from the flagellum originally possessed by the "female" gamete, as it corresponds in position with the flagellum of the "male" gamete.

In *Urospora* the flagellum of the "male" gamete arises from the region of the centrosome at the apex of the cone. Similar conical projections, with apical centrosomes, have

been described in the formation of the gametes of both sexes of various other Sporozoa, e. g. *Aggregata* (21b). The "male" gamete of *Lithocystis*, in which apparently the flagellum does not develop, has no doubt lost its conical process at an early stage of development, and its centrosome has possibly passed inwards with the nucleus.

SYSTEMATIC.

Below are given the characteristics on which these genera were originally established. It will be noticed that in neither case is any mention made of the movements of the parasites. Other features of the trophozoites of these monocystid Gregarines are of very little value from a systematic point of view. The only given characteristic which is of any use for this purpose is, I think, the filiform tail of the *Urospora* spores in contradistinction to the tubular ones of *Lithocystis*. The latter tend to become flattened and easily recognisable, though even here the narrow tubular tail presented by *L. microspora* approaches that of *Urospora*, in which the apparently filiform tail has presumably a tubular origin.

It is with a considerable amount of hesitation that I formulate the flagellated nature of the gametes and zygotes among the characteristics of the genus *Urospora* because I have not had as yet the opportunity of investigating certain forms which have been ascribed to the genus. Some of the results of my search after these are recorded below, and I fully realise that it may be years before gametes and zygotes of all these elusive forms can be found and studied. However, should such flagellated gametes and zygotes be found later not to be constantly present in *Urospora* and absent in *Lithocystis*, it seems to me that the latter genus would be superfluous, and that all the five forms described should be included in the older genus *Urospora*.

Genus *Lithocystis*, Giard, 1876 (Characteristics given by Labbé) (12a, p. 42).—"Individus de grande taille, ovoïdes ou cylindriques avec entoplasm remplie de cristaux clino-

ombliques d'ovalité de chaux. Kystes sphériques, spores, longuement ovoïdes, tronquées à une des extrémités : épispore formant un tube à parois délicates, très allongé et sinueux: Toutes les spores sont rangées dans le kyste en groupes, radiamment autour de centres communs (sporozoites 8?).”

Extended Characteristics.—Monocystid Engregarines, with elongated trophozoites continually changing their shape, and containing rapidly flowing protoplasm; cysts spherical, gametes non-motile, slightly anisogamous. Prozygotes motionless, with short tubular tails. Spores when ripe tend to form rosettes. Epispore produced into a funnel at one end, through which the eight sporozoites escape, and into a tubular, but generally flattened, tail at the other.

(1) *Lithocystis schneideri*, Giard.—Trophozoites may attain a length of 4 or 5 mm. Cysts 1–2 mm. in diameter. Prozygote bi-lobed. Spores generally 22–24 μ long and 8–9 μ wide, with tail about four times this length, but narrower than the spore.

Hosts.—*Echinocardium*, Naples and Channel, and *Spatangus purpureus* from the Channel.

(2) *Lithocystis foliacea*, n. sp.—Trophozoites smaller than *L. schneideri*; maximum length 2–3 mm. Cysts generally about .6 mm. in diameter. Zygote top-shaped. Spores about 24 \times 9 μ , but their tails are only three times as long, and widen out towards their distal end to form a leaf-like expansion, which may be as much as 15 μ wide.

Hosts.—*Echinocardium cordatum*, Naples, Plymouth.

(3) *Lithocystis microspora*, n. sp.—Trophozoites 1 mm., or less, in length. Cysts .1–.3 mm. in diameter. Zygotes small; spores generally 12–13 μ long and 6–7 μ wide, with tail two to three times this length, narrow, and tapering.

Host.—*Spatangus purpureus*, Channel.

Genus *Urospora* (Aimé Schneider), 1875 (23).—“Monocystidée de forme allongée, terminée en pointe aiguë en arrière, arrondie en avant et légèrement micronée au pôle supérieur. Epicyte à simple contour. Entocyte à grains très-fins.

Kystes à sporulation complète, déhiscentes par simple rupture. Spores pourvues d'un appendice immobile, filiform, environ de la longueur de la spore, et inséré sur son extrémité la plus large. Cette spore contenant, à l'état de maturité, six ou sept corpuscles falciformes très-allongés et diversement groupés à son intérieur, offrant en outre un nucleus de reliquat au centre ou à la base des corpuscles."

Emended Characteristics.—Monocystid Eugregarines. Trophozoites elongated, and coarsely granular. Somewhat sinuous movements with slight change of outline. Anisogamy, ♂ gametes with a long flagellum, ♀ non-motile. Early zygotes (Prozygotes) motile retaining flagellum until after beginning of the formation of the tail. Spores with eight sporozoites. The epispore produced into a funnel for the escape of the sporozoites at one end, and at the other into a filamentous tail of varying length.

(1) *Urospora neopolitana*, n. sp.—Trophozoite small, about 200–300 μ long, and 40 μ wide. Cysts 100–200 μ in diameter. Prozygotes motile retaining flagellum of ♂ gamete until corkscrew-like tail is well developed (fig. 20). Adult spores about 12 μ long and 7 μ wide. Tails about twenty times the length of the spores, but generally tightly coiled (fig. 23).

Host.—*Echinocardium cordatum*, Naples.

(2) *Urospora echinocardii*, n. sp.—Trophozoites and cysts indistinguishable from those of *Urospora neapolitana*. Prozygote flagellated and with a pointed or fusiform tail (figs. 24–26). Spores may be as long as 19 μ . Their tails are six or seven times this length and never form a tight coil as in *Urospora neapolitana* (fig. 27).

Hosts.—*Echinocardium* and *Spatangus*, Plymouth.

OBSERVATIONS ON CERTAIN PARASITES INCLUDED BY LABBÉ
AND OTHERS AS UROSPORA.

(1) On the so-called "*Urospora sænuridis*" (Köll.) from *Tubifex*. References taken from Labbé (12a, p. 43).

1843. Gregarina s., Kölliker (11 (a), p. 12).

1882. *Urospora* s., Nasse (21a, p. 26).
 1882. „ „ Bütschli (3, p. 557).
 1872. „ „ Lankester (12b, p. 348).

After studying Kölliker's description and figures I quite agreed with Hesse (10, p. 43, footnote) that the gregarine there described has no connection with *Urospora*, but is a *Monocystis*.

Schneider was apparently the first to suggest that the parasite described by Lankester in 1872 should be included in his new genus *Urospora*, and Labbé definitely included this form among *Urospora* without expressing any doubt, and so have other modern authors. Lankester recorded (12b, p. 348) that he only found one specimen of *Tubifex* infected with ripe spores and no doubt purposely refrained from giving any name to the parasite. He, however, pointed out the resemblance to the psorosperms of fish and to this group of Sporozoa (*Neosporidia*) it undoubtedly belongs. The presence of a tail to the spore probably induced some observers to rush to the conclusion that it was a species of *Urospora*, but I have no doubt that this rather rare parasite is identical with *Myxocystis ciliata* described by Mrazek (20) from *Limnodrilus*. The results of studying this parasite, which presents some interesting problems, I hope to publish shortly.

The other two supposed references to this hypothetical species, *Urospora sænuridis*, can be rapidly dealt with. Nasse's observations (21a, p. 26) undoubtedly refer to the neosporidian *Myxocystis ciliata*, and Bütschli mentioned (3, p. 55, footnote) that he had not seen the parasite, but only judged it to be *Urospora* from Nasse's figures.

Thus this "species" of *Urospora* may be considered as truly a myth!

(2) *Urospora nemertis* (Köll.). References taken from Labbé (12a, p. 43).

1845. *Gregarina* n., Kölliker (11 (b), p. 100).
 1848. „ „ „ (11 (a), p. 1).
 1867. „ „ McIntosh (16, p. 38).
 1875. *Urospora* n., Schneider (23, p. 597).
 ? 1893. „ „ Bürger (1, p. 208).

So far I have searched without success for this parasite which Schneider said was rare in Valenciennia at Roscoff. Kölliker's gregarine came from *Nemertes delineatus* (*Polia delineata*) from Naples. It seems somewhat doubtful, however, judging from his description (11 (a), p. 1) whether he was dealing with *Urospora*. I have been more fortunate in finding the parasite in *Lineus gessensis* (*Borlasia olivacea*) described by McIntosh in 1867. From this early description alone it is quite clear that it is a very different parasite from *Urospora* with which, so far as I know, McIntosh has never suggested its affinities. The thick gelatinous cyst (staining bright blue with Nigrosene) separates it clearly, but I have not yet been able to study it fully owing to its rarity. Bürger's parasite, briefly recorded in 1893 (1), and again in his monograph of the Nemerteans, 1895 (2), is likely to be the same as McIntosh's parasite. In 1896 Gravier (9, p. 307) recorded a parasite from a Phyllodocid (*Eulalia punctifera*, Grube), and this has also been included among the *Urospora*. It may be of interest to mention in this connection that at Plymouth in September I found a *Lineus* which had swallowed a whole Phyllodoce nearly as big as itself. It seems to be so very important that records of the natural food of all hosts should be kept if progress with the life-histories of many parasitic protozoa is to be made.

When the spores of *U. nemertis* are again found it will be interesting to see whether their tails are as short as described and figured. The thin filamentous ends of the tails in *Urospora* are difficult to see in the living and practically invisible when mounted in Canada balsam unless they have previously been deeply stained. Consequently, spores which have been differentiated to show the contained sporozoite nuclei have the appearance shown in Pl. 8, fig. 29 with only the thick proximal part of the tail at all visible, and this is very similar to Schneider's figure of *U. nemertis* (23, Pl. 21, fig. 4 a-c).

(3) *U. sipunculi* from *Sipunculus nudus* and *U. synaptæ* from *Synapta inhærens*, and *S. digitata*

are well established species that require much further study.

(4) *Urospora lagidis*, St. Joseph (22); Brasil (4 and 5).

Host.—*Pectinaria* (*Lagis*) *koreni*.

From Brasil's interesting description of *Urospora lagidis*, the gametes seemed to have a great resemblance to those of *Lithocystis* and as he did not figure the spore I made strenuous efforts to re-obtain the parasite. Six specimens of *Pectinaria* that were procured from the only collecting ground at Plymouth proved to be uninfected, but nearly all the specimens from Llanfairfechan had a good infection and from them I have been able to obtain other stages besides spores. These have been studied chiefly in a living condition, whereas Brasil studied fixed material.

The milk-white trophozoites can be seen through the body-wall of living *Pectinaria* roaming about in the cœlom and encysted specimens form spheres about 1 mm. in diameter which are generally carried forwards towards the oral end of the host. Here, as they ripen, they gradually become massed together connected by the amœbocytes on their surfaces and often suspended from some of the viscera or the body wall.

The spore (Pl. 8, fig. 30) proved to have a tail much more like that of *Lithocystis* than *Urospora*. At its other end there is a deep funnel (not two spines as previously described). I have not, so far, been fortunate enough to obtain living gametes, but the zygotes that have been met with several times have been quite motionless.

All these details point to the fact that the *Pectinaria* parasite should be regarded as *Lithocystis* rather than *Urospora*. I would not venture, however, to make this alteration without the most conclusive evidence, and for this, as pointed out on p. 95, it will be necessary to study not only the living gametes of this species, but also those of *U. nemertis*, *U. sipunculi*, and *U. synaptæ*.

SUMMARY.

(1) The sporozoa of Spatangoids, previously referred to *Lithocystis schneideri*, Giard, include at least five species. In addition to Giard's original species of *Lithocystis* it has been necessary to establish two others, namely, *L. foliacea*, n. sp., and *L. microspora*, n. sp. Further, the genus *Urospora* is also represented by two species which have not been recorded before, namely, *U. neapolitana*, n. sp., from Naples, and *U. echinocardii*, n. sp., from Plymouth. On the other hand, it has been shown that there is no such species as "*Urospora sænuridis*," which has been ascribed to *Tubifex* by some authors.

(2) The instances of so-called "solitary encystment," of which *Lithocystis* is quoted in text-books as furnishing a clear case, are shown not to be normal stages at all, but necrotic specimens attacked by the phagocytes of the host.

(3) In both *Lithocystis* and *Urospora* there is intercalated a stage—Prozygote—in which the cytoplasm of the gametes has fused and the tail of the spore has appeared, but the nuclei have not yet combined to form the synkaryon of the true zygote.

(4) In *Urospora*, both the "male" gamete and the prozygote are flagellated and motile.

October, 1914.

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EXPLANATION OF PLATE 8,

Illustrating Mrs. Helen L. M. Pixell-Goodrich’s paper on
 “The Life History of the Sporozoa of Spatangoids with
 Observations on some Allied Forms.”

[Unless otherwise stated, the parasites figured came from *Echinocardium cordatum*, Naples. Preparations were stained with iron-hæmatoxylin and drawn at an approximate magnification of 2000, except where otherwise indicated.]

Fig. 1.—*Lithocystis schneideri* rounding itself off and still un-
 attacked by amœbocytes. Drawn from the living. $\times 16$.

Fig. 2.—Female gamete of *L. foliacea* stained Carm Alum.

Fig. 3.—Male gamete of *L. foliacea* stained Carm Alum.

Fig. 4.—Uniting gametes of *L. foliacea* stained Carm. Alum.

Fig. 5.—Prozygote of *L. foliacea*.

Fig. 6.—True zygote of *L. foliacea* after the formation of the Syn-
 karyon.

Fig. 7.—Uninucleate sporoblast of *L. foliacea*. $\times 1000$.

Fig. 8.—Adult spore of *L. foliacea*. $\times 1000$.

Fig. 9.—Prozygote of *L. schneideri*, living specimen.

Fig. 10.—Prozygote of *L. schneideri* from *Spatangus pur-
 pureus*, Plymouth. Drawn from the living.

Fig. 11.—Later stages of *L. schneideri* showing the proximal ends
 only of tails; (a) Prozygote and (b) true zygote, after the formation
 of the sinkaryon. $\times 1000$

Fig. 12.—Sporoblast of *L. schneideri*. $\times 1000$.

Fig. 13.—Spore head with sporozoites and refringent residuum. Drawn from the living. $\times 1500$.

Fig. 14.—Adult spore of *L. schneideri*. $\times 1000$.

Fig. 15.—Adult spore of *L. microspora* from *Spatangus*, Plymouth.

Fig. 16.—Zygote of *L. microspora* from *Spatangus*, Plymouth.

Fig. 17.—Trophozoite of *U. echinocardii* from *Spatangus*, Plymouth. $\times 160$.

Fig. 18.—Encysted associates of *U. neapolitana* (a) ♀ with large nuclei; (b) ♂ with small, more chromatic nuclei. $\times 260$.

Fig. 19.—Flagellated gamete (♂) of *U. neapolitana*. Drawn free-hand from the living by E. S. G. Magnification slightly over 2000.

Fig. 20.—Flagellated Prozygote of *U. neapolitana*. Drawn similarly to fig. 19.

Fig. 21.—Zygote of *U. neapolitana* after loss of flagellum.

Fig. 22.—Uninucleate sporoblast of *U. neapolitana*.

Fig. 23.—Adult spora of *U. neapolitana* (a) with tail tightly coiled; (b) with tail partly uncoiled.

Fig. 24.—Living Prozygote of *U. echinocardii* from *Echinocardium*, Plymouth.

Fig. 25.—Living prozygote of *U. echinocardii* (?) *Spatangus*, Plymouth.

Fig. 26.—The same Prozygote, showing nuclei not fused; stained iron hæmatoxylin and Orange G.

Fig. 27.—Adult spore of *U. echinocardii* from *Echinocardium*, Plymouth.

Fig. 28.—Sporoblast of *U. echinocardii* from *Spatangus*, Plymouth, stained iron hæmatoxylin and Orange G.

Fig. 29.—Young spore of *U. echinocardii* with four nuclei; from *Spatangus*, Plymouth.

Fig. 30.—Ripe spore of *Urospora lagidis* from *Pectinaria* (*Lagis*) *koreni*.

Studies on Parasitic Protozoa.

III.

- (a) Notes on the Flagellate *Embadomonas*.
- (b) The Multiplication Cysts of a *Trichomastigine*.

By

Doris L. Mackinnon, D.Sc.,

Assistant in the Zoology Department, University College, Dundee.

With Plate 9.

(a) THE FLAGELLATE *EMBADOMONAS*.

Introductory.

IN 1911, I recorded from the intestine of trichopterous larvæ, a slipper-shaped flagellate with a relatively enormous cytostome. I named the organism *Embadomonas*, and pointed out that it showed affinities with *Chilomastix Alexeieff*, 1911 (*Syn. Macrostoma*, Alex., *Tetramitus* Alex.), from which it differed in its general form, and in the number of the flagella. The species from trichoptera I called *E. agilis*.

I have since found *Embadomonas* in far greater quantity in the intestine of the crane-fly larva (Mackinnon, 1912). Here there are two distinct species; one is morphologically indistinguishable from *E. agilis*, but the other and more abundant is a much larger, more robust form in which the details of structure, rather obscure in *E. agilis*, can be studied with ease. As I have also found dividing and encysted stages, I am now in a position to supplement my former description. I do so the more readily that I deplore the hasty and incomplete accounts of "new" protozoa of which the literature

is already only too largely composed. Sometimes these "descriptions" are not illustrated by a single drawing: sometimes a figure of some selected stage is given, so little typical that it helps other observers scarcely at all in their work of identification. The classification of the flagellate protozoa is admittedly full of anomalies and confusions. The best way to set about clearing away some of these is to work out carefully, and as fully as may be, the structure and life-history of every "new" form described, the account to be accompanied in every case by an adequate and representative series of drawings. In this way comparison between different forms becomes at least possible, and in time a better understanding may lead to a more natural grouping. The present tendency, however, seems to be in the opposite direction. It is the fashion to start with a theory, and then go out in search of organisms wherewith to illustrate it; the mind and eye thus prejudiced are aware of and record only those cases that do what is expected of them, by conforming to the views of the particular school to which the so-called scientist belongs. The "centriole question" with its attendant absurdities and exaggerations is a case in point.

The Genus *Embadomonas*, Mackinnon, 1911.

Diagnosis.—This genus contains small slipper-shaped flagellates, characterised by a very large cytostome bordered by prominent lips, which are more or less siderophilous, and two flagella, not so long as the body, one acting as an organ of locomotion, and the other lying in the cytostome; the spherical nucleus is placed at the anterior end of the body; the two basal granules, from which rise the flagella, lie at the anterior border of the cytostome. There is a definite periplast, which prevents deformation of the body. The anterior part of the body shows a well-marked torsion. The cysts are relatively small, and are ovoid in form.

As "species characters" may be used: (1) The form of the body; (2) the nature of the periplast; (3) the degree of development of the cytostome and its lips; (4) the size of the cysts.

Embadomonas agilis, Mackinnon, 1911 (Pl. 9, fig. 21-26).

Exceedingly slender, slipper-shaped body, with the anterior end bent back, and the posterior end sharply pointed. There is a delicate periplast. The cytostome is large, but its borders only feebly siderophilous. The flagellum within the cytostome is exceedingly delicate and inconspicuous: it cannot be made out at all in any but the largest individuals.¹ The nucleus is spherical, with a central group of chromatin granules, and some peripheral chromatin; the nuclear membrane is often very indefinite.

Dimensions of flagellates: $4\mu \times 1.5\mu$ to $11\mu \times 3\mu$.

Dimensions of cysts: $3.5\mu \times 3\mu$ to $4\mu \times 3\mu$.

Habitat.—Intestine of trichopterous larvæ and larvæ of *Tipula*.

Embadomonas alexeieffi. Mackinnon, 1912 (Pl. 9, figs. 1-20).

Of much heavier, more "robust" build than the preceding species, the anterior end only slightly bent back, if at all—the posterior end nearly always rounded and blunt.² The periplast is relatively thick and well-developed. The cytostome is large, with prominent lips, which are very markedly siderophilous. The two flagella are well

¹ I overlooked the presence of this second flagellum until quite recently.

² The form of *E. agilis* may be compared to a lady's slipper with a high heel, that of *E. alexeieffi* to a clumsy sabot.

developed; the one lying in the cytostome often stains more deeply than the other. The spherical nucleus has an exceedingly definite outline; the chromatin is very variously disposed, but most common is the arrangement with a central group of granules and the peripheral chromatin mainly concentrated into a crescent-shaped mass at one side of the nuclear border.

Dimensions of flagellates: $7\ \mu \times 5\ \mu$ to $16\ \mu \times 9\ \mu$.

Dimensions of cysts: $5\ \mu \times 4\ \mu$ to $6\ \mu \times 5\ \mu$.

Habitat.—Intestine of larvæ of *Tipula*.

Division.

I have been able to find only a few individuals of *Embadomonas* (*E. alexeieffi*) in division, though I have searched through many preparations crowded with the organism. Few though these are, I think they are representative enough to show the general process of division. This is of some interest since we know practically nothing of the division in the allied flagellates, *Chilomastix* and *Fana-pepea*.

The nucleus elongates (Pl. 9, figs. 4 and 5), and forms a spindle at the poles of which lie the basal granules supporting the flagella. The chromatin is disposed over the spindle in more or less definite granules, which travel to the poles as the spindle elongates, without first forming an equatorial plate, as far as I have been able to discover (Pl. 9, figs. 6, 7, and 8). The spindle thins and disappears (Pl. 9, fig. 9). Fig. 10, pl. 9, shows daughter nuclei concentrating, one at each side of the broadened organism, while the last trace of the spindle lies between them. The daughter nuclei now seem to go through a stage in which they appear as clear vesicles, with the chromatin in peripheral blocks and strands. Later, they take on the more usual form, with a central mass of granules and a peripheral layer.

A very marked constriction now appears midway between the nuclei (Pl. 9, figs. 11–15), and the two halves separate,

hanging end to end till the uniting isthmus breaks. Flagellates recently emerged from division may have the posterior end drawn out into a point.

The fate of the cytostome and the flagella is very variable. In some rare cases the cytostome seems to widen out laterally, and to be divided between the daughter individuals by the new constriction that separates them one from another (Pl. 9, fig. 11). But as a rule (Pl. 9, figs. 6-10) it disappears, and apparently is reformed in each daughter individual. This second condition of things is said by Alexeieff to occur in *Chilomastix caulleryi*, and Kuczynski figures the same thing for *Chilomastix intestinalis*.

One flagellum may pass with its basal granule to each end of the spindle (Pl. 9, fig. 5); sometimes the basal granule divides there almost at once (Pl. 9, fig. 8), and the second flagellum grows out very early; at other times the formation of the second flagellum may not have taken place even at quite a late stage (Pl. 9, fig. 13).

On two or three occasions I found individuals, such as that shown on Pl. 9, fig. 2, in which there were two nuclei. These nuclei did not occupy the opposite sides of the organism, as one would expect if they were the product of a division, but lay pressed close together at the anterior end. They were each below the normal size for an organism of the size in which they occurred. Probably such individuals should be regarded as "freak" forms, but the possibility of a sexual process should be borne in mind.

Encystment.

The cysts of *Embadomonas* are very abundant in the larvæ of *Tipula*. The encysting individual ceases to feed and loses its flagella, its cytoplasm shrinks away from the pellicula, which surrounds the cyst like a loose coat and persists for a long time (Pl. 9, fig. 17). The cytoplasm becomes more compact and dark-staining, especially around the margins, and there it forms a thick and definite cyst wall, "à double contour" (Pl. 9, fig. 18). The cyst is from the

first ovoid, but this shape becomes more marked in the later stages. Within the cyst the borders of the cytostome remain as dark-staining, loop-shaped strands. The nuclear membrane disintegrates and the chromatin escapes in groups of granules (Pl. 9, figs. 19 and 20). N_{α} multiplication seems to take place within the cyst, though probably there is some important readjustment of the nuclear substances. In this encysted state the organism is probably transferred from host to host through contaminated food. Very small individuals can sometimes be seen, measuring about 3.5μ to 4μ in length, with a vesicular nucleus, relatively very large cytostome, and only one long flagellum visible (Pl. 9, fig. 16). It is possible that these are individuals recently emerged from the cysts.

The Affinities of *Embadomonas*.

Embadomonas is certainly allied to *Chilomastix* Alexeieff, and to *Fanapepea* Prowazek.¹ In *Chilomastix* there are three anteriorly directed flagella, and the fourth lies in the cytostome: in *Fanapepea* there are only two anterior flagella, and the third in the cytostome. The number of the flagella and something in the shape of the organism seems to have impressed observers such as Prowazek, Wenyon, Alexeieff and Kuczynski with the idea that there must be a close affinity between these organisms and the trichomonads. In recent classifications (Doflein, 1911, and Alexeieff, 1914), this seems to be taken for granted; Kuczynski (1914) includes *Chilomastix intestinalis* in a study on the trichomonads; Prowazek goes so far as to declare that alongside his *Fanapepea* he "once" (!) found a species of *Trichomonas* with a short undulating membrane "die phylogenetisch zu der folgenden neuen Flagellatenart (*Fanapepea intestinalis*) führt." He gives one small and unconvincing figure of this

¹ It is not easy to make out, from Prowazek's very poor figures, in how far the genera *Chilomastix* and *Fanapepea* really differ. Alexeieff (1914) suggests that these are different names for the same organism, but if that be so, then Prowazek must have overlooked one of the anterior flagella.

new *Trichomonas*, which is like nothing in the world so much as *Chilomastix*, and which excuses Alexeieff's scepticism with regard to *Fanapepea*. And then he goes off into the favourite phylogenetic speculations: "Diese Flagellatenform deutet daraufhin dass die undulierende Membran der Trichomonaden phylogenetisch anders abzuleiten ist als die undulierende Membran der Trypanosomen, die nur eine Art Periplast-lamelle darstellt. . . . Die undulierende Membran der Trichomonaden stand dagegen ursprünglich als ein Strudel- und Lippen-organell direkt im Dienste der Nahrungsaufnahme, und trat erst, etc." All of which may be true, but there is literally nothing to prove it.

Now I think that, even if *Chilomastix* and its allies be related in some degree to the trichomonads, the resemblance is very largely superficial, and that if *Embadomonas*, with its two flagella, had been the genus first examined the closeness of the relationship would not have been so strongly insisted on.¹ For consider: The axostyle is a characteristic possession of the trichomonads; there is no trace of such a structure in *Chilomastix* or *Embadomonas*, nor in *Fanapepea*, for the matter of that. There is a cytostome in both kinds of flagellate, it is true, but in the trichomonads it is a comparatively small and insignificant, and sometimes a transient structure, while in the chilomastigines it is half as long as the body at least, extremely definite and constant, and bordered by prominent lips. The trichomonads are characteristically "naked" and extremely plastic²: the chilomastigines have a well-developed periplast "Der Körper ist von einer Pellicula bedeckt," Prowazek. The nucleus of the chilomastigines is not at all like that of trichomonads. Nor are the division phenomena very similar in the two groups, so far, at least, as I know them. The cysts of *Chilomastix* and *Embadomonas* resemble one another in essential points,

¹ For one might almost as plausibly derive *Chilomastix* through *Embadomonas* from a *Bodo*!

² Kuczynski (1914) mentions an exceedingly delicate periplast in trichomonads, demonstrable by special methods.

but are not like the cysts of trichomonads, so far as these have been described.

In fact, in the trichomonads and chilomastigines, we are dealing with two very highly specialised groups of flagellates, both of which must have diverged very considerably from some much simpler type, and neither of which is in the least likely to be the ancestor of the other.

As observations on the chilomastigines seem to be increasing, and as the references are scattered through the literature, I give a list of the species hitherto described.

CHILOMASTIX Alexeieff (1911), (*Macrostoma* Alex. (1909), *Tetramitus* Alex.) with one flagellum in the cytostome, and three others directed forwards.

Chilomastix caulleryi (Alexeieff, 1909) from the intestine of the frog tadpole.

*C. mesnili*¹ (Wenyon), 1910, from the intestine of man.

C. bocis Brumpt, from *Box salpa*.

C. gallinarum Martin and Robertson (1911) from the rectal cæca of the fowl.

C. intestinalis Kuczynski (1914) from the intestine of the guinea-pig.

FANAPEPEA v. Prowazek (1911) with one flagellum in the cytostome, and two others directed forwards.

F. intestinalis v. Prowazek (1911) from the intestine of a baboon, and from human fæces.

EMBADOMONAS, Mackinnon (1911) with one flagellum in the cytostome, and one other directed forwards.

E. agilis, Mackinnon (1911) from intestine of larval trichoptera and *Tipula*.

¹ Alexeieff (1914) thinks that these forms, *C. caulleryi* and *C. mesnili*, may be identical. This author strongly advances the opinion, which I share, that in the case of intestinal flagellates, at any rate, the "parasitic specificity" is by no means so close as used to be supposed. A number of protozoa, which are saprophytes rather than parasites in the strict sense, are capable of existing in the food-canal of widely different sorts of animals, and do so if the animals have access to the same contaminated food-supply. This fact should be kept in mind when new species are described.

E. alexeieffi, Mackinnon (1912) from intestine of larval *Tipula*.

(b) MULTIPLICATION CYSTS OF A TRICHOMASTIGINE.

It is almost certain that delicate intestinal flagellates must usually be transferred from host to host protected from desiccation by a cyst. The information concerning the cysts of trichomonads is scanty, and much of it has been rendered suspect since Alexeieff (1912) showed that the so-called cysts of *Trichomonas intestinalis* have nothing to do with any flagellate, but are stages in the life-history of the organism that he calls *Blastocystis enterocola*.

Dobell (1909) figured and described two undivided protection cysts of *Trichomonas ranarum*, but his material was admittedly very scanty. Since then authors have again and again casually remarked that they have seen the cysts of one or another species, but they give no details and no figures.

Martin and Robertson (1911) found the cysts of the trichomonads of the fowl, while Kuczynski (1914), who has recently made an exhaustive study of a number of trichomonads, seems to have had no difficulty in obtaining the cysts, though he also refrains from giving any helpful pictures. He, indeed, goes on to quote Escomel (1913), who, speaking of his cultures of a trichomonad, says: "Le parasite qui a pris la forme ovale, s'enkyste. En cet état il peut vivre longtemps avant de se diviser. Lorsque les conditions du milieu sont favorables, la division s'opère et les jeunes *Trichomonas* s'échappent du kyste rompu."

In 1911 I described and figured what I considered to be the multiplication cysts of *Trichomastix trichopterorum*, Alexeieff (1914) has found very similar cysts in the intestine of *Box salpa*, and he ascribes them to *Trichomastix salpæ*, Alexeieff. His figures, inadequate though they are, show that he is dealing with division cysts comparable with those I described from trichoptera.

The published descriptions, then, being insufficient for

purposes of identification, I think it helpful to give here a series of figures (Pl. 9, figs. 27-36) showing the division in the cysts of the trichomastigines from *Tipula*. In a general way these resemble very closely the cysts of *Trichomastix trichopterorum* Mackinnon, but I have obtained a much more complete series. There are two trichomastigines in *Tipula*, one a typical *Trichomastix*, which I could not distinguish from *T. trichopterorum*, and the other a form with an additional flagellum, *Tetratrachomastix parisii* Mackinnon. I am unable to say to which of these the cysts belong.

The flagellate rounds itself off, and the flagella show a strong tendency to adhere to the sides of the body¹ (Pl. 9, fig. 27). The cysts are almost spherical when fully formed, with a diametrical measurement of 4 to 5 μ . They have a relatively thick wall. Within the cyst the flagella may persist as dark coiled lines lying superficially in the cytoplasm. The cytoplasm is otherwise remarkably clear, and free from granules. The axostyle disappears. The basal granules separate, and between them extends a centrodesmosis (Pl. 9, fig. 28) on which the nucleus comes to lie. The nuclear membrane has disappeared, and the chromatin breaks up into four large masses, approximately spherical in form; the basal granules move to opposite poles of the cyst, and the nucleus lies suspended between them (Pl. 9, figs. 29-31). The four chromosomes, if one may call them so, apparently divide, though I have not found a stage showing the process. The next stage available shows two daughter-nuclear masses, each containing four smaller chromatin clumps (Pl. 9, figs. 32 and 33); these move to opposite poles. The centrodesmosis draws out longer and longer, so that the two nuclear masses approach one another once more and lie superficially at one side of the cyst. Below them extends the loop of the centrodesmosis (Pl. 9, fig. 34), which breaks at last into two, each half forming the axostyle of one of the two

¹ Cf. Martin and Robertson's "swathed forms" of *Trichomastix gallinarum*.

new daughter-flagellates, as in the free-swimming stage.¹ Some of the flagella of the mother organism seem to persist through the entire process—others are re-grown (Pl. 9, figs. 34-36). In late stages the cyst tends to lose its spherical form, while the cytoplasm begins to divide into two, and the nuclei show the adult flagellate structure. There is no trace of any sexual process.

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¹ A great deal of discussion has centered round the division phenomena of the trichomonads, and the accounts of the formation of the axostyle are very contradictory. I sometimes wonder whether the slender, dark-staining line that forms the axostyle of *Trichomastix trichopterorum*, *T. salpæ*, *Tetratrichomastix parisii*, etc., is strictly homologous with the stout, non-siderophilous, supporting rod of *Trichomonas eberthi*, *Trichomastix gallinarum*, etc. The homology is always assumed, but supposing it not really to exist, then the discrepancies between the accounts of the behaviour of “axostyles” in division might be explained to some extent.

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EXPLANATION OF PLATE 9,

Illustrating Miss D. L. Mackinnon's paper on “(a) The Flagellate *Embadomonas*; (b) Multiplication Cysts of a *Trichomastigine*.”

[Figures drawn to scale ($\times 3900$ ca.) under Zeiss comp. oc. 12 and 2 mm. apochromat. The stain employed was Heidenhain's iron-haematoxylin, after fixation with sublimate-alcohol.]

Figs. 1-20.—*Embadomonas alexeieffi*.

Fig. 1.—Typical flagellate, with large cytostome (*c.*), anteriorly placed nucleus (*n.*), two flagella (*f.*), two basal granules (*b.g.*), and numerous ingested bacteria (*b.b.*).

Fig. 2.—Large individual with pointed extremity and two half-sized nuclei.

Figs. 3 *a, b, c.*—Three types of nucleus. In 3*c* the basal granules are further from the cytostome margin than usual; this gives a false appearance of the flagella springing from the nucleus.

Fig. 4.—Rounded-off individual with elongated nucleus. The beginning of division?

Fig. 5.—The division spindle forming, with the basal granules at the poles. The new flagella have already been re-grown; the cytostome persists.

Fig. 6.—A stage comparable with fig. 5, but with both the flagella at one end of the spindle; the cytostome has completely disappeared.

Fig. 7.—The division spindle extending right across the rounded-off organism. The cytostome has disappeared and one new flagella has grown out.

Fig. 8.—A slightly later stage than fig. 7. The chromatin is concentrating at the poles; one of the basal granules has divided; the new flagella have both grown.

Fig. 9.—The drawn-out spindle shows a constriction in the middle.

Fig. 10.—The spindle is drawn out in the middle almost to breaking point; the nuclei are forming at each end; the cytostome of one of the daughter-individuals is appearing.

Fig. 11.—The cytoplasm begins to divide by a median constriction: the nuclei takes on the adult form. In this case the cytostome seems to be dividing in two. The new flagella have not yet been re-grown.

Fig. 12.—A slightly later stage than 11, in which the cytostomes are complete, and there is the full complement of flagella.

Fig. 13.—An individual with two nuclei recently emerged from a division, but with one large cytostome only, and no flagella.

Fig. 14.—The daughter flagellates separating.

Fig. 15.—Only a narrow strand of cytoplasm connects the separating individuals. In one of these the cytostome is only just beginning to appear even at this late stage.

Fig. 16.—A very small individual, with relatively large cytostome and one long flagellum. Young form recently emerged from cyst?

Fig. 17.—Encysting individual. The cytoplasm has shrunk away from the periplast (*p.*); the chromatin is escaping from the nucleus.

Fig. 18.—The cyst-wall is formed; the periplast (*p.*) still invests the whole as a loose envelope. The border of the cytostome and one of the flagella can be seen inside the cyst.

Figs. 19 and 20.—Slightly later stages, in which the nuclear membrane has disappeared, and the chromatin has escaped in clumps into the cytoplasm.

Figs. 21-26.—*Embadomonas agilis*.

Figs. 21, 22, 23.—Cysts of *E. agilis*.

Figs. 24, 25, 26.—Flagellate individuals. In the very small one, fig. 25, only one flagella is visible.

Figs. 27-36.—Encystment of a *Trichomastigine*.

Fig. 27.—*Trichomastix* rounding itself off. The flagella show a tendency to wrap round and adhere to the body of the organism.

Fig. 28.—The flagellate completely rounded off, but the cyst-wall not yet formed; the axostyle is disappearing; the basal granules have separated; between them lies a centrodesmosis.

Fig. 29.—The cysts formed. Within can be seen the nucleus and a complex interweaving of dark strands—probably the persisting flagella.

Fig. 30.—The nucleus, suspended on the centrodesmosis, divides into four large masses. The cyst-wall has not yet been formed in this case.

Fig. 31.—A stage comparable with fig. 30, except that the cyst-wall is complete.

Figs. 32 and 33.—The nucleus has divided into two, each containing four smaller chromatin masses. The centrodesmosis persists.

Figs. 34, 35, and 36.—The nuclei move nearer and nearer one another and seem to lie at one side of the cyst. The drawn-out centrodesmosis forms the axostyles of the new individuals. Fresh flagella are seen growing out from the basal granules. In fig. 36 the cytoplasm is beginning to divide.

Note on the Relation of Spermatozoa to Electrolytes and its bearing on the Problem of Fertilization.

By

James Gray, B.A.,

Fellow of King's College, Cambridge.

IN June 1913, a number of large male *Luidia* were obtained at Plymouth, and to all external appearances the gonads appeared to be perfectly ripe; when, however, the spermatic fluid in sea-water was examined under the microscope, the spermatozoa were found to be quite motionless, and repeated experiments showed them to be quite incapable of fertilising ripe eggs of the same species. It was found, however, that the addition of a few drops of $\frac{N}{10}$ NaOH to a suspension of sperm in sea-water immediately caused active movement. When such "activated" sperm was added to eggs from the same female as before, every egg was quickly fertilised and large numbers of healthy larvæ were obtained from the culture.¹

It might be supposed that the activation of the sperm by alkali was essentially an analogous phenomenon to the ripening of starfish eggs, which is dependent upon the alkalinity of the surrounding sea-water. It should be noted, however, that the spermatozoa began to move immediately the alkalinity of the solution was raised. Again, the following experiments dealing with the behaviour of Echinoid sperm, support the conclusion that active movement is dependent upon the alkalinity of the surrounding fluid, even when the spermatozoa are known to be perfectly ripe.

¹ An essentially similar phenomenon was observed this year in the case of several male *Asterias glacialis*.

(1) THE BEHAVIOUR OF ECHINUS ACUTUS SPERMATOZOA IN ACID SEA-WATER.

Solution.	Behaviour of sperm.
(1) Pure sea-water	Active movements for at least two hours.
(2) 5 c.c. sea-water + 1 drop N/10 HCl.	Active movement, which almost ceased after thirty minutes.
(3) 5 c.c. sea-water + 2 drops N/10 HCl.	Active movement, which almost ceased after seventeen minutes.
(4) 5 c.c. sea-water + 3 drops N/10 HCl.	Active movement, greatly reduced after four minutes, very slow after fourteen minutes.
(5) 5 c.c. sea-water + 4 drops N/10 HCl.	No movement (no agglutination).
(6) 5 c.c. sea-water + 5 drops N/10 HCl.	
(7) 5 c.c. sea-water + 6 drops N/10 HCl.	
(8) 5 c.c. sea-water + 7 drops N/10 HCl.	

(2) THE BEHAVIOUR OF E. ACUTUS SPERMATOZOA IN ALKALINE SEA-WATER.

Solution.	Behaviour of sperm.
(1) Sea-water (which was faintly alkaline).	Prolonged active movement.
(2) 5 c.c. sea-water + 1 drop N/10 NaOH.	Active movement.
(3) 5 c.c. sea-water + 2 drops N/10 NaOH.	
(4) 5 c.c. sea-water + 3 drops N/10 NaOH.	
(5) 5 c.c. sea-water + 4 drops N/10 NaOH.	
(6) 5 c.c. sea-water + 5 drops N/10 NaOH.	Movement slower, some agglutination.
(7) 5 c.c. sea-water + 6 drops N/10 NaOH.	
(8) 5 c.c. sea-water + 7 drops N/10 NaOH.	Movement slower, considerable agglutination.

The effect of excess of alkali in the water is to bring the spermatozoa to rest by agglutination. About a minute after

the addition of sperm to such a solution, the liquid becomes cloudy, and if examined under the microscope, it is found that most of the spermatozoa are inactive and aggregated into clumps—those which are agglutinated are usually active. This agglutination phenomenon is never produced by acids.

Similar experiments were performed last year, using artificial sea-water free from carbonates and phosphates. Identical results were obtained, but smaller quantities of acid and alkali were required than in the original experiments.

From these experiments it appears that spermatozoa are much more sensitive in their behaviour to acid than to alkali, and an attempt was made to determine whether a certain concentration of OH is necessary in order that the sperm may exhibit its usual activity.

It has been shown that the spermatozoa did not move in acid sea-water; if this cessation of movement is due to the absence of hydroxyl-ion in the surrounding medium, then by increasing the alkalinity of the water movement of the sperm should again take place. This is, undoubtedly, what takes place, as is shown by the following experiments:

(1) A drop of sperm was put into samples of sea-water containing various concentrations of $N/10$ HCl (such as previous experiments had shown caused cessation of movement); when all movement had ceased in the solutions one or more drops of $N/10$ NaOH were added, and the behaviour of the sperm watched under the microscope.

Solution.	No. of drops $N/10$ NaOH added.	Behaviour of sperm.
5 c.c. sea-water + 1 drop $N/10$ HCl.	1	Active movement re-commenced. No agglutination.
5 c.c. sea-water + 2 drops $N/10$ HCl.	1	Rapid movement began. Some agglutination.
5 c.c. sea-water + 3 drops $N/10$ HCl.	2	Movement greatly increased. Some agglutination.
5 c.c. sea-water + 4 drops $N/10$ HCl.	1-6	Sperm did not move on addition of the alkali, but rapid agglu- tination of all the spermatozoa took place.
5 c.c. sea-water + 5 drops $N/10$ HCl.		
5 c.c. sea-water + 6 drops $N/10$ HCl.		

This experiment was repeated several times with the same results, viz. that sperm which is motionless in "acid" sea-water, can be rendered motile by the addition of alkali, if the concentration of acid used in the experiment is not too great. Agglutination of spermatozoa is only caused by alkali, and not by acid, although the latter be of relatively high concentration. The cessation of movement in acid and that in alkaline sea-water are two separate phenomena; in the former case the spermatozoa never agglutinate, and the addition of alkali can remove the effect of the acid. In alkaline solutions, however, only relatively high concentrations produce cessation of movement, and this is closely connected with the agglutination phenomenon; the agglutination by alkali cannot be reversed by the addition of acid.

The behaviour of the spermatozoa of *Echinus* in acid and alkaline solutions is apparently identical with that of *Nereis* and *Arbacia* sperm as described by F. R. Lillie (1). This author, however, does not mention that the effect of acid can be removed by the addition of alkali. This fact, however, leads to a consideration of the possibility that the movement of spermatozoa is dependent upon the electromotive properties of the cell and of its medium. This suggestion is supported by two series of experiments: (1) The behaviour of spermatozoa in an electric field, (2) the behaviour of spermatozoa in the presence of trivalent ions.

(1) THE BEHAVIOUR OF SPERMATOZOA IN AN ELECTRIC FIELD.

If spermatozoa are suspended in an isotonic cane-sugar solution, their activity is lost, but if a trace of alkali is present, they again become motile. When an electric current is passed through a neutral suspension of spermatozoa in cane-sugar, the sperm travels rapidly to the positive pole where it accumulates¹; round the negative pole, however, the sperma-

¹ This was also observed by R. S. Lillie (2) in the case of the spermatozoa of the frog.

tozoa become exceedingly active. The behaviour of the sperm round the negative pole is apparently due to the liberation of alkali, which can be detected by means of an indicator in the solution. If, however, spermatozoa are suspended in a faintly acid solution of cane-sugar, no migration takes place to the positive pole, and no activation was observed at the negative pole; in such a suspension, the electric current causes the sperm to form a retiform aggregation throughout the solution. These facts appear, then, to show that motile spermatozoa possess a negative charge on their surface, and that this charge is lost in the presence of free hydrogen ions.

(2) THE BEHAVIOUR OF SPERMATOOA IN THE PRESENCE OF TRIVALENT IONS.

The effect of simple trivalent kat-ions upon spermatozoa is very remarkable. If a drop or two of a very weak solution of cerous chloride is added to a suspension of *Arbacia* sperm in sea-water the spermatozoa become intensely active, and rapidly aggregate into clumps, which soon become visible to the naked eye. Examined under the microscope, these clumps are seen to consist of extremely active spermatozoa. After some time the aggregated spermatozoa become motionless, but those which are not aggregated continue to swim actively.

The "aggregation" by means of cerium appears to be partly reversible by mechanical agitation, if the aggregation has not existed for more than a few minutes. If a suspension of "aggregated" spermatozoa be agitated in a phial, the fluid becomes quite homogeneous to the naked eye. Under the microscope, however, small "aggregates" are still found to be present. After a few minutes, macroscopic aggregates again become visible. Finally, all the spermatozoa come to rest, and are mostly "aggregated" together into small clumps. It is remarkable that the final effects of the cerium ions¹ is the same as that of hydroxyl ions, viz. an

¹ The solution of cerium was not alkaline in solution, being faintly acid to neutral red and phenolphthalein.

almost complete agglutination of all the spermatozoa. It resembles, however, the effect of the hydrogen ion in the formation of aggregated masses of living spermatozoa, although this phenomenon is very much less obvious in the case of the hydrogen ion.

If a piece of filter-paper previously soaked in a solution of cerium be put into a dilute suspension of sperm in sea-water, the spermatozoa are rapidly attracted to the paper. They aggregate round the edges and are at first exceedingly motile, after a time, however, they become quiescent, and the paper is covered by a thick felt of agglutinated sperm.

Experiments with neodymium nitrate gave results exactly similar to those with cerous chloride.

It is important to note that the effect of these trivalent positive ions is completely removed by means of sodium citrate. In other words, there is little or no doubt that these experiments afford another fact in favour of the belief that trivalent ions affect living organisms by virtue of their electrical charge.

Now, the "aggregation" phenomena of spermatozoa has been observed by F. R. Lillie (1) in the case of a suspension of *Nereis* sperm in normal sea-water. From his account I conclude that the behaviour of a normal suspension of *Nereis* sperm is very similar to that of *Arbacia* sperm in the presence of cerium ions. Lillie concludes that the behaviour in the case of *Nereis* sperm is due to positive chemiotaxis towards a CO_2 gradient. He also observed that sea-water which had been in contact with unfertilised eggs has a marked effect on the activity of spermatozoa. He found that such sea-water had a threefold action on the sperm: (1) It greatly increases their activity; (2) it aggregates them; (3) agglutinates them. Lillie regards these effects as due to an "agglutin" which has been extracted from the eggs with which the water was in contact. Further, this "agglutin" cannot be obtained from any other tissue except the ripe ovaries. From its properties it may be inferred that the substance is of a colloidal nature.

The effects of trivalent positive ions and of "egg-agglutins"

upon living spermatozoa appeared to be so nearly identical that considerable care was taken to test this conclusion by the method of examination used by Lillie (1). The result of injecting a drop of cerous chloride or neodymium nitrate into a suspension of sperm under a cover-glass was apparently identical with that described by Lillie on p. 550 of his paper, when using egg-extractive. It should be noted also that both trivalent, positive ions and egg-extractive are efficient at extremely low dilutions.

It would thus appear that the behaviour of spermatozoa towards "agglutin" is identical with its behaviour towards a trivalent kat-ion.¹

P.S.—It had been hoped that circumstances would permit of a more detailed investigation of the above facts in the near future. This, however, is no longer the case, and the present note is published merely to suggest that the nature of the electric charges upon the surface of the gametes may play an important rôle in the behaviour of these cells.

Note.—The inactivity of *Luidia* sperm in normal sea-water is all the more remarkable from the following considerations. The habitat of this species is given by Crawshay² as being 42–50 fathoms. Now, the hydroxyl-ion concentration of sea-water at this depth is appreciably lower than that of the surface water which was used in the laboratory. Unfortunately, a complete investigation of the alkalinity of the water in the English Channel is not available, but the available data afford no evidence for supposing that the alkalinity of the sea-water ever reaches the value which is necessary to effect activation of the sperm under experimental conditions. It would be exceedingly interesting to know whether the *Luidia* which are dredged from the Rame Eddy-stone grounds at Plymouth ever breed, or whether the limit of migration of this species is fixed by the alkalinity of the sea-

¹ I understand that Prof. Lillie intends to investigate whether the behaviour of spermatozoa to egg-extractives and to trivalent-positive ions are really identical phenomenon or not; further discussion of the point must be reserved until absolute certainty is established.

² 'Journ, M. B. A.,' vol. ix, p. 334, 1912.

water. It is almost certain that the true "home" of this Asteroid lies considerably further west of Plymouth.

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Klossiella muris.

By

A. C. Stevenson, M.B., D.P.H.,
University College Hospital Medical School.

With Plate 10.

THIS parasite was first described by Smith and Johnson in 1902. There are only two other papers I know of on the subject, one by Woodcock of a critical nature, and one by Sangiorgi containing experimental facts of interest.¹ In all the above the only phases of the parasite described and mentioned are those in the glomeruli of the kidney, the epithelium of the tubules, and the urine. The first describers consider the schizogony that takes place in the glomeruli as a formation of merozoites, and the tubular phase as sporogony leading on to spores and sporozoites in the urine. Woodcock, on the analogy of *Caryotropha mesnili*, suggests that the tubular phase is one of schizogony leading to merozoites and that possibly the glomerular phase is one of schizogony leading to gametocytes. Sangiorgi, in a paper of which I have received an abstract from Woodcock, holds the same view with regard to the tubular phase, and says that he has infected mice with urine which contained cysts, but he seems unaware that these cysts contained definite products of division. Sangiorgi also states that he could not infect mice from mash of kidney-substance, and states that he considers the cysts in the urine to be oöcysts and describes the glomeruli of the kidney as containing bodies which he describes as sporozoites in a membrane or spore-capsule. He says that difficulty

¹ In addition to these Seidelin has lately published a paper describing a similar parasite in the guinea-pig in Africa, but mentions no other forms than those found by Smith and Johnson.

arises as to the phase of the parasite in which a new host is infected.

As will be seen, I consider the original describer is right in thinking the stage in the tubules to be sporogony, since cysts found freely in the urine of infected mice are practically in the same condition as those seen in the last stages of development in the kidneys. Sangiorgi's first experiment confirms this, but a criticism on the experiment must be raised as to whether his mice were previously infected or not. Examination of the urine would prove nothing in the early stages. I have several times missed infections in fresh specimens of the kidney, and found the parasite when I cut sections, cysts being nowhere present.

If the cysts are not spores, it is difficult to imagine how another host is to be infected on the analogy of coccidium. If cysts contain gametocytes and an insect host is concerned, there is only one I have heard of that is definitely attracted by urine, ants.

The schizogony in the glomeruli I consider to be one that produces gametocytes and to be secondary to the one producing merozoites which takes place elsewhere.

My great difficulty is the type of syngamy that takes place, but I have no doubt that this stage takes place in the cells of the kidney tubules.

I have found the parasite in about 40 per cent. of the white mice examined (25). I have an impression that it occurs more frequently during the summer months, but this may be due to differences in the source of supply of the mice. It is, however, possible that infection is brought about by an invertebrate host prevalent in summer, and, from what I have seen of the organism, I think this may take place as well as infection by the normal casual coccidial method.

FORMS OBSERVED.

What is possibly the earliest form I have seen was in a mouse, which post-mortem showed no parasites in the kidney,

but had lived in a cage with others that were passing cysts. It consisted of a small gregariniform body about $12\ \mu$ long, free in the peripheral blood (Pl. 10, fig. 1). When stained by Giemsa, however, its morphology very closely resembles that of the merozoites of an intestinal coccidium of the mouse, and it is quite possible that one of these might have found its way into the blood-stream. I do not know if this mouse had coccidiosis of the intestine, but a very large percentage have.

The next stage I have seen was one in an arteriole of the kidney (Pl. 10, fig. 2). This consisted of a round body in an endothelial cell of the arteriole, with three nuclei in process of division. The diameter is about $8\ \mu$. It may, however, be an early instance of either of the following types of schizogony.

In a similar position I have found a parasite definitely divided up into eight to twelve daughter individuals, these still being contained in an envelope formed by the remains of the host-cell (Pl. 10, fig. 3). This I take to be the schizogony of the parasite into merozoites. I have searched many sections of kidneys and other organs both in infected and uninfected mice, but up to the present I have not come across any other instances of the above stages. The daughter individuals in the above case have a definite nucleus with a well-marked karyosome. As the division in the other type of schizogony and also that into sporozoites are characterised by the great numbers of individuals produced, it is possible that this first type is comparatively infrequent in occurrence.

The second type of schizogony is very different, the daughter individuals being very numerous, fifty to sixty being a moderate estimate. This division takes place usually in the cells forming the capillaries in the glomeruli of the kidneys, but on rare occasions (three)¹ I have seen it occurring in the endothelial cells of the arterioles of those organs (Pl. 10, figs. 9, 10).

As first seen, the parasite in this stage is a small uni-nucleate body bulging into the capillary, the granules in it giving it a greenish-grey tinge when stained with iron-

¹ Since the above was written, I have seen two other instances; in one the parasite had only two nuclei, in the other division was complete.

haematoxylin and orange (Pl. 10, figs. 4, 5, 6). This nucleus divides apparently fairly rapidly till we get a large mass occupying quite a large portion of Bowman's capsule in section, and containing nuclei too numerous to count easily, but about fifty to sixty in number (Pl. 10, figs. 7, 8). Around these the protoplasm segments, and we get a large number of gregariniform bodies, each with a nuclear area, containing two small masses of chromatin, and some residual protoplasm (Pl. 10, figs. 11, 12, 13). These chromatin granules are marked by their ovoid shape, and lie side by side with their long axes parallel to that of the organism. The mass of merozoites lies in a capsule formed by the thinned-out remnants of the host-cell, and on the bursting of this into Bowman's capsule the organisms are set free to wander down the tubule of the kidney, where they enter the cells of the convoluted portion in, I believe, the condition of gametocytes or gametes.

The next stage is the one somewhat difficult to interpret. There is no doubt in my mind that the normal condition is a double infection of the cells, the two individuals being at first indistinguishable from one another. Single infections are also found, but I think the balance of evidence is that these do not develop farther in a normal way. There is certainly often found growth, which is sometimes marked, and even budding apparently occurs, but this latter is generally internal instead of the normal external type.

Again, in advanced cases where spores are freely found in the urine, one frequently finds cast-off epithelial cells containing uninucleate small parasites. Another point in favour of this view is that in tubules containing parasites well on in the sporoblastic stage and further, it is common to find cells containing small uninucleate parasites. When one considers that the parasites of the epithelium of the tubule must arrive there practically simultaneously, for it is rare to find two bodies undergoing schizogony in one Malpighian tuft, it is difficult to understand the great difference of development unless due to syngamy having occurred in the advanced cases.

What is the type of syngamy? There seem two possibilities. First, though the observed instances are not too numerous, that it resembles that of *Adelea ovata*,¹ where the nucleus of one of the two associated and closely applied individuals divides into microgametes (four), one of which fertilises the other individual.

Second, that complete fusion takes place between the cytoplasm of the individuals, and their nuclei divide, though fusion of the chromatin does not take place till a much later stage.

That anisogamy of the type of coccidium with many microgametes formed separately from the macrogametes does not take place, I am practically certain.

The evidence for either of the above two views is not very definite. In favour of the first are the facts that forms in one cell sometimes show differences of nucleus and cytoplasm, the one showing signs of division in one and not in the other, while the latter is more finely granular in the parasite with the dividing nucleus and coarse in the other (Pl. 10, figs. 17, 18, 19). In later stages when apparent fusion has taken place and division has proceeded, there can sometimes be observed a body closely resembling a bud, but more definitely separated from the main mass, which contains irregular masses of a chromatin-like staining substance (Pl. 10, figs. 22, 23, 26, 29).

In favour of the second view is the fact that all through the enlargement of the parasite after fusion there seems to be two types of chromatin present, shown by different staining reactions, the one retaining iron-hæmatoxylin well, and staining deeply with acid hæmalum, while in the other the staining intensity is less marked. These masses often lie close to one another in pairs, and the dark stained one seems to divide first. This condition is retained until the bud is finally separated from the mother individual. This, however, may be due to differences in the condition of the chromatin, division of nucleus, etc. (Pl. 10, figs. 26, 27).

Whichever of the above views is right, there is no doubt

¹ Further observations tend to this view.

that fusion or association of two gametocytes takes place, and that after this nuclear division follows until there are twelve to sixteen nuclei arranged round the periphery of the parasite. The nuclei gradually travel outside the line of general contour of the parasite (Pl. 10, fig. 30) and along with some of the cytoplasm are finally budded off one by one. There can often be found a renal cell containing two or three buds and the remainder of the parasite still undivided (Pl. 10, fig. 31). By the time all the buds have formed, which are generally twelve to sixteen in number, the renal cell has become tremendously enlarged and often dilates the renal tubule to many times its original diameter. It is still, however, attached to the basement membrane by a fine point of apparently altered protoplasm. This fine attachment is seen at very early stages of infection of the cell; in fact, nearly all infected cells project freely into the lumen of the tubule (Pl. 10, figs. 15, 32).

The nuclei in these buds or sporoblasts then divide, enlargement takes place, and a cyst wall forms round the sporoblast till we finally get a spherical body containing about twenty-five nuclei, each of which latter consists of three granules of chromatin arranged in a line about an equator of the sphere and at right angles to the plane of that equator (Pl. 10, fig. 35). Around each three granules of chromatin the protoplasm segments, and we get a spore cyst containing about twenty-five sporozoites with a certain amount of residual matter left at one pole of the cyst (Pl. 10, figs. 36, 37, 38). The capsule containing these spores then bursts, and the spores travel down the tubules through the papilla and ureter to the bladder in which situations (except the ureter) I have found them in sections. In the urine they can be easily found if the infection is at all heavy.

GENERAL.

The later stages closely resemble those described by Christophers in the sporogony stage of *Leucocytozoon*

Canis in its invertebrate host. Miss Porter also in her description of the *Leucocytozoon* of the mouse mentions having found a schizogony stage in the bone-marrow. Is it possible that the organism we are dealing with is the same as this latter *Leucocytozoon*? We have only to postulate that in a large infection we might get a large number of parasites undergoing the gametocyte-forming schizogony in the arterioles, and when these were set free in the blood-stream they would probably be taken up by leucocytes. From Miss Porter's description the schizogony stage she saw was possibly that into merozoites.

Another interesting supposition also arises in regard to this parasite. Have we here a link between the hæmogregarines and the coccidia? We have only to presuppose that gametocyte formation takes place largely in the arterioles instead of in the Malpighian tuft capillaries, and we get the blood-stream charged with parasites in a condition suitable for sporogony in a blood-sucking host. The question of the greater (?) incidence of the parasite in summer also bears on this point.

From the medical standpoint of view the possibility of a protozoon being a parasite of the arteries seems to me to be of great importance.

METHODS.

Fresh specimens were always examined, the tissue being teased in normal saline. This was useful for general diagnosis, but slight or early infections might easily be overlooked.

Smears, after teasing, were also made and fixed by Schaudinn's method. These were good for small forms.

Most of the work was done with serial sections, the tissue being fixed with corrosive sublimate and glacial acetic. The staining of these and the smears was with iron-hæmatoxylin or acid hæmalum.

EXPLANATION OF PLATE 10,

Illustrating Mr. A. C. Stevenson's paper on "*Klossiella muris*."

REFERENCE LETTERS.

P. Parasite in its various forms. *C.* Host-cell or its remains. *N.* Nucleus of host-cell. *N'*. Possible degenerate nucleus of host-cell. *R.* Red blood-corpuscles. *tn.* Nuclei of tissue-cells. *m.* Possible remains of microgametocyte. *Bc.* Bowman's capsule.

[Magnification $\times 1200$.]

SCHIZOGONY CYCLE.

Fig. 1.—Free merozoite? in the blood-stream of a mouse which had been exposed to infection.

Figs. 2, 2*a*, 2*b*.—Young form in endothelial cell of kidney arteriole, showing division of nuclei. Three sections through parasite. This may be a form leading to that shown in fig. 3 or those in figs. 9 and 10.

Fig. 3.—Complete division of parasite into merozoites in endothelial cell of arteriole of kidney.

Figs. 4, 5, 6.—Small forms in the cells of capillaries of a glomerulus.

Fig. 7.—The same larger, nuclear increase.

Fig. 8.—Large form.

Figs. 9, 10.—Forms as in fig. 8, but with more nuclei, in the endothelial cells of the arterioles of a kidney.

Fig. 11.—Commencement of division forming gametes.

Fig. 12.—Complete division.

Fig. 13.—Same from a smear, showing residual protoplasm.

SPOROLOGY CYCLE.

Figs. 14, 15, 16.—Showing double infection of the cells of the convoluted tubule of a kidney by small forms.

Figs. 17, 18, 19.—Double forms of larger size, showing slight differences in the cytoplasm and nucleus.

Figs. 20, 21.—Single forms in a cell.

Fig. 22.—Single form in a cell: early nuclear division. At one side of the parasite is a mass of homogeneous protoplasm containing chromatin granules possibly the remains of a microgametocyte. Similar masses are seen in figs. 23, 26, 29.

Fig. 23.—Form with two nuclei.

Figs. 24, 25.—Two sections through one parasite. Chromatin mostly in granules, some suggestions of a mitotic figure.

Figs. 26, 27.—Sections through two parasites in one tubule, both in the same section, showing differences of nuclear staining.

Figs. 28, 29, 30.—Increase of nuclei and commencement of budding.

Fig. 31.—Sporoblasts separating from the main mass of a parasite.

Figs. 32, 33.—Complete separation into sporoblasts. Increase of nuclei.

Figs. 34, 35.—Further increase of nuclei. In fig. 35 arrangement of nuclei about the equator of a sporoblast.

Figs. 36, 37, 38.—Sections through three spores showing sporozoites.

The Chorda Tympani and Middle Ear in Reptiles, Birds, and Mammals.

By

Edwin S. Goodrich, F.R.S.,
Fellow of Merton College, Oxford.

With Plates 11, 12 and 13, and 5 Text-figures.

A GREAT deal has been written of late years about the development and homology of the columella auris of reptiles and the chain of auditory ossicles of mammals; a mass of evidence has gradually been gathered from all sides supporting the view put forward by Reichert that the stapes and the columella are derived from the dorsal end of the hyoid arch, and that the incus and malleus, derived from the mandibular arch, correspond to the quadrate and articular. In the search for evidence not only has the development of the skeletal elements been studied, but also the origin of the tympanum and tympanic cavity, and the disposition of the blood-vessels, muscles, and nerves of the middle ear. It was with the intention of comparing the exact relation of these various parts in reptiles, birds, and mammals that the present work was undertaken. Since it was begun, however, so admirable and convincing a summary of the facts in favour of Reichert's view has been given by Gaupp (17) that it would seem as if the question were finally settled and little remained to be said. Yet some doubts and obscurities still remain, especially with regard to the exact relation of the chorda tympani to the first gill-slit, tympanum, and surrounding structures; so I decided to publish this paper as a small contribution to the discussion of a most important morphological problem. The

results of these researches have mostly been given in figures of reconstructions in which I have endeavoured to represent clearly the true relation of the parts dealt with, and to enable the reader easily to compare the different forms studied. The figures are no mere diagrams, but carefully made graphic reconstructions of transverse or longitudinal sections drawn with the camera lucida. As far as possible they have been shown from corresponding points of view, in uniform style, with consistent colouring; unessential details and irregularities being omitted to avoid unnecessary complication. I am indebted to various friends for the opportunity of studying many series of sections besides my own. Dr. Versluys I have to thank for lending me a series of sections of an embryo *Platydaetylus*, and Prof. Dendy for sections of *Sphenodon*; Dr. Jenkinson for the loan of series of lizard, chick, and mouse embryos (from which figs. 1, 2, 3, 13, 22-26, were drawn), and Prof. J. Hill for valuable series of *Ornithorhynchus* and *Trichosurus* embryos of various stages (figs. 5, 14-21).

The chorda tympani, a twig of the main or hyomandibular branch of the facial nerve, supplies the organs of taste near the base of the tongue and the salivary glands in the region of the lower jaw. In adult mammals it issues from the seventh nerve behind the tympanic cavity, turns forward over this cavity, and makes its way to the lower jaw, passing below the chain of ossicles. Thus the chorda tympani runs downwards anterior to the tympanum and tympanic cavity and posterior to the incus and malleus. While the importance of the chorda in determining the homology of the parts of the middle ear has become more and more apparent through the work of Gaupp (16, 17), Kingsley (25), and others, there has been considerable confusion about its development and homology. It can easily be identified in birds and reptiles where it follows much the same course as in mammals; but although in these it has the same origin and destination, it always passes over the columella, being anterior to it and the tympanum, and posterior to the quadrate and articular. Stannius first suggested that the chorda tympani is homo-

logus with the post-spiracular ramus mandibularis internus of the facial nerve in Amphibia and Pisces. Balfour (1) and Dixon (7), however, compare it to a pre-spiracular branch, being doubtless misled by the then prevailing view that the tympanum represents the closing membrane of the spiracular slit. So we find Cole (11) and Herrick (20) arguing that, in spite of its similar function and peripheral distribution, the chorda cannot be homologous with the ramus mandibularis internus, because the latter is post-spiracular in position. However, it is now known that the tympanum is not developed at the point of closure of the first gill-slit, but behind it; so that a nerve may be post-spiracular and yet pre-tympanic. Long ago Froriep (15) correctly described the chorda tympani in an embryo calf as post-spiracular in origin, and the same result was recorded by Kastschenko in the pig (23), by Eumel (13) in *Microtus*, and Drüner in the mouse (10), while Hoffmann (21) and Versluys (31) have shown that in lizards it develops in the same way. That the chorda tympani is really homologous with the post-spiracular ramus mandibularis internus of fish and amphibians may now be considered as established, chiefly owing to the work of Gaupp (16) and Bender (2). In his recently published monograph Bender traces this nerve through the whole vertebrate series, and his conclusion is further strengthened by the results of Strong (30), Herrick (20), and others who have shown that gustatory fibres pass up this ramus in the lower forms.

My own observations on the development of the chorda, and the various reconstructions figured on Pls. 11, 12, 13 may now be described.

Reptilia.—*Lacerta* is the type studied. At a stage when the spiracular gill-cleft still opens to the exterior by a small pore at the dorsal edge of the first gill-pouch (Pl. 11, fig. 3), the skeleton of the first two visceral arches becomes visible as a vaguely defined blastema extending along the mandibular bar in front of the first gill-pouch, and a similar blastema in the hyoid bar behind the gill-pouch. The latter blastema reaches up to the scarcely yet defined blastema of the auditory cap-

sule, pushing in the posterior dorsal surface of the pouch and passing inwards between the facial nerve and vena capitis lateralis above and the internal carotid below. This stage corresponds nearly to that described in *Platydactylus* by Versluys (31), though perhaps a little earlier. Later on, as so well shown by Versluys, the continuous hyoid blastema bends sharply to form the horizontal columellar region and the more ventral cornual region curved backwards and downwards. For a considerable time these two regions remain connected by a band of blastema in *Lacerta* (Pl. 11, fig. 8), though eventually separating. The more dorsal columellar region develops into a stout cartilage rod, with a foot or stapedial base fitting into the fenestra ovalis of the auditory capsule, and an expanded extra-columella applied to that region of the body-wall which will form the middle part of the tympanum (Pl. 11, figs. 6, 8, 11; Pl. 13, fig. 30). On the columella¹ develops about midway an upstanding dorsal process and an anterior internal process. The former has a swollen dorsal extremity which later separates off from the columella and becomes fixed on to the parotic process of the skull (Pl. 11, fig. 8). A fine ligament remains, indicating the original cartilaginous connection of this "intercalary" with the columella. The internal process (figs. 6, 9-11, 29, 30) projects towards the quadrate, with which it becomes connected by ligament.

Meanwhile, the mandibular blastema has given rise to the quadrate and Meckel's cartilage. The quadrate articulates with the parotic process above, the intercalary being wedged in between them at this point. Extending downwards to meet the articular region of Meckel's cartilage the quadrate passes outside the facial nerve, vena capitis lateralis, and facial artery (Pl. 11, figs. 7, 9).

In the earliest stage here figured, the first or spiracular

¹ The term "columella" is used to denote the whole rod, stretching from tympanum to fenestra ovalis. From the point where the remainder of the hyoid arch separates off, the inner or stapedial portion runs inwards, and the outer or extra-columellar portion runs outwards.

gill-split is widely open to the exterior and the first gill-pouch is in the form of a wide, somewhat obliquely flattened outgrowth (Pl. 11, figs. 1, 2). As explained above, by the time the hyoid blastema is differentiated the slit has almost closed, but still remains open by a small pore at the upper corner of the pouch (Pl. 11, fig. 3). Now, it is well known that the ganglia of the fifth, seventh, ninth, and tenth cranial nerves receive contributions from the epiblast at the top edge of the corresponding gill-slits. These are the so-called branchial sense organs (Froiep, 15; Kastschenko, 23a). Such an epidermal thickening can be seen above the first gill-slit in *Lacerta* in early stages (Pl. 11, figs. 1, 3; Pl. 13, fig. 27), and later on will sink in, contributing to form the geniculate ganglion. It therefore marks a fixed point very useful in the comparison both of different stages and of different animals. In *Lacerta* it can be followed for some little time after the closure of the first gill-slit and the separation of the first gill-pouch from the epiblast, and is seen to correspond to the anterior dorsal, inner or medial, corner of the pouch which gives rise to the recessus medialis (fig. 6 *air.*). A careful description of the development of the tympanic cavity in *Lacerta* has been given by El. Cords (12), and my own observations are in agreement with hers, as shown in Pl. 11, figs. 6, 8-11; Pl. 13, figs. 29 and 30. The flattened first gill-pouch separates off from the epiblast from below upwards. At the same time the lower posterior region grows outwards and forwards, and pushing inwards the original upper part of the pouch which last opened to the exterior it expands behind the quadrate to form the adult tympanic cavity. Three outgrowths or recesses of the cavity tend to surround the columella. These are an anterior inner or medial, an anterior outer or lateral, and a posterior recess. The two first grow upwards and then backwards over the columella, having between them the dorsal process, the internal process, and the chorda tympani (figs. 6 and 29). At a later stage the lateral recess meeting and opening into the posterior recess enables the tympanic cavity to completely surround the extra-columella.

By a thinning-out of the mesenchymatous wall separating the tympanic diverticulum of the gill-pouch from the superficial epidermis the tympanic membrane is formed. The tympanum is really developed, then, not from a membrane closing the spiracular slit, but, as clearly stated by Versluys (31), by the outgrowth of the first gill-pouch posterior and somewhat ventral to the spiracle and immediately in front of the hyoid arch. This outgrowth we may call the tympanic diverticulum (see diagram A, B, and C). The whole columella, and the hyoid cornu as well, are morphologically posterior to the tympanum and tympanic cavity.

Coming now to the blood-vessels, we find that the vena capitis lateralis passes forwards outside the tenth, ninth, and seventh nerves, above the columella, and on the inner side of the fifth nerve (Pl. 11, figs. 2, 3, 9). It runs on the inner side of the process dorsalis in the space included between the quadrate and the auditory capsule (Pl. 11, fig. 9; Pl. 13, figs. 29, 30). Accompanying the vein for this part of its course is the facial or stapedia artery (figs. 3, 6, 8, 9, 30). Starting from the internal carotid, which, of course, runs above the pharynx and gill-pouches, this artery passes outwards and forwards over the columella, then upwards and forwards on the outer side of the vena capitis lateralis and below the articulation of the quadrate with the skull. Versluys (31) has shown that in *Platydictylus* the facial artery pierces the foot of the columella, and his suggestion that it is homologous with the stapedia artery of the mammal seems to be fully justified, since it develops like the latter from the top end of the hyoid arterial arch and has the same relations (figs. 3, 14).

The position of the seventh or facial nerve in relation to the above-mentioned structures is well shown in Pl. 11, figs. 6, 8, 9, and Pl. 13, figs. 29, 30. Passing outwards and backwards from its ganglionic masses in front of the auditory capsule, the main or hyomandibular branch of the facial is seen to run across and under the vena capitis lateralis just dorsal to the first gill-slit or pouch, and then backwards and downwards behind the spiracular slit into the hyoid bar. As

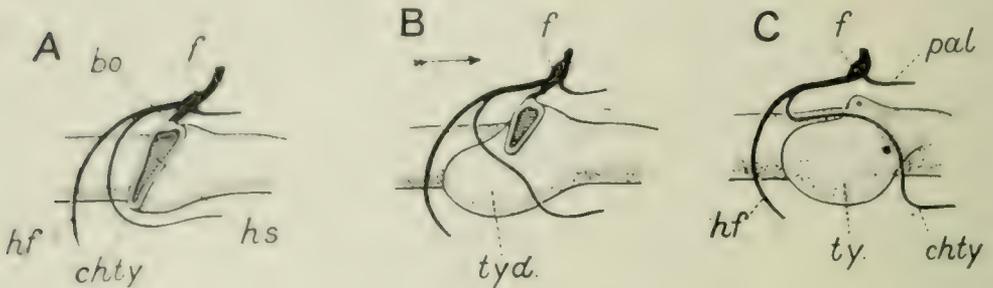
already described, a connection exists in early stages between the facial and inner dorsal region of the epiblastic ingrowth forming the opening of the first gill-slit. An epidermal proliferation here contributes to the geniculate ganglion, and immediately in front of it runs forward the incipient palatine nerve. At a stage when the spiracular slit is still widely open (figs. 1, 2), the chorda tympani is already seen to arise from the hyomandibular branch of the facial behind the opening, and to run below the opening obliquely downwards and forwards into the mandibular bar. The chorda tympani is, therefore, as described in lizards by Hoffmann (21) and Versluys (31), distinctly a post-spiracular or post-trematic nerve. It first runs near the epidermis until it passes the lower edge of the gill-slit, when it turns inwards and runs along the mandibular bar and close to the floor of the buccal cavity, to which most of its fibres are doubtless distributed.

As the spiracular slit closes from below upwards the chorda tympani keeps near its lower edge, passing at first diagonally downwards to the lower jaw across the future tympanic region (figs. 3, 27). When the first gill-slit separates off from the epidermis, and as the tympanic diverticulum enlarges, the chorda tympani becomes more and more pushed up in front of the developing tympanic membrane (diagrams A, B, and C). Finally it slips inwards, so to speak, over the tympanic cavity so as to pass over the extra-columella on the inner side of the lateral recess, but outside both the dorsal and the internal process of the columella. The chorda tympani in reptiles is, therefore, primarily post-trematic and pre-tympanic, being situated between the opening of the first gill-slit and the tympanic membrane (diagram D, p. 153) as described by Versluys in *Platydactylus*.

A muscle has been described by Killian (24) in reptiles extending backwards from the extra-columellar to the parotic process of the skull. He calls it the stapedial muscle, and homologises it with the muscle of the same name in the Mammalia. It is the muscle called "m. extra-columellaris" by Versluys (31) in adult *Geckonidæ*, and also in the embryo

Lacerta, and found by him to be derived from the facialis musculature (depressor mandibulae). Both Versluys and Gaupp accept Killian's conclusion, but it is difficult to see how this muscle, which passes outside the facial nerve (as shown in Pl. 11, fig. 8), can be homologous with the mammalian stapelial muscle situated on the inner side of the nerve (Pl. 12, fig. 16, and Pl. 13, fig. 25).

TEXT-FIG. 1.



A, B, and C, Diagrams illustrating the relation of the chorda tympani nerve to the first gill-slit and the tympanum in reptiles, birds, and mammals, during ontogeny. A shows an early stage in which the slit is widely open; C, a later stage, in which the slit has closed, the gill-pouch has separated off from the epidermis, and the tympanic diverticulum has developed. B is an intermediate stage. The arrow points forwards. *bo*. Epidermal thickening ("branchial sense organ") at top of first gill-slit. *f*. Facial nerve and ganglion. *chty*. Chorda tympani. *hf*. Hyomandibular branch of facial nerve. *hs*. Hyoid or spiracular gill-slit. *pal*. Palatine nerve. *ty*. Tympanum. *tyd*. Tympanic diverticulum. (Right-side view.)

Sections of early stages of *Sphenodon* show that the chorda tympani develops in the same way, and bears the same relation to the first gill-slit and other structures as in *Lacerta* or *Platydactylus*. The condition described above may, therefore, be considered as typical of reptiles generally.

Aves.—So far as I am aware, the development of the chorda tympani has not yet been described in early stages of birds. Both Kastschenko (23a) and Neumayer (27) failed to find it in chick embryos less than seven and a half days old, by which time the spiracular cleft is, of course, closed and the first gill-pouch has separated from the epiblast. No

doubt these observers looked for the nerve where we should expect to find it, but where, as a matter of fact, it is not in the chick, as will be explained below.

In addition to the chick I have studied early stages in the duck, and find that this bird agrees in every essential with *Lacerta*. Fig. 4, Pl. 11, is a reconstruction of a four and a half-day duck embryo, where the spiracular region of the first gill-pouch is still quite continuous with the epidermis, but the skeletal blastema can hardly yet be distinguished. The vena capitis lateralis and the internal carotid bear the same relation to surrounding structures as in *Lacerta*. A beginning of the facial artery can be seen arising from the root of the reduced hyoid arterial arch. The outer lower region of the geniculate ganglion is attached by an epidermal proliferation, the so-called branchial sense-organ, to the dorsal part of the first gill-pouch, and the hyomandibular branch of the facial nerve passes out below the vena capitis lateralis and down into the hyoid bar (Pl. 13, fig. 28). Coming off close to the origin of the facial can be seen the chorda tympani running behind the spiracular slit, and pursuing a curved course below it into the mandibular bar, along which it can be traced near the floor of the buccal cavity. Behind the chorda tympani the first gill-pouch is growing out to form the tympanic diverticulum. Since the position of the chorda tympani in the adult duck agrees with that found in the majority of birds—Magnien (26), Bender (2), Smith (29)—we may assume that this is the normal structure and development of these parts in the Aves. The chorda tympani in every respect behaves as in *Lacerta*, passing over the extra-columella (extra-stapedial cartilage) and distal to the dorsal process (supra-stapedial), Smith (29).

Strangely enough, in the fowl quite another relation is borne by the chorda tympani to surrounding structures. To begin with, Hasse has shown that in the adult *Gallus* (19), and Magnien (26) that in the adult turkey (*Meleagris gallopavo*), this nerve takes a very unusual course. Originating quite far forwards from the geniculate ganglion, it passes

almost vertically downwards in front of the tympanic cavity to reach the articulation of the lower jaw, along which it runs as usual. Hasse and Smith both consider, I believe rightly, that this is a secondary condition; but they did not study the development, and, as already mentioned, Kastschenko and Neumayer failed to discover the chorda tympani in early stages. I have been able, however, to find it in sections of seven- and six-day chicks, and even to trace it back to the five-day chick, when the first gill-pouch is still continuous with the epiblast (Pl. 11, fig. 12). In these early stages the chorda tympani still occupies approximately the same position as in the adult bird, passing in front of the first gill-pouch from the geniculate ganglion to the mandibular bar. A minute nervous filament can be seen in the same place even in the four and a half-day chick, when the spiracle is open to the exterior.

Never at any stage have I found a chorda tympani in the chick taking the usual course behind and below the first gill-slit. Moreover, even in the earliest stages, it arises dorsally, not from the hyomandibular branch of the facial, but from the ganglionic proliferation at the dorsal edge of the spiracular slit (Pl. 11, fig. 12; Pl. 12, fig. 13).

It would appear, then, that in gallinaceous birds, alone among the amniote vertebrates, the chorda tympani is from its earliest appearance in the embryo a pre-trematic branch of the facial nerve. Considering how constant is the relation of nerves to surrounding structures, it is very difficult to account for this strange exception to such a general rule. The peripheral distribution of the chorda in the turkey is just like that in other birds according to Magnien (26). But perhaps the chorda tympani of the gallinaceous birds is not strictly homologous with that of other vertebrates, and it is to be noticed that it comes into relation dorsally with a complex system of slender nerves, partly sympathetic and partly palatine, which I have not followed out in detail, but have indicated in Pl. 12, fig. 13; or possibly there has been some sort of secondary short-circuiting in front of the spiracle. In any case the anomalous disposition does not appear to be due

to a mere shifting of the chorda tympani, as suggested by Smith (29), analogous to the shifting inwards of the nerve accompanying the reduction of the dorsal and internal processes of the columella found to occur in the Lacertilia, and so well described by Versluys (31). The whole question requires further investigation, and it would be interesting to know whether the chorda tympani develops in front of the first gill-slit in any other birds.

The development of the skeletal elements of the hyoid bar in the chick agrees with that described above in *Lacerta*. The earliest appearance of the columella has recently been studied by Smith (29), whose conclusions I can confirm. About the fifth and sixth day there is a continuous blastema representing the hyoid arch; passing below and on the inner side of the vena capitis lateralis it merges into the blastema of the auditory capsule above. Later on the stapedial and extra-columellar regions and the various processes of the columella chondrify in this hyoid blastema. Fig. 13, Pl. 12, shows the condition in an eight-day chick: the stapedial region is in the form of a stout plug fitting into the fenestra ovalis, and continued outwards into the extra-columella. From this latter region extends downwards a long infra-stapedial or stylohyal process, almost continuous below with a small cartilage separate at this stage. The cornu of the hyoid has already become detached, and taken up a position behind the auditory region. The stylohyal and the little detached cartilage approach the posterior process of the articular cartilage of the lower jaw, and remind one of the interhyal and epihyal cartilages described by Parker in the crocodile, where they become secondarily connected with Meckel's cartilage.

This reconstruction of an eight-day chick also shows a distinct "stapedial muscle" attached to the infra-stapedial process, and occupying a position on the inner side of the hyomandibular nerve. It corresponds, therefore, to the mammalian stapedial muscle in this respect, but not to the reptilian "extra-columellar muscle."

The first gill-pouch is also seen, now, of course, detached from the epidermis. The tympanic diverticulum, however, is but little developed, the mesenchymatous tissue, into which extends the extra-columellar, between it and the ingrowing external auditory meatus, being still quite thick.

Mammalia.—As already mentioned (p. 139) the position of the chorda tympani was correctly figured and described by Fricap in early calf embryos as long ago as 1885. Broman (4), Emmel (13), and others, have since confirmed this observation, and it may now be considered as firmly established that, just as in the Reptilia so in the Mammalia, the chorda tympani arises as a post-trematic branch of the facial nerve.

My own observations fully support this conclusion. As seen in the reconstructions of early stages of *Trichosurus*, when the gill-pouch is still in continuity with the epidermis at a point corresponding to the open spiracular cleft of lower vertebrates, the chorda passes from the hyomandibular branch of the facial nerve round behind and below the gill-pouch to reach its destination in the mandibular arch (figs. 5 and 15). At a later stage, when the first gill-pouch is separating off from the epidermis, the chorda tympani still occupies much the same position; and it is only later when the tympanic diverticulum develops and expands that the nerve is pushed upwards and forwards just as in *Lacerta* (Pl. 12, figs. 14 and 16).

Little remains to be said about the development of the auditory ossicles of the Mammalia, which has been lately so accurately described by Dreyfuss (8), Broman (4), Jenkinson (22) and others. My observations entirely support the views of these and other authors who contend that the stapes is derived from the upper end of the hyoid arch, and the incus and malleus from the upper end of the mandibular arch; as against Fuchs and others who believe otherwise (see Gaupp (17)). From the time when it first appears as a blastema in the mouse the stapes is distinct from the auditory capsule and continuous with the blastema of the remainder of the hyoid arch, passing as usual below the vena capitis

lateralis. This continuity is preserved for some time, and can still be seen quite distinctly in a 9.5 mm. *Trichosurus* embryo (Pl. 12, fig. 21), and a mouse (Pl. 12, fig. 22). On the other hand, it is not continuous at these early stages with the blastema of the processus longus incudis. Soon, however, the stapes separates off from the more ventral and distal region of the hyoid arch, and chondrifies separately round the stapedia artery (figs. 22, 24). Meanwhile that region of the arch ventral to the stapes and dorsal to the future cornu, loses its connection with the stapes owing to the disappearance of the intervening zone of blastema, and then grows up round and outside the vena capitis lateralis, to become joined on to the paroccipital process of the skull (Pl. 12, figs. 16, 20; Pl. 13, figs. 23, 24). It is across this region of the hyoid that the chorda tympani passes on its way from the hyomandibular branch of the facial to the lower jaw (fig. 17). Named intercalare by Dreyfuss (8), and laterohyal by Bröman (4), it has been aptly compared by Versluys (31) and Gaupp (17) to the intercalary cartilage developed from the dorsal process of the columella in reptiles. The two structures have the same origin, occupy the same position relative to other parts, and are probably homologous (Pl. 12, fig. 20).

In a 17 mm. *Trichosurus* embryo a slender cartilaginous hyoid extends continuously up to the skull (Pl. 12, fig. 16). Later on the upper region degenerates, and the more ventral part remains as the cornu of the hyoid. The developing stapes of *Ornithorhynchus* shows just the same relations as that of the mouse or *Trichosurus*, and is at first pierced by the stapedia artery.

In the mandibular bar the dorsal proximal region of the blastema differentiates as a separate cartilaginous element, the incus, and the more ventral region as Meckel's cartilage. Subsequently, as is well known, the more dorsal or articular region of this cartilage separates off as the malleus (figs. 22, 23, 24). There can now be hardly any doubt that the incus and malleus of the mammal represent the quadrate

and articular of the reptile. In the 17 mm. embryo of *Trichosurus* (Pl. 12, fig. 16), the incus is comparatively large and remarkably like the reptilian quadrate in its relations. Dorsally, by means of the processus brevis, it reaches up to the auditory capsule, and may be said to articulate with it, as described in the pig by Kingsley and Ruddick (25); below it articulates with the malleus. The processus longus of the incus is stout and long, and although apparently quite separate from the stapedia blastema in the earliest stages, it comes into close connection later on with the distal end of the stapes (figs. 19, 23, 25). This connection seems to me quite comparable to that established between the columella and the quadrate by means of the processus internus in reptiles, and possibly is represented by Platner's ligament in birds. In Amphibia also the stapes becomes connected with the quadrate either by cartilage or by ligament.

The incus lies outside the vena capitis lateralis, the stapedia artery, and the hyomandibular branch of the facial, all of which pass between it and the auditory capsule, just as they pass between the quadrate and the capsule in reptiles and birds. A comparison of transverse sections of this region in these various groups brings out this remarkable uniformity (Pl. 12, figs. 18, 20; Pl. 13, figs. 29, 30). A manubrium develops from the malleus, extending downwards in the tympanum between the first gill-pouch and the ingrowing external auditory meatus (Pl. 12, figs. 16, 19). The chorda tympani, having passed round outside the laterohyal, runs forwards and inwards to the inner face of Meckel's cartilage, usually between the crus longus incudis and the manubrium. In *Trichosurus* its course seems to be more ventral than usual in mammals, as it runs well below the incus even in the newborn young (Pl. 12, fig. 19).

The development of the first gill-pouch is very similar to that described in *Lacerta*. In early stages it is continuous with the epiblast along an extensive region, but soon begins to separate off, and in 5 and 6 mm. *Trichosurus* embryos it is only connected with the epiblast dorsally at a point corresponding

to the closing slit in *Lacerta* (Pl. 12, fig. 14). Here arises the epiblastic proliferation which contributes to the geniculate ganglion, and marks the exact spot comparable in mammals, birds, and reptiles. The tympanic diverticulum grows out as usual from the more ventral and posterior region of the gill-pouch, while the dorsal apex, becoming quite detached, moves inwards, losing its connection both with the epiblast and with the ganglion. Subsequently various outgrowths of the wall of the tympanic cavity grow round the stapes and incus and malleus, leaving mesentery-like folds suspending these ossicles to the wall. The stapes may be said to push into the enlarging tympanic cavity from behind and above, while the incus and malleus push in from in front. The details of the development of the tympanic cavity have been described and illustrated very elaborately by Hammar (18) and Drüner (10), and need not detain us here. Gradually the mesoblastic tissue between the outgrowing tympanic diverticulum and the ingrowing external auditory meatus becomes thinned out, forming the tympanic membrane enclosing the manubrium of the malleus (Pl. 12, fig. 16).

The stapedial muscle can be seen extending back from the stapes to the skull-wall, below and on the inner side of the hyomandibular branch of the facial nerve (figs. 16, 23, and 25). Derived from the hyoid musculature this muscle is supplied by the seventh nerve (Killian (24)). On the contrary, the tensor tympani, derived from the pterygoid musculature of lower forms, is situated in front of the tympanic cavity, and is innervated from the fifth nerve. The tensor tympani of *Mammalia* stretches inwards from the malleus to the skull-wall; but the relation to the chorda tympani varies in different groups. For instance, the nerve passes above or dorsal to the tensor ligament in *Man* and *Macacus*; ventral or below the ligament in *Sus*, *Canis*, *Arvicola*, and *Mus*; while in *Equus* and *Sciurus* it passes through the ligament, according to the observations of Eschweiler (14) and Bondy (3). Doubtless the first position is the most primitive. It is that found in *Trichosurus* (fig. 17), and in

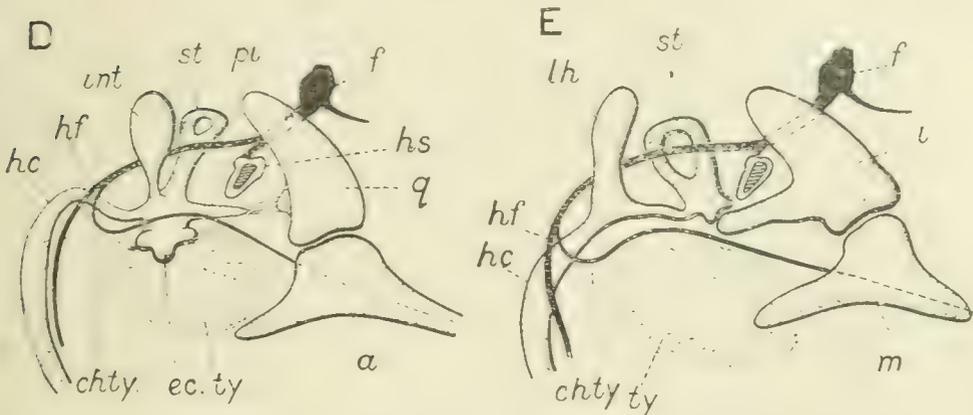
Mus (fig. 26), and corresponds to the relation of nerve and muscle in reptiles.

Conclusion.—In the preceding pages it has been shown that the chorda tympani in reptiles, birds, and mammals, develops as a post-trematic branch of the facial nerve behind the first gill-slit. As may be seen in the diagrams A, B, and C (p. 144), owing to the outgrowth of the tympanic diverticulum from the hinder region of the first gill-pouch, the chorda comes to lie in front of this pouch when the slit closes and the pouch separates off from the epidermis. The only known exception to this rule is that of the gallinaceous birds, in which the chorda is found to pass in front of the first gill-pouch from its very first appearance. In the adult amniote the chorda necessarily passes down anterior to the tympanum. Although in certain forms like *Sphenodon*, where the membrane is not very clearly delimited, the chorda may seem to pass across the dorsal and anterior region of the tympanum, the relative position of these parts remains essentially unaltered.

It has also been shown how very constant throughout ontogeny are the relations of the spiracular slit, vena capitis lateralis, facial or stapedial artery, facial nerve, and chorda tympani, in mammals, birds, and reptiles. Indeed, the early stages of the lizard, duck, and *Trichosurus*, when the first gill-pouch is still continuous with the epidermis, are so similar that figures of any one of them would apply almost equally well to the other two. Only unimportant differences of relative size and proportion can be detected between them. Later on divergencies occur owing to the great development of the extra-columella in birds and reptiles, and to the position taken up in mammals by the incus and malleus, where they come to lie between the stapes and the tympanum; but even then the blood-vessels, muscles, nerves, and skeletal parts retain their essential morphological relations. Only on the supposition that the incus represents the quadrate, and the malleus the articular, is this structure intelligible. As a glance at diagrams D and E will show, the chorda tympani

really follows essentially the same course in reptiles and mammals. In both the hyoid arch, at its dorsal end, has two diverging branches: a stapedia fitting into the fenestra ovalis and an intercalary branch coming into contact with the skull. The hyomandibular branch of the facial nerve passes back over the stapes, and the chorda tympani runs from it outside the arch, then forwards between the spiracular

TEXT-FIG. 2.



D and E, Diagrams showing the relation of the first and second visceral arches and the branches of the facial nerve to the closing first gill-slit and the developing tympanum in a reptile (D) and a mammal (E). *a*. Articular cartilage. *chty*. Chorda tympani. *ec.ty*. Extra columella. *f*. Facial ganglion. *hc*. Hyoid cornu. *hf*. Hyomandibular branch of facial nerve. *hs*. Hyoid or spiracular gill-slit. *i*. Incus. *int*. Intercalary cartilage of processus dorsalis. *lh*. Intercalary or laterohyal cartilage. *m*. Malleus. *pt*. Processus internus. *q*. Quadrate. *st*. Stapes. *ty*. Tympanum. (Right-side view.)

opening (virtual in mammals) and the tympanum to the inner side of the articular or malleus. But, whereas in the reptile the columella has given rise to an extra-columella which grows into the tympanum below the chorda, in the mammal no such extra-columella is developed, and it is the malleus which comes into relation with the tympanum. Thus the relation of the chorda to the hyoid arch is really the same in the two cases. The articulation of the incus with the stapes is paralleled by the connection, cartilaginous or ligamentous, so frequently established between the columella and the

quadrate in reptiles and birds, and even in Amphibia. Whether the extra-columella be primitive among Reptilia is a doubtful point ; but on the whole it seems probable that the Mammalia have lost it, on the gradual assumption of its functions by the incus and malleus. Accompanying this modification of the quadrate and articular to transmit vibrations from the tympanum, the jaws in mammals must, of course, have acquired a new mode of articulation. But this difficulty often urged against Reichert's theory may now be said to have disappeared owing chiefly to the discoveries of Seeley and Broom (5, 6). There is no need to go into this question in this paper. But it may be pointed out that these authors have shown that, in fossil Reptilia related to the ancestors of the Mammalia, the squamosal and dentary bones have gradually increased in importance, contributing more and more to the support of the lower jaw ; while the quadrate and articular have dwindled in size, become loosened from the surrounding bones, and have, so to speak, been drawn into the service of the middle ear.

While the homology of these structures may now be considered as well established in the amniote vertebrates, their disposition in the Amphibia still presents serious difficulties. For in the only group which possesses a tympanum, the Anura, the ramus mandibularis internus (chorda tympani) is posterior to it. Drüner, indeed, concludes that the tympanum and tympanic cavity of the Amphibia and Amniota are not homologous (9). The evidence for such an extreme view seems quite insufficient, and Bender (2) has brought forward important facts with regard to the nerve-supply of the wall of the tympanic cavity, which go far to prove that it is homologous throughout the terrestrial vertebrates and with the spiracular slit of fishes, a conclusion which is in agreement with the results of embryology. While accepting Bender's conclusion Gaupp still considers that the tympanum itself has become independently developed in Amphibia, reptiles, and mammals (17). But, while admitting that the position of the chorda is a serious difficulty in comparing the amphibian with the

reptilian structure, it would seem much more probable that there has been some relative shifting of parts. Moreover, the presence of a characteristic notch behind the quadrate in fossil *Stegocephalia* indicates the possession of a tympanum, and those modern forms (*Apoda* and *Urodela*) which do not possess one have probably lost it, being secondarily adapted to a burrowing or aquatic mode of life. The view of Gaupp that the tympanum of *Reptilia* is not homologous with that of *Mammalia*, chiefly because the former is situated above the Meckelian cartilage and the latter below it, seems to me greatly to exaggerate the importance of a comparatively trivial difference. If the manubrium of the malleus represents the posterior process of the articular, the tympanum extends both above and below it, and the difference between the two types is small, and just such as we should expect to find accompanying the change of size and function of the incus and malleus. Rather should we consider the modern reptilian and mammalian plan as showing two divergent types derived from some intermediate plan of structure perhaps to be discovered among the *Theromorpha*.

Summary.—A comparison of the development of the various structures of the middle-ear region in the lizard, duck, and mammal, shows a remarkable uniformity in their origin and relation. The first gill-pouch separates off from the epidermis from below upwards; at its dorsal edge is an epiblastic proliferation contributing to the geniculate ganglion. The tympanum is formed between the outer epidermis and an outgrowing diverticulum of the hinder lower region of the first gill-pouch. The chorda tympani is a post-trematic branch of the facial nerve, developing behind the first or spiracular gill-slit, and passing down to the lower jaw between the tympanum and the closing spiracle. The relation of these parts to the skeleton and blood-vessels is (with the exception mentioned below) constant throughout the *Amniota*, and is only intelligible on the view of Reichert that the proximal region of the columella corresponds to the stapes, the quadrate to the incus, and the articular to the malleus.

In the chick the chorda tympani develops as a pre-trematic branch of the facial nerve from its first appearance. In adult gallinaceous birds the chorda passes down directly from the geniculate ganglion in front of the tympanic cavity. This exceptional position is probably due to some secondary modification at present unexplained.

September 2nd, 1914.

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EXPLANATION OF PLATES 11, 12, AND 13,

Illustrating Mr. Edwin S. Goodrich's paper on "The Chorda Tympani and Middle Ear in Reptiles, Birds, and Mammals."

LETTERING OF PLATES.

a. a. Aortic arch. *a. c.* Auditory capsule. *a. i. r.* Anterior inner recess of tympanic cavity. *a. o. r.* Anterior outer recess. *art.* Articular cartilage. *a. s.* Auditory sac. *b.* Blastema extending from stapes to hyoid. *bo.* Epiblastic proliferation or branchial "sense organ" of facial nerve. *bra.* Branchial arch. *bs 1-4.* First to fourth branchial slits. *c.* Small cartilage of hyoid arch, = epihyal? *car.* Internal carotid artery. *col.* Columella auris. *eam.* Invagination to form external auditory meatus and external wall of tympanum. *ep.* Epipterygoid. *et.* Eustachian tube. *f.* Facial ganglion and nerve. *fb.* Forebrain. *gl.* Glosso-pharyngeal ganglion and nerve. *h.* Hyoid bar. *ha.* Blastema of hyoid arch. *hart.* Hyoid arterial arch. *hb.* Hindbrain. *hc.* Hyoid cornu. *hf.* Hyomandibular branch of facial nerve. *hs.* Hyoid or spiracular slit. *hsp.* Pouch of hyoid slit. *intc.* Intercalary cartilage from dorsal process. *lsh.* Lateral cranial wall. *man.* Manubrium mallei. *md.* Mandibular bar. *mda.* Blastema of mandibular arch. *mk.* Meckel's cartilage. *ms.* Extra-columellar muscle. *oc.* Occipital cartilage. *os.* Optic stalk. *par.* Paroccipital process. *pb.* Processus brevis incudis. *pl.* Processus longus incudis. *ppart.* Posterior process of articular cartilage. *pr.* Posterior recess of tympanic cavity. *prant.* Anterior process of columella. *prp.* Posterior process of extra-columella. *psk.* Process of skull. *quad.* Quadrangle cartilage. *sta.* Stapedial artery or facial artery. *sth.* Stylohyal cartilage. *stm.* Stapedial muscle. *tr.* Trigeminal nerve and ganglion. *tt.* Tensor tympani muscle. *tyd.* Tympanic diverticulum. *v.* Vagus ganglion and nerve. *vel.* Vena capitis lateralis.

[In the reconstructions here figured the nerves are in black; the pharynx, gill-pouches, and tympanic cavity in green; the veins in blue; the arteries in red; the cartilage in purple. In the earliest stages the blastema representing the skeleton is indicated by purple dots.]

PLATE 11.

Fig. 1.—Reconstruction of a portion of the right side of the head of a *Lacerta* embryo at an early stage when the first or spiracular slit is still widely open to the exterior. The nerves and gill-slits are reconstructed on the outline of a section near the middle line.

Fig. 2.—The same with the blood-vessels added.

Fig. 3.—Similar view of a later stage of *Lacerta*, when the first gill-slit is partially closed, and the blastemata of the mandibular and hyoid arches are visible.

Fig. 4.—Similar view of a corresponding stage of an embryo duck (four and a half days). In this, in fig. 5, and in some of the following figures, the epidermis indicating the surface of the embryo has been shown.

Fig. 5.—Reconstruction of a portion of the right side of an embryo *Trichosurus vulpecula* 5 mm. long (I, A, '01). The endodermal pouch of the first slit is still continuous with the epidermis at a point not represented in the figure, the surface of the head having been cut away.

Fig. 6.—Reconstruction of the right side of a thick horizontal section of the head of *Lacerta* at a stage when the hyoid arch is still continuous with the columella by means of procartilage. View from above (dorsal) the vena capitis lateralis having been removed. The first gill-pouch has separated from the epidermis.

Fig. 7.—Right side of the head of an older *Lacerta* embryo. Only the skeletal and nervous systems have been reconstructed, but the pharynx and blood-vessels are shown as cut in the section nearest the median line. For the sake of clearness the distal region of the columella has been cut away.

Fig. 8.—More complete reconstruction of a portion of the same specimen as shown in fig. 7, with the complete columella, the arteries and the gill-pouch. A narrow strip of vaguely defined tissue still connects the top of the hyoid cornu with the extra-columella.

Figs. 9, 10, 11.—Reconstructions of the quadrate region of a late *Lacerta* embryo seen from behind (posterior view). In figs. 10 and 11 only the skeleton and tympanic cavity are shown, the columella being completed in both. Fig. 11 fits on to fig. 10, and shows the posterior upper part of the quadrate and the tympanic recesses. Fig. 9 resembles fig. 10, but has the nerves, tympanic cavity, and arteries included. The position of the vena capitis lateralis is indicated by a dotted ring.

Fig. 12.—Reconstruction of the right auditory region of a five-day chick embryo. The first gill-pouch is still continuous with the epiblast, and the chorda tympani runs down anterior to it. The skeleton is present only as a vaguely defined blastema at this stage.

PLATE 12.

Fig. 13.—The right auditory region of an eight-day chick embryo. In the thick slice reconstructed only the inner region of the ingrowing external auditory meatus appears. The upper posterior region of the quadrate has been cut away to expose the underlying structures.

Fig. 14.—Reconstruction of a portion of the right side of the head of an embryo of *Trichosurus vulpecula* 7.25 mm. long (XII, A, '01). The first gill-pouch has just separated from the epiblast at a point below the "branchial sense organ" *bo*.

Fig. 15.—Dorsal view of a reconstruction from transverse sections of the right side of the head of an embryo *Trichosurus vulpecula* 6 mm. long. The first gill-pouch is still continuous with the epiblast, and the chorda tympani passes behind and below it. The thick slice does not include the whole vena capitis lateralis, and only the most ventral part of the auditory sac lying above the other structures.

Figs. 16 and 17.—Reconstructions of the right auditory regions of the head of an embryo *Trichosurus vulpecula* 17 mm. long. To expose the stapes and other structures the incus and malleus have not been included in fig. 17.

Figs. 18, 19, 20.—Reconstruction of three consecutive thick transverse slices through the left auditory region of the head of a newborn *Trichosurus vulpecula*. The reconstructions are seen from in front (anterior view), and the hind surface of the slices 18 and 19 fit on to the front surface of the slices 19 and 20 respectively.

Fig. 21.—Ventral view of a thick slice of the right auditory region of the head of an embryo of *Trichosurus vulpecula* 9.5 mm. long, reconstructed from transverse sections. The skeleton is procartilagenous. The stapes is seen through the first gill-pouch, and is continuous with the procartilage of the hyoid arch.

Fig. 22.—Similar view, but from the dorsal surface, of the left auditory region of an embryo mouse at a slightly later stage.

PLATE 13.

Figs. 23 and 24.—Similar dorsal views of the same region of a fourteen-day embryo mouse. A portion of the skull has been cut away to expose the stapedial muscle in fig. 23. In fig. 24 the skeleton of the visceral arches and the blood-vessels are shown.

Figs. 25 and 26.—Two consecutive thick slices of the right auditory region of an older embryo mouse 22 mm. long, viewed from the dorsal surface and reconstructed from transverse sections.

Fig. 27.—Longitudinal sagittal section through the middle-ear region of the *Lacerta* embryo drawn in fig. 3. *Cam.*

Fig. 28.—Similar section of the embryo duck drawn in fig. 4. *Cam.*

Figs. 29 and 30.—Transverse sections of the ear region of the *Lacerta* embryo shown in fig. 9. Fig. 29 represents a section in front of the columella, and fig. 30 through the columella. *Cam.*

Studies on the Turbellaria.

Part III.—Didymorchis.

By

W. A. Haswell, M.A., D.Sc., F.R.S.,
Challis Professor of Zoology, University of Sydney.

With Plate 14, and 1 Text-figure.

OF the many and varied inhabitants of the branchial cavities of Australasian crayfishes the Rhabdocœles are not the least remarkable. They are very minute colourless forms, which only occur in association with the crayfishes, and present certain adaptations to their special mode of life. They are able to adhere tenaciously by means of an apparatus having the function of a sucker to the filaments of the gills or to the walls of the branchial chamber, and are thus not liable to be swept out by the respiratory current or dislodged by the movements of the podobranchs; and, with cilia only developed ventrally, they glide along in close contact with the substratum, so that they can instantaneously anchor themselves when any movement takes place that might displace them.

One of these, which occurs on *Paranephrops neozelanicus* of New Zealand, I described some years ago (5) under the name of *Didymorchis paranephropis*. *Didymorchis* belongs to Graff's family *Dalyellidæ* (formerly *Derostomidæ*), and differs from the other genera of that family in the character of the excretory system and in the restriction of the cilia to the ventral surface.

In the two common crayfishes of Eastern Australia,

Astacopsis serratus and *Cheraps bicarinatus*, *Rhabdocœles* are constantly present. Of these there are, as far as I have been able to determine, only two species, one confined to each of the two crayfishes in question. They both belong to the *Dalyellidae*, and resemble *Didymorchis* in many points; but the excretory system is constructed on entirely different lines. In this respect—the arrangement of the main trunks of the excretory system—the two Australian forms are at one, though they differ from one another in certain features of the reproductive apparatus. On the whole I think it better, instead of coining a new generic name or names, to regard them as a species of *Didymorchis*.¹

The species living in the branchial cavities of *Cheraps bicarinatus* I propose to name *D. cherapsis*. When the gills and branchiostegites of the crayfish are removed and placed in water in a glass vessel, a small number of the *Rhabdocœles* will always be found among the crowd of actively moving animals—chiefly *Craspedellæ*, together with a *Nematode*—which soon begin to become separated out from the gills. When among the gill-filaments the *Rhabdocœles* readily clamber from one to another by looping movements. On the surface of the glass, when undisturbed, they glide evenly along through the action of the cilia of the ventral surface; when disturbed by a touch or by an attempt to draw them into a pipette, they instantly attach themselves to the surface with considerable tenacity.

Didymorchis cherapsis (Pl. 14, fig. 1) is, like *D. paranephropis*, a very small *Rhabdocœle*, not exceeding a millimetre in length. When gliding along freely it becomes relatively long and narrow, with the anterior end truncate and slightly more expanded than the rest, the posterior end narrower and slightly emarginate. The largest specimens are of a very light greenish-yellow colour: smaller specimens

¹ I have a note also on an allied form occurring in *A. australiensis* of Tasmania, but without details sufficient to determine its relationship to the others.

are quite colourless. There is a small pair of eyes of crescentic shape situated about one sixth of the total length from the anterior end, and separated from one another by an interval of about a half to a third of the total breadth. The mouth is some little distance—about one eighth to one sixth of the total length from the anterior end. The single reproductive aperture is a similar distance from the posterior end.

At the posterior end on the ventral surface is a sub-crescentic elevated area with the concavity forwards (Pl. 14, fig. 2). The surface of this is non-ciliated, but is dotted with numerous minute bright spots, which indicate the openings of the ducts of integumentary glands. This elevation appears to play the part of a sucker.

The epidermis (*ep.*), which varies somewhat in thickness in different parts, is ciliated only on a definitely limited area of the surface on the ventral side. In this respect, as well as in the other features of the integument, *D. cherapsis* resembles *D. paranephropis*. A yet more remarkable feature of this layer in *Didymorchis* is that it is entirely devoid both of cell-boundaries and of nuclei. This is a very exceptional condition. I do not know of any other instance of it except Plehn's genus *Sanguinicola*, which lives as an internal parasite in the blood-vascular system of Cyprinid fishes (6).

Within the epidermis is a basement-membrane which becomes very conspicuous in hæmatoxylin-stained sections (Pl. 14, figs. 3–5), owing to its affinity for the dye. Within this again are the muscular layers usual in *Rhabdocœles*—an external circular and an internal longitudinal. The former is very feebly developed; it is not shown in the figures of sections.

The openings of the ducts of the integumentary glands are confined mainly to two localities—the anterior and posterior extremities of the ventral surface. In the latter position in the middle line behind the testes, numerous ducts open close together on a small area, which is capable of being depressed into a deep pit, occupying the middle of the crescentic area

already referred to. In the terminal portions of the ducts, where they pass between the testes, masses of the secretion collect ready to be discharged instantaneously. It is mainly this arrangement that enables the animal to adhere firmly to a surface in the manner already described. At the anterior end, where the adhesive power is much feebler, there is less concentration of the ducts and their apertures, but the latter are mainly concentrated on two areas at the sides of the mouth and in front of it.

The mouth leads into a narrow passage—the anterior part of the pharyngeal sac—opening into the pharynx behind. The latter is of Graff's "doliiform" type, and has the shape of a cylinder which is about twice as long as it is wide. The unicellular glands in the substance of its walls are very conspicuous in the living animal; the ducts of all of them, much convoluted in the ordinary retracted condition of the organ, straightened out in the protracted state, open round the anterior margin.

The pharynx is freely eversible, becoming protruded to as much as a third of its extent through the mouth, its thin, circular, anterior margin projecting beyond the anterior end of the body.

Posteriorly it opens directly into the intestine—an œsophagus not being differentiated. Around the opening are about six œsophageal glands, the secretion of which has the appearance of extremely minute spherules.

The intestine (Pl. 14; fig. 4) has no epithelium and no lumen. It is merely a vacuolated protoplasmic mass with irregularly distributed nuclei; numerous bright spherical granules or droplets, some of relatively large size, are embedded in it. In some series of sections the intestine is quite solid without any internal cavity. In others irregular spaces occur. Externally the intestinal syncytium is not definitely circumscribed, and no definite limit can be recognised between it and the general mesenchyme or parenchyma.

This syncytial condition of the intestine appears to be specially characteristic of the Dalyellidæ. When I found

such a condition occurring in *Anomalocœlus* (4), I was under the impression that it was a very exceptional one. But Hallez (3) describes exactly the same state of things in *Proderostoma*, and, in this case at least, it is clear enough that the syncytial condition is not a secondary one; there never is an epithelium at any stage in *Proderostoma*. In all probability the same holds good of the others; certainly there is no appearance of an epithelium in the youngest specimens of *Anomalocœlus* and *Didymorchis* examined.¹

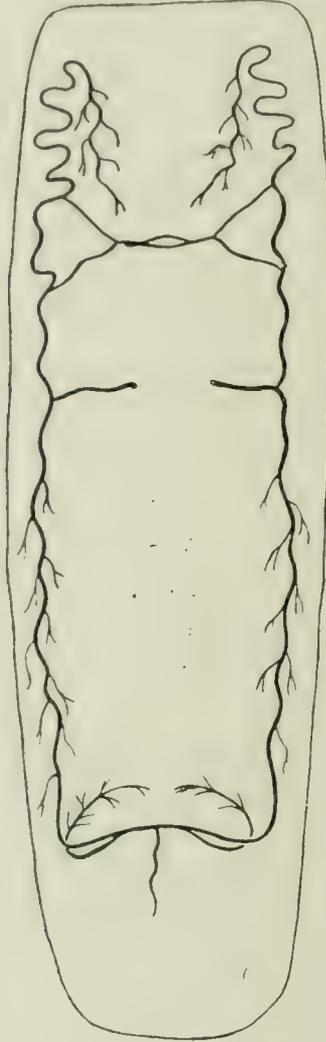
There is no doubt, however, that Hallez goes too far in concluding that the condition observed in *Proderostoma* is general among the Rhabdocœles. He says: "On voit que von Ihering a raison en ce sens que l'intestin des Rhabdocœles est ces l'embryon un organ massif et chez l'adulte un plasmode comme chez les Acœles. Une lumiere n'en existe pas moins chez l'adulte, mais celle est un simple vacuole du syncytium." In von Graff's 'Monographie' (1) there are a number of figures showing enteric epithelium and a definite enteric lumen in members of various families of Rhabdocœles. In fact, the only member of the group in which that distinguished author recognises a plasmodial condition is *Plicastomum bimaculatum* (loc. cit., p. 2126, also 2, p. 2132).

There are two excretory apertures, situated on the ventral surface towards the middle of the body and some distance behind the posterior end of the pharynx. Each of the apertures (Text-fig. 1), which are separated from one another by a considerable space, leads directly, without the intercalation of any excretory sac or vesicle, into a transverse main vessel

¹ It was partly on account of this feature, supposed to be exceptional, that I proposed that *Anomalocœlus* should be regarded as the type of a distinct family. Von Graff, however, states (2, p. 1993) that *Anomalocœlus* approximates so closely to *Phænocora* (*Derostoma*) as scarcely to demand a separate generic name. But he overlooks the fact that in the latter the testes are compact, as in the rest of the *Dalyellidæ*, while in the former they are of the diffuse type—a distinction which elsewhere he treats as an important one in his scheme of classification.

of considerable dimensions. This divides towards the margin of the body into anterior and posterior longitudinal trunks. The anterior runs forwards towards the anterior extremity and then bends back upon itself and breaks up into a number

TEXT-FIG. 1.



of branches in the anterior region of the body. From it as it runs forwards are given off internally two large branches, which, widely separated at their origin, converge and unite near the middle line on the ventral side below the middle of the pharynx to form a short, transverse commissural vessel having corresponding relations on the opposite side.

The posterior longitudinal vessel bends inwards towards the posterior extremity of the body, and, behind the genital aperture, passes into the posterior longitudinal vessel of the opposite side. It gives off, not far from the middle line, a large branch which runs outwards in close apposition with it for some distance, and then bends inwards and breaks up into branches in the reproductive region.

The above-described arrangement of the excretory vessels and their apertures differs completely from that which occurs in *D. paranephropis*, and appears to approximate more closely to that observable in *Phænocora stagnalis*, as described by Fuhrmann (see 2, p. 2147), than to that occurring in any other member of the *Dalyellidæ*.

The reproductive apparatus (Pl. 14, fig. 1) is very similar to that of *D. paranephropis* with certain important points of difference. The genital aperture leads into a genital cloaca into which the free end of the penis projects on the left, while the female aperture is situated towards the right. The penis (Pl. 14, fig. 6) is a chitinous cylinder narrowing somewhat towards its free end, where it possesses an introvert armed with a number of fine spines. At its base is a rounded bulb into which numerous ducts of granule glands open, and from which is given off a short rounded ejaculatory sac. The granule glands are situated chiefly on the left side between the left testis and the intestine. With the bulb of the penis is connected by a narrow neck the large pyriform vesicula seminalis, into which the two vasa deferentia open. There are two compact, somewhat reniform testes situated in the posterior region of the body and coming very close to one another posteriorly.

The female aperture leads into a rounded chamber, the vagina. In the walls of this are situated two, or, in the largest specimens, three groups of chitinous teeth. Each group is supported on a muscular cushion. In each group there are six rows, the outermost row containing about ten teeth—the number in each row decreasing from the outer towards the inner rows. In large specimens the teeth are

more closely aggregated, and the division into rows becomes less definite.

The succeeding division of the female duct (ootype) is not definitely dilated; eggs have never been found in it, but there are a number of unicellular glands (shell-glands) around it. The anterior part of the female duct (oviduct) usually contains a small number of actively moving spermatozoa. It receives the two vitelline ducts and gives off in front, in about the middle line of the body, a rounded sac, the receptaculum, which varies greatly in size in different individuals, and usually contains a quantity of granular matter with embedded inert spermatozoa. The ovary, on the right side of the body, is of the same type as that of *D. paranephropis*—compact, oval, containing only a small number of relatively large ova.

The vitelline glands are a pair of solid cylinders, which extend forward on each side to about the line of junction between the pharynx and the intestine. Posteriorly they converge, become narrowed into the form of ducts, and open together into the oviduct close to the mouth of the receptaculum and not far from the ovary.

The form which occurs in the spiny crayfish, and which I propose to call *D. astacopsidis*, resembles *D. cherapsis* in all the most essential features. It is usually more abruptly truncate at the ends, the posterior end being more or less emarginate. The eyes are wider apart. The pharynx is relatively shorter. The penis (Pl. 14, fig. 7) has a somewhat longer introvert with longer spines. The vagina is entirely devoid of teeth.

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EXPLANATION OF PLATE 14,

Illustrating Mr. W. A. Haswell's paper “Studies on the Turbellaria.”

LETTERING.

ap. Reproductive aperture. *bm.* Basement membrane. *e.* Eyes. *ep.* Epidermis. *ex.* Excretory vessels. *i.* Intestine. *lm.* Longitudinal muscular layer. *n.* Fibrous mass of brain. *od.* Oviduct. *ov.* Ovary. *p.* Penis. *ph.* Pharynx. *r.* Receptaculum. *t.* Teeth in vagina. *te.* Testes. *v.* Vitelline glands.

Fig. 1.—General view (semi-diagrammatic) of the organisation of *Didymorchis cherapsis*, magnified 140 times.

Fig. 2.—Ventral surface of posterior end with adhesive apparatus.

Fig. 3.—Transverse section passing through brain and eyes.

Fig. 4.—Transverse section through intestinal region, showing the absence of epithelium and of lumen. The cilia of the surface are not shown.

Fig. 5.—Transverse section a little behind the genital aperture.

Fig. 6.—Penis of *D. cherapsis*.

Fig. 7.—Penis of *D. astacopsidis*.

The Placenta of a Lemur.

By

J. W. Jenkinson, M.A., D.Sc.,

University Lecturer in Embryology, Oxford; Late Fellow of
Exeter College.

With Plates 15, 16, and 17, and 7 Text-figures.

OUR knowledge of the placentation of the lemurs begins with the publication, in 1875, by A. Milne-Edwards and A. Grandidier, in their 'Historie naturelle des Mammifères de Madagascar,' of an account of the placenta in *Propithecus* and some other forms.

In this memoir the placenta is described as being diffuse and "en cloche" or bell-shaped, because villi are absent at one end of the chorion, namely, that towards which the head of the embryo is turned:

The uterus is bicornuate, the embryo lodged in one cornu, which is consequently much enlarged. The other, though invisible from the outside, is distinguishable internally, being marked off from the pregnant cornu by a fold (Text-fig. 6, *f*); it is occupied by an extension of the allantois and of the placenta.

The mucosa is folded, except near the cervix. The folds radiate from smooth areas in which open the mouths of glands, from 6 to 8 mm. in length. The chorion is correspondingly folded, except opposite these smooth areas and the cervix.

After pulling away the foetal "villi," that is the folds, and injecting the maternal vessels, it is observed that none of

the injectum fluid escapes. It is concluded that the placenta is indeciduate.

The allantois is very large, with two or three cornua, and sometimes some small stalked diverticula as well.

In *Avalis* and *Indris* the structure of the placenta and foetal membranes is similar.

In the following year Sir William Turner presented to the Royal Society a description of the placenta in *Propithecus diadema* and *Lemur rufipes*.

In the first of these the foetus was found to be lodged in the corpus uteri and left cornu.

The mucosa is folded, except in the numerous (twenty) smooth areas on which the glands open. On and between these folds are crypts, in which Turner states that he saw a persistent uterine epithelium. The chorion is covered with ridges, bearing villi which fit in between the folds and into the crypts respectively. Only at the cervical end is the chorion non-villous. In *Lemur rufipes* Turner observed a similar arrangement of folds, crypts, and villi, but there was a smooth non-villous area on the posterior side, and other such areas elsewhere in the cornu. The allantois was very large. Some years later the same author added a brief account of a full-time foetus and placenta of *Lemur xanthomystax*. The foetus was lodged in the left cornu, but the right cornu was also dilated, though less so.

In structure the placenta resembled that of *Lemur rufipes*, with smooth, non-placental regions in various parts.

The next author to deal with the subject was Hubrecht, who in his 'Spolia nemoris' (1894) showed that in the Mayalan species, *Nycticebus tardigradus*, the placenta was essentially similar in structure to that possessed by the Madagascan forms. In early stages the villi were seen to be short and cylindrical, later somewhat folded and wrinkled. They folded into crypts lined by a persistent uterine epithelium. "Chorionic vesicles" were also found—that is, recesses produced by an in-pushing of the chorion, with villi depend-

ing into them. In later stages the chorion was observed to be non-villous at the cervical end and also in a patch on the anterior side.

Next comes Strubl's memoir on the African species, *Galago agisymbanus*.

In the possession of short cylindrical villi this form resembles *Nycticebus*.

The villi are situated in crypts lined by a persistent uterine epithelium, and are covered by a columnar or cubical trophoblast. At the summit of each is a depression into which the uterine epithelium does not enter. In this depression a greenish substance is found.¹

There are also "chorionic vesicles," again as in *Nycticebus*; with branched vascular villi hanging into them. The wall of vesicle is backed by a layer of smooth muscle fibres and some loose connective tissue.

Opposite each vesicle is a depressed "Turnerian" area, at the bottom of which open the uterine glands. Both this depression and the overlying vesicle are filled with a granular matter, in which leucocytes are intermingled, presumably the secretion of the glands.

The yolk-sac was found in early stages. The allantois is very large and covers the whole of the inner surface of the chorion. In later stages its cavity is subdivided by septa, in which travel the blood-vessels of the placenta.

Strubl has more recently (1905) investigated the placenta in *Propithecus coronatus* and *Lemur albimanus* and *mongoz*.

In the first of these genera the earlier description of the villi as folds is confirmed.

These folds are wavy, and later secondarily folded and lobed. The uterine epithelium persists. There are non-villous areas. In *Lemur*, on the other hand, the villi are not long ridges, but leaf-like, each with a narrow base. The uterine epithelium persists here also.

¹ This may be a hæmoglobin derivative, from the digestion of extravasated maternal hæmatids.

The short communication of Anthony (1908) on the placenta of *Propithecus varreauxii typicus* adds little to what was already known. An interesting point is that the allantois does not extend over the whole of the inner surface of the chorion. The villi are long ridges. An internal fold marks the separation of the cornua.

It is sufficiently clear from these several accounts that the placenta in the Lemuroidea is of the so-called deciduate type, and it might well be supposed that there is very little for another author to add. The histological details given are, however, if not meagre (except in Strubl's memoir on *Galago*), at least not such as might be obtained by a more modern technique, and I therefore propose in the present paper to fill this lacuna as far as possible.

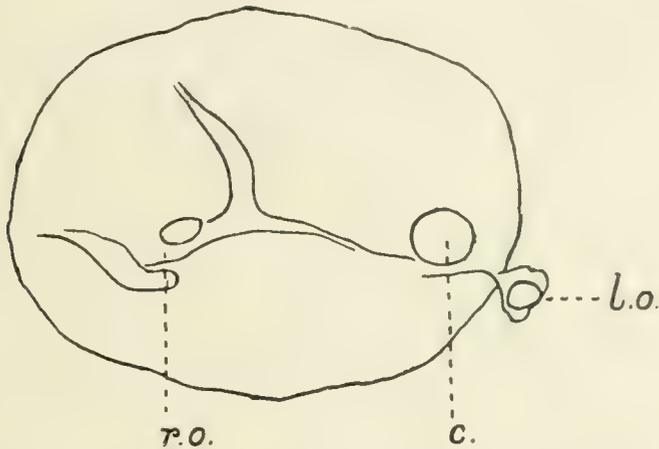
I have, unfortunately, only one stage at my disposal, the apparently full-time embryo and placenta of a Madagascan lemur, which I received a year or so ago through Sir Ray Lankester, from Mr. A. Dobrée. According to Mr. Dobrée's statement this uterus belongs to a grey lemur, known by the natives as "Bohengy," and was obtained on the west side of the island, about thirty miles from the coast in the Menabe district on the Tsuribilima river.

The fœtus evidently is a member of the sub-family Lemurinae (the toes are not webbed and the cæcum not long), and possibly belongs to the genus *Lepidolemur* (*Lepilemur*), since I find that A. Milne-Edwards states that the native name for *Lepilemur ruficaudatus* is "Bouenghé." Beyond this I cannot identify it.

The gravid uterus (Text-fig. 1) is an oval sac measuring $2\frac{3}{4}$ in. by $2\frac{1}{5}$ in. Towards one end is the cut cervix, through which protrudes the chorion. At the same end, which I take to be the left, is one ovary and Fallopian tube: the other ovary and tube are about mid-way between the middle and the opposite end. Pieces of the broad ligament (mesometrium) remain, and its line of insertion on the uterus can be distinguished. This line runs on what is presumably the dorsal side of the cervix. The oval sac, as will be shown later,

is divisible, by an internal fold, into two cornua, of which one, the right, is much larger than the other, and contains the bulk of the embryo.

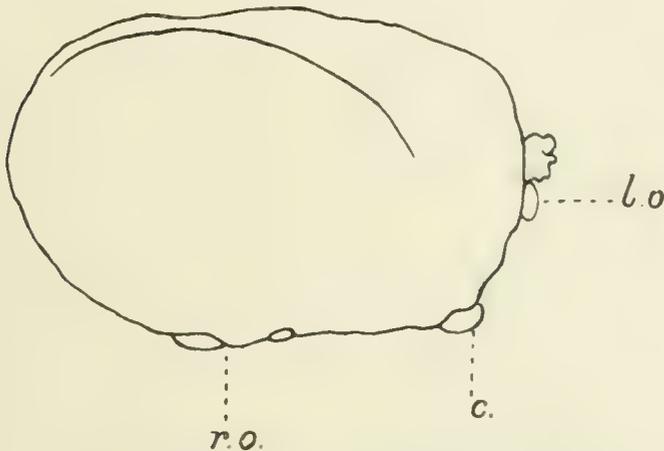
TEXT-FIG. 1.



Gravid uterus seen from the posterior (cervical) side: *c.* Cervix.
r.o. Right ovary and tube. *l.o.* Left ovary and tube.

[Turner states that in *Lemur xanthomystax*, and also in *Propithecus diadema*, the fœtus is lodged mainly in the

TEXT-FIG. 2.

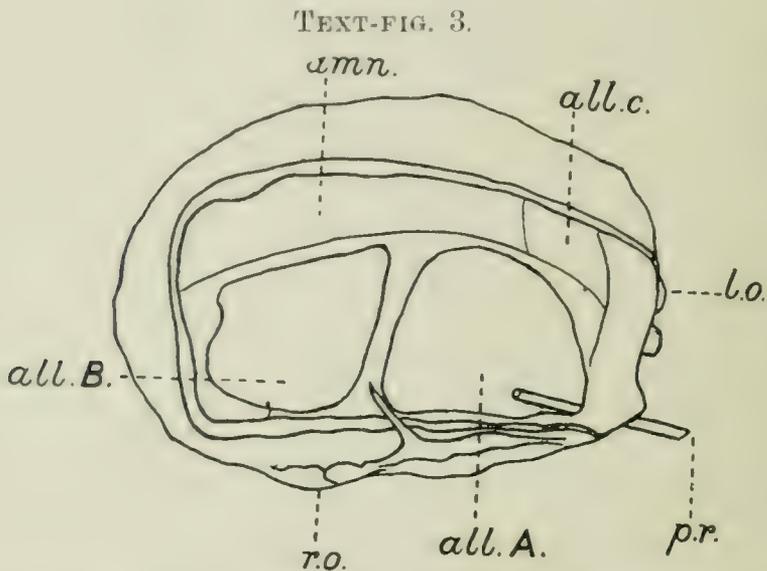


The same seen from the ventral aspect.

left cornu, which is more dilated than the right. That may be so in the present case. Orientation of the excised uterus is uncertain.]

On the ventral side a slight fold of peritoneum runs obliquely from the anterior right to the posterior left end (Text-fig. 2).

The uterus was opened from the ventral aspect by the removal of almost the whole of the wall on this side (Text-fig. 3). With the wall was removed the placenta, and the amnion and allantois were laid bare, the fœtus being indistinctly visible through them.



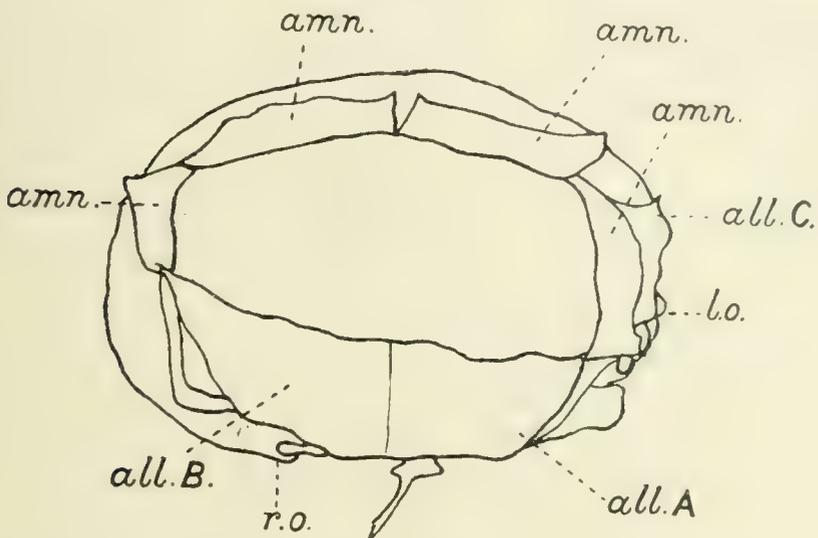
A rectangular piece of the ventral wall has been removed displaying the amnion, *amn.*, and the allantois, *all. A*, *B*, and *C*. *pr.* Probe passed from the allantoic cavity *A* into the cervical extension of this cavity. The outer wall has been removed from cavities *A* and *B*.

The allantois comes up on this, the ventral side only half-way towards the anterior end; at which end only the amnion intervenes between the embryo and the chorion.

Removal of the outer wall of that part of the allantois exposed showed it to be here divided by an antero-posterior septum into two compartments: *A* on the left and *B* on the right. A probe was passed from cavity *A* into the cervical extension. A third division of the allantois (*C*) was laid open in a later stage of the dissection. This lies on the left, and towards the anterior end.

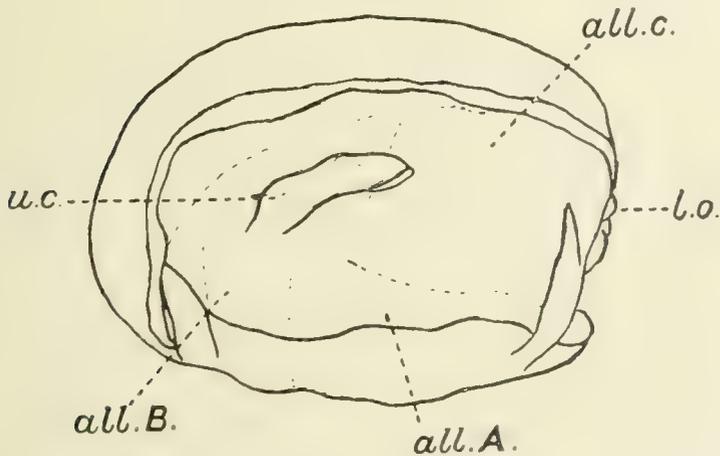
An incision was now made along the extreme margin of the allantois and the latter turned back posteriorly (Text-fig. 4).

TEXT-FIG. 4.



The amnion and allantois have been folded back, exposing the foetus. The outer wall of allantoic cavity C has been folded back.

TEXT-FIG. 5.



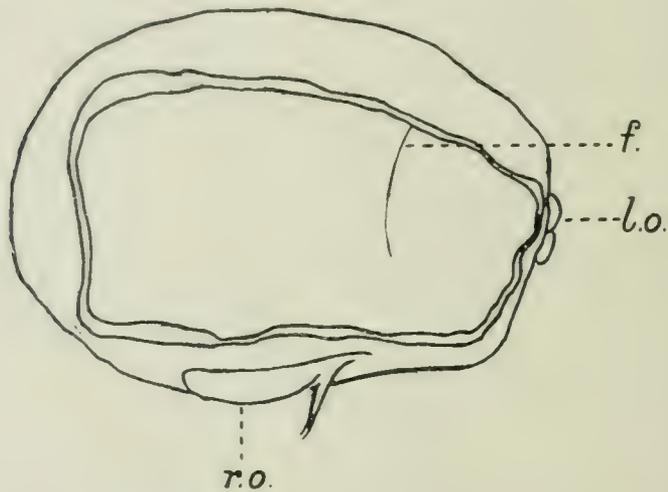
The foetus has been removed, exposing the interior of the amniotic cavity. The dotted lines mark the limits of the allantois and its compartments A, B, and c, which are seen, of course, through the amnion. *u. c.* Umbilical cord.

The third division (C) of the allantois was turned back to the left, the flaps of the amnion to the front and to the right.

The body of the foetus, its head to the left, was now exposed to view and could be easily extracted, and the umbilical cord severed (Text-fig. 5). The extent of the space occupied by the allantois could now be seen. On the dorsal side it reaches almost to the anterior end on the left, but in the other direction reaches neither to the anterior end nor to the right-hand side.

Divisions *A* and *C* of its cavity are separated by a septum which runs obliquely from the left towards the umbilical

TEXT-FIG. 6.



The rest of the amnion, together with the inner wall of the allantois, have been removed. The inner surface of the wall of the uterus is now seen (through the outer wall of the allantois and through the chorion). *f.* Fold marking the division of the cornua from one another.

cord, *C* and *B* by one coming from the anterior margin on the dorsal side to the same point, *A* and *B* by one coming from the anterior margin on the ventral side, and passing posteriorly towards the insertion of the cord.

The relation of these cavities can be seen better when the inner wall of the allantois has been removed by a marginal incision (Pl. 15, figs. 1, 2).

The inner side of the dorsal wall of the uterus is now displayed to view, with only the thin amnion, or outer wall of the allantois, as the case may be, intervening, and the slight

internal fold which separates the more distended from the less distended cornu can be seen (Text-fig. 6, *f*). Examination of this wall by reflected light shows that there are thin patches, especially on either side of the inter-cornual fold. In these thin patches the placenta is ill-developed, as is made

TEXT-FIG. 7.

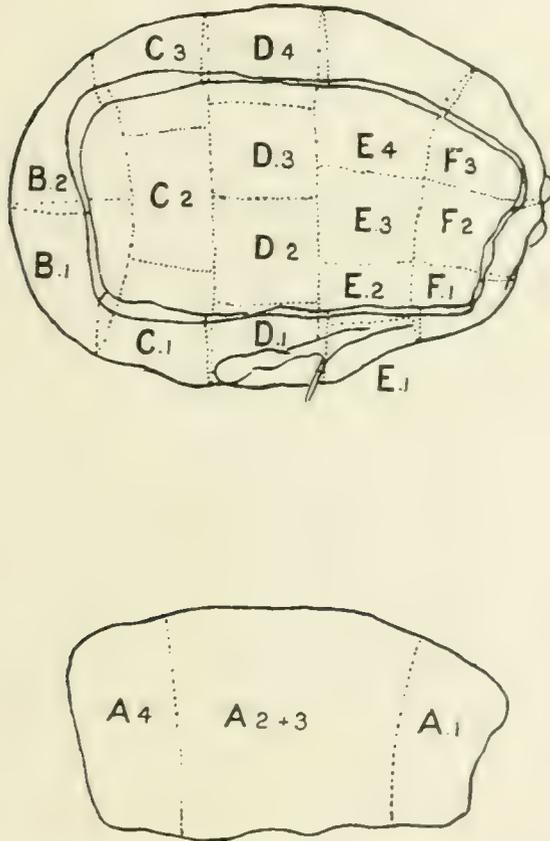


Diagram of the pieces into which the wall of the uterus was cut up.

clear by microscopical examination. For this purpose the whole uterine wall, including the piece excised from the ventral side, was cut up into small pieces, lettered and numbered as in the diagram (Text-fig. 7), and consigned to the microtome.

The sections show a well-developed indeciduate placenta—composed of branching villi dipping into crypts of a corresponding form, and lined by a persistent uterine epithelium—in the thick parts, while in the thinner parts the placenta is

much reduced, or absent, the trophoblast in the latter case not being produced into villi.

The fully-developed placenta is found in all regions except A 1, F 1, and parts of A 2 + 3, A 4, B 1, D 1, E 1, E 2, E 3, E 4, F 1, F 2, and F 3. In A 1, A 2 + 3, D 1, E 1, E 2, E 4, F 1 the villi are small, while in A 4, A 2 + 3, B 1, D 1, E 3, and F 1, F 2, F 3 there are places where the trophoblast is not folded at all.

We may begin with the simpler conditions in the non-placental regions.

The trophoblast is a columnar epithelium of coarsely vacuolated cells (Pl. 15, fig. 3), with frequently a denser region—with thicker walls to the vacuoles—round the nucleus or near the basal end. At this end is a basement membrane, and behind this branched connective-tissue cells. The free ends of the trophoblast cells appear to be amœboid, and protruded into a striated or granular (Pl. 15, figs. 4, 5) coagulum, apparently a secretion of the uterine epithelium. Some of these cells appear to be glandular, at least they contain an internal mass of fine granules, which appears to be poured out into the space between them and the uterus (Pl. 15, fig. 5). The uterine epithelium is composed of shorter columnar cells, with scattered vacuoles in the densely staining cytoplasm, and amongst these elements are goblet gland cells (Pl. 15, fig. 3). Underneath it is a layer of dense fibrous tissue.

In many cases the uterine epithelium may be folded while the trophoblast remains simple (Pl. 17, fig. 15). The folding may be trifling or considerable, and the folded part may be itself folded over an adjacent portion of the wall (Pl. 16, fig. 7).

Between the epithelium and the muscularis are the glands. These are lined by columnar cells, with broad stout necks, through which the granular secretion is ejected (Pl. 16, fig. 8, seen in section in fig. 8, *a*). Round the basal nuclei are some deeply staining granules.

The glands were found to open at the base of depressions.

The trophoblast is here non-villous. In the placental regions the trophoblast is thrown into folds or villi, which are lodged in uterine crypts. In the thinner parts the folds are exceedingly slight and the crypts correspondingly shallow: in the fully developed placenta, villi and crypts branch in the most complicated way, and the placenta becomes very thick (up to 3.5 mm.) (Pl. 17, figs. 18-19).

Every transition is found between the two extremes, and every transition from the columnar trophoblast and columnar uterine epithelium of the non-placental to the flat layers which cover the villi and line the crypts of the placental regions (Pl. 16, figs. 9, A-C, 10). The cytoplasm of the trophoblast still remains vacuolated, and the uterine epithelium dense. Everywhere in the crypts both are found, and closely adherent to one another.

The villi are leaf-like, with irregular branches perpendicular to their surface. In vertical sections a main villus is frequently cut throughout its length (Pl. 17, fig. 19), the flattened leaf form is best seen in tangential section (Pl. 17, fig. 20).

The maternal connective tissue is dense and fibrous, the foetal composed of loose stellate cells often very abundantly branched and of large size (Pl. 16, fig. 10). In addition there are numerous aggregations of smaller vacuolated elements (Pl. 16, fig. 10a).

The foetal blood-vessels are lined by a simple endothelium. In the connective tissue between the amnion and allantois are found large cells, stuffed with globules, and some of them containing pigment; the pigment may be a hæmaglobin derivative. In any case the cells are probably phagocytic (Pl. 16, fig. 12).

The allantoic epithelium is very flat and covered by a cuticular layer (Pl. 16, fig. 13).

The amniotic epithelium is exceeding flat (Pl. 16, fig. 14).

In the umbilical cord are two arteries and two veins, and the stalk of the allantois.

No trace of the yolk-sac was found.

It is evident the placenta and foetal membranes of this species of Lemur conform in structure to those of other members of the Lemuroidea, with the exception of *Tarsius*. In the Lemuroid placenta the relation between foetal and maternal circulations is brought about by the production of vascular trophoblastic villi, which fit into crypts lined by a persistent uterine epithelium. So the placenta therefore resembles that of an Ungulate: it is of the so-called "Indeciduate" type. The allantois is large and occupies a great deal, if not the whole, of the space between the amnion and the chorion.

In *Tarsius*, on the other hand, the uterine epithelium disappears at the point of formation and attachment of the placenta, and the latter consists of a thickened trophoblast excavated by the lacunæ in which the maternal blood circulates, and penetrated on the foetal side by the capillaries of the allantois. Of this type are the placentas of the Anthropoid Primates, of Rodentia, Insectivora, and Cheiroptera. In *Tarsius*, further, the allantois is rudimentary, while at an early stage there are a diminutive yolk-sac and a precociously developed extra-embryonic cœlom. In these respects also *Tarsius* resembles the Anthropoids. But while these resemblances very strongly support the view that *Tarsius*, an aberrant Lemur at best, should be ranked with monkeys and man, it is not, of course, to be concluded that the other Lemurs should be separated from the Primates and grouped with other "Indeciduata."

The Lemuroid placenta seems indeed to have developed features of its own with variations characteristic of each sub-family. Thus, while in the Galaginæ and Lorisinæ the villi are roughly cylindrical, in the Lemurinæ and Indrisinæ they are ridges, ridges which in the former are broken up into short leaves, as in the genus, *Lepidolemur*, described above.

But such general characters as the Lemuroid placenta does possess in common with that of Ungulates, Cetacea, Sirenia, and perhaps some Edentata, may be the inheritance from a common ancestral "indeciduate" type. And in that case we

should probably have to believe that the "deciduate" type observed in monkeys and man has been developed—independently of that of rodents and other orders—from the simpler Lemuroid form.

EXPLANATION OF PLATES 15, 16, AND 17,
Illustrating Mr. J. W. Jenkinson's paper, "The Placenta
of a Lemur."

PLATE 15.

Fig. 1.—The inner wall of the allantois removed, viewed from the amniotic side, except for the reflected portion at the posterior end. *A*, *B*, and *C* the three cavities. *ce*. Cervical extension of cavity *A*.

Fig. 2.—The same seen from the allantoic side.

Fig. 3.—Simple columnar trophoblast, *tr*, and simple columnar uterine epithelium, *ut. ep.*, from a non-placental region. Between the two a striated coagulum, the secretion of the uterus.

Fig. 4.—Examples of highly columnar trophoblast cells with amœboid free ends, from non-placental regions.

Fig. 5.—Columnar trophoblast, *tr.*, and slightly folded uterine epithelium, *ut. ep.*, from a non-placental region. Between the two a granular coagulum. Gland-cells may be seen in the trophoblast.

Fig. 6.—Slightly folded uterine epithelium, from a non-placental region, with its striated coagulated secretion.

PLATE 16.

Fig. 7.—Simple trophoblast, and complexly folded uterine epithelium, from a non-placental region.

Fig. 8.—Gland cells of a uterine gland. *a*. Transverse section of mouth of a gland cell.

Fig. 9.—Transition from the columnar trophoblast and uterine epithelium of the non-placental regions. *A*. To the flattened layers of the placental region. *B*, *C*. Intermediate condition.

Fig. 10.—Trophoblast and uterine epithelium in the completely placental region. *f. c. t.* Branched foetal connective-tissue cells. *f. b. v.* Foetal capillaries. *m. b. v.* Maternal capillaries. *tr.* Trophoblast. *ut. ep.* Uterine epithelium.

Fig. 10a.—Small vacuolated fetal connective-tissue cells.

Fig. 11.—Small fetal blood-vessel, with granular and fibrous connective-tissue cells.

Fig. 12.—Large fetal connective-tissue cells, filled with globules, and in two cases pigment granules.

Fig. 13.—Allantoic epithelium, with its cuticle. Below, connective-tissue cells.

Fig. 14.—Amniotic epithelium, with underlying fibrous tissue.

PLATE 17.

Fig. 15.—Thin portion of the uterine wall, where there is no placenta, the trophoblast being simple, the uterine epithelium only slightly folded. *a.* Allantoic epithelium. *tr.* Trophoblast. *ut. ep.* Uterine epithelium. *gl.* glands. In this and the following figures the trophoblast is stippled, the uterine epithelium black.

Fig. 16.—Thin part of the uterine wall where the trophoblast is slightly folded. Both it and the uterine epithelium are very thin.

Fig. 17.—The uterine wall is slightly thicker, the folds of trophoblast and uterus better developed.

Fig. 18.—The edge of a placental region; the folds of the trophoblast and of the uterus are much better developed. Letters as in fig. 15.

Fig. 19.—Section through the thickest part of the placenta. *a.* Allantoic epithelium. *f. c. t.* Fœtal connective-tissue in the villi. *m. c. t.* Maternal connective-tissue in the walls of the crypts. *gl.* Glands. *m.* Muscularis.

Fig. 20.—Tangential section across one of the leaf-like villi, showing the lateral leaf-like branches.

On a New Species of Pentastomid from a N.
African Snake (*Zamenis ravigieri*).

By

Mary L. Hett, B.Sc.,

Demonstrator of Zoology, Bedford College, University of London.

With 5 Text-figs.

THE specimens of Pentastomid described in this paper were given to me in the spring of 1913 by Mrs. Pixell Goodrich, who obtained them from the body cavity of *Zamenis ravigieri* while studying the hæmogregarine of that snake in the Sahara.

My thanks are due to Mrs. Goodrich for placing the material at my disposal and I should also like to take this opportunity of acknowledging my indebtedness to Dr. Marett Tims for his kind advice and encouragement.

There were two specimens, one ♂ and one ♀, the former being about 10 mm. and the latter about 40 mm. in length. I showed the ♂ to Dr. Sambon who believed it to be identical with a new species he had recently described¹ from *Bitis gabonica* and *B. arietans* under the name of *Porocephalus boulengeri*, and for which he proposed to create a new genus *Raillietia*, characterised as follows: "A bifid posterior extremity, three vesicular prominences round each hook-pit, the female sexual opening at the anterior end of the body."

On investigation of the internal anatomy of the African specimens, I found considerable differences of arrangement as compared with species hitherto described, the variation

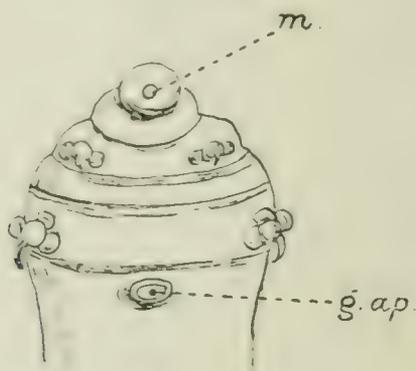
¹ 'Trans. Trop. Med.,' vol. iii, 1910, p. 40.

being especially noticeable in the reproductive organs. These differences of internal structure, combined with the external features quoted above, appear quite sufficient to justify the establishment of the new genus.

EXTERNAL FEATURES.

The ♂ is 10 mm. the ♀ 40 mm. in length. In both cases the posterior extremity is bifurcated and the anterior end is almost triangular in shape. The cephalothorax is distin-

TEXT-FIG. 1.



Cephalothorax. *m.* Mouth. *g.ap.* Genital aperture.

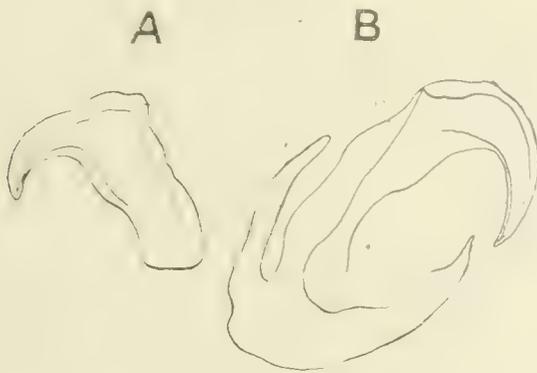
guished by the prominences which surround the hooks and mouth. The latter lies at the base of a circular depression on the ventral side of a large protruberance situated at the extreme anterior end of the cephalothorax (Text-fig. 1).

The hooks are simple and sharply curved, one pair lying in front of the other, and rather nearer to the middle line. The anterior hooks measure 26 mm. in length, the posterior 32 mm. (Text-fig. 2). Each hook is surrounded by three protuberances, one anterior and two lateral, those of the posterior pair of hooks being much more conspicuous than those of the anterior pair.

The annulation is difficult to determine. In the ♀ specimen the rings are practically obliterated; in the ♂ there appear to be about fifty body rings. In Dr. Sambon's species the annulation was indistinct, but he believed the rings to

number twenty-five to thirty.¹ In addition to the individuals described in this paper, I have had the opportunity of examining fourteen others (12 ♂, 2 ♀), obtained from African snakes which have died in the Zoological Society's Gardens, and apparently belonging to the same species. The rings were in nearly all cases distinct, and in the females numbered from thirty to forty. One of the males had thirty, the other thirty-five. It is quite possible that the number of rings for the male, given above as fifty, may be an error, since the body contracts in such a way as to make each ring appear

TEXT-FIG. 2.



Hooks. A. Anterior. B. Posterior.

double, but the mistake cannot now be rectified, as the specimen has been sectioned. Thus the number seems to vary between thirty and forty, in most cases being less than forty. These figures refer to body rings only, that is to say, rings behind the cephalothorax, which, however, is not clearly marked off in this species. I have regarded it as extending to and including the posterior pair of hooks. There are in addition three or four rings on the cephalothorax, well-marked, and nearly as wide as those on the body.

The anus opens between the two caudal processes.

The genital aperture is anterior both in the ♂ and ♀, and lies just behind the cephalothorax in the mid-ventral line, opening in the centre of a rounded papilla (Text-fig. 1).

¹ *Loc. cit.*, p. 132.

In both specimens the integument was semi-transparent, so that the internal organs could be seen through it in places. A dark streak was observable in both, extending almost from end to end. This proved to be the contents of the intestine showing through the thin wall.

INTERNAL ANATOMY.

Alimentary Canal.

The mouth opens on the papilla before mentioned. Here the epithelium is considerably thickened and contains a number of gland-cells. The mouth-cavity is fairly large and lined with a deeply staining cuticle continuous with that of the external body layer. A mass of tissue projects into it from the roof, and is pierced by the pharynx. This leads upwards and backwards from the mouth-cavity, and is lined with similar cuticle, thin at first, but soon becoming thicker, especially on the posterior wall, which bulges out into the pharynx, making the lumen a narrow crescent-shape in cross-section, the horns pointing ventrally. Posteriorly the cuticle thins again. In the female a single layer of columnar epithelium can be seen beneath the cuticle, except just below the bulge of the posterior wall, where it is stratified, forming a thick mass. There is also a little mass of stratified epithelium in the anterior wall just opposite.

The pharynx becomes almost triangular in section at its posterior end, and then passes into the œsophagus.

The œsophagus is a dorso-ventrally compressed tube lined by a single layer of columnar epithelium. The cuticular layer is continued throughout, but is thinner and not so deeply staining. About halfway the œsophagus gives off a narrow dorsal diverticulum, and then becomes almost circular in outline. Finally it meets the antero-ventral wall of the intestine, into which it opens through an inwardly-projecting papilla.

The intestine or mid-gut extends from a point shortly in front of the posterior end of the œsophagus to the caudal end of the body. It is a relatively wide tube, which gradually

decreases in size posteriorly, and terminates in a very narrow rectum leading to the anus. The gut wall is composed of muscle layers, within which is a single layer of epithelium. This is not folded to any extent, as described in other species, but many of the cells are considerably elongated and produced into finger-like processes which project into the gut lumen. This is particularly noticeable in the female on the ventral side, and in both specimens the epithelium is much thickened in this region, especially in the posterior half of the body. The gut epithelium is characterised by its extremely glandular nature, especially in the thickened portions. The cells are crowded with a glandular secretion, which is extruded in little spherical masses to the gut lumen, where they are found in large numbers. In the male there are apparently a pair of lateral diverticula given off in the mid-region of the intestine, but this may possibly be due to shrinkage, as all the walls are thin and delicate and consequently liable to collapse.

The gut contained a large quantity of blood from the host in various stages of digestion, while a considerable number of hæmogregarines and of the granules mentioned above were found distributed through the mass of blood débris.

Secretory System.

Stigmata are present, but not very numerous.

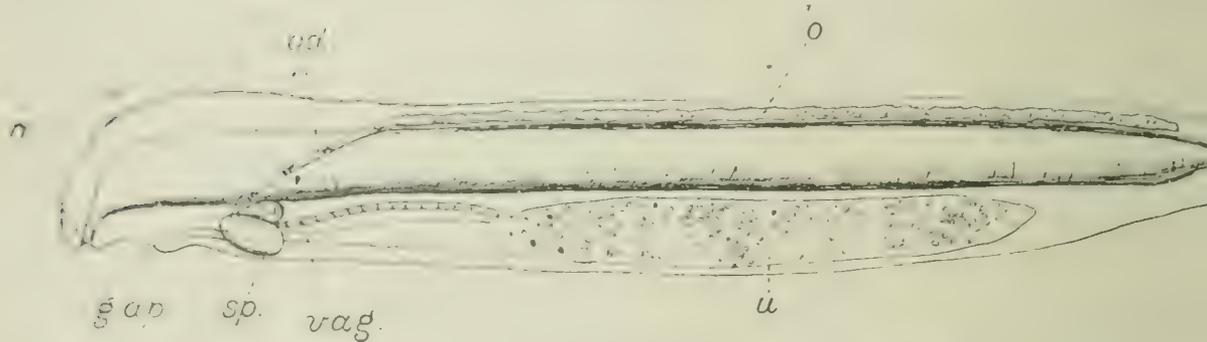
The head, hook, and parietal glands differ in no important respect from those of other species. The head and hook glands are not very clearly marked off from one another, and the latter are comparatively small, only stretching back for about a quarter of the length of the body. Parietal glands are present, and there is a certain amount of glandular tissue round the dilator-rod sac in the male and round the spermathecæ in the female. In addition to these there is in both sexes a glandular mass with very large cells lying dorsal to and partly enclosing the pharynx. It appears to be part of the head gland, and disappears where the pharynx passes into the œsophagus.

Nervous System.

The nervous system is typical. There is a large bilobed mass lying below and partly enclosing the œsophagus.

Near its anterior end it gives off a commissure which encircles the latter organ. The mass is obviously paired, and divides posteriorly into two separate halves for a short distance. The two halves then gradually approach again in the middle line and unite. Beyond this point they again diverge, and are continued backwards as two fairly stout nerve strands, rather wide apart and extending nearly to the

TEXT-FIG. 3.



♀ reproductive organs. *g.ap.* Genital aperture. *m.* Mouth. *o.* Ovary.
od. Oviduct. *u.* Uterus. *vag.* Vagina.

posterior end of the body. A series of paired nerves are given off from the sub-œsophageal mass to the muscles and papillæ.

Reproductive Organs.

Female.—The female reproductive system differs in several respects from any species of *Porocephalus* hitherto described.

The ovary is quite typical, and extends through the greater part of the body, lying dorsal to the intestine (Text-fig. 3). It is visible through the body wall as a narrow brown band, the colour being due to the outer egg-envelopes, which are here thick and of a deep reddish-brown. In section the ovary shows a structure much like that figured by

Spencer,¹ viz., a central tube, on the sides of which the ova are developed, forming two projecting lateral masses. The wall of the ovary is, however, unilaminar throughout except just at the base of the mesentery, and there is no trace of the dorsal syncytium or "lateral crests" described by Spencer. The cells of the ventral wall are somewhat columnar, and those of the dorsal wall are rounded and not clearly marked off from one another. The mesentery which connects the ovary with the dorsal body wall is median and unpaired. It is very narrow, and composed of fibres and connective tissue cells, with a small aggregation of the latter just at the point where it leaves the wall of the ovary.

The eggs in the ovary are relatively large and occur in various stages of development. Anteriorly the ovary passes into a rather wide oviduct with a similar histological structure. It is unpaired and found on the left side only, leading forwards and downwards round the intestine. I was unable to detect any traces of a right oviduct, either in the sections or in the dissection of another mature female specimen.

The duct is distended at intervals by batches of eggs making their way from the ovary to the uterus, and single ova may be seen at intervals in the lumen of the ovary itself. At its lower end the oviduct turns backward in the mid-ventral line and becomes much enlarged. The wall is here composed of columnar cells with a thin internal cuticle and an outer layer of circular muscles. The duct narrows somewhat and is joined by a pair of ducts from the spermathecæ, one on each side.

The spermathecæ are paired egg-shaped structures extending backwards on either side of the ventral median line. A short and comparatively wide duct leads from the anterior end of each spermatheca to the oviduct. The whole structure is like that described both by Leuckart² and Spencer (*loc. cit.*), except that the papilla through which the duct opens into the spermatheca is almost entirely in-

¹ 'Quart. Journ. Micr. Sci.,' vol. 34, 1892, p. 1.

² 'Bau u. Entwicklungsgeschichte der Pentastomen,' Leipzig, 1860.

vaginated into the spermathecal lumen. Also the histological structure of the spermathecal wall is somewhat different. There is no columnar epithelium to be seen at any point, but there is an internal layer of rather flattened epithelial cells with an outer muscular sheath. Apparently there is no cuticular lining. The epithelium appears slightly glandular in places especially on the outer wall, and, as already mentioned, there is a definite glandular mass apposed to the dorsal wall of each spermatheca. Both the spermathecæ were full of ripe spermatozoa. Accessory glands are present, and as in *P. tannioides*¹ they appear as forwardly pointing diverticula from the anterior walls of the spermathecal ducts. These latter and the accessory glands have a similar histological structure to that of the median oviduct; and they are all embedded in a glandular connective tissue, which is continued into the spermathecal papillæ. Posterior to the union of the spermathecal ducts the oviduct passes into the uterus.

The uterus (Text-fig. 3) runs backwards below the intestine and consists of three parts:

(1) A relatively narrow tube, just large enough to allow for the passage of one egg at a time. The eggs can be seen passing down it singly and separated by regular intervals. This duct in no way differs in structure from the median part of the oviduct. It soon passes ventrally into the uterus proper, the opening being marked by a ring of long columnar cells.

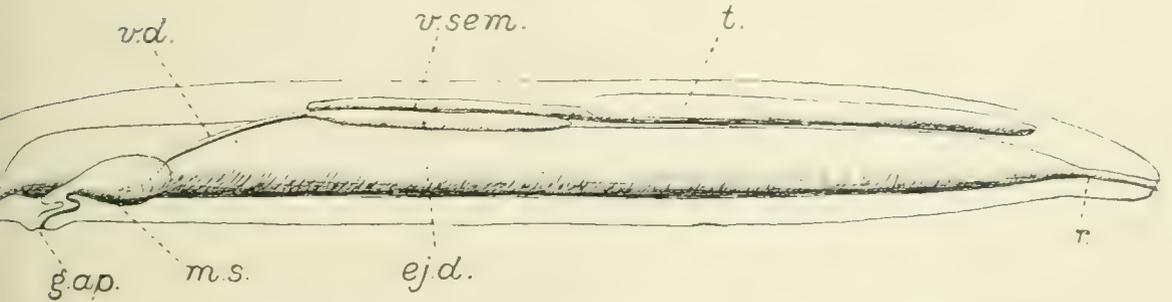
(2) The main portion of the uterus is extensive, being large and thin-walled, but not coiled. It occupies nearly half the body cavity, in some places pushing the gut to one side. The wall consists of a layer of very flattened cells with a thin cuticular lining. Outside this there appears to be a layer of circular muscle-fibres, but it is difficult to determine, as the whole wall is so attenuated. The uterus extends backwards and forwards from the union with the narrow tube, stretching from the spermathecæ nearly to the anus, and is crowded with

¹ Loc. cit.

embryos in different stages of development. Anteriorly it narrows to form the vagina.

(3) The vagina possesses a very thick cuticular lining, the innermost layer of which is deeply staining and produced into small processes. No radial striation is visible in the cuticle. The cells of the wall are columnar, with irregular outlines, and they are surrounded externally by a thick sheath of circular muscles. The cuticle does not extend right to the opening, but disappears shortly before this point. The vagina runs forward below the spermathecae in the middle line, widening again slightly and finally opening on the

TEXT-FIG. 4.



♂ reproductive organs. *ej. d.* Ejaculatory duct. *g. ap.* Genital aperture. *m.* Mouth. *m. s.* Muscular sac. *t.* Testis. *v. d.* Vas deferens. *v. sem.* Vesicula seminalis.

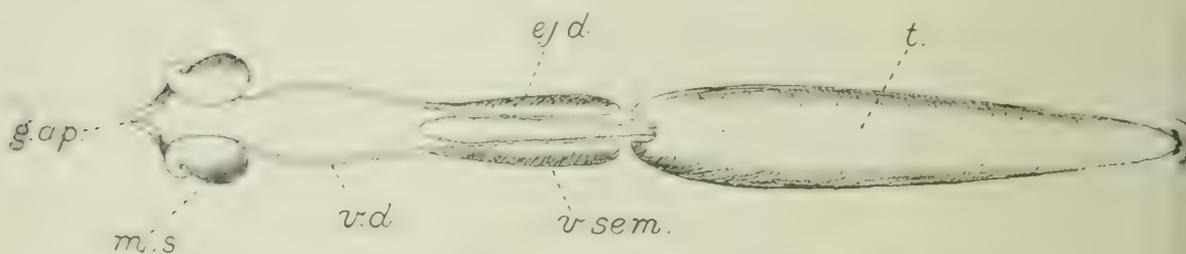
papilla mentioned above, just behind the posterior pair of hooks.

Male.—The male reproductive system is relatively simpler than in other species. It consists of two parts apparently unconnected. One comprises the testis and vesicula seminalis, the other the vasa deferentia, ejaculatory ducts, cirrus, cirrus bulb, dilator-rod sac, and common duct (Text-fig. 4).

The testis occupies the same relative position as the ovary in the female, and like it extends through the greater part of the body. It is a median, unpaired, tubular structure of considerable size, at its widest point occupying about a third of the body cavity. The thin outer wall is clearly distinguishable, but the inner syncytial layer has broken up into rounded masses. The whole testis is filled with spermatozoa

in all stages of development. Anteriorly it is produced into two forwardly-pointing lobes, between which runs a median structure, apparently the vesicula seminalis (Text-fig. 5). If such be the case, this organ is remarkable in being unpaired. The anterior end terminates blindly, and I could trace no communication between it and the vasa deferentia. It passes backwards for a short distance as a relatively wide tube, the structure of whose wall is like that figured by Spencer, viz., an inner layer of columnar epithelium with gland cells and an outer sheath of circular muscles, the whole being enclosed in a thin layer of connective tissue. The

TEXT-FIG. 5.



♂ reproductive organs seen from above. Lettering as in Text-fig. 4. The words *v. sem.* refer to the median structure.

diameter is nearly uniform until towards the posterior end, where it narrows considerably, and a little mass of connective tissue containing fibres begins to project inwards on the ventral side till it occupies half the cavity. In this projection a duct soon appears, whose wall consists of a single layer of columnar epithelium. As it passes backwards the dorsal half of the original lumen becomes filled with very glandular tissue, indistinguishable from that forming the inner wall of the testis, so that in this way the original cavity of the vesicula seminalis is altogether occluded. The whole structure becomes further reduced in size till only the ventral duct is recognisable, and this is gradually merged in the substance of the testis. From the position and structure of this median tube there seems little doubt that it represents the vesicula seminalis, though there were no sperms to be found at any

point in its course. There was a little coagulum observable in one or two places.

The vasa deferentia are paired. Each vas deferens is a narrow duct, terminating posteriorly on a level with the anterior end of the vesicula seminalis, dorsal to the intestine. No communication can be traced with the vesicula seminalis, but each vas deferens gives off posteriorly an ejaculatory duct which thus lies dorsal to the gut and lateral to the vesicula seminalis, with which it is practically co-extensive (Text-figs. 4 and 5). The vasa deferentia appear to be continued for a short distance into the ejaculatory ducts as narrow tubes lined with chitin, opening on a papilla into the lumen of the latter organs. The walls of the ejaculatory ducts consist of an inner layer of columnar cells with nuclei at their outer ends. These cells rest on a thin basement membrane, external to which is a layer of thick radial muscles. These are covered by a layer of connective tissue, the whole being enclosed in an outer sheath of very thin flattened cells. No cuticular lining was apparent.

From the junction with the ejaculatory ducts the vasa deferentia run forwards and downwards, encircling the intestine exactly in the same way as the oviduct of the ♀. At the lower end each becomes swollen to form a muscular bulb = the cirrus bulb which opens on the ventral side of a large muscular sac. In its passage down the side of the intestine the vas deferens runs on the inner side of this sac, lying between it and the intestine. The structure of the vasa deferentia is simple, the wall being composed of a single layer of columnar epithelium with large nuclei and a thin cuticular lining.

The muscular sac is a large structure with very thick walls and an internal chitinous lining. Anteriorly the wall is composed of enormously thick columnar epithelium, enclosing a fairly wide lumen, through which runs a small chitinous tube, apparently the cirrus. Farther back the lumen of this muscular sac becomes filled with muscular tissue, which gradually comes to occupy the whole cavity,

and the epithelial walls become indistinguishable. At this point the sac divides into two portions, the smaller ventral part forming the cirrus bulb, the larger dorsal part constituting the muscular sac proper. It is a little difficult to harmonise the conditions in this specimen with those found in other members of the group, but there seems little doubt that the muscular sac represents the dilator rod sac of other authors. Here, as in other cases, the walls, unlike those of the cirrus sac, are very thick and muscular, and there is a narrow semi-annular cavity lined with very deeply staining cuticle. From this there appears to project a small dilator rod connected with the cirrus. Further, the sac lies in the same position relative to the cirrus bulb and to the intestine as does the dilator rod sac. Anteriorly each sac is continuous with an S-shaped duct, whose course lies in the sagittal plane. Each duct passes forwards for a short distance, then bends back on itself ventrally, and finally takes another turn forward in the same plane. This backwardly turning loop seems to represent a rudimentary cirrus sac, and the cirrus cannot be traced forwards beyond this point. Anteriorly each duct bends inwards, and unites with its fellow of the opposite side in the ventral middle line to form a common genital duct opening by the genital aperture. The common duct is lined by a very thick, slightly staining cuticle.

The question arises as to whether the condition of the male reproductive organs is due to immaturity or whether the simplification is a distinguishing feature of the species or genus.

In favour of the view that it is immature we have the facts:

(a) That no sperms are to be seen in the vasa deferentia or vesicula seminales;

(b) that no communication could be traced between the vesicula seminales and vasa deferentia.

A similar condition to this last was described by Hoyle¹ for *P. protelis*. Stiles² suggests that the inability to

¹ 'Trans. R. Soc. Edin.,' xxxii, 1883, p. 183.

² 'Zeitschr. f. wiss. Zool.,' 52, 1891, p. 138.

find the communication in his specimen was due to the bad preservation of the material, as the duct is very small. On the other hand, *P. protelis* is an immature form, and Spencer¹ attributes the condition observed to this fact. As the two parts of the male reproductive system apparently arise independently the communication must be established later, and he suggests that at this period of development such communication has not yet taken place. In the specimen under discussion the condition might be due to either of these causes. The walls of the two ducts—viz., the vas deferens and the vesicula seminales—lie in close apposition at one point, and it is possible that a section may have been lost with the communicating passage, though it looks more as if the two walls were just in act of fusion and had not yet broken down.

Still even if not actually mature, the specimen cannot be far from maturity since some of the sperms in the testis are fully developed. Moreover, Leuckart states² that the first signs of spermatogenesis are coincident with the formation of the cirrus, and in the fully-formed larva of *P. proboscideum* Stiles³ describes the cirrus sac as already present when apparently spermatogenesis is only in its early stages. In our specimen spermatogenesis is far advanced and yet the cirrus sac is in a very rudimentary condition.

Hence the evidence seems to point to the conclusion that the specimen has almost reached sexual maturity, and the simplification of its parts must be regarded as a primitive feature. This simplification involves the straight or only slightly coiled cirrus, the slight development of the cirrus sac and the dilator rod, and the direct course of the vasa differentia comparable to that of the oviducts in the female. The median unpaired vesicula seminales may also be a primitive feature, but opinion on that point will differ according as to

¹ 'Quart. Journ. Micr. Sci.,' vol. 34, 1892, p. 1.

² 'Bau u. Entwicklungsgeschichte der Pentastomen.' Leipzig, 1860, p. 143.

³ 'Zeitschr. f. wiss. Zool., 52, 1891, pp. 131-135.

whether we regard the testis as paired or unpaired in origin.

When we consider the arrangement of the female reproductive organs the evidence in favour of a primitive condition is strengthened. It had already been noted by Leuckart that the reproductive organs in the two sexes are built on a fundamentally similar plan, that in the early stages of development they are indistinguishable and that it is only later that the peculiar features of the two systems can be recognised. In this case the adult male and female organs correspond more closely than usual. The paired vasa deferentia probably represent a more primitive condition than the single oviduct, but, however that may be, the oviduct is in a relatively similar position to the corresponding vas deferens, the latter encircling the intestine and passing from the dorsal to the ventral side instead of being entirely ventral as in other cases. Here it may be noted that Leuckart¹ describes a similar condition in the earliest stages of the development of the ♀ *Pent. taenioides*, the vasa deferentia being originally laid down in this position. It is not quite clear how they come eventually to lie entirely ventral to the intestine, but apparently they are carried downwards by the growth of the vesiculæ seminales with which they are bound up, although not in actual communication with them.

Another point of resemblance between the two sexes is the position of the genital opening. In other species the adult ♀ genital aperture is posterior, quite close to the anus, while in the ♂ it is anterior, lying close behind the last pair of hooks. In this specimen both the ♂ and ♀ genital apertures are anterior, which, according to Leuckart, is the original position, the two sexes being indistinguishable at first, when a pair of simple ducts (representing the future vasa deferentia or oviducts) pass down on either side of the intestine and open immediately below on a ventral papilla. This position is retained in the male, but in the female the aperture is carried

¹ 'Bau u. Entwicklungsgeschichte der Pentastomen,' Leipzig, 1860, p. 134.

back to the posterior end. Leuckart suggests that this is the result of a difference of growth of the body-wall in the two sexes, the male increasing in length entirely by growth at a point behind the genital aperture, the ♀ by growth entirely in front of it. In commenting on this, Stiles¹ remarks that if this is the case in *Pent. proboscideum* the change must have begun at a very early stage, as the difference of position of the apertures is obvious in the fourth week. But this would be in accordance with Leuckart's observations, since the shifting of the genital aperture would keep pace with the backward growth of the uterus, which begins very early. It would, therefore, seem most reasonable to accept Leuckart's suggestion and to consider the posterior position of the ♀ genital aperture as due to unequal growth of the body-wall, possibly brought about, in the first instance, by the backward growth of the uterus.

The arrangement in our specimen looks like a more primitive one, as the uterus has only grown back a short distance, forming a V-shaped tube, and the rest of the posterior extension consists of a very long diverticulum from the posterior wall. The uterus is here very thin walled, and instead of being a long, coiled structure it provides the necessary space by great development of the diameter of the diverticulum, which in some parts occupies half the body cavity, pushing the intestine to one side.

Thus, to sum up, apart from histological differences, this species differs from a true *Porocephalus* in the following particulars :

- (1) The bifid caudal extremity.
- (2) Anterior mouth opening.
- (3) Vesicular protuberances round the hooks.
- (4) A relatively lower degree of development in the ♂ genital system.
- (5) Unpaired vesicula seminales lying dorsal to the gut in the ♂.

¹ 'Zeitsch. f. wiss. Zool.,' 52, 1891, p. 141.

(6) Correlated dorsal extension of the vasa deferentia and dorsal position of the ejaculatory ducts.

(7) Uncoiled uterus in the ♀.

(8) Anterior position of the ♀ genital aperture with correlated differences of arrangement in the uterus and vagina.

Naturally the distinguishing features of the internal anatomy cannot be established as diagnostic characters without investigation of the other species referred to this genus, but there is little doubt that the general arrangement will be found to be the same, especially as in the ♀ it is partly dependent on the anterior position of the genital aperture, which is a constant feature for the genus.

I have no further remarks to add to those of Dr. Sambon on the specific value of *P. boulengeri*, but I should like to note that I have examined two other apparently undescribed forms of this genus—one from India, the other from S. Europe—of which I hope to give an account later. They both differ from *P. boulengeri* in quite small, but perfectly constant and recognisable details.

NOTE.—Since writing the above I have described the two new forms there mentioned in the 'Proceedings of the Zoological Society' for March, 1915.

Review.

TEXT-BOOK OF EMBRYOLOGY: Vol. I, Invertebrata. By E. W. MACBRIDE, M.A., D.Sc., LL.D., F.R.S., Professor of Zoology at the Imperial College of Science and Technology, South Kensington. (Macmillan & Co., London.)

PROF. MACBRIDE is to be congratulated on having successfully completed a very difficult and laborious task. This volume, which is to be followed by one on the Embryology of the Lower Vertebrata by Prof. Graham Kerr of Glasgow and another on the Embryology of Mammals by Mr. Richard Assheton, gives a comprehensive account of the embryology of the various invertebrate classes. We here find careful and fully illustrated accounts of the most recent and trustworthy descriptions of the growth from the egg of a large series of invertebrate animals. The method adopted by the author is, so far as possible, to select one type in each class, the embryology of which has been fully worked out, and to give full details and ample illustrations taken from the latest sources of information. Then additional diverging histories are given, and, at the close of the larger chapters, the general bearing of the embryological facts upon the ancestral history, and the interpretation of the structural peculiarities of the assemblage of groups dealt with in it, is discussed. The remarkable results of the modern study of cell-lineage in such groups as the Leeches, the Mollusca, the Platyhelminthes and the Annelida are fully set forth and illustrated by first-rate diagrams. Frequent reference is made to the results of experiments on embryonic forms and the artificial interference with their normal growth, and clear accounts of such work are given.

Such a book disarms criticism. It is a fine and successful effort to place the student and investigator of embryology in

possession of the most important facts of his study. At the same time by citation of the titles of original memoirs the reader is enabled to yet further amplify his information.

Modern embryology grew up in the last third of the nineteenth century under the influence of Darwin's theory and the doctrine of "recapitulation" as a new and exciting branch of inquiry. Previously confined to the area of the higher Vertebrata, it was then extended to the whole animal kingdom and by parallel enterprise to the vegetable kingdom also.

Enthusiasts are apt to overlook the fact that such special departments of research do not really form distinct branches of science. Embryology is only the study of morphology, more or less arbitrarily limited to the earlier stages of growth. Just as the description of organic form should be called "morphography" and the word "morphology" applied only to the attempt to account for the facts recorded, so the detailed description of embryonic growth is rather "embryography" than "embryology." In fact, there can be no embryology of animals apart from the "morphology" of animals—of which it is an important and inseparable part. It would, no doubt, be possible to write a treatise considering the facts of growth from the egg from the point of view of morphology. Professor MacBride's treatise aims rather at being a store-house of embryographical fact. Nevertheless he introduces from time to time appropriate morphological disquisitions. We do not complain that these are not longer and more complete, for his main task has been to render accessible to the student a vast and complicated body of embryographical record. We must not expect to find here extended discussions of the morphological significance of the various kinds of renal tubes, of the coelom and the blood-vascular system, or of the persistence of the blastopore here as anus and there as mouth. Such questions are briefly touched on, but cannot be given the leading place in a treatise planned as this is to place before the reader as much detail as possible of observed fact in all groups of invertebrate animals.

It perhaps follows naturally from the fact that Prof.

MacBride has himself made very important original studies on the development of the Echinoderms, that his chapter on that group is the most original and interesting in the book, though all maintain a high quality of thoroughness and lucidity.

Some of us would perhaps have liked to see something more of a historical method pursued in the exposition of the origin of terms and ideas which are now the commonplaces of embryology. Naturally the older author who first saw and described some important fact is passed over without citation because a worker of a later generation has gone over the same ground and published figures more suitable for reproduction and has, as well, added facts to and corrected errors in the older work. Prof. MacBride is as generous as his space allows him to be to those who set the embryological top spinning. He is necessarily concerned with its present buzz and excursions. Nevertheless a valuable kind of teaching is that which traces out the history of ideas and terms which have become incorporated in a branch of science and are apt to be taken as a matter of course. Kowalewsky, whose discovery of the kinship of Ascidiæ and Vertebrates was reported on forty-five years ago in Vol. X of this JOURNAL by Michael Foster, was the founder of modern comparative embryology. Foster had Balfour for his pupil, Balfour taught Sedgwick, and Sedgwick taught MacBride, as his heart-felt dedication of this volume tells. A study of the growth of embryology as associated with these and other names, would be a more valuable training in scientific method for a young student than a knowledge of the innumerable details which the record of embryography comprises. But that does not diminish the value of Prof. MacBride's fine book, nor lessen our indebtedness to him for having produced it.

The other possible treatise, which we should like to see, would correct the erroneous notion, prevalent in this country, that the important advances in embryology, of the last fifty years, have been due to the industrious people (now at war with us) who have worked up details and published the latest

illustrations embalmed in text-books. It is the citation of figures and details from German sources unaccompanied by a careful history of essential ideas and fundamental observations made at an earlier date by English, Russian, and French investigators which does injustice to the latter and makes a new and authoritative history of embryology still necessary.

The Gregarines of *Glycera siphonostoma*.

By

Helen L. M. Pixell-Goodrich, D.Sc.,
Beit Memorial Research Fellow.

With Plate 18.

THE large Polychaet, *Glycera siphonostoma* D. Ch. (sometimes placed in a separate genus *Rhynchobolus*), is at times infested with numerous gregarines, both intestinal and coelomic. On this account it is somewhat difficult to differentiate the stages in the life-history of anyone of them. Out of fifty-two specimens examined at Naples in March and April, 1914, twelve (I to IX, XII, XIV, and XXVI) were infected with a species of *Gonospora* which presented some interesting points. The affinities of this form with previously established species will be discussed later. It does not agree in detail with the published account of any, notwithstanding the fact that Léger (11) has already recorded *Gonospora sparsa* as occurring in some undetermined species of *Glycera* at Belle Isle.

A specimen of *Gl. siphonostoma* infected with *Gonospora* can generally be detected in the living, for, through its body-wall, both the large attached trophozoites and free cysts can be distinguished. The latter move backwards and forwards suspended in the coelomic fluid, as the host expands and contracts. The large trophozoites are attached to the thick muscular pharynx from the region of the jaws to the intestine (Pl. 18, fig. 1). The numbers and size of the individual parasites vary very much in different specimens. Sometimes only one or two occur, but in the case illustrated (VI) they

were numerous towards the posterior half of the pharynx. In another (IX) a fringe of trophozoites was found just in front of the jaws. The length of the single trophozoites varies from about 1 mm. to 4 or 5 mm. in length. Narrow at the attached end, they gradually widen out, and then taper to a blunt point. The smallest specimen shown in Pl. 18, fig. 1, is at *c*. The small projections at *f* were thought to be possibly young forms, but on cutting sections of this part they were discovered to be only the remains of the attached ends of associates which had become free. The nucleus is spherical and near the widest part of each individual; it contains generally four or five caryosomes (Plate 18, fig. 4). Each of the parasites is covered with a layer of the host's coelomic epithelium, thicker in some parts than in others. It seems clear, as will be further explained later, that the parasites of a certain length, having reached the coelom, attach themselves to the pharynx. Thereupon they penetrate a little into the host's tissue, and the peritoneum, greatly increasing in the neighbourhood, rapidly grows round the parasite, forming a layer in contact with, but not attached to, the exterior cuticle of the gregarine. This covering of host's cells does not keep pace with the growth of the trophozoite, which consequently has to become bent on itself to some extent, especially at its narrow attached end (Plate 18, fig. 4).

The trophozoites evidently revolve to a certain extent about their points of attachment, and in this way the free extremities of two forms may come together as at *a* and *b* (Pl. 18, fig. 1), when association proceeds to take place. The layer of host tissue is eliminated from between the contiguous extremities, and the end of one associate projects into the end of the other, which consequently becomes cup-shaped, and thus union of the two associates is made secure (Pl. 18, figs. 2 and 3). This dove-tail arrangement reminds one very forcibly of the cup and ball structure described by Huxley (10) in *Ganymedes*, and I would suggest that in this gregarine also the distinctive ends, which the author states he could not always find, were probably only temporary forms taken on by the parasites at

the beginning of association. That is at any rate the case here. Before association all the parasites have regularly tapering ends. A somewhat similar mode of association of two or several individuals was described by Caullery and Mesnil (3) in *Gonospora longissima*, where they state that the extremity of one associate sometimes forces itself into the other, invaginating it "en doigt de gant." They compare this with the similar phenomenon in the *Didymophyes* of Stein. In this polycystid gregarine, however, it is the satellite which attaches itself to the primate of a syzygy in this way (17, Taf. IX, fig. 40), and therefore necessarily the attachment is by opposite ends. In the *Gonospora* from *Gl. siphonostoma* it is union of similar ends that is affected in this secure way. Following this association the pair continue to rotate, and since the proximal ends are still attached to the pharynx they become much convoluted and shortened (Pl. 18, figs. 2 and 3). It would have been interesting to see what would have happened during shortening of the attached ends in the case of the associates in Pl. 18, fig. 1, marked *a* and *b*, which, it will be seen, have become intertwined. In Pl. 18, fig. 2, is shown a case of attempted triple association. Evidently after the firm union of two specimens a third has become attached, and is seeking to come into closer relation, though as yet separated by much host tissue. In normal cases the associated ends gradually enlarge and become rounded off, forming a spherical cyst, into which is drawn up most of the protoplasm from the attached ends. A little, however, may be left with its covering of host tissue outside the cyst, as in *Kalpidorhynchus*¹ (7). The cuticle of the trophozoite may be seen to be closely ribbed by examining the ball end of an associate. After formation of the cyst the cup and ball arrangement disappears and the thin partition between the gametocytes becomes straightened out, the remaining cuticle thickening slightly to form the cyst wall. Meanwhile the covering provided by the host forms a thick wall round the associates, and its proximal parts shorten and

¹ The correct generic name of this parasite is discussed on p. 213.

thicken, but continue to attach the spherical cyst to the pharynx for some time. There is no organic connection between parasite and host cyst. The latter may easily be removed at any time (Pl. 18, fig. 3).

During the changes recorded above nuclear division has been proceeding in each gametocyte. The stage showing the first nuclear division has not been seen. It would take place in a couple at a stage between those represented in Pl. 18, fig. 1, by *a* and *b* on the one hand, and *c* on the other. For in the pair of associates *a* and *b* there were only the trophozoite nuclei in each, while in *c* there were several nuclei which were still dividing. Here it may be mentioned that although all these nuclei appear similar, there is at this early stage a much greater number in one associate than in the other. A similar observation is recorded by Cunningham (7, p. 205) in the case of his *Kalpidorhynchus arenicolæ*, namely, that one gametocyte has fewer nuclei than the other. This difference is here at any rate only transitory, for at the stage represented by *d* (Pl. 18, fig. 1) there seems to be the same number of nuclei in each gametocyte; and there is still no difference to be distinguished in the size or appearance of the nuclei of the two gametocytes. However, Brasil (1) stated that in *G. varia* the difference in appearance of the nuclei of the two gametocytes only appeared distinctly on the passage of the nuclei to the surface formed by their convolutions (1, p. 31). Unfortunately these later stages are wanting among my cysts. Both the formation and fusion of the gametes appear to be gone through rapidly at the time when the cyst breaks away from the pharynx and becomes free in the cœlom. The youngest of these cysts obtained free in the cœlom still had processes at either side where the host's cyst had broken off from the short stumps *f* (Pl. 18, fig. 1) left attached to the pharynx. This cyst contained, besides some residual protoplasm, numerous young spores each with an undivided syncaryon (Pl. 18, fig. 5). Two older cysts containing ripe spores had become completely spherical, showing no signs of having been attached. Round

these the host cells formed a colourless layer of uniform thickness.

SPORES.

The spore is provided with a very characteristic wall composed of endospore and exospore, and having a funnel at one end (Pl. 18, fig. 6). The exospore is thick and transparent, and is supported by processes running through it from the endospore. Similar, though longer, processes run up and support the sides of the funnel. The appearance of the latter is evidently what has given rise to the general statement that in *Gonospora* the spore is terminated by a crown of spines—"couronne de fines pointes hyalines," Léger (11, p. 156) and Brasil (1, p. 20). When unstained, or washed out, the endospore and its processes are refringent, but they can be shown up much more clearly by overstaining with iron-hæmatoxylin. The endospore measures about $10\ \mu$ by $8\ \mu$. The exospore is very delicate and easily overlooked unless the illumination is very good. The processes of the endospore enlarge slightly towards their outer ends, sometimes appearing to have globular extremities. They might almost be minute canals, out of which a sticky fluid was oozing. At any rate, granules in the neighbourhood adhere easily to them. Ripe spores contain eight sporozoites, which escape through the funnel. The thickness of the fully-formed spore-coat makes it difficult for reagents to penetrate it and stain the contents (Pl. 18, fig. 6).

Only on the one occasion, cited above, were cysts with ripe spores found free in the cavity, and those had evidently only just become detached from the pharynx. In all other cases such cysts were embedded in brown masses composed of host phagocytes. Their brown colour was due to granules and other waste products removed from the cœlom. These masses were sometimes very large, one, in specimen VI, was 9 mm. long by 1 mm. wide, and contained embedded in it about a dozen cysts with ripe spores. More usually they measured less than 2 or 3 mm. in any direction; sometimes

they contained small trophozoites in addition to spores, at other times necrotic Nematodes and their eggs, and very often old broken setae. They give, in fact, evidence of the very effective way in which the host is destroying its parasites together with other useless matter. The ultimate destruction of these masses in *Glycera siphonostoma* has been described by Goodrich (9, p. 456), as taking place in the nephridial sacs. Much has been written also on general phagocytic action of the leucocytes of annelids, and destruction of parasites by them has been described by Siedlicki (15), Cuenot (5 and 6), Caullery and Mesnil (2), and others. I am unable, however, to find any recorded instance of phagocytes having to attack a layer of their own host's tissue in order to reach their quarry. In *Gl. siphonostoma* they appear to attack first any free foreign bodies, and it is only after dealing effectively with these that an onslaught is made on attached forms. The attacking phagocytes can easily be distinguished from the covering cells of ordinary cœlomic epithelium by their larger size, branching character, and brown colour due to enclosed granules. In one specimen (IX) a brown mass free in the cœlom was found to contain four trophozoites and four cysts, all in a necrotic condition. The only other parasites were three or four small trophozoites attached quite close to the jaws. Two of these were being held together laterally by a mass of phagocytes with the usual brown granules. These two trophozoites, in spite of their covering of colourless host cells, were evidently in process of being detached from the pharynx and destined to ultimate destruction by phagocytes. This is the only sign of anything like lateral association that I have been able to observe in this form, and of course here it is really nothing of the kind, but merely a case of two small trophozoites being conveniently destroyed at the same time by the host. Léger, however, described lateral association in *G. sparsa*, a form found by him in *Glycera* sp. This makes me hesitate to connect this parasite from *Gl. siphonostoma* with that from other Glycerids until such time as it may be possible to

compare the living forms. To prevent confusion, therefore, it is perhaps well to refer to the form under consideration as *G. glyceræ*.

SYSTEMATIC POSITION OF GONOSPORA GLYCERÆ N.SP.

The genus *Gonospora* was established by Schneider (14, p. 597) in 1875 to include a species found by him in "*Audouinia lamarkii* at Roscoff and also in Terebellids." The trophozoite of this form is described as elongated, broader at one end than the other and the spores as oval, without processes. This would appear to be not unlike Léger's *G. varia* from *Audouinia* (see below). Unfortunately Schneider called this species *G. terebellæ* Köll., although he stated immediately after that he dared not affirm that his species was the same as Kölliker's *Gregarina* (*Monocystis*) *terebellæ*. If, however, the description and figure of the latter given by Kölliker (10a, fig. 6) be studied it will be clear that this form should be included in the genus *Selenidium*. This fact has already been pointed out by Dogiel (8) in his interesting work on the gregarines.

On the whole, then, it seems reasonably clear that the type species on which Schneider founded the genus *Gonospora* was the one subsequently named *G. varia* by Léger in 1892. The chief known characteristics of this and the other two species hitherto described (all apparently forms free in the cœlom) may be summed up as follows:

(1) *G. varia*, Léger, from the cœlom of *Audouinia* (11, p. 157), further described by Brasil (1, p. 21). Trophozoites may be 2 mm. long; association terminal; gametes anisogamous; spores oval, 18 to 21 μ long.

(2) *G. sparsa*, Léger, from the cœlom of *Phyllodoce* and *Glycera* sp. (11). Trophozoites elongated, attaining a length of 1 mm.; association lateral; spores 10 μ long, nearly spherical with "couronne de pointes hyalines."

(3) *G. longissima* C. and M. (2 and 3) from cœlom of *Dodecaceria*. Trophozoites very large; association terminal and intimate; spore apparently oval (not clearly figured).

In none of the above cases, I venture to think, has the spore been studied at a sufficiently high magnification to reveal its true characteristics. Dogiel (8) has recorded the existence of a funnel, and it seems possible that there may also be found a more complicated coat than has been described. In the meanwhile it may be pointed out that the spore of the species from *Gl. siphonostoma* in size and shape much resembles *G. sparsa*; but for the present its distinguishing characteristics may be summarised as follows:

(4) *G. glyceræ* n. sp. from the cœlom of *Glyceria siphonostoma* D. Ch. Trophozoites attached to pharynx during the greater part of their life and covered with a layer of the host's cœlomic epithelium; association terminal and made secure by a dovetail arrangement; spores with a refringent endospore which gives off processes supporting the thick transparent exospore with its funnel.

In addition to *Gonospora* several other gregarines have been found in *Gl. siphonostoma* which have not been shown to have any connection with it. A few observations, however, on these may be of assistance to anyone who should have a chance of obtaining this polychæte at Naples and wish to continue the study of the parasites. I have not been able to obtain any living specimen since leaving Naples, and equally unsuccessful have been efforts to obtain from the British coasts *Gl. gigantea*, *Quatrefages*, which McIntosh (12) maintains is the same species.

(1) *Cystobia intestinalis* Ssok. was obtained in three specimens (namely, IV, XXII, and LII). This parasite was described by Ssokoloff (16) from specimens occurring in a preserved intestine of a *Gl. siphonostoma* (*Rhynchobolus*) which he received from Naples. In this case the whole of the sporogony is described as taking place in the intestinal wall. In my specimen of *Gl. siphonostoma* with the best infection, while there are numerous trophozoites single or associated in the intestinal wall (Pl. 18, fig. 7), there are also very many free. Among these the only pair of associates found in which nuclear division has started is free in the

lumen. Unfortunately no later stages are represented at all, so that I am unable to confirm the account given by Ssokoloff from his somewhat inadequate material. This author does not refer to the peculiar dense meshwork of the ectoplasm which contains small deeply staining granules, although it can be distinguished in one of his micro-photographs.

In his classification (p. 227) Ssokoloff has omitted *C. minchinii*, established by Woodcock (18) as a species occurring in *Cucumaria*, but has included the badly-defined *C. schneideri* Ming., which was stated by Cuenot (4, p. 4), so early as 1892, to be identical with *C. holothuriæ*. He has also included Cunningham's *Kalpidorhynchus arenicolæ* among *Cystobia*, as advocated by Dogiel (8).¹

(2) Another gregarine occurring abundantly in at least three specimens (II, VIII, and XXIV) of *Gl. siphono-*

¹ That this last parasite is a monocystid Gregarine is quite clear from an examination of specimens which can easily be found in *Arenicola ecaudata* at Plymouth. Since it does not undergo neogamy (i. e. precocious association, Woodcock (18)), it should be included in the genus *Gonospora* rather than in *Cystobia*. The same is probably true of the parasite under consideration called *Cystobia intestinalis* by Ssokoloff, but its life history requires confirmation.

The characteristics of the spores of Gregarines are much more important, from a systematic point of view, than such details in their life history as the manner of association (whether terminal or lateral), or the time at which it takes place (whether neogamous or normal). I cannot, therefore, agree with Woodcock as to the necessity of the genera *Diplodina* (Woodcock, 1906), and *Cystobia* (Ming., 1891), in which, with one exception mentioned below, the spores are practically identical with those of *Gonospora*. The occurrence of neogamy, though of much interest, is not of systematic value. Woodcock himself points out that this condition is developed to a varying extent even in a single species, viz. *Diplodina irregularis* (18, pp. 60 and 63), and also that the condition occurs to some extent in other genera such as *Diplocystis*, *Zygocystis* (pp. 62 and 63). This being the case it seems preferable to place the two species of *Diplodina*, viz. *D. (Cystobia) irregularis* and *D. (Cystobia) minchinii*, in the genus *Gonospora*, and *Cystobia holothuriæ* (the spore of which is provided with a short flattened tail as well as a funnel) in the genus *Lithocystis* (13a). Woodcock (18, p. 60) fully realised that such were their relationships.

stoma is a cœlomic form always seen in pairs (Pl. 18, fig. 8). These are generally very much smaller than the *Gonospora* trophozoites attached to the pharynx, and have no apparent relationship with them. Specimens measured varied between .2 and 1.6 mm. in length and were never more than 1 mm. wide. They were attached to the body-wall, retractor muscles, or intestinal wall, never to the pharynx. The two individuals of a pair are held together and attached to the host by a homogeneous secretion (Pl. 18, fig. 8, *m*), which extends between them for about one third of their length from the attached extremities. The cuticle is thin and the protoplasm finely granular. The large, oval nucleus varies in position, but is generally nearer the distal end; it is limited by a well-marked membrane and sometimes contains a couple of caryosomes.

Presumably this form has no more connection with *C. intestinalis* than with *Gonospora glyceræ*, and I merely make these few observations in case they may be of use in subsequent investigations.

(3) In two specimens (II and IV) free or attached monocyetid gregarines were found in the intestine, especially towards the posterior end. There was nothing especially characteristic about these, and it is unlikely that they should have any connection with the previously described forms.

(4) In one specimen (III) small active gregarines were found in the anterior region of the intestine. These were probably the sporozoites of one of the above forms, although no spores were seen.

SUMMARY.

(1) There appear to be four different gregarines parasitic in *Gl. siphonostoma*, including at least one species of *Gonospora*.

(2) *Gonospora glyceræ* n. sp. is surrounded throughout the greater part of its existence by a layer of host epithelium. Association is made secure by means of a dovetail arrangement. The spores, under a high magnification, reveal a more

complicated structure than has previously been described in the genus.

THE MUSEUMS, OXFORD;
July 16th, 1915.

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EXPLANATION OF PLATE 18,

Illustrating Mrs. Helen L. M. Pixell-Goodrich's paper on
“The Gregarines of *Glycera siphonostoma*.”

(All preparations, unless otherwise stated, were stained with iron-hæmatoxylin and drawn with the aid of a camera lucida.)

Fig. 1.—Posterior region of pharynx of *Glycera siphonostoma* showing attachment of single and associated Gonospora. *a, b, c, d*, pairs of associates in order of development; *e*, young trophozoite; *f*, remains of attached ends left by liberated cysts. Drawn after fixation in corrosive acetic mixture. $\times 8$.

Fig. 2.—Optical section of distal ends of two associates, *a* and *b*, showing intimate union between them, and a third specimen, *c*, attempting to effect multiple association. Stained iron-hæmatoxylin. $\times 23$.

Fig. 3.—A pair of associates slightly compressed, after removal of envelope of host cells. Drawn from the living. $\times 23$.

Fig. 4.—Optical section of a single trophozoite, after being detached from pharynx, showing small nucleus with several caryosomes. *h*, Covering of host's cells; *p*, proximal end bent on itself. $\times 75$.

Fig. 5.—Optical section of young spore, with characteristic coat just formed. The nucleus (syncaryon) starting to divide, but much overstained. $\times 2000$.

Fig. 6.—Ripe spore showing characteristic endospore with processes, faintly staining exospore, and funnel supported by slightly longer processes. $\times 2000$.

Fig. 7.—*Cystobia intestinalis* Ssok. Section through trophozoite at base of intestinal cells. *n*, Remains of hypertrophied nucleus of host cell. *ec*, Dense ectoplasm with deeply staining granules. $\times 500$.

Fig. 8.—A pair of cœlomic gregarines held together by a structureless membrane (*m*), extending to nearly half their length from the attached ends. $\times 250$.

Observations on the Insect Parasites of some Coccidæ.

By

A. D. Imms, M.A., D.Sc.,

Reader in Agricultural Entomology in the Victoria University
of Manchester.

I.—On *Aphelinus mytilaspidis* Le Baron, a Chalcid Parasite of the Mussel Scale (*Lepidosaphes ulmi* L.).

With Plates 19 and 20, and 5 Text-figures.

CONTENTS.

	PAGE
1. INTRODUCTION	218
2. REMARKS ON THE BIOLOGY AND FECUNDITY OF THE HOST	219
3. HISTORICAL	221
4. SYSTEMATIC POSITION	222
5. DISTRIBUTION AND HABITS OF THE APHELININÆ	224
6. THE FEMALE	225
7. THE MALE	232
8. REACTIONS TO EXTERNAL STIMULI	234
9. PARTHENOGENESIS	235
10. OOGENESIS	235
11. OVIPOSITION	237
12. THE EGG	240
13. THE NEWLY HATCHED LARVA	241
14. THE FULLY GROWN LARVA	241
15. THE PUPA	244
16. LIFE-HISTORY	246
17. ECONOMIC STATUS AS A PARASITE	262
18. SUMMARY OF CONCLUSIONS	268
19. BIBLIOGRAPHY	270
20. EXPLANATION OF THE PLATES	272

INTRODUCTION.

THE present paper is intended as the first of a series dealing with the biology of the principal insect parasites of certain common British species of Coccidæ. The object of these researches is to ascertain the relative values of such insects as natural controlling agents of a family embracing many species injurious to the operations of man. That certain of the British Coccidæ are extensively parasitised is well known, but the effects of parasitism, as a factor limiting their abundance, has in almost all cases remained unstudied. In some instances it is undoubtedly great, but until the essential features of the relationships between the various species of host and parasites are understood, it is not possible to estimate their value from the economic standpoint. As an example may be mentioned that the second generation of a species of Chalcididæ attacking *Lecanium capreæ* parasitises the latter to an enormous extent. Its effects on the species, however, is but slight, owing to the fact that it destroys its host as a rule only after the latter has laid most of its eggs. On the other hand, the first generation of the same parasite destroys upwards of 50 per cent. of the *Lecanium* while the latter is still immature. Judged merely from the external evidences of parasitism, the palm of efficiency would have been awarded to the second generation of the Chalcid.

It has been pointed out (Embleton, 1902, p. 221) that a colony of Coccidæ may be extensively parasitised, and yet betray no obvious manifestation of the fact except to the trained and experienced observer. In such case it may be highly injurious and superfluous to apply insecticides, as they also destroy the beneficial insects which are already acting as a check upon the injurious species. A prevalent belief is that the methods of applied entomology only concern the destruction of injurious insects. An equally important aspect is the investigation of those species which are either directly or indirectly beneficial to the human race.

Aphelinus mytilaspidis Le Baron ranks as one of the

principal parasites of the "mussel" scale¹ (*Lepidosaphes ulmi* L.), and the present paper is intended as a contribution towards a knowledge of its biology and relationship to its host. The investigation has been mainly carried out in the Department of Agricultural Entomology, Manchester University. To Prof. S. J. Hickson, F.R.S., I owe a debt of gratitude for the use of a Zeiss-Greil drawing apparatus and for various other facilities. I am also indebted to Mr. T. J. Young, Principal of the College of Agriculture, Holmes Chapel, Cheshire, for allowing me to carry out field observations on the lands under his charge. Dr. L. O. Howard, Chief of the U.S. Bureau of Entomology, has kindly aided me in the identification of this and other species of Chalcididæ. Also Mr. T. J. Wadsworth, research assistant in my department, has rendered valuable help in the counting and measuring of large numbers of the host mussel scale. Much assistance in procuring material from various localities has been obtained from several sources, and is acknowledged in a later portion of this paper.

Owing to the marked difference in size of the insect in its later stages of the two generations, it is necessary to add that the descriptions of the fully grown larva, the pupa, and the adults were drawn up from observations made on individuals of the first generation.

REMARKS ON THE BIOLOGY AND FECUNDITY OF THE HOST.

Lepidosaphes ulmi L.² is the commonest of the injurious Coccidæ found in the British Isles. It is nearly world-wide in distribution as a pest of cultivated fruits and nursery stock, and has been largely disseminated through the agency of man. Its favourite food-plant is the apple, but many other species are utilised, Quaintance and Sasscer

¹ Formerly known as *Mytilaspis pomorum* (Bouché).

² The expressions "scale insect" and "mussel scale" are used in this paper with reference to the insect as a whole, while the term "scale" is restricted to its shield-like outer covering.

(1910, p. 5) recording over 118 host plants, and a few more have been added since.

From observations conducted at Holmes Chapel, in Cheshire, during the years 1913-15 it was found that the females commenced to lay their eggs on or about August 17th, and continued oviposition on into September. By the end of October the majority of the parent *Lepidosaphes* were dead, their scales alone remaining as a protection to the eggs. Newly hatched larvæ were first noticed on May 21st of the following year, and the developmental cycle was completed by the end of July or beginning of August. In Great Britain the insect is single brooded, but there are two broods in certain parts of N. America. No males were met with, and, according to Newstead (1901, p. 198), they are seldom found; parthenogenesis, therefore, appears to be frequent.

The adult female *Lepidosaphes* vary very considerably in size, and the following observations were made on 75 specimens obtained from three different localities. The size of a scale was judged by its length rather than by the breadth.

Locality.	Food-plant.	Scales examined.	Maximum size.	Minimum size.
Northenden (Cheshire)	Apple	25	2.88 × .85 mm.	1.63 × .58 mm.
Warford (Cheshire)	Apple	25	3.07 × .78 mm.	1.37 × .65 mm.
Aspley Guise (Beds.)	Apple	25	2.68 × .72 mm.	1.44 × .72 mm.

The number of ova laid varies very considerably in different individuals. In America it appears to be much higher than in the British Isles. Girault (1909, p. 357) states that the average number of eggs on poplar in Illinois is 85.6, the numbers varying in different individuals from 48 to 120. Quaintance and Sasser (1910, p. 2) mention that it varies between 40 and 100. In England, according to my experience, the number is much lower, and from observations made

on 75 scales the average found was 37·2 eggs per female. The counts yielded the following figures, the food-plant in all cases being apple :

Locality.	Scales counted.	Eggs present.	Maximum number.	Minimum number.	Average number.
Warford (Cheshire)	25	533	33	10	21·3
Holmes Chapel (Cheshire)	25	1186	66	26	47·4
Aspley Guise (Beds.)	25	1071	62	11	42·8
Totals	75	2790	66	10	37·2

As a general rule it was found that the size of the parent scale bears some relation to the number of eggs produced. Measurements were made of 30 scales taken at random, and obtained from two widely separated localities. The number of eggs deposited was noted in each case, and the counts were made after the females were dead, thus ensuring that they had laid their full complement. It was found that the largest scales usually sheltered the greatest number of eggs beneath them.

HISTORICAL.

So far as I am aware, Fitch (1855, p. 36) was the first to observe what was most probably the *Aphelinus* parasite in any of its stages. He states that he repeatedly noticed a small, honey-yellow larva, three hundredths of an inch long beneath the mussel scale. He further adds that it is probably the larva of some minute Hymenopterous insect, "specially designed by Providence for destroying the eggs of the bark louse." The perfect insect, he believes, makes its exit by perforating a small round hole through the scales, such holes being frequently met with, and on p. 35 of his Report one of these holes is figured. Walsh (1867, p. 45) refers to Fitch's

observations, mentioning that he had also noted round holes in the scales, and similarly regarded them as being made by a parasitic fly, belonging probably either to the Chalcididæ or Proctotrypidæ. Walsh, however, did not observe the yellow larva referred to by Fitch. The adult insect remained undiscovered until three years later, when Le Baron (1870, p. 360) described the female. He gives outline figures of the insect and its larva with some observations on its life-history and habits, and the extent of its parasitism. He concludes that the insect has two broods in the year, and remarks that by the middle of September many of the present year's scales are pierced with round holes, through which the first brood of the Chalcid has escaped; while late in the fall, under about an equal number of scales, the fully-grown larvæ of the second brood are to be found. The second brood, he adds, must appear in the winged state early enough the next summer to deposit the eggs from which the first brood of next year will proceed. He also deals with the insect in his first Report as State Entomologist of Illinois (1871, pp. 34-39). Riley (1873, p. 87) quotes Le Baron's observations, but does not add any new information concerning the insect. The male insect was not discovered until 1881, when it was described by Howard (1881, p. 354), who also mentions that his observations seem to confirm Le Baron in the supposition that there are two broods of the Chalcid in the course of a year. Twenty-eight years later Marchal (1909, p. 1223) published some interesting observations on the process of oviposition and certain habits associated therewith, but I am not aware that any further contributions have been made towards a knowledge of the biology of this insect.

SYSTEMATIC POSITION.

The genus *Aphelinus* is a member of the great group of parasitic Hymenoptera placed by Ashmead (1904) in his super-family of the Chalcidoidea, and in the family Eulophidæ. It belongs to the sub-family Aphelininæ, which are

regarded by many authorities as being allied to the Encyrtinæ. Ashmead (loc. cit., p. 344) states that they are clearly a component of the Eulophidæ as is shown by the structural characters of the meso-thorax, and Schmiedenknecht (1909, p. 390) adopts this same view. Morley, in his Catalogue (1910, p. 25), places them between the Encyrtinæ and the Pireninæ.

The Aphelininæ are distinguished by Howard (1895A, p. 6) by the following characters: The mesopleura are divided, the middle legs are not specially developed for saltatory purposes (although the insects jump well), and the first tarsal joint of the middle legs is not incrassate; the antennæ are not more than eight-jointed, and the parapsidal sutures are distinct. The mandibles are small, two to three dentate; the maxillary palpi are three-jointed, and the labial palpi are represented by an elongate tubercle. The antennæ are inserted near the clypeus, and the scape is long and slender. The fore-wings lack the post-marginal vein, and the abdomen is broadly sessile.

The genus *Aphelinus* was erected by Dalman in 1820, and according to Howard (loc. cit., pp. 23-24) may be separated from other genera of the sub-family by the following characters: The oblique hairless line of the fore-wings is very distinct. The ovipositor is very slightly extruded or is entirely hidden. The fringed apical cilia of the fore-wings are very short; the body is robust, eyes naked in the yellow species and hairy in the black species. The posterior border of the mesocutellum is rounded, and the anterior border is bounded by three straight lines. The antennæ are six-jointed, scape long and slender, pedicel normal, joints 1 and 2 of the funicle very short, joint 3 about as long as or a little longer than the pedicel, club compact, not jointed, subellipsoidal. The middle tibial spur is very pronounced, mesoscutar parapsides rather small, marginal vein very long, longer than submarginal; stigmal and post-marginal short.

Schmiedenknecht (1909, pp. 451-453) catalogues forty-two

species of the genus, and additional forms have been described since, making the known species rather more than sixty in number. Of these, Morley (1910, pp. 25-26) catalogues fourteen species as occurring in the British Isles, excluding *A. mytilaspidis*, which was not known to him as a British insect.

DISTRIBUTION AND HABITS OF THE APHELININÆ.

This sub-family is nearly world-wide in range, species being known from almost all parts of the globe, with the exception of the colder temperate and polar regions. *Aphelinus mytilaspidis* occurs in many parts of the United States and Canada, also in France (Marchal, 1909) and in Italy, according to Masi (1911) and Voglino (1913). In the British Isles the only previous record is that of the Duke of Bedford and Pickering (1906, p. 7), who found it at Woburn (Beds.) during the course of their experiments on the effects of insecticides on the mussel scale. During the years 1913-15 I have obtained a large number of branches and twigs of apple attacked by mussel scale and have bred out this Chalcid from the following localities:—SURREY: Kew Gardens, Merton, Oxshott, Cobham, Wisley, and Woking. DEVONSHIRE: Plymouth, Bere Alston. BEDFORDSHIRE: Aspley Guise. GLOUCESTERSHIRE: Mickleton, Slough. HAMPSHIRE: Botley, Bishop's Waltham. SOMERSETSHIRE: Long Ashton. WORCESTERSHIRE: Badsey. LEICESTERSHIRE: Meatham. SHROPSHIRE: Newport. CHESHIRE: Holmes Chapel, Northenden, Northen Etchells. LANCASHIRE: West Didsbury near Manchester. CUMBERLAND: Carlisle. It may be said, therefore, that *Aphelinus mytilaspidis* is generally distributed in England. I have not, however, been able to obtain any records for Scotland or Ireland, but it doubtlessly occurs in both countries.

In their habits the larvæ of the Aphelinæ are either exclusively parasitic, or parasitic and partially predaceous. They may devour both their hosts and the eggs of the latter, and

confine their attack almost exclusively to the Rhynchota. Their principal hosts are Coccidæ (particularly the Diaspinæ) and Aphididæ; less frequently they attack the Aleurodidæ. Outside the Rhynchota, Giraud ('Verh. zool. bot. Ges. Wien.,' xiii, 1864, p. 1278) has recorded *Aphelinus locustarum* (Gir.) from the Orthopteron *Xiphidium fuscum*, and Rondani ('Arch. p. l. Zool.,' 1870, pp. 12 and 15) has described *A. nemoranæ* (Rond.) from a Lepidopterous insect, *Xylopoda nemorana*.

Aphelinus mytilaspidis, in addition to parasitising *Lepidosaphes ulmi*, has also been recorded, according to Howard (1895A, p. 11), from the following Coccidæ in America: *Chionaspis salicis*, *Diaspis carueli*, and *Aspidiotus perniciosus*. Girault (1911, p. 184) also records it from the latter host on plum in Illinois. In France, Marchal (1909) mentions it utilising *Aspidiotus ostræformis* as a host. In Italy, Voglino (1913, p. 1004) mentions it parasitising *L. ulmi* on Canadian poplar, and Masi (1911, p. 158) records it from *Aspidiotus betulæ* and *A. hederæ* in various localities. In Great Britain it is only known from *Lepidosaphes ulmi*.

THE FEMALE.

Coloration.—Pale lemon yellow with the ocelli crimson and the eyes black. The antennæ are yellow with a slight smoky suffusion, and the mandibles and the stylets of the ovipositor are brown owing to their greater degree of chitinisation. The legs are entirely yellow, the wings hyaline, and in some examples they are slightly tinged with yellow. The wing veins are distinctly lemon yellow. In newly emerged individuals the yellow coloration is paler, and the eyes are usually green, not having yet attained their black pigmentation.

The Head.—The head is of about equal width to the thorax, and is invested dorsally with a number of short setæ. The antennæ (Pl. 19, fig. 5) measure ·38 mm. in length

and arise from the head a very short distance (.01 mm.) in front of the mandibles. They are seven-jointed, the respective joints being related to one another in length in the proportion of 1:14:5:1:1:3:11. Howard (1895, p. 24) states that in the genus *Aphelinus* the antennæ are six-jointed. In the present species, however, there is a small though well-defined basal joint (*b.*) which previous writers have regarded as belonging to the scape (*s.*). The eyes are slightly hairy; each hair is very minute and arises from the point where three ommatidial facets are in contact with one another. The ocelli are disposed almost to form the extremities of the sides of an equilateral triangle. The two posterior ocelli are situated nearer to the eyes than to the median and anterior ocellus (Pl. 19, fig. 1). The mandibles (Pl. 19, fig. 4) are relatively stout and measure .05 mm. long \times .04 mm. wide. They are both similar in form, and each is armed by three teeth, of which the two outer are prolonged obliquely across the jaw in the form of a pair of outstanding ridges. Situated dorsally to the mandibles is a transverse chitinised bar which serves to give rigidity to the anterior margin of the head and support to the jaws. It is more slender than its counterpart figured by Bugnion (1890, Pl. 24, fig. 44) in *Encyrtus fuscicollis* and is, furthermore, attached to the head skeleton by a pair of backwardly directed arms not referred to by that observer.

The first maxillæ (Pl. 19, fig. 2) are delicate and membranous; each measures .09 mm. in maximum length and .02 mm. in width. Its morphology is not clear, but probably the basal portion (*b.*) is to be regarded as corresponding to an undifferentiated cardo and stipes. Arising from it is an undivided lobe (*a.*) bearing hairs along its anterior margin. The maxillary palp (*mx. p.*) is a slender appendage measuring .03 mm. long and is two-jointed, the joints being related to one another in the proportion of 2:3. The second joint is terminated by a slender drawn out seta, longer than itself. The labium (second maxillæ) (Pl. 19, fig. 3) is a small median structure fitting in between the two

maxillæ, but not extending so far backwards as the hind margin of the latter. The body of the labium (*m.*) is probably to be regarded as the mentum, and it measures ·03 mm. to ·04 mm. long and ·02 mm. in width. At its distal end it carries a membranous lobe, ·01 mm. long, and apparently representing the ligula (*l.*). The latter structure bears along its anterior margin several, usually four, peg-like organs possibly of sensory function. The labial palpi (*l. p.*) are extremely small and delicate organs and are easily overlooked. They are setiform, ·02 mm. in length, and composed of a single joint terminated by a seta of about equal length.

The Thorax (Pl. 19, figs. 1 and 6).—In describing the sclerites of the thorax I have followed the nomenclature adopted by Howard (1881, p. 352), and also used by him in his Revision of the Aphelinæ (1895).

The pronotum (*pn.*) is narrow and band-like and bears five bristles on either side, the external one being the most prominent. The mesonotum consists of (1) the mesoscutum with its lateral plates or parapsides, and (2) the mesoscutellum, together with the scapulæ. The mesoscutum (*mtm.*) is a large sclerite forming the greater part of the anterior half of the mesonotum. It is armed with five to seven bristles on either side of the middle line, whose arrangement can be readily understood by referring to Pl. 19, fig. 6. When the lower number is present the second bristle from the median line in the anterior row on either side is absent, and likewise the anterior pair of the four bristles occupying the centre of the thorax. The remaining bristles are constant in all specimens that I have examined. The parapsides (*pr.*) form the sides of the mesoscutum; they are very narrow posteriorly, broadening out anteriorly and becoming somewhat laterally extended. They carry a pair of small setæ on their anterior border. The mesoscutellum (*mlm.*) forms the posterior half of the mesonotum; its hind margin is prominently rounded, and it carries a pair of conspicuous backwardly directed dorso-lateral bristles on either side. Its scapulæ (*scp.*) are roughly triangular in form, and extend

forwards to the commencement of the lateral widening of the parapsides. Each scapula carries a single small bristle. Arising from the mesoscutellum is an internal chitinous plate, developed from the vertical intersegmental fold between the meso- and metanotum. It is a characteristic feature in many species of *Aphelinus*, and is best seen in specimens mounted in balsam. This structure also occurs in the Encyrtinæ, and has been probably correctly identified by Bugnion (1890, p. 506) as the mesophragma. It measures .19 mm. long and .11 mm. wide, and extends backwards to as far as the second abdominal segment. Its function is to give attachment to the large longitudinal muscles of the thorax. The metanotum (*mn.*) is very narrow and band-like, expanding somewhat laterally on either side. The propodeum (*i. t.*) is about three times the depth of the metanotum, and, similarly to the latter, carries no bristles.

The fore-wings (Pl. 19, fig. 1) are finely pilose except for an oblique hairless tract measuring .03 mm. in greatest breadth, which extends backwards from the costal margin, just in front of the stigmal vein, to the posterior margin of the wing. On the proximal side of this tract, the fine hairs investing the wing membrane are nearly twice the length of those on the distal portion. They are arranged in seven or more irregular rows disposed more or less parallel to the hairless tract, but are absent from a small area at the base of the wing. The sub-marginal vein is much shorter than the marginal, the post-marginal vein is practically absent, and the stigmal vein small and inconspicuous. Arising from the posterior border of the sub-marginal vein and the base of the marginal vein, is a row of about twenty-two minute rounded peg-like processes (Pl. 19, fig. 7). The marginal hairs are fine and short; they commence at a point on the costal margin where the marginal vein terminates, and extend round the wing to a point almost opposite it on the hind margin. From this position, where the marginal hairs cease to be present, to as far back as the hairless tract (already referred to), the hind margin of the wing is strength-

ened by the presence of a kind of chitinous rim or thickening (Pl. 19, fig. 1).

In the hind-wings the submarginal vein presents about sixteen projecting points (Pl. 19, fig. 8) very similar to those on the fore-wing. The marginal vein is a little shorter than that of the fore-wing, and there is no stigmal vein; at its apex is a pair of hooked hair-like processes which fit into the chitinous rim of the fore-wing and thereby hold the two wings together when in flight. The remaining features of the hind-wings are sufficiently evident on referring to Pl. 19, fig. 1 to need no further reference.

The legs are tolerably long and slender. The fore legs (Pl. 19, fig. 9) are the shortest and differ from the succeeding pairs in having short and somewhat swollen tibiæ. The fore tibia is not as long as the tarsus, and is armed with a single curved spine or spur at its apex. The tarsal joints (excluding the claws) are related to one another in length in the proportion of 5:4:2:2:3. The middle legs (Pl. 19, fig. 10) differ in their much longer and more slender tibiæ, which slightly exceed the tarsi in length; they are armed on the inner side of their apices with a prominent terminal spur .07 mm. long. The tarsal joints are mutually related in length as 8:4:4:2:3. The hind legs (Pl. 19, fig. 11) are the longest of the three pairs, and their tibiæ are considerably longer than the tarsi. The tibial spur is small and inconspicuous, measuring only .02 mm. long. The tarsal joints are related to one another in length as 7:4:3:3:5.

The Abdomen.—The abdomen measures on an average .5 mm. long and is not separated from the thorax by any marked constriction or petiole. It consists of seven evident segments (Pl. 19, fig. 1), of which the first five carry a group of postero-lateral setæ on either side. On the hind margin of the sixth segment there is a complete dorsal band of similar setæ and likewise a band across the middle of the terminal segment. Situated at the base of the apical segment of the abdomen, and fitting into a lateral sinus on the hind border of the preceding segment, is a small sensory plate, .015

mm. in diameter (Pl. 19, fig. 13). Each plate carries three delicate setæ, of which the longest measures .12 mm. in length. These plates are very characteristic structures among Aphelinæ and Encyrtinæ, and the setæ to which they give basal support are apparently sensory in function. On the ventral aspect of the abdomen the most important structure is the ovipositor (Pl. 19, fig. 12); as it scarcely projects beyond the apex of the abdomen it is frequently invisible dorsally. It consists of the following parts: (A) A pair of extremely fine stylets (*sty.*), .33 mm. long, each having a diameter of .002 mm. The two stylets fit together in exact juxtaposition to form a very delicate rigid piercing needle. Basally they divaricate in the form of a letter V, and the two arms thus formed (*sty.*₁) also curve prominently upwards in the vertical plane. In the preparation represented in Pl. 19, fig. 12, these two curved arms are pressed outwards, so as to lie in the same plane as the other parts owing to the weight exerted by the cover-glass. (B) The sheath (*sh.*) (gorgeret of Bugnion), which takes the form of two very elongate pieces united together in the middle line to form a groove. This groove faces ventralwards, and within it are lodged the stylets. It is practically of the same length as the latter and measures .013 mm. in width. On the right side its apex is sharply pointed and armed with five extremely minute teeth (Pl. 19, fig. 14). The two parts forming the sheath divaricate basally and are also curved dorsalwards in a manner similar to the stylets. At its innermost extremity each arm of the sheath is fused with a somewhat triangular supporting plate (*t. p.*). The curved portions of the sheath (*sh.*₁) are thickened and groove-like, and within each trough so formed lies the arm of the stylet of its side. At its point of bifurcation the sheath is strengthened dorsally by means of a transverse chitinous support or bridge (*c. sh.*), only partially visible from the ventral side. (C) Situated on either side of the sheath is an elongate inner plate (*pl.*₁), .30 mm. long, and movably articulated with the apex of the latter is a single-jointed palp-like appendage (*ap.*). This carries a

small number of hairs, some of which being possibly tactile in function. Basally, the inner plate articulates by means of a chitinised area (*s. pl.*₁), with the support (*c. sh.*) of the ovipositor sheath. The plate is strengthened by means of a median rib of chitin (*r.*₁), and this structure fans out basally to fit in contact with the thickened edge (*sh.*₁) of the arm of the sheath of the ovipositor. By means of a concavity near the base of the median rib, the inner plate movably articulates with the rounded inner process of the supporting plate (*t. p.*). (D) Partially overlying the inner plates is a pair of much wider outer plates (*pl.*₂) measuring .22 mm. long and .1 mm. in greatest breadth. Each outer plate is similarly supported and strengthened by a median rib (*r.*₂), which bifurcates into two branches directly from the base. The proximal extremity of the rib is hollowed out to present a surface for articulation with the rounded outer surface of the supporting plate (*t. p.*). Bugnion (1890, p. 514, and *y'* in Pl. 25, fig. 52) describes in *Encyrtus fuscicollis* a third pair of plates or "ecailles chitineuses," which appear to have no counterpart in *A. mytilaspidis*. The only other account of the structure of the ovipositor in a Chalcid with which I am acquainted is that of Miss Embleton, who studied it in *Comys infelix* Embl. From a perusal of the authoress' paper it is clear that the ovipositor of *Aphelinus* agrees more closely with that of *E. fuscicollis* than with the former species. (E) The supporting plate (*t. p.*) measures .04 mm. × .03 mm. and articulates, as already described, with the arms of the ovipositor sheath and the median ribs of the outer and inner plates. It is of great importance as a fulcrum upon which the movement of these parts is effected. I may add that the ovipositor, on account of its minuteness, and its associated parts, on account of their extreme delicacy and transparency, have proved very difficult to investigate. It was not until after more than thirty preparations had been made, by various methods of technique, that it was found possible to understand the relations of the different parts connected with it.

Size.—The following measurements were made from two average-sized examples taken from each generation. It will be noted that there is a relatively marked difference in size between individuals of the two generations. Out of forty examples of the second generation (from two localities) the largest measured .67 and .77 mm. in length respectively. The wing measurements in all cases include the marginal hairs.

	First generation.		Second generation.	
Length to apex of ovipositor93 mm.	.83 mm.	.55 mm.	.59 mm.
Length of head and thorax44 "	.40 "	.26 "	.29 "
Length of abdomen .	.49 "	.43 "	.29 "	.30 "
Breadth of thorax .	.36 "	.28 "	.23 "	.20 "
Length of fore-wing	1.02 "	.81 "	.59 "	.59 "
Width of fore-wing .	.36 "	.33 "	.27 "	.19 "
Length of hind-wing	.72 "	.68 "	.44 "	.49 "
Width of hind-wing .	.17 "	.17 "	.11 "	.15 "
Expanse of fore-wings from tip to tip .	2.40 "	1.90 "	1.41 "	1.38 "

THE MALE.

The male is very much rarer than the female, a feature which is of common occurrence among the Chalcididæ. Out of over 750 specimens bred between May, 1914, and September, 1915, I only obtained ten male individuals. Dr. L. O. Howard (1881, p. 354) was the first to describe the male of *A. mytilaspidis*, and he mentions that it is so similar to the female as to be indistinguishable from the latter unless the genitalia be examined. The males are generally smaller in size than the females. The joints of the antennæ are mutually related in length in the proportion of 6 : 27 : 13 : 3 : 3 : 7 : 23. In most examples the scape is a little shorter than in the female, and Howard states that the club of the antenna is more truncated at its apex. The genitalia (Pl. 19, fig. 15), however, are the only organs to which it is necessary to refer in any detail. From the apex of the penis

to the base of its associated parts the genitalia measures $\cdot 13$ to $\cdot 15$ mm. in length.

The penis (*p.*) is a cylindrical organ $\cdot 068$ mm. long and $\cdot 015$ mm. in greatest diameter. At its apex it is provided with about eight minute papillæ (*p.p.*). Bugnion (1890, Pl. XXV, fig. 51, p. 511) has figured very similar structures in *Encyrtus fuscicollis*, and regards them as being sensory in function. Miss Embleton, in her study of *Comys infelix* (1904, p. 251, Pl. 12, fig. 45), mentions that the penis in that Chalcid has minute papillæ which are the openings of the ducts leading from the gonads. They are situated in a very similar position on the apex of the penis as the papillæ referred to herewith. The sides of the penis are strengthened by a pair of flattened chitinous rods (*r.p.*), which are continued forwards into the basal portion of the genitalia. Arising near the base of the penis is a pair of claspers (*c.*) or "harpons mobiles" (Bugnion); these structures measure $\cdot 068$ mm. in length, and each carries at its apex a minute outwardly directed tooth. Both the penis and claspers are attached to an unpaired basal portion into which the penis can be partially withdrawn. Ventrally near the bases of the claspers is a pair of minute papillæ (*v.p.*), each surmounted by a small seta.

During copulation the penis and claspers are protruded beyond the apex of the abdomen and introduced into the genital orifice of the female. By means of the contractions of muscle fibres situated at the base of the claspers the latter organs are drawn far apart so as to form a wide V with one another. In this way they apparently function in maintaining the penis within the genital aperture of the female.

The following measurements were made on a typical specimen; those concerning the wings include the marginal fringe of hairs:

Length to apex of abdomen	$\cdot 8$	mm.
Length of head and thorax	$\cdot 4$	"
Length of abdomen	$\cdot 32$	"
Breadth of thorax	$\cdot 29$	"

Length of fore-wing	·8 mm.
Breadth of fore-wing	·27 „
Length of hind-wing	·69 „
Breadth of hind-wing	·19 „
Expanse of fore-wings from tip to tip	1·89 „

MOVEMENTS AND REACTIONS TO EXTERNAL STIMULI.

The adult insect moves from one portion of a branch to another almost entirely by running; when disturbed it may leap to a distance of $1\frac{1}{2}$ to $2\frac{1}{2}$ in., its progress being aided by the wings, but true flight, in the ordinary sense of the term, seems to be seldom resorted to. Owing to the limited powers of migration of the winged insects, parasitised scales frequently occur in patches on the branches, and for this reason it is necessary to examine a large number of twigs and branches to form an accurate estimate of the degree of parasitism present. The migratory powers of the insect are, therefore, extremely limited. The chief agent in distributing the parasite from one tree to another is wind, which is also the principal factor in spreading the young larvæ of the host.

The insect avoids darkness and instinctively steers its course towards the light, being therefore positively phototropic. It is in virtue of this habit that examples, which emerge in darkened breeding cages, congregate within the glass tubes inserted in the sides of the latter as described on p. 247, thus affording a ready method of collecting them. They do not exhibit marked response to geotropism, and parasitised scale insects are not restricted to the apices or other portions of a branch. Tower (1914, p. 432) states that *Prospaltella perniciosi*, a parasite of the San Jose Scale (*Aspidiosus perniciosus* Comst.), exhibits both positive phototropism and geotropism, and that scale insects situated on the smaller and outermost branches and twigs are well parasitised. A large number of Chalcids are known to fly towards the light when reared in breeding cages, but Miss

Embleton (1904, p. 235) states that *Comys infelix* is negatively phototrophic, and the Proctotripid, *Eumicrosoma benefica* Gahan, according to McColloch (1915, p. 260), also exhibits a similar reaction.

PARTHENOGENESIS.

A notable feature in the biology of this Chalcid is the great scarcity of males, only ten being bred from among over 750 specimens. This fact naturally suggested the probability of parthenogenesis occurring in the species. In order to ascertain whether it obtained or not, seven mussel scales each sheltering a pupa of the *Aphelinus* were isolated in separate phials. When the adults emerged they were transferred to freshly cut twigs of apple, bearing numerous examples of the host in its younger stages. These twigs were enclosed in glass tubes and the Chalcids were observed under a binocular microscope depositing their eggs beneath the scales on the same day (July 17th, 1915). Five of these eggs (laid by three separate females) were transferred to watch-glasses and kept under close observation in a moist chamber. Four of the eggs hatched in from nine to eleven days in the laboratory at an average temperature of 63° F., and one of the resulting larvæ is represented on Pl. 20, fig. 20. It is noteworthy that Quayle (1910, p. 461) has recorded parthenogenesis in *Aphelinus diaspidis* and found no males in that species. Parthenogenesis is also known to occur in other Chalcididæ, and it is probably of wide occurrence in that family.

OOGENESIS.

In specimens freshly killed, by means of chloroform vapour, the ovaries can be dissected out with comparatively little difficulty. Each ovary (Pl. 20, fig. 22) measures on an average .4 mm. in length and consists of five ovarioles, though the latter number is not absolutely constant. The oviduct (*od.*) is extremely short and unites almost immediately with its fellow of the opposite side to form a median unpaired

vagina. Each ovariole is invested externally with a very delicate membranous coat (*w. o.*), in which nuclei are only discernible here and there. The ovariole ends distally in a germarium or terminal chamber (*g.*) containing a small number of rounded undifferentiated cells. Lower down, the ovariole exhibits a differentiation into egg chambers and nutritive chambers. In the egg chambers (*e. c.*) certain of the cells enlarge greatly, yolk material accumulates therein, and they become clearly marked out as future eggs. Each is surrounded by a layer of follicle cells. Alternating with the egg chambers are groups of smaller cells with prominent nuclei and bearing considerable resemblance to the cells of the germarium. These aggregations of cells constitute what are usually regarded as nutritive chambers (*n. c.*). Still further down, the nutritive chambers are no longer in evidence, and we find a succession of eggs (usually three) in later phases of development. The yolk has greatly accumulated in their protoplasm, obscuring the germinal vesicle from view, the latter being only discernible after treatment with appropriate reagents. The follicle cells at this stage having subserved their function are no longer in evidence, the eggs lying free within the ovariole.

In general structure, therefore, the ovarioles pertain to the meroistic type, in which the ovarian and nutritive (or vitellogenous) chambers alternate with each other. Such a method of arrangement is the rule among the majority of Hymenoptera. The largest ovarian eggs measured .20 mm. in length and .05 mm. in greatest breadth; they are elongate oval in shape, often somewhat constricted in the middle (Pl. 20, fig. 22). An attempt was made to compute the number of eggs capable of being laid by each female, from an examination of the ovaries. In certain of the Cynipidæ, for instance, the eggs attain maturity very nearly at the same time, which lends value to this method of estimation. In the present species, however, it has been found to be unreliable, as the eggs mature in succession from the bases of the ovarioles upwards. It may be stated, however, that not less than thirty

eggs are produced in the two ovaries, but probably double that number is nearer the correct average figure. The fecundity, nevertheless, is relatively low, and it is a well-known fact that in most insects the number of eggs which develop is considerably in excess of those which are actually laid.

OVIPOSITION.

Oviposition was observed from July 10th to 14th in 1914, and from July 15th to 19th in 1915, both in the laboratory and out in the field. For the purpose of noting the details of the process, bred females were transferred to small, freshly cut branches of apple, which were abundantly attacked by the mussel scale. The whole course of oviposition may then be readily observed under a Zeiss binocular microscope. The insects were seen running actively along a branch searching for suitable hosts wherein to deposit their ova. They surveyed the surfaces of likely scale insects by means of a kind of tapping action of the antennæ, and when an apparently suitable host was found, a closer examination followed as a preliminary to the actual process of egg-laying. When the latter commences, the insect directs its abdomen towards the scale with its head pointing outwards—it seldom takes up a position parallel with the length of the scale. The wings are folded over the back, the long axis of the insect is inclined slightly upwards, and the legs are extended backwards. The antennæ are no longer in activity and hang downwards in front of the face. The movable appendages of the inner plates of the ovipositor (*ap.*, in Pl. 19, fig. 12) first come into use, and appear to play a part in selecting the actual place where the perforation of the scale is to occur. When the ovipositor comes into work, the stylets and sheath together form a piercing dart with which the insect makes a minute hole in the scaly covering of the host. After an opening is made, the sheath of the ovipositor is withdrawn, leaving the stylets in situ. At first the body of the insect undergoes a slow backward and forward motion, but the final act is almost motion-

less to the observer. The stylets are so far inserted that the ventral side of the abdomen is in contact with the surface of the scale. The eggs are apparently forced down the incomplete canal formed by the stylets by means of the contractions of muscle fibres situated in the walls of the oviducts. Probably their passage is facilitated by the secretion of certain associated glands which may act as a lubricant, but it has not been possible to definitely verify this suggestion.

Each individual act of egg-laying may last from about fifty seconds up to as much as ten minutes; about six minutes may be taken as the average time occupied in the process. One female was observed to execute as many as eight apparent acts of oviposition in one scale insect, each act involving perforation of the outer covering of the latter in a fresh position. As the result, however, only one egg was found to have been deposited. Five and six perforations were also noted on other occasions, but in not a single instance was there more than one egg deposited in association with each host. Frequently the process of oviposition was apparently gone through and no egg laid, the insect having found the particular host selected unsuitable for some reason. It is evident, therefore, that the antennæ only function in making a preliminary survey of each host, and that it requires the insertion of the ovipositor to finally decide whether the selection has been opportune or not.

The egg is invariably laid beneath the scale on the body of the host and not within the latter, the scaly covering alone being perforated. It may be placed either on the dorsal or ventral surface of the host, and was never found adhering to the scaly investment of the latter. Hosts which have recently undergone ecdysis are, in my experience, invariably selected; the exuvium is then lying free from the body, and the new cuticle is still thin and colourless. Such hosts appear white to the eye, and in no instance did the Chalcid select an example whose cuticle was becoming slightly brown and the hardening process setting in preparatory to ecdysis. The average size of the scale selected is about

.8-1 mm. long and .4 or .5 mm. broad. Hosts much smaller than this, however, though in other ways apparently suitable, were never selected. Most probably they would provide too little sustenance for the parasitic larva. Out of 324 scale insects of the size usually selected by the *Aphelinus* for the purposes of oviposition, 254 had turned brown, and no ova of the parasites were found in association with them. The remaining 70 insects were white with a thin and transparent cuticle, and of these 41 carried an egg or young larva of the parasite. The above observations agree in essential points with those of Quayle (1910, p. 399) made upon *Aphelinus diaspidis* How., a common parasite of the red or orange scale (*Chrysomphalus aurantii* Mask.) in America.

Marchal (1909, p. 1223) has made some interesting observations on the egg-laying and other habits of *A. mytilaspidis*, the host in this instance being another species of Coccid, viz., *Aspidiotus ostreæformis*. He states that he has seen this Chalcid pierce the same *Aspidiotus* eight times, and each time apply its head to the wound to lick the liquid that issued. "It is very certain," he adds, "that each thrust of the ovipositor does not correspond to the deposition of an egg." Howard (1910, p. 257) summarises the evidence of four different observations, upon four different species of parasites and hosts, dealing with this curious habit of Chalcids feeding at puncture holes made by the ovipositor. He suggests that it seems more probable that the habit will be found to be widespread. With reference to Howard's suggestion, I kept watch over a number of females of the *Aphelinus* and observed the curious trait described by Marchal. One example pierced an individual scale six times, and after each act applied its mouth-parts to the perforations. Whether the body of the host was actually pierced, and it imbibed any liquid or not, I am unable to say, as the attitude assumed by the insect precluded exact observation on this point. This act was noted in two other specimens, and it is noteworthy that all three were freshly

emerged individuals and had probably not previously taken any nutriment. I may add that these same three insects subsequently laid several eggs, and did not afterwards repeat the act just described during the time I had them under observation. Quayle (1910, p. 400), in his account of *Aphelinus diaspidis*, mentions that it was only on two or three occasions that he observed any indication of this habit. He remarks that it was not certain whether the Chalcids fed at the puncture hole or sealed it, and at any rate it cannot be counted a common habit with that species. An apparently similar habit has been observed by Newstead (1903, pp. 66 and 251) in a Chalcid of the genus *Blastothrix*, probably *B. sericea*, which lays its eggs in *Pulvinaria vitis*, var. *ribesiæ*. This author remarks that when a suitable host was found "the parasite turned its head towards the anterior extremity of the Coccid and, resting with all its feet upon the body of the latter, inserted its ovipositor into the centre of the thoracic area; it then slowly moved its abdomen up and down, and apparently laid its eggs in the puncture; the parasite then withdrew its ovipositor, and, turning round abruptly, feeling its way again with its antennæ, seized with its jaws the lips of the wound made by the ovipositor, and distinctly closed them upon it and apparently pressed the edges together; finally it passed the palpi over the wound, and then left the coccid to its fate. I subsequently saw the process of ovipositing repeated by three individuals, each one acting precisely the same as the first."

THE EGG.

The eggs vary in size from $\cdot 13$ to $\cdot 15$ mm. in length and from $\cdot 06$ to $\cdot 11$ mm. in diameter. They are oval in shape (Pl. 20, fig. 21) and pale yellowish white in colour. The surface of the chorion is smooth and glistening, and at one pole of the egg is a somewhat shrivelled hooked process. In the laboratory, the average time taken before hatching by eggs laid by females of the first generation, was nine to

eleven days. When viewed by means of transmitted light the chorion is sufficiently transparent to allow of the inner contents being clearly seen. Two days or so before hatching the egg swells slightly in size, and the young larva can be clearly seen within. Its mandibles were observed occasionally undergoing movement, and the main lateral tracheal trunks, together with the spiracles, are visible under a high power magnification.

THE NEWLY HATCHED LARVA.

The larva on emergence from the ovum is nearly spherical in form when viewed from above, and is somewhat flattened in the dorso-ventral plane. In size it averages .17 mm. in length and .15 mm. in greatest breadth. It is pale yellowish in colour and is clearly divisible into a head region and thirteen segments (Pl. 20, fig. 20). The mid-gut (*m. g.*) contains a considerable amount of residual food yolk and is clearly discernible as a dark central area. Eight pairs of spiracles are present, and they are situated on the same segments as in the fully developed larva. The tracheal system consists of a main lateral trunk running down either side of the body (*m. t.*), an anterior and posterior commissure (*a. c.* and *p. c.*), and a transverse tracheal branch (*t. t.*) leading from each spiracle to the main lateral trunk of its side. None of the smaller tracheal branches of the older larvæ were to be observed. The mandibles are very similar in form to those of the fully grown larva, but are correspondingly smaller in size. The fat-body is very little developed, and consequently the young larva is more transparent than the later stages. In other essential details of structure it differs very little from the older larvæ, with the exception of the absence of the conspicuous imaginal discs.

THE FULLY GROWN LARVA.

Coloration.—Fully developed larvæ are uniformly bright lemon yellow with a faint greenish tinge in some

specimens. The mid-gut or stomach shows through the body-wall as a conspicuous oval sac, and appears pale brown in virtue of the ingested food contents. The cuticle is colourless, smooth, and shining.

Size.—From measurements made on a dozen fully grown larvæ, the latter were found to vary in length from ·56 to ·96 mm., and from ·48 to ·60 in maximum breadth. Their average size is ·8 mm. long and ·5 mm. broad.

External Morphology.—In shape the larva is broadly oval and slightly flattened; when fully extended it tapers slightly towards both extremities, but more especially at the posterior end. It is divisible into a head and twelve complete segments, with no differentiation into thoracic and abdominal regions (Pl. 20, figs. 17 and 18). There is a diversity of statement with regard to the number of segments in the larvæ of *Aphelinus*. Quayle (1910, p. 398) states that in *A. diaspidis* How. there are "fourteen indistinct segments including the button at the tip." In *A. mytilaspidis*, Howard (1881, p. 354) states that the dividing lines of twelve segments can be observed with some difficulty. From observations made on sixteen larvæ I find twelve segments to be constant in every instance. The first two segments are the broadest, the anal segment is the smallest, and the intervening somites are sub-equal. The head is much modified, antennæ and eyes are absent, and its most prominent organs are the mandibles (Pl. 20, fig. 19). These structures are minute objects measuring ·01 mm. from apex to base, and approximately the same in breadth across the widest part. They are of the usual form common to Chalcid larvæ and can be withdrawn within the pharynx. They are adapted solely for piercing the eggs or tissues of the host and maintaining a hold thereon. The mouth (*mo.*) is surrounded by a somewhat thickened chitinous rim, and along the upper portion of the latter is a row of six minute papillæ (Pl. 20, fig. 19). There are also a series of five larger papillæ (*pp.*) on either side of the antero-ventral region of the head.

There are eight pairs of spiracles (Pl. 20, fig. 18), the

first pair being situated on the anterior border of the first segment. The second segment contains no spiracles, but a pair is present on each of the seven succeeding segments. The spiracles are exceedingly simple in structure, being merely funnel-like depressions of the cuticle receiving the tracheæ at their inner and narrower ends.

Internal Morphology.—As it is not my object to deal with this species anatomically, only a few features of its internal structure will be referred to. The tracheal system is well developed, but its tubes are of extremely narrow diameter. A pair of main lateral tracheal trunks (*m. t.* in Pl. 20, fig. 18) are present, and they differ but little in their calibre from that of their principal branches. The two main trunks are united with one another by a transverse commissure (*a. c.*) situated in the first segment of the larva, and by means of a posterior commissure (*p. c.*) situated in or near the ninth segment. From each spiracle a relatively long and narrow trachea (*t. t.*) passes to the main trunk of its side, and at the point of junction an inwardly directed branch (*v. t.*) passes to the ventral region of its segment. Arising from the branch *v. t.*, or in some cases at the point where it joins the main lateral trunk, is a second branch (*d. t.*), which is mainly dorsal in its distribution. The head receives its tracheal supply by means of a pair of branches originating from the anterior transverse commissure.

The digestive system is extremely simple (Pl. 20, fig. 18). The mouth leads into the pharynx, which passes into a very short œsophagus. At the point where the latter joins the mid-gut or stomach a simple valve is present. The mid-gut is an extensive ovoid sac occupying a large proportion of the hæmocœlic space. The hind-gut passes directly to the posterior extremity of the body, and at its commencement are placed two Malpighian tubes. A pair of salivary glands are present, and their two ducts converge and unite in the first segment, and the main duct so formed passes along the mid-ventral line of the head, beneath the œsophagus, to open on the floor of the mouth (Pl. 20, fig. 19).

The nervous system consists of a brain or supra-oesophageal ganglion, an infra-oesophageal ganglion, and a ventral nerve cord. The latter is a relatively thick linear structure, and exhibits no obvious differentiation into ganglia and connectives.

The fat-body (Pl. 20, fig. 18) is very extensively developed. It is markedly lobulated, and occupies almost the whole of the hæmocœlic cavity between the body-wall and the alimentary canal. It contains a large number of bright yellow globules apparently of a fatty nature, and it is to their presence that the yellow coloration of the larva is mainly due.

THE PUPA.

Coloration.—Dorsally the pupa is lemon colour with a slight greenish tinge. The anterior portion of the head, the frontal margin of the thorax, and the fore-wings are smoky. Ventrally the head, antennæ, wings, thoracic sterna, and legs are smoky, and also the developing abdominal plates. The remainder of the pupa is lemon yellow. As development proceeds the smoky coloration extends and deepens to black.

Size.—In length it measures 1 mm. and .48 mm. in greatest breadth. Owing to being greatly flattened in the dorso-ventral plane the distance between the upper and lower surfaces is reduced to .2 mm.

Morphology.—On its dorsal aspect the pupa exhibits but few distinguishing features. It is divisible into head, thorax, and abdomen; the numerical proportions in length of these regions are respectively as 2:3:7. The only head appendages visible are the antennal sheaths, which project slightly laterally. The thorax consists of but a single segment, and exhibits none of the subdivisions seen in the perfect insect. The sheaths of the fore-wings extend backwards to the second abdominal segment, but only the base of the sheaths of the hind-wings are visible. The abdomen is divisible into seven segments, whose limits can only be made with difficulty.

Viewed from the ventral aspect, the following features are most noticeable (Pl. 20, fig. 16). The antennal sheaths (*an. s.*) are elbowed and two-jointed; they extend on to the thorax and lie between the proximal portions of the first two pairs of legs, separating the latter from each other. The bases of the antennal sheaths are placed widely apart, the internal being equal to about half the width of the head. The mandibular sheaths (*md. s.*) lie between the bases of the sheaths of the antennæ, on the same level with the latter. The maxillary sheaths (*mx. s.*) are in contact with each other basally and diverge in a V-shaped manner. Each is two-jointed, the distal joint being very narrow and truncated. The labial sheath (*lm. s.*) lies in the space between the diverging sheaths of the maxillæ and is bounded by the latter. It is subtriangular in form, and in contact with it is a pair of very small distal sheaths which enclose the developing labial palpi.

In the thorax the sterna are very conspicuous as a pair of large bilobed plates occupying the greater part of the mid-ventral area. The sheaths of the fore-legs (*l.₁ s.*) are single-jointed, and their basal attachment is situated very far forwards. The sheaths of the middle pair of legs (*l.₂ s.*) are, in most specimens, two-jointed, and a prominent inward projection encloses the long tibial spine of the adult. The sheaths of the hind-legs (*l.₃ s.*) are very elongated and reach backwards to the penultimate segment of the abdomen. The proximal half of each sheath is entirely concealed by the sheath of the fore-wing of its side. The sheaths of the fore-wings (*f. w.s.*) extend posteriorly as far as the frontal border of the second abdominal segment, almost entirely concealing those of the hind-wings (*h. w.s.*), only the apices of the latter being visible. The abdomen does not offer any special characters for description; the most conspicuous feature is the developing ovipositor in the case of the female and the penis in the male.

The foregoing description refers to pupæ of the first generation; those of the second generation are markedly

smaller in size, averaging .64 to .7 mm. in length and .28 to .31 mm. in maximum breadth, but do not differ in any other respect.

LIFE-HISTORY.

Material and Methods.—In order to ascertain the distribution of the Chalcid and the extent of its parasitism, branches of apple infected with the mussel scale were obtained from various parts of England. These were cut up into convenient lengths and placed in breeding-cages. Wherever desirable, the cut ends of the branches and twigs were sealed up with wax. It is obvious that this method is only effective from about the end of September until the commencement of the following May, when the host is in the egg stage and is not dependent upon living plant tissues for its sustenance.

In obtaining an adequate supply of the mussel scale from as many localities as possible I have been generously aided by Mr. J. C. F. Fryer, Entomologist to the Board of Agriculture. Further material was also sent by Messrs. P. Hedworth Foulkes, A. S. Horne, W. Laurance, W. H. Nield, A. G. L. Rogers, J. T. Wadsworth, C. B. Williams, B. L. Wolf, and others, to all of whom my thanks are due. Mr. A. Calderbank, of the Holmes Chapel Agricultural College, has also aided me in various ways.

In order to rear and breed out the *Aphelinus* under as nearly as circumstances allow to natural conditions an open insectary was utilised (Text-fig. 1). This structure was erected in the Manchester University Biological Experiment Ground at Fallowfield. It is constructed of wood, with a galvanised corrugated iron sloping roof. Two glass skylights were let into the latter in order to admit a certain amount of light from above. It measures 20 ft. long, 9 ft. wide, and 10 ft. high to the centre of the ridge of the roof. Apart from the framework, the walls and door of the insectary are entirely open to the weather, only being protected by wire rabbit netting of large mesh.

Twenty-three breeding cages were utilised. Each cage was constructed in the form of a tall wooden box, measuring 39·5 cm. by 23 cm. and 73·5 cm. high (Text-fig. 2). For the purpose

TEXT-FIG. 1.¹



View of the interior of insectary; on the tabling to the left-hand side is seen a row of breeding cages used in rearing *Aphelinus mytilaspidis*.

of attracting the insects towards the light and collecting them as they emerged, ten round holes were made in its front wall, and into each of these apertures a glass tube, 10 cm. long and

¹ I am indebted to Mr. J. T. Wadsworth for taking the photographs reproduced in Text-figs. 1-5.

1.6 cm. in diameter, was inserted. Each cage was closed by a closely-fitting sheet of glass resting on an inner ledge,

TEXT-FIG. 2.



A breeding cage used in rearing *Aphelinus mytilaspidis*.
About one eleventh actual size.

situated just below the opening of the box, and light was excluded by means of a wooden lid. The cages were elevated from the surface of the tabling upon which they stood by means of wooden squares, 1.5 cm. in thickness. In the middle of the floor of each box an aperture, 6.25 cm. square, was cut;

this was closed by a piece of very fine bolting silk whose meshes averaged .06 mm. across. By this means a certain amount of air was admitted into the interior of the cage, but at the same time only a negligible amount of light could enter, and the escape of any parasites was effectively precluded. By the time the last insect had emerged in each cage, a small amount of débris had accumulated on the floor of the latter. This was collected and examined under a binocular microscope for any dead Chalcids it might contain. From twenty-three cages it was found that only thirteen *A. mytilaspidis* failed to find their way into the glass tubes—a much lower proportion than had been anticipated.

In order to carry out field observations, and to ensure an adequate supply of material at every stage for laboratory work, three cages were erected over selected apple trees on the lands belonging to the Agricultural College at Holmes Chapel (Cheshire). These were constructed of a wooden framework, and across their sides and roof was stretched wire rabbit netting, in order to exclude tits and other birds which prey upon Coccidæ. Two of the cages were erected in a tolerably open though sheltered situation (Text-fig. 3), and the remaining cage was constructed against a warm south wall (Text-fig. 4), which is a situation specially favoured by scale insects.

On account of the extensive use of nicotine spraying very few of the trees on the lands of the Holmes Chapel Agricultural College were found to be attacked by the mussel scale. For the purpose of following the life-history of the parasite, four perfectly clean young trees were infected on April 30th and May 21st, 1914, with parasitised material obtained from Aspley Guise (Beds.) and Newport (Salop). The host rapidly spread over its food plants, especially in the case of the trees shown in Text-fig. 4, and the parasite was found to have thoroughly established itself. It maintained its existence through three generations, after which no further observations were conducted.

The First Generation.—The first generation of the

TEXT-FIG. 3.



Cage at Holmes Chapel (Cheshire) used for screening apple trees bearing the mussel scale and its parasite. It measures 5 ft. 6 in. square and 7 ft. in height.

TEXT-FIG. 4.



Cage at Holmes Chapel (Cheshire) erected against a south wall over apple trees bearing the mussel scale and its parasite. The cage measures 4 ft. 6 in. long, 7 ft. high, and 1 ft. deep.

Chalcid of each year are derived from larvæ which have passed through the winter beneath fully-grown mussel scales. When the time for emergence arrives, the parasite cuts an

TEXT-FIG. 5.



A group of mussel scales (*Lepidosaphes ulmi*) in situ on a branch of apple. About half of the scales show emergence holes made by *Aphelinus mytilaspidis* (first generation).
× 8.

aperture in the scale by means of its mandibles, and thereby effects its escape into the outer world (Text-fig. 5). These emergence holes can be seen with the unaided eye, and are seldom totally absent from any colony of the mussel scale.

Their position on the scale varies, and is dependent upon whether the pupa of the parasite lay with its head directed towards the anterior or posterior extremity of the scale. In those cases where the pupal head was directed anteriorly, the emerging Chalcid usually made the hole about the middle or nearer to the anterior end of the scale. On the other hand, if the pupa lay with its head directed towards the broad end of the scale, the emergence hole was situated nearer that extremity of its host.

The first generation consists almost entirely of females. Out of over 700 examples reared from various localities, only four were males. These four specimens were bred out singly from four different localities on the following dates during the year 1914: Aspley Guise (Beds.), July 2nd; Cobham (Surrey), August 11th; Oxshott (Surrey), June 4th; Newport (Salop), July 25th. Records were kept of the approximate dates of emergence of 681 females reared from material obtained from fifteen different localities. They commenced appearing in the breeding cages from May 24th, and continued emerging until August 9th; over 79 per cent., however, appeared between June 21st and July 8th. The primary factors which cause the times of emergence to vary between such wide limits I have been unable to definitely discover. The prevailing climatic conditions certainly exercise a marked influence, especially upon the pupa, and a good deal also depends upon the time when the larvæ became full-fed, some of the latter pupating very much later than others. Reproduction takes place almost entirely by means of parthenogenesis. In Lancashire and Cheshire egg-laying occurs principally during the first three weeks of July. On hatching the young larvæ attach themselves to the body of their hosts, and remain as external parasites during the whole of that period of their existence. They maintain a firm hold on the cuticle of the host by means of their sharp-pointed mandibles, and gradually imbibe its juices until they become full-fed. The nutriment appears to be entirely absorbed by means of a combined sucking and pumping action of the pharynx and

cesophagus. Parasitised hosts undergo no further growth or ecdysis, and their scaly investment remains in a condition similar to that which obtained when the Chalcid larvæ emerged from the egg. A host harbouring the larvæ of the parasite may be frequently recognised by the fact that the latter shows through the partially developed scale as a faint yellowish object. The *Lepidosaphes* continues to imbibe nourishment from the tissues of the food-plant, but it invariably dies as the result of the parasitism and, as a rule, long before it has become capable of depositing any ova. Although I have examined considerably over 1000 parasitic larvæ belonging to the two generations, I have never found more than one parasite in association with an individual host. In America, Le Baron states that he never met with more than two.

In working out the life-history of this species a practical difficulty confronts the observer, inasmuch as it is not possible to study the same individual specimen continuously through its life-cycle. This is sufficiently obvious when taking into account the fact that the host must be exposed by the removal of its scale. Also, in order to ascertain when the young parasitic larva has emerged from the egg a microscopic examination is necessary owing to its minute size. This entails cutting off the twig of the tree bearing it, and it has not been possible to keep the twig sufficiently fresh to enable the host to remain alive the requisite time necessary to support its parasite until the latter pupates. By examining a large number of specimens, however, a very close approximation to the truth can be arrived at.

The period spent in the larval condition varies from about twenty-three days to rather more than one month. In Cheshire the first young larvæ were observed on July 15th, 1915, and the earliest date pupæ were noted was August 12th. When fully fed the larva discharges the contents of the alimentary canal, no excreta having been voided previously. It then passes into a pro-nymph (or semi-pupa), which subsequently transforms into the fully formed pupa. The larva constructs nothing of the nature of a cocoon, and the

pupa lies on its ventral surface beneath the host scale with its head directed towards the broad end of the latter. The period passed in the pupal stage averages from about twenty-one to thirty days, though it may be prolonged to as long as two months. The latter duration happened in the case of larvæ which were late in pupating, and a long period of cold and wet weather prevailed afterwards.

The Second Generation.—The perfect insects of the second generation usually make their exit into the outer world by pushing their way out from beneath the host scales. In some cases, however, the scale adheres with greater firmness to its resting surface, and under these circumstances the parasite effects its emergence by gnawing away with its mandibles a portion of the hinder end of the scale. The dorsal emergence holes seen in Text-fig. 5, with one exception, were not observed in connection with the second generation of the *Aphelinus*. In the case of material obtained from Kew (Surrey) the mussel scale was in a more advanced stage of development than in Lancashire and Cheshire. Here and there among the living mussel scales were, apparently fresh, empty scales perforated with the dorsal emergence holes of the parasite. Some of the perforated scales overlaid living scale insects, and I believe, in this particular instance, there was no doubt that all the hosts, both parasitised and unparasitised, were derived from the present year's parents.

It has been mentioned (p. 232) that the winged insects of this generation are markedly smaller than those of the preceding one, and it appears probable that the difference in size is mainly determined by the food supply. The first generation of adults spent some eight months in the larval condition, and subsisted upon the greater food supply afforded by the fully-grown adult hosts; whereas in the case of the second generation only about three weeks or one month is spent in the larval stage, and each larva has for its sustenance a single immature host averaging 1 mm. long. The latter when parasitised undergoes no further growth,

and is only able to support itself and its parasite until the latter is about to pupate.

The average duration of life of the adult parasites is apparently very short, for when freshly emerged examples were placed upon twigs, which were kept moistened in a cool room, they failed to survive longer than five days. In Lancashire and Cheshire the first adult parasite appeared in the breeding-cages on August 26th in 1914 and August 31st in 1915; but in warm, sunny situations they commence to emerge at least one week earlier than the first-mentioned date. From the south of England (Plymouth) I have obtained evidence indicating that they commence appearing during the first week in August. The period of emergence covers about one month, and the latest date upon which any specimens were bred out was September 17th. A large number of examples failed to emerge at all, having died in the pupal stage. Altogether only fifty-one specimens were bred out from three localities, situated respectively in Devon, Lancashire, and Cheshire. Of these six were males, which appeared between August 26th and September 10th, 1914, from mussel scale obtained from Plymouth. Copulation was not observed, and the females are apparently parthenogenetic, as in the first generation.

The eggs of the parasite are laid on the fully grown, sexually mature hosts at the time when the latter have commenced to deposit their own ova, or a few days previous to that event. The scaly covering, only, is perforated in the act of oviposition, and the egg is placed on the body of the host, most usually on the dorsal surface. The young larva behaves, at any rate during the first period of its life, in a manner similar to that described for the first generation, and the nutriment afforded by the host is sufficient to support it until it has attained its full size towards the end of October or the beginning of November. The host by this time becomes completely exhausted and soon dies.

The parasitic larva, when disturbed or irritated, is capable of some slight movement, both the head and anal extremities

admitting of a certain amount of protrusion and retraction. At times, also, a kind of slow "peristaltic" movement passes over the body from one end to the other, and is accompanied occasionally by a slight rotatory or twisting motion first to one side and then back again to the other. Under ordinary circumstances, however, the fully grown larva appears practically motionless, with the head end of the body somewhat retracted into the region which immediately follows.

During the winter and a portion of the following spring the larva hibernates beneath the covering scale of its dead host. Before pupation it discharges the contents of its digestive system as a series of dark brown, or black, fusiform bodies, very definite in both shape and size. They vary up to about eighteen in number, and average .14 mm. \times .05 mm. in dimensions. They are found accompanying the pupa also, but the larvæ were never observed to evacuate the contents of the alimentary canal during earlier stages in their life. Very similar bodies have been noted by Quayle (1910, p. 399) in *Aphelinus diaspidis* How., and the habit of a single evacuation of excrementous material, at the end of the larval stage, is of frequent occurrence among the various groups of parasitic Hymenoptera.

A pre-pupal or pro-nymph stage (semi-pupa of Packard) follows that of the larva, and is of short duration. The insect then measures .73 to about .83 mm. in length, and .35 mm. wide across the thoracic region. This stage is intermediate between that of the larva and pupa, and has been described and figured in the Chalcididæ by Bugnion (1890) for *Encyrtus fuscicollis* and by Miss Embleton (1904) for *Comys infelix*. It is also known in numerous other Hymenoptera. In the present species it is pale yellowish white in colour and enclosed in a definite cuticle; the appendages of the imago are already clearly defined, although in a less advanced condition than occurs in the pupa. The head is clearly marked off from the rest of the insect, the thorax less perfectly so, and there are only faint indications of segmentation in the abdomen.

The earliest date upon which the pupa was observed was May 10th, 1914, while some of the larvæ remained as late as June 30th before assuming the pre-pupal condition. On an average twenty-one to thirty days are spent in the pupa, though damp and cold weather may prolong the period for upwards of two months. No cocoon is constructed, and the pupa lies on its back beneath the scale with its head directed, in some cases anteriorly and in others posteriorly, with reference to its host.

While following up the life-history of the second generation the question arose as to whether the larvæ, after the death of the host, became predaceous upon the eggs of the latter. Some 365 parasitised hosts were examined at various periods, and the number of eggs found in association with them was recorded in each instance. The average was found to be 3.4 eggs per host (Table I). Further details are given in Table II, and it will be noted that in 109 of the hosts no

TABLE I.—Based on an Examination of 365 Parasitised Females of *Lepidosaphes ulmi*, indicating the Average Number of Ova which were found to be present.

Locality.	Dates of examination.	Parasitised hosts examined.	Number of ova present.	Average number of ova per female.
Oxshott (Surrey)	Feb. 24th-Mar. 1st, 1914	121	356	2.9
Bere Alston (Devon)	Dec. 1st, 1914	23	56	2.4
Holmes Chapel (Cheshire)	Oct. 28th-Nov. 11th, 1914	188	705	3.7
Holmes Chapel (Cheshire)	Feb. 17th, 1915	33	155	4.7
Totals		365	1272	3.4

NOTE.—The two counts made on material from Holmes Chapel were from two different branches of the same tree.

TABLE II.—Detailed Analysis of Preceding Table, showing the Number of Eggs present in each of the 365 Parasitised Hosts.

Locality.	Dates of observations.	Hosts examined.	Number of eggs present.																
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Over 15
Oxshott (Surrey)	Feb. 24th— Mar. 1st, 1914	121	42	6	20	15	14	6	5	3	3	1	2	1	—	—	2	—	1
Bere Alston (Devon)	Dec. 1st, 1914	23	10	2	3	4	—	1	1	1	1	—	—	—	—	—	—	—	—
Holmes Chapel (Cheshire)	Oct. 28th—Nov. 11th, 1914	188	52	15	27	17	19	15	15	7	10	3	8	2	—	3	2	—	8
Holmes Chapel (Cheshire)	Feb. 17th, 1915	33	5	4	6	2	4	2	1	1	1	2	1	1	—	1	—	1	1
Totals	365	109	27	56	38	37	24	14	15	9	10	5	1	4	2	3	1	10

eggs were present, 56 hosts contained two eggs each, while only 74 hosts, or 20·2 per cent., contained more than five eggs apiece, the approximate reduction in the number of eggs, as compared with the average number laid by unparasitised hosts, being 91 per cent. This essential difference in the rate of increase appears to be due to either (a) the destruction of ova by the larval parasite, or (b) to the parasite inhibiting the egg-laying process, or (c) to a combination of both these factors.

With regard to the possibility of the ova being destroyed by the parasite, I may add that I have never observed the larvæ of *mytilaspidis* actually preying upon the eggs of its host, notwithstanding having examined over 500 parasitised scale insects for the purpose. On the other hand, Fitch (1885), Le Baron (1870), Howard (1881), the Duke of Bedford and Pickering (1906 and 1908), and others have published statements to the effect that the larvæ feed upon the eggs of their host. It is true that, after the destruction of the female mussel scale, the larval parasite is usually to be found in close proximity to the eggs, and frequently its head end is in contact with the latter, although on other occasions its posterior extremity was observed to be nearest the eggs. Furthermore, shrivelled eggs are of frequent occurrence beneath parasitised scales, suggesting that the larvæ of *mytilaspidis* are the causative agents. In this connection, however, it is noteworthy that shrivelled eggs and empty shells are also often to be met with among the healthy eggs of unparasitised scale insects, and their presence is not necessarily associated with that of the parasite. Several writers have recorded Acari as attacking the eggs of the mussel scale. Not infrequently, during the present investigations, they were found beneath scales containing both shrivelled and empty ova, and were responsible for the destruction of a certain number of them. In other cases no assignable cause could be discovered to account for the destruction of the eggs. If the larvæ of *mytilaspidis* are the primary agents entailing their destruction, it would be reasonable to expect

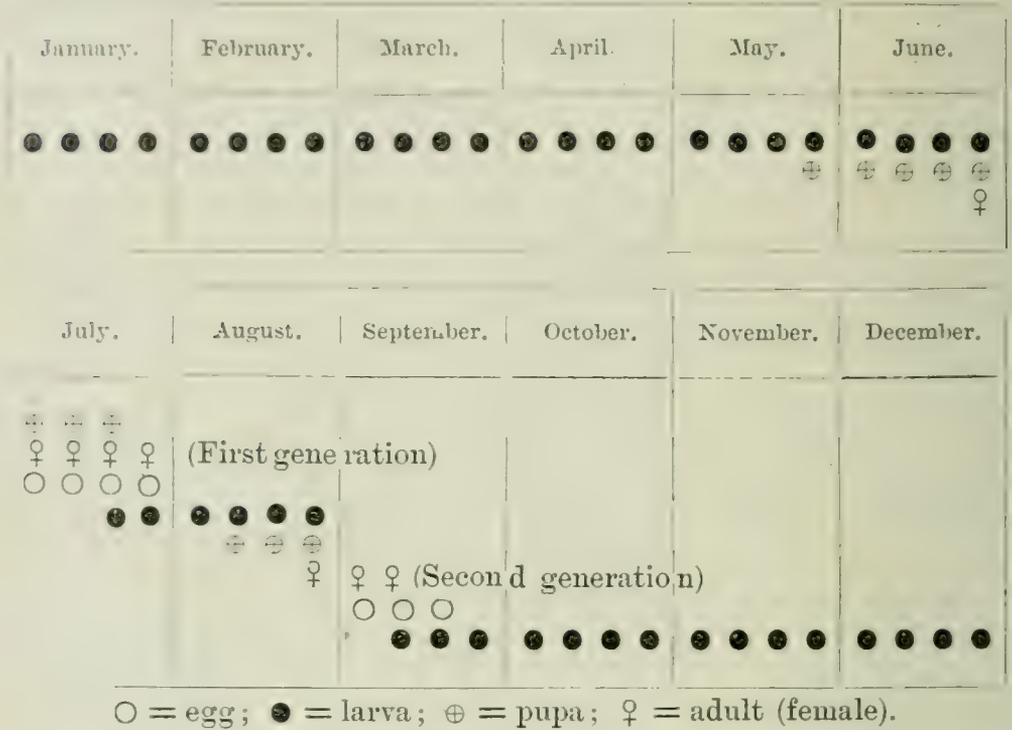
to find eggs beneath parasitised scales to become gradually reduced in numbers as the season progresses. On the contrary, if reference be made to Tables II and III it will be seen that there is no evidence in favour of such a contention.

The evidence supporting the possibility of the reduction in the number of eggs being due to the parasite exercising an inhibitory effect upon oviposition is much more conclusive. Firstly, the food supply afforded by the adult host is sufficient to enable the larval parasite to attain its full size by the end of October or beginning of November. A series of measurements made upon larvæ the following spring indicated that no further increase in their dimensions appear to take place. Secondly, it is noteworthy that over 27 per cent. of 188 parasitised hosts examined between October 28th and November 11th, 1914 (Table II), sheltered fully grown larvæ of the *Aphelinus*, but no eggs were present, and the hosts were dead in every case. Twenty of these larvæ were kept under laboratory conditions, and nine of them pupated the following spring, while the remaining eleven were attacked by fungi and died. It appears, therefore, that the eggs are not essential for the nutrition of the parasite. Also, if the latter does not inhibit oviposition, we have to assume that each parasite destroyed in these instances an average of thirty-seven eggs during less than one eighth of the span of its larval existence, which is beyond the bounds of probability. Furthermore, no shrivelled remains of eggs were found beneath the scales in any instance, which might indicate that such destruction had taken place.

It may be claimed, therefore, that one of the effects of the parasitism is to inhibit oviposition, and this fact is the primary cause of the essential reduction in the number of eggs found in association with affected hosts. It is not unlikely that the parasitic larvæ may devour a small number of eggs, but quite an inadequate quantity to bring about the observed reduction in their numbers.

Summary of the Life-History.—The complete life-history, based upon observations conducted in Lancashire

and Cheshire during the years 1913-15, may be summarised in a symbolical form as follows :



In this diagram it will be noted that the signs are grouped in fours, representing four weeks to each month. As an example, it will be seen that the earliest date the adults of the first generation are likely to occur is the last week in June, and they are to be met with up to the last week in July, and so on.

ECONOMIC STATUS AS A PARASITE.

The earliest writer, with whom I am acquainted, dealing with the economic status of the Aphelininæ is Le Baron (1870, p. 362). This observer states that *A. mytilaspidis* was destroying in Illinois in 1870 twice as many of its host as all other agencies together. During the winter of 1871-72 he transported half a dozen scale infested twigs, known to be parasitised, from Geneva (Illinois) to Galena, where the parasite seemed to be lacking. These branches were fastened to infected trees in three different orchards. At the end of

the season evidence was obtained which tended to show that the parasites had established themselves. No exact details of the experiment were given, and it cannot be regarded as one of very much value. Riley (1873, p. 90) adds: "To colonise the parasite all that is necessary is to tie such parasitised twigs on to trees which it is required to protect, and the microscopic flies will issue at the proper season and carry on their good work." In Canada, Fletcher (1904, p. 188) states that the parasite is sometimes so abundant that it destroys more than half of the scale insects present, and that it has occurred in all parts of Canada, but never seems to remain long in any district. Howard (1907, p. 69) states that the Aphelininæ are by far the most important parasites of the Diaspinæ (to which the mussel scale belongs), and that in the twelve years which have elapsed since the publication of his 'Revision' their economic importance has become even more evident.

The Duke of Bedford and Pickering (1906, p. 7; 1908, p. 35) mention that larvæ of this insect are often found in large numbers under the scales, feeding on the eggs. They remark that they are very localised, and that an abundance of the food supply does not appear to ensure the spread and multiplication of the Chalcid, which may be absent on one branch and plentiful on another. Quaintance and Sasser remark (1910, p. 6) that parasitic Hymenoptera are often efficient enemies of the mussel scale, and in some localities they apparently hold it in check. They quote seven species of parasites of this insect, but give no details with regard to their relative efficiency. Sherman (1913, p. 15) mentions that in the State of North Carolina it is known that the parasites of *Lepidosaphes ulmi* are very actively at work, and that in some cases at least they are a decided factor in holding that insect in check. In a foot-note he adds that specimens of parasites bred from infested twigs and submitted to Washington proved to be exclusively *A. mytilaspidis*. Cæsar (1914, p. 30) states that in some localities in Ontario as high as 50 per cent. or more of the scales have emergence

holes of this parasite, and though it does not destroy all the eggs beneath a scale, it must be of considerable aid in keeping down the rate of increase. Additional references to its occurrence as a parasite are to be found, especially in North American literature, but as they do not contain any further information it is not necessary to deal with them here.

The value and efficiency of any given parasite from the economic standpoint depends upon numerous factors. The all-important test is afforded by ascertaining the extent by which it reduces the normal rate of increase of its host. In so far as I am aware, only two attempts have been made to apply this criterion in the case of *Aphelinus mytilaspidis*. Le Baron (1870, p. 362) states that from four twigs infested with mussel scale of that year, obtained from four different apple trees in two gardens remote from each other, he found the following:

Whole number of scales	330
Round holes made by <i>Chalcis</i> fly	116
Larvæ of the <i>Chalcis</i> under the scales	95
Ragged holes made by <i>Coccinellidæ</i>	7
Shrunken and discoloured eggs	81
Acari found under the scales	4
Scales containing eggs not damaged	27

It will be seen, therefore, the *Chalcis* (e. g. *Aphelinus mytilaspidis*) attacked 63·9 per cent. of the total number of the host.

From mussel scale collected from different localities in Kane and Du Page counties he obtained the following results:

Whole number of scales examined	844
Number destroyed by <i>Chalcids</i>	533
Destroyed by Acari and unknown causes	234
Scales containing more or less eggs	57

In this case it appears that *A. mytilaspidis* attacked 63·1 per cent. of the total number of scale insects.

Sherman (1913, pp. 16-17) gives a table showing that out of a total of 584 scales examined during March and April,

1913, from six different localities, 56 showed attacks by parasites, the greater number of which appeared to be *A. mytilaspidis*. This gives 9.6 per cent. as the average number killed by the Chalcid. The obvious criticism of the counts made by these two observers is that they deal with too small a number of the host to be regarded as being really representative. From experience gained during the past two years, I have been able to satisfy myself that estimates of parasitism based upon small numbers of counts are often misleading. This is owing to the fact that the parasite, in virtue of its limited powers of migration, is extremely localised, even with reference to a single branch of a tree. One small portion of a branch may be heavily parasitised and the remainder contain no parasites whatever.

The practice of searching for the emergence holes of the parasite is at once the readiest method, and the easiest to carry out in so far as the second generation is concerned. This method of estimation, however, is open to the objection that it is not usually possible to distinguish between the scales of the present and previous years, as the latter may adhere to the bark of the twigs for several successive seasons. Furthermore, unparasitised scales are liable to fall off more readily owing to the practice of the young larvæ, as they emerge, of pushing their way out from beneath the scales, thus loosening the latter in the process. It will, therefore be evident that the proportion of parasitised to unparasitised scales is liable to appear greater than is actually the case. The most reliable method is to confine the examination exclusively to living specimens, and to turn them over by the aid of a needle under a binocular microscope, recording at the same time the number which harbour the *Aphelinus* larvæ beneath them. This method was partially resorted to by Le Barou and Sherman, and has been adopted throughout the present investigation for both generations of the parasite. In the case of the first generation, material was obtained from fewer localities than had been anticipated. This was largely due to the inspectors of the Board of Agriculture being

occupied with additional duties owing to the European War, and were unable in consequence to give the necessary assistance in procuring it. Also, at this time of the year the trees are in fruit, which offers an obvious difficulty to the removal of branches therefrom. Scale infested branches, containing parasites of the first generation, were obtained during August, 1915, from three localities, and the average parasitism was found to be 4·1 per cent. The results obtained are tabulated as follows:

Locality.	Hosts examined.	Hosts parasitised.	Percentage of parasitism.
Kew Gardens (Surrey)	583	27	4·6 per cent.
West Didsbury (Lancashire)	1853	104	5·6 per cent.
Holmes Chapel (Cheshire)	1491	33	2·2 per cent.
Totals	3927	164	4·1 per cent. (Average parasitism).

In the case of the second generation an abundance of material was secured during the years 1914 and 1915, and the parasitism was found to vary from ·1 per cent. to 11·2 per cent. in different localities. The average parasitism, based upon an examination of 14,155 examples of the host, worked out at 3·2 per cent. (vide Table III), and in only three localities did it exceed 2·2 per cent.

The first generation of the *Aphelinus* attacks the mussel scale in the early stages of the latter. The result of the parasitism is complete, for the affected hosts invariably die in consequence. The attacks of the second generation of the parasite occur when the mussel scale is sexually mature and commencing to lay its eggs. As previously mentioned (p. 258), although each affected host is killed, an average of 3·4 of its eggs remain to help to maintain the species, and in this respect, therefore, the result of the parasitism is only

TABLE III.—Showing the number of *Lepidosaphes ulmi* attacked by *Aphelinus mytilaspidis*, based on material obtained from various parts of England.

Locality.	Dates of examination.	Hosts examined.	Hosts parasitised.	Percentage of parasitism.
Plymouth (Devon)	Oct. 23rd–26th, 1914	1080	3	·2 per cent.
Bere Alston (Devon)	Dec. 1st, 1913	1020	23	2·2 per cent.
Oxshott (Surrey)	Feb. 24th–Mar. 1st, 1915	1076	121	11·2 per cent.
Slough (Glos.)	May 30th, 1914	230	23	10 per cent.
Aspley Guise (Beds.)	April 29th–May 10th, 1914	2716	27	1 per cent.
Meatham (Leices.)	May 19th, 1914	1025	9	·3 per cent.
Newport (Salop.)	May 5th, 1914	1000	4	·4 per cent.
Northen Etchells (Ches.)	May 15th, 1914	122	1	·8 per cent.
Northenden (Ches.)	Oct. 23rd, 1914	380	5	1·3 per cent.
Holmes Chapel (Ches.)	Oct. 28th–Nov. 11th, 1914	2016	209	10·3 per cent.
Holmes Chapel (Ches.)	Nov. 6th–10th, 1914	2027	4	·2 per cent.
Holmes Chapel (Ches.)	Feb. 17th, 1915	1463	33	2·2 per cent.
Totals		14,155	462	3·2 per cent. (Average parasitism)

NOTE.—The Holmes Chapel material was obtained from three different localities situated less than two miles from each other.

partial and not complete. The effect of one year's parasitism on the natural rate of increase of the host can be more easily represented by taking a hypothetical instance than by any other method. According to the foregoing investigations, out of every 1000 examples of the mussel scale forty-one are destroyed by the larvæ of the first generation of the *Aphe-*

linus. The remaining 959 hosts become sexually mature and commence laying their eggs. They are then attacked to the extent of 3·2 per cent. of their numbers by the larvæ of the second generation of the parasite—in other words, thirty-one examples will be affected. Now, taking the actual number of eggs laid by each individual host as being 37·2 (vide p. 221), the number laid by 928 examples will be 34,522, while the thirty-one parasitised individuals will among them lay 105 eggs (vide p. 258). The total number of eggs laid by the hosts will, therefore, be 34,627. But 1000 unparasitised hosts lay on an average 37,200 eggs, so that the net result of a year's parasitism will be a reduction of 2573 in the number of eggs laid, or 7 per cent.

It is not unlikely that in very favourable seasons, more especially in the south of England, this parasite may be more abundant and entail a greater reduction in the rate of increase of its host. Even allowing its effectiveness to be increased by 100 per cent., its capability as an inhibiting agent would not justify special measures being taken to attempt its preservation and increase, since the net results of its parasitism would be far below those of the most effective insecticides. It is noteworthy that hyperparasites do not appear to be a factor entering into the economy of *A. mytilaspidis*. Not a single hyperparasite was reared during these investigations, and none have been recorded as enemies of this species. Four important factors appear to be responsible for the relatively low degree of efficiency of this parasite: firstly, its extremely limited powers of migration, which prevent it from becoming quickly disseminated in the event of any local increase in its numbers; secondly, its relatively low fecundity; thirdly, its marked susceptibility to the influence of unfavourable climatic conditions; fourthly, the fact that the effects of its second annual generation of parasitism are only partial and incomplete.

SUMMARY OF CONCLUSIONS.

- (1) *Aphelinus mytilaspidis* Le Baron, a Chalcid

belonging to the sub-family Aphelininæ, is the principal parasite of the mussel scale (*Lepidosaphes ulmi* L.) in England. Material obtained from various parts of the country indicates that it is generally distributed. Detailed descriptions are given of the insect in all its stages.

(2) The parasite passes through two generations in the year, and the adults consist almost entirely of females. Out of over 750 bred specimens only 10, or approximately 1 per cent., were males. Parthenogenesis is definitely proved to occur, and is probably the usual method of reproduction.

(3) The adult insects seldom resort to flight, and have extremely limited powers of migration. They are positively phototropic, but exhibit no marked response to geotropism.

(4) In the first generation the adults appear in greatest frequency between the third week in June and the middle of July. The female lays a single egg on the dorsal or ventral surface of the body of the immature host, only the scaly covering of the latter being perforated. The newly hatched larva closely resembles the fully grown stage in form, and during larval life the insect is an ectoparasite of its host. The second generation of adults mostly appear between the middle of August and the first week in September. They parasitise the sexually mature hosts, and the resulting larvæ hibernate through the winter, giving rise to the first generation of adults of the following year.

(5) The results of the first generation of parasitism are complete, the affected hosts invariably dying in consequence. In the second generation of parasitism the affected hosts usually deposit a small number of eggs before succumbing; its results, therefore, are partial and incomplete. The parasite exercises an inhibitory effect upon oviposition, the essential reduction in the number of eggs not being primarily due, as stated by previous observers, to their destruction by the *Aphelinus* larvæ.

(6) Assuming that every 1000 hosts lay on an average 37,200 eggs, the net results of a year's parasitism entails a reduction of about 2600 in the number of eggs laid, or 7 per

cent. The efficiency of the parasite, therefore, is far below that of the most effective insecticides. This is primarily due to four factors: (1) its extremely limited powers of migration; (2) its relatively low fecundity; (3) its marked susceptibility to the influence of unfavourable climatic conditions; (4) the effects of the second annual generation of parasitism being only partial and incomplete.

September, 1915.

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EXPLANATION OF PLATES 19 AND 20.

Illustrating Dr. A. D. Imms’ paper on “Observations on the Insect Parasites of some Coccidæ.—I: On *Aphelinus mytilaspidis* Le Baron, a Chalcid Parasite of the Mussel Scale (*Lepidosaphes ulmi* L.)”

PLATE 19.

[With the exception of Fig. 15, the drawings were made from the female imago.]

Fig. 1.—Adult female of *Aphelinus mytilaspidis* Le Baron. × 50.

Fig. 2.—First maxilla. × 533. *a.* Lobe of maxilla, possibly representing an undivided galea and lacinia. *b.* Basal portion, probably representing an undifferentiated cardo and stripes. *ma. p.* Maxillary palp.

Fig. 3.—Second maxillæ (labium) viewed from the dorsal or pharyngeal aspect. × 900. *l.* Ligula. *l. p.* Labial palp. *m.* Mentum.

Fig. 4.—Right mandible. × 650.

Fig. 5.—Left antenna viewed from the ventral surface. × 220. *a. s.* Socket of antenna. *b.* Basal joint. *p.* Pedicel. *s.* Scape.

Fig. 6.—Dorsal aspect of the thorax. $\times 180$. *f. v.* Base of the right fore-wing. *h. w.* Base of the right hind-wing. *i. t.* Propodeum. *mlm.* Mesoscutellum. *mn.* Metanotum. *mtn.* Mesoscutum. *p. n.* Pronotum. *pr.* Parapsides (right). *scp.* Scapula (left).

Fig. 7.—Submarginal vein of the left fore-wing. $\times 440$.

Fig. 8.—Submarginal vein of the left hind-wing. $\times 440$.

Fig. 9.—Right anterior leg. $\times 250$. *f.* Femur. *t. s.* Tibial spur (or spine).

Fig. 10.—Left middle leg. $\times 250$. *f.* Femur. *t. s.* Tibial spur (or spine).

Fig. 11.—Right posterior leg. $\times 250$. *f.* Femur. *t. s.* Tibial spur (or spine).

Fig. 12.—Ventral aspect of the ovipositor and associated parts. From a preparation stained with a 1 per cent. aqueous solution of fuchsin and mounted in glycerine. $\times 235$. *ap.* Appendage of right inner plate. *b. pl.₁* Basal portion of the median rib of the left inner plate. *c. sh.* Transverse chitinous support of the sheath of the ovipositor. *pl.₁* Inner plate (left). *pl.₂* Outer plate (left). *r.₁* Median rib of left inner plate. *r.₂* Median or basal portion of supporting ribs of the left outer plate. *sh.* Sheath of ovipositor. *sh.₁* Thickened rim or edge of the left arm of the sheath of the ovipositor. *s. pl.₁* Chitinised area of left inner plate, by means of which the latter articulates with the transverse support (*c. sh.*) of the ovipositor sheath. *sty.* Stylets. *sty.₁* Curved arm of the left stylet. *t. p.* Supporting plate.

Fig. 13.—Abdominal sensory plate of the left side. From a preparation stained with a 1 per cent. aqueous solution of fuchsin and mounted in Canada balsam. \times circa 900.

Fig. 14.—Apex of the sheath of the ovipositor seen from the right side. From a specimen mounted in Canada balsam. $\times 1000$.

Fig. 15.—Ventral aspect of the penis and associated parts. From a preparation stained with 1 per cent. aqueous solution of fuchsin and mounted in Canada balsam. $\times 680$. *b. p.* Basal portion. *c.* Clasper (left). *p.* Penis. *p. p.* Apical papillæ of the penis. *r. p.* Rod-like support (left). *v. p.* Ventral papilla (left).

PLATE 20.

[With the exception of fig. 23, the drawings were made from different stages in the first generation of the *Aphelinus*.]

Fig. 16.—Pupa (female) of *Aphelinus mytilaspidis*, seen from the ventral aspect. From a living specimen. $\times 95$. *an. s.* Antennal sheath. *f. w. s.* Sheath of fore-wing. *h. w. s.* Sheath of hind-wing.

l.₁s. Sheath of anterior leg. *l.₂s.* Sheath of middle leg. *l.₃s.* Sheath of posterior leg. *lm. s.* Sheath of labium. *md. s.* Sheath of mandible. *mx. s.* Sheath of 1st maxilla. *ov. pl.* Developing plates of ovipositor. *st. pl.* Developing plates of abdominal sterna.

Fig. 17.—A fully-grown larva, dorsal aspect. From a living specimen placed on a dark background and viewed with reflected light. The larva is seen extended to its fullest extent. $\times 50$.

Fig. 18.—A fully-grown larva, dorsal aspect. From a living specimen placed in normal salt solution and examined under a cover glass. Slight pressure has been applied to the cover glass, extending the larva to its fullest degree. The course of the fore and hind gut is represented by the double series of dotted lines. $\times 120$. *a. c.* Anterior tracheal commissure. *d. t.* Dorsal segmental tracheal branch. *f. b.* Fat body. *md.* Mandible. *m. g.* Mid-gut. *mo.* Mouth. *m. t.* Main lateral tracheal trunk. *p. c.* Posterior tracheal commissure. *sp.* Spiracle. *t. t.* Transverse segmental tracheal trunk leading to spiracle. *v. t.* Ventral segmental tracheal branch.

Fig. 19.—Ventral aspect of the head of a fully-grown larva. $\times 950$. *c. s.* Supporting skeleton of head. *mo.* Mouth. *md.* Mandible. *m. s. d.* Median salivary duct. *r. s. d.* Salivary duct from gland of the right side. *ph.* Pharynx. *pp.* Ventral cuticular papillæ.

Fig. 20.—A youngest stage larva, about an hour after emergence from the egg. From a living specimen mounted in salt solution and viewed under a cover glass. $\times 630$. *a. c.* Anterior tracheal commissure. *md.* Mandible. *m. g.* Mid-gut. *m. s. d.* Median salivary duct. *m. t.* Main lateral tracheal trunk. *p. c.* Posterior tracheal commissure. *sp.* Spiracle. *t. t.* Transverse segmental tracheal trunk leading to spiracle.

Fig. 21.—Ovum, about six hours after being laid by a parthenogenetic female. $\times 550$.

Fig. 22.—Right ovary of a parthenogenetic female insect. The latter had deposited eight ova, and the ovaries were then teased out in normal salt solution. The preparation was afterwards treated with acetic acid, but was unstained. $\times 225$. *e.₁, e.₂, e.₃* Different stages of developing ova. *e. c.* Egg chamber with young oocyte surrounded by follicular cells. *g.* Germarium. *n. c.* Nutritive chamber. *od.* Oviduct. *w. o.* Wall of ovariole.

Fig. 23.—Dorsal view of a young adult female *Lepidosaphes ulmi* parasitised by a larva of *Aphelinus mytilaspidis* (second generation). The latter is in an early stage of development, and measured .25 mm. in length. Drawn from the living objects, after removal of the scale investing the host. $\times 35$. *p.* Parasitic larva.

The Transition of Peritoneal Epithelial Cells
into Germ Cells in some Amphibia Anura,
especially in *Rana temporaria*.

By

J. Bronté Gatenby,
Exhibitioner of Jesus College, Oxford.

With Plates 21 and 22, and 5 Text-figures.

INTRODUCTION.

THE theory, that the germ cells are derived from the cœlomic epithelial cells, known as the germinal epithelium theory, was advanced by Waldeyer (1) in 1870. It has been believed by Kolliker, Balfour, Semon, Semper, and many others. At about the same period (1880) Nussbaum (2) advanced a rival theory that germ cells are not derived from the soma, but that they are early differentiated as segmentation products which do not take part in body formation, and do not give up their embryonic character.

Since the discovery of the migration of the primordial germ cells, Waldeyer's theory has lost favour with many embryologists. Beard writes (3): "For myself, in reviewing the actual facts of my observations, I most emphatically endorse the correctness of his (Nussbaum's) brilliant idea. There is no real evidence showing that germ cells are ever formed from any part of the embryo." In another part of his paper Beard makes the following statement: "The

transition stages from epithelial cell to germ cell, of which, among others, Semon speaks so confidently without figuring a single one, are in the skate only conspicuous by total absence."

At this juncture it should be pointed out that Beard mentions embryos only up to 42 mm. in length, and he draws none of his conclusions from adult or intermediate ovaries. Not only this, but the demonstration of the migration of germ cells during one short part of any animal's life does not prove that germ cells are never afterwards derived from soma cells.

For the Amphibia Miss King (4) has declared in a recent paper on *Bufo lentiginosus* that transition stages between germ cell and peritoneal cell do not exist. In this she is supported by Allen (5). On the other hand, Semon (6), Bouin (7), Dustin (8), Kuschakewitsch (9), and Champy (10), are all supporters of the germinal epithelium theory. Indeed, it may fairly be stated that most workers on Amphibian germ cells are believers in Waldeyer's theory.

Beard says in his paper: "The change from epithelial cell to germ cell, though asserted times without number, has never really been depicted, and in all probability has never actually been observed. Indeed, it does not exist."

I firmly believe that the peritoneum does produce germ cells, and in this paper I have endeavoured to make good the omission of those of my predecessors who were followers of Waldeyer and Balfour, in publishing drawings of transition stages between peritoneal cell and germ cell, and in bringing evidence of a new character not from the tadpole only, but especially from the adult frog, to show that the epithelium surrounding the ovary is truly germinal.

Whilst admitting that some germ cells possibly migrate from the endoderm of the yolk sac, I feel sure that during life very large additional reinforcements of germ cells arise in the epithelium of the gonad of Amphibia.

It is my pleasant duty to thank Prof. Bourne for allowing me to work in the laboratory during the vacations. Dr.

Jenkinson¹ has helped me greatly by his continual interest in my work. I have also to thank Mr. Goodrich for kind criticism, advice, and encouragement. For the opinions hereafter expressed I am alone responsible.

TECHNIQUE AND MATERIAL.

Ovaries of frogs and toads were collected during every month of the year. Bouin, corrosive acetic, strong Flemming and Perenyi were used as fixatives. Sections were cut from 3 μ –9 μ , and were stained on the slide or in bulk with iron hæmatoxylin and orange G, Erlich's hæmatoxylin and eosin, borax carmine or paracarmine, and picro-indo-carmine or picro-nigrosin. Silver nitrate impregnations were also used for whole mounts of epithelium. The eggs, in the ovary of frogs from 10 mm. to 40 mm. in length, were counted under a dissecting microscope—the large ones being removed first, and the smaller ones, after maceration in osmic acid or corrosive sublimate, being shaken and teased apart and counted under a low-power microscope.

Dr. Jenkinson also placed at my disposal a good collection of frog ovaries, and allowed me to examine his fine series of newt, frog, toad, salamander, and axolotl ovaries and testes. Though this paper is mainly concerned with *R. temporaria*, it should be stated that my remarks and observations for the latter accord in all the main points with *Salamandra*, *Triton* (*Molge*), and *Amblystoma*, though the series in some cases is not complete enough to allow me to speak so confidently as I feel able to do for *Rana*. The latter closely resembles *Bufo* in the history of germ cell production.

¹ Since this was written, news has arrived of Dr. Jenkinson's sad death at the Dardanelles. I shall always be grateful for the privilege I have enjoyed in working under Dr. Jenkinson; his inspiring example will ever remain one of the most valuable benefits I have received during my stay at Oxford, and any small merit which might possibly be attached to this paper is largely traceable to the foundation laid while I was a student in Dr. Jenkinson's class.

THE SPAWNING OF SOME ANURA.

A frog must have some means of providing itself with fresh supplies of eggs when the stock is finished; for instance, a 40 mm. frog or toad, which I believe would be about two years old, has only from 10,000 to 16,000 ova in its ovaries. This figure is derived from a count of the contents of the gonad of September frogs and toads of that length. One must remember that the Anurous Amphibia lay many eggs at a spawning, and were not a means of replenishment at hand the animal, after a small number of spawnings, would be unable to lay further eggs. Frogs and toads have been kept for years and are known to spawn regularly. The following are some figures of the number of eggs laid at one spawning by several species of Anura (Gadow, 'Cambridge Natural History'):

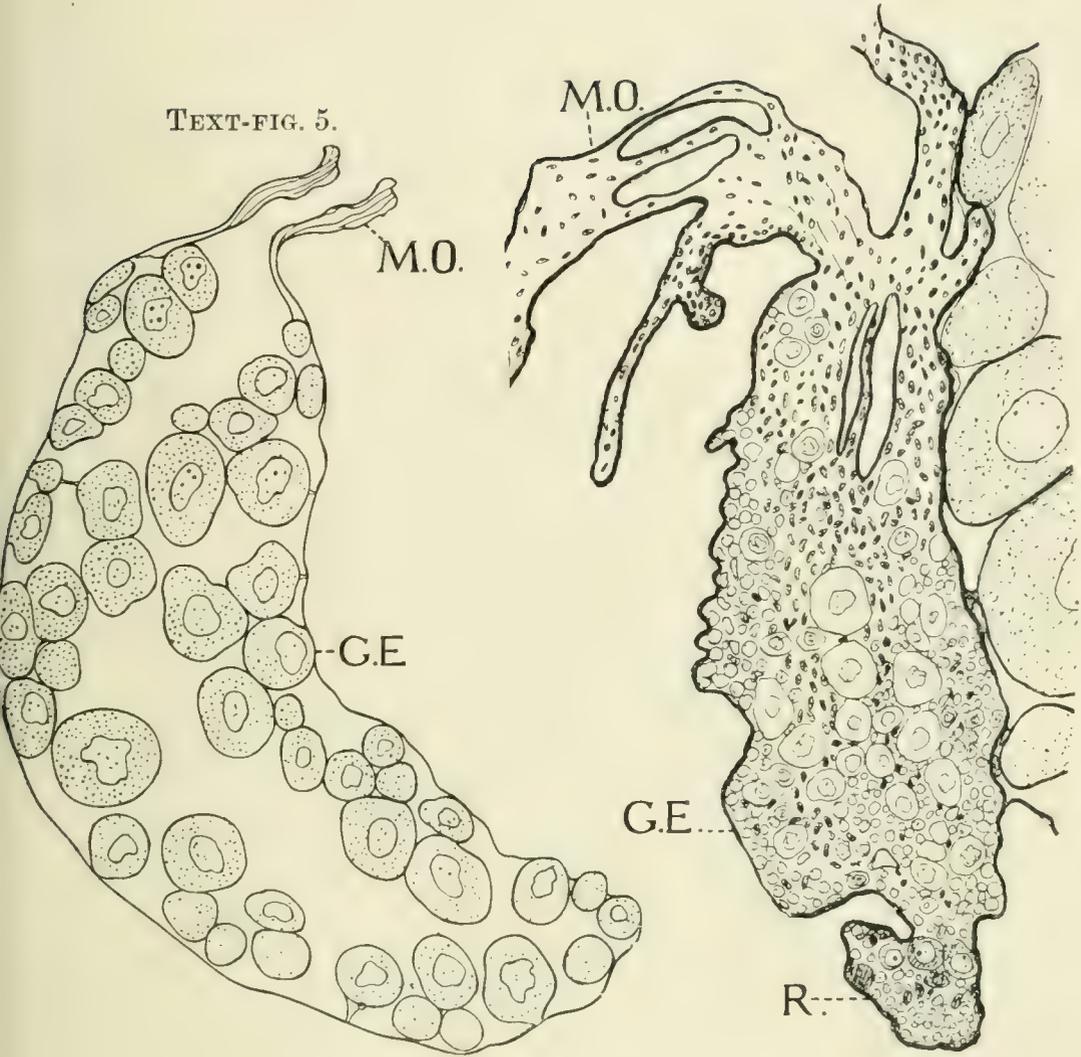
"*Hyla arborea* 1000; *Rana temporaria* 3000; *Bufo*

TEXT-FIG. 1.—Diagrammatic transverse section of ovaries and the surrounding organs in the frog. The dotted line (*P.E.* and *G.E.*) represents the pleuroperitoneal epithelium. That part surrounding and enclosing the ovary (*G.E.*), according to the theory adopted in this paper, is concerned throughout the life of the Amphibian in the production of a constant stream of new germ cells. On the left an egg is drawn diagrammatically to show the mode of attachment to the germinal epithelium by the thecal stalk (*TH. S.*). On the right an ovary is drawn to show the essential arrangement of septa (*S.P.*) and of eggs (*OV.*). *AO.* Aorta. *F.* Follicle, represented by a wavy line. *G.* Gut. *I. V. C.* Inferior vena cava. *K.* Kidney. *M. O.* Mesovarium. *MS.* Mesentery. *OV.* Ovum. *S. J.* Region where septa join the germinal epithelium (*G. E.*). *SP.* Septum. *TH.* Theca. *TH. S.* Thecal stalk or pedicle. *V. C.* Vertebral centrum. [Partly after Bourne (11).]

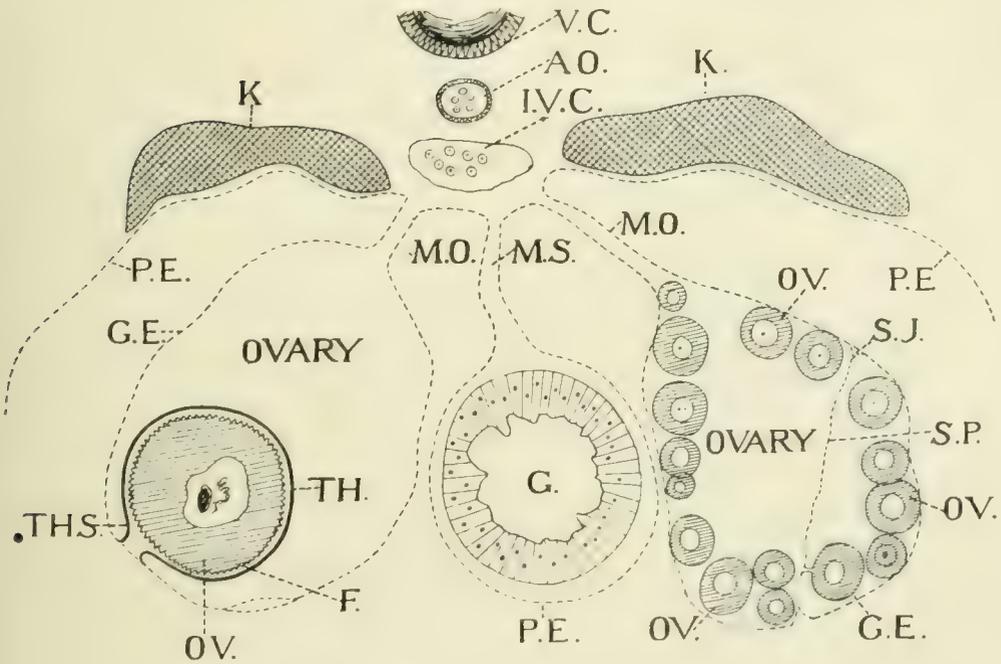
TEXT-FIG. 4.—Large ovarian germinal area about four or five months old, found in the ovary of an adult frog killed in late May. This thickening hung close up to the inferior vena cava. *M. O.* mesovarium, *R.* refers to Pl. 21, fig. 7, where this region is drawn on a larger scale. This figure is specially drawn for comparison with the next text-figure. Both have been drawn to the same scale, with camera lucida.

TEXT-FIG. 5.—Ovary of a frog, 40 mm. in length, killed in August. Both Text-fig. 4 and this figure are transverse sections, and serve to draw attention to the fact that in spring and summer the germinal epithelium of the frog ovary contains active germinal islands as large as, and containing more cells than, the ovary of a young frog. These islands are absent in the late winter months.

TEXT-FIG. 4.



TEXT-FIG. 1.



vulgaris averages 5000; *Rana esculenta* up to 10,000 and more. T. H. Morgan observed a *Bufo lentiginosus* which laid 28,000 eggs within ten hours."

In one of his essays Weismann (10a) gives the age to which a toad may grow as forty years. Supposing it began to spawn from four years after birth and kept on till death, it might lay 180,000 eggs during its life. This total is very probably larger than the correct average one, but serves to draw attention to the huge number of eggs some *Anura* must lay during life. The enormous number of eggs laid at a single spawning by some fish is also a good example of the fertility of some *Vertebrata*, and illustrates my contention that a germ cell organ must actively work during adult life.

THE GERMINAL EPITHELIUM OF R. TEMPORARIA AND THE CHANGES UNDERGONE BY IT DURING THE YEAR.

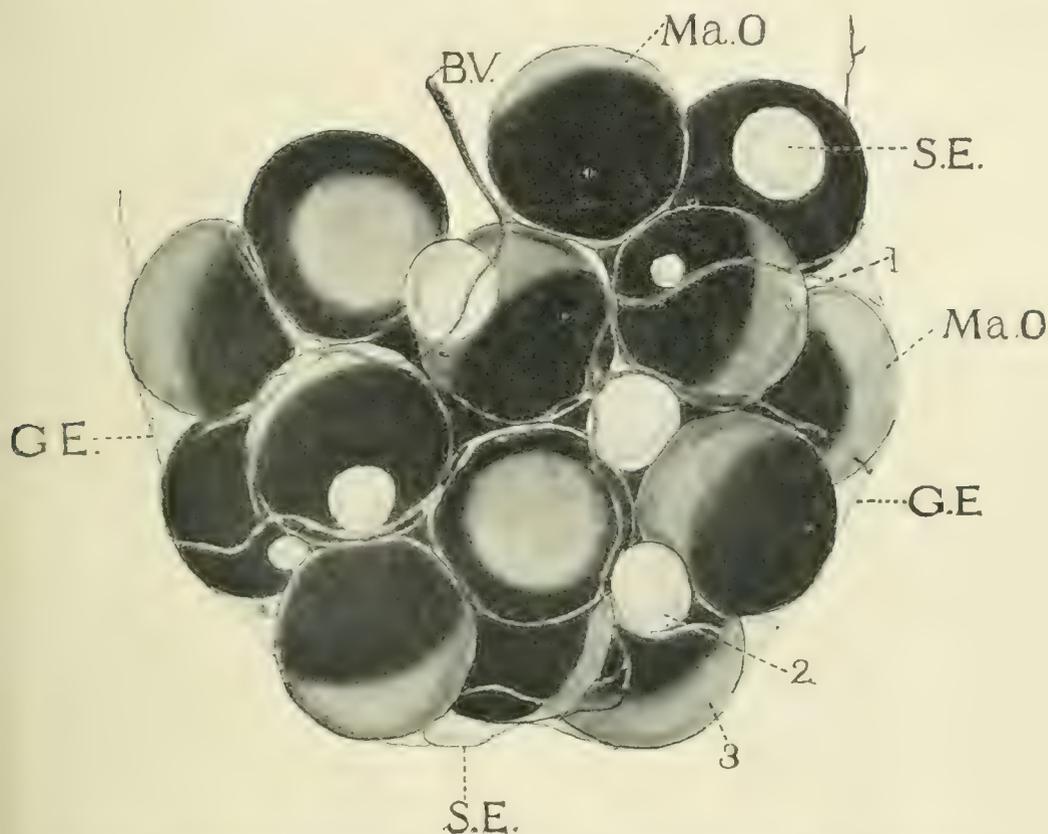
In Text-fig 1 is drawn a diagrammatic section showing the relation of peritoneal epithelium to the viscera. The ova are seen to be suspended in a reflection of the peritoneal membrane, the mesovarium, which "passes right round them," Bourne (11). Similarly the gut is suspended by the mesentery. Waldeyer and his followers believe that the part of the peritoneum enclosing the gonad differs from the rest of the pleuro-peritoneal lining in possessing the power of proliferating germ cells. This is denied by Nussbaum's school, which asserts that the peritoneal fold enclosing the ova merely functions as a suspensory sac.

The ova are connected to the germinal epithelium or to the ovarian septa (*S. P.*) by the pedicle of the theca (*TH. S.*). Inside the thecal membrane is the follicle (*F*), which wraps round the ovum itself. The egg thus has two membranes. Blood-vessels connect to the egg from the germinal epithelium by means of the pedicle. Capillaries run between follicle and theca, and by this means every egg is in connection with the blood system.

Examined in winter ovaries, the germinal epithelium is

found to consist of a membrane made up of at least two layers of cells, and often as many as three or four. In some places the epithelium is much thickened, the cells being generally about twelve in the layer. There is nothing peculiar in these thickened areas in the epithelium, the

TEXT-FIG. 2.

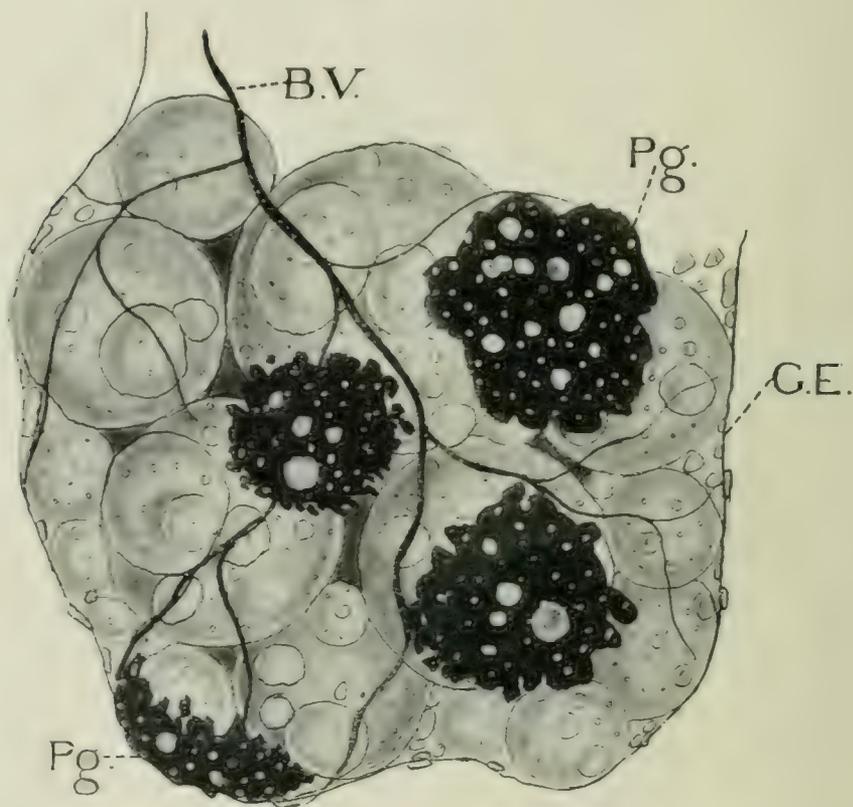


Part of the ovary in late December about three months before spawning. The large eggs *Ma. O.* are the ones which will be extruded and spawned. Of the smaller eggs (*S. E.*), which are of different sizes (1, 2), some will degenerate after the large ones have left the ovary, and will form the little black specks drawn in Text-fig. 3, *Pg.* Others will continue to grow during spring and summer after spawning, and will partly be ready for next year's spawning. Those still left in the ovary after spawning are soon reinforced by large numbers of small oocytes derived from the germinal epithelium (*G. E.*), as is shown in Text-fig. 3.

nuclei are not different from those in the ordinary epithelium, and the cell outline, examined in silver nitrate, is found to be more or less wavy, as is characteristic of other peritoneal cells. In Pl. 21, fig. 15, there is drawn one of these thickenings,

about four cells in depth. Near *G. E.* the epithelium is of the usual thickness. At the edges where the septa, which form compartments in the ovary, meet the germinal epithelium (Text-fig. 1, *S. J.*), and close up near the insertion of the ovary

TEXT-FIG. 3.



Part of the ovary in May, after spawning, and during the most active period in the history of the ovary. This figure is drawn on a scale about twice as large as that of Text-fig. 2. The black masses (*Pg.*) are degenerating ova of the size drawn in Text-fig. 2 at *S. E.* More especially on the surface of these black masses, but really over the whole surface of the ovary, the germinal epithelium (*G. E.*) is seen to be full of germ cells in every stage of metamorphosis. The new eggs appear more clearly over the pigment masses because of the black background. The edges of the pigment masses are frayed and the process of disintegration is well advanced. Before autumn these masses almost completely disappear. The pigmented hemisphere has not yet appeared in any eggs in this figure.

beneath the aorta, occur large thickenings and areas where numerous cells are collected together. The mesovarian thickenings as well as those near the septa are intended to

support and bind the ovary into a compact whole (Text-fig. 4, *M. O.*). Blood-vessels occur here and there over the surface of the epithelium and pass by means of the septa and thecal stalks to the ova.

In the tadpole the peritoneum is formed at an early age from mesodermal cells derived from the retro-peritoneal mass beneath and alongside the aorta or from cells of the mesentery. Most authors are in accord with the conclusion that the peritoneal epithelium is derived from mesoderm, and in view of the evidence brought forward in this paper, the origin of the peritoneum has no small importance.

Text-fig. 2 depicts an ovary in late December.

In the winter months the contents of the gonad consists of very large, almost if not quite, mature ova (*Ma. O.*) and other smaller eggs, the largest of which is generally about one fourth the size of the mature ovum (*S. E.*). The smaller eggs are not pigmented, being a greyish-white in colour.

When spawning time arrives, about March, the large ova are extruded and the ovary shrinks greatly in size, containing only the smaller eggs (*S. E.*) and a few larger eggs not successfully extruded. These latter soon degenerate together with a large proportion of the smaller ova (*S. E.*). In the process of disintegration the inner egg membrane or follicle breaks up, and its cells get mixed up in the lumps of dark yolk (Pl. 21, fig. 6, *F. N.*), and evidently assist in some way or other in removing the waste matter. Very soon the yolk, at first grey, becomes yellow, then brown, and finally turns black. The ovary then has a very characteristic speckled appearance, shown in Text-fig. 3, which is drawn to a larger scale than Text-fig. 2.

The black irregular masses (*Pg.*) are pigment produced from degenerate ova. On them small white masses of various sizes can be seen. These are very young oocytes appearing in the epithelium, which stretches over the black masses. These small white masses appear all over the ovary after spawning, but are seen well only near the pigment masses, since the black background shows them clearly. No

pigment has yet made its appearance in the growing eggs in Pl. 21, fig. 3. The pigment appears much later in the year. After spawning the history of the ovary consists roughly in the growth of the remaining oocytes and the formation of others, as explained below.

Towards the end of winter and in early spring the frog emerges from its hibernating place, and if it is not already near a pond or ditch travels towards one. If the ovary at this period, and some time before spawning, be examined the eggs will be found to be similar in size to those described above for late December. It is in the germinal epithelium that changes have taken place already, or are about to occur, and this period in the history of the germinal epithelium is of great importance.

If the epithelium be examined under a low power and attention be concentrated on any thickened areas, many germinal islands, such as is drawn in Pl. 21, fig. 11, will be discovered. The appearance of these islands and their staining reactions are so characteristic that one cannot overlook them. They are swollen areas in the epithelium, containing several cells with nuclei larger and staining less chromatically than the ordinary peritoneal cell nucleus. In the latter the chromatin is gathered in irregular lumps not apparently connected by a continuous reticulum, and a nucleolus can often be discovered towards the middle of the nucleus (Pl. 22, fig. 19).

The chromatin stains darkly with nuclear stains. This description would apply equally well to the follicle and thecal nuclei, which are evidently of quite the same nature and origin as those in the peritoneum. In Pl. 21, fig. 11, there will be seen three nuclei marked *T. S.* The right hand one is bilobed. Each of these nuclei stains less chromatically than the other peritoneal nuclei in the thickening, and, what is more important, each has a distinct granular protoplasmic zone around its periphery. This zone tends to give the nuclei such a curious appearance that their instant detection is not difficult. Viewed from surface preparations of the

whole peritoneum, the nucleus and granular area appears as in Pl. 21, fig. 9 (*G. A.*). Occasionally, nuclei may be found in the peritoneum which stain as lightly as those in Pl. 21, fig. 11, but without the zone. These latter are not destined to become germ cells unless a granular area can be discovered around them. I have looked for this characteristic nucleus and granular zone in several other Amphibians. It is very clear in Salamandra and the Axolotl. That this peripheral granulation is derived from the nucleus appears probable, both because it stains with chromatin dyes and because it first appears closely applied around the nuclear outline.

The fact that the zone at first stains clearly in chromatin dyes also suggests that it might be the expelled chromatic material of the nucleus, which latter, it may be remarked, is now becoming more and more faintly staining.

These cells containing nuclei wrapped around by a granular zone become characteristic of the ovary about spawning time and soon cause numerous thickened germ pockets to appear. The further history of these future germ nuclei will be resumed at a later stage.

THE HISTOLOGICAL CONDITION OF GERMINAL THICKENINGS AND THE FIRST TRACES OF THEIR APPEARANCE.

Germ islands vary greatly in size. Some are large tracts plastered on the side of the ovary and, after staining, quite visible to the naked eye.

Very large thickenings, such as that depicted in Text-fig. 4, never appear in other parts of the peritoneum except near the mesovarium (Text-fig. 1, *M. O.*) or near the septal joinings (Text-fig. 1, *S. J.*). In all probability the reason for this is that no other part of the peritoneal membrane is strong enough to support such a large area as that of Text-fig. 4. When one remembers that the germ island in Text-fig. 4, could be easily seen by the naked eye and was almost as big as the whole ovary of a 40 mm. frog (Text-fig. 5), this reason will be better understood. The thickening in Text-fig. 4 would

be about five months old, assuming that it arose in January or thereabouts. The largest oocytes in it are approximately the same size as the largest oocytes in a five-months-old tadpole ovary.

More commonly ovarian thickenings, instead of containing many thousands of differentiating germ cells, often only contain from twenty to a hundred. One most curious fact must be mentioned with regard to many of the smaller thickenings—it is, that some of them bear such a remarkably complete resemblance to the tadpole ovary that one would have difficulty in distinguishing them were examples of each set side by side under two microscopes. In the later embryonic ovary yolk discs may be present, these never appearing in germ islands.

We speak of the genital ridge of a tadpole. The germinal epithelium of the adult frog develops every year hundreds of genital ridges, some so large that the whole transverse section of an eight-day tadpole would hardly cover them. The migration of primordial germ cells is a curious and interesting fact, but when compared with the annual production of germ cells from the peritoneal epithelium it falls into insignificance.

In the early spring there will be found on the peritoneal epithelium little undifferentiated thickenings such as drawn in Pl. 21, fig. 15. The nuclei composing these areas are quite similar in all details to the ordinary peritoneal nuclei, and the cell wall is wavy in outline, as is usual with peritoneal epithelium. From their subsequent history it seems certain that generally the inner cells of these thickenings metamorphose into germ cells. The outer ones form ovarian membranes—follicle and theca—after the germ cells have metamorphosed into oocytes. In the first instance, these undifferentiated islands arise through the rapid division of pre-existing peritoneal nuclei. Whether this division is mitotic or amitotic is a moot point, but my observations lead me to think that the divisions are amitotic. I do not intend to bring forward my evidence for this in the present paper.

However, the nuclei of the cells in these thickenings do certainly arise from peritoneal nuclei, have the same chromatic arrangement, stain in exactly the same manner, and in short allow of no avoidance of the truth that they are somatic and not germ cells. The germ cell nucleus has a different chromatic arrangement from that of the peritoneal, thecal, or follicle nucleus, and it is quite easy to discriminate between these nuclei.

I wish to make an important point of this, because it is anticipated that attempts will be made to show that the peritoneal islands contain undifferentiated stored-up germ cells which have been dormant till the need for them arose. It is, therefore, very fortunate that almost all ovaries taken in winter have no properly differentiated germ islands, and not only this, but in spring all the intermediate stages between peritoneal cell and germ cell are beautifully apparent in suitable material.

The following changes are undergone in the transformation of the somatic cell into the germ cell. These are the much-discussed stages whose presence has been so confidently denied by Beard and others, and which were believed in by Balfour and Semon.

The peritoneal nucleus has the structure depicted in Pl. 22, figs. 17 and 18, and Pl. 21, fig. 16.

The filaments and lumps of chromatin are coarser in some nuclei than in others. A karyosome is nearly always present, and there are often two. At no stage in the history of the first primordial germ cell is the arrangement of chromatin, such as that of the peritoneal nucleus to be discovered. The shape of the nucleus, its staining power and chromatic arrangement are highly characteristic of the germinal epithelial cell. Not only this, but the cell outline is extremely characteristic examined in silver nitrate preparations. The very earliest sign of a change in the nucleus is detected in a slight loss of shape. The outline becomes less even and somewhat polymorphic. Such nuclei are drawn in Pl. 21, figs. 16B, 16C, and Pl. 22, fig. 20.

The nucleus at this stage grows larger, and little by little loses its staining power (Pl. 22, fig. 21 ; Pl. 21, fig. 16D). The chromatic lumps and the karyosome do not take the stain so sharply as in other unaffected nuclei, and consequent upon this loss of staining power comes the appearance of the granular zone around the periphery of the nucleus (Pl. 21, fig. 16c and fig. 11).

Step by step as the nucleus loses its staining power the peritoneal cell cytoplasm, before clear and staining exclusively with plasma dyes, becomes more and more granular. The loss of staining power seems not only traceable to the chromatic network, but also to the nuclear sap, or fluid contents of the nucleus (see Pl. 22, figs. 17, 18, 19, 20, etc.).

If the ovary be preserved in corrosive acetic and stained in borax carmine and picro-indigo carmine the peritoneal cytoplasm stains pale greenish blue, the granular zone pink, and the nucleus a little darker, while red blood corpuscles stain dark red in the nucleus and orange yellow in the cytoplasm. In iron hæmatoxylin the granular zone is picked out, even after prolonged differentiation (Pl. 21, fig. 12). With Ehrlich's hæmatoxylin the zone appears a light reddish purple (Pl. 21, fig. 16c). At this stage the future germ cells begin to lose their flattened shape, and tend to form cysts as drawn in Pl. 21, fig. 14 or fig. 12. Cyst formation is just beginning in fig. 11 ; certain peritoneal cells quite near are already becoming turned in the correct direction (*P. C.*).

During the loss in staining power of the future germ nucleus the chromatin lumps seem to become more finely distributed and smaller (Pl. 22, fig. 20). At the same time a faint reticulum can be made out. Whether this was present before the loss of staining power began and invisible because of the coarseness and denseness of the chromatin, or whether it appears secondarily, I cannot decide for certain. In some nuclei at this period a reticulum is quite clear, while in others it seems less easily demonstrable.

In Pl. 21, figs. 16A, B, C, D, are drawn some stages in the transformation of a somatic cell into a germ cell (stained in

Erhlich). The loss of staining power is clearer after using a selective stain like Erhlich's hæmatoxylin and eosin than with iron hæmatoxylin. In Pl. 22, figs. 17, 18, 19, etc., such stages are drawn from iron hæmatoxylin preparations. The nucleoli, after prolonged staining, differentiate quite black. In the polymorphic mulberry shaped germ nucleus (Pl. 22, figs. 22 and 25) many nucleoli may be seen. I believe that these nucleoli may be budded off from the original large nucleoli or nucleolus (Pl. 22, figs. 19 and 21). Each fold of the polymorphic nucleus almost always contains a nucleolus, and very often several. Pl. 22, fig. 24, depicts a different form of nucleus. I think that this is derived from one of the mulberry shaped nuclei, and is really a part of one of the latter after it has divided amitotically into several parts. Later on the reticulum of the nucleus (Pl. 22, fig. 24) becomes coarser, and chromosomes are formed, and a mitotic oogonial division ensues (Pl. 22, fig. 26). In all large germinal thickenings a cavity sooner or later appears and the same process as that undergone in the embryonic ovary takes place—i. e., the ovary, once many oocytes in depth, becomes hollow, and the walls sag down till a depth of only one or two oocytes are found on the walls (Text-fig. 5). During the appearance of the cavity or cavities (for there may be more than one) septa are preserved between the peritoneum and new walls of the cavities, and, these persisting, later on form compartments in the ovary. The lobations and sacculations of the adult ovary are directly traceable to thickenings in the peritoneum, as are also the septal walls of the compartments.

Germ cells do not exclusively appear in the peritoneal epithelium, but they seldom become differentiated inside the ovary on the septa (Text-fig. 1, *SP.*).

In Pl. 21, fig. 6, is drawn a small degenerating oocyte; follicle nuclei (*F. N.*) are mixed up in the pigment masses. The theca is still intact (*TH.*). At *XX* a pocket of cells has appeared. These are future germ cells, and the nuclei have already assumed the irregular shape, and the cytoplasm has

become granular. Such nests and cysts as this are not uncommon during the months when many oocytes are degenerating. In fig. 3 of the same plate a part of an April ovary is shown with three old follicle sacs (*P. S.*). In the wall of two of them at *XX* appear several germ cysts. This is quite common in April months. In both this case and the one cited above the germ pocket arises from follicle or thecal cells. In Pl. 21, fig. 6, the new germ cells appear inside the old degenerating oocyte—a remarkable fact.

After spawning many eggs degenerate, as already explained, but those left (Text-fig. 2, *S. E.*) are destined to form the eggs to be laid next year. Though one cannot be certain, I believe that an oocyte takes two years at least, and more probably three, to become mature. It is evident, therefore, that the young oocytes formed in April or May in the adult will not be used for spawning next March, but certainly for a spawning several years ahead. The first ova derived from primordial germ cells would not be spawned till three years after the hatching of the tadpole, since the frogs around Oxford seem to become mature in three years. The tadpole born in March is a metamorphosed frog 25 mm. in length by September. By September of next year it is 40 mm., and by the September of the year after it is almost full grown (70 mm.—80 mm.), and mature enough to spawn. I cannot be absolutely certain for these figures, but they accord with my results for other observations. Very probably the condition of the weather and of the food supply has considerable effect upon the time at which a young frog reaches maturity.

An approximate table of the changes in the ovary of *Rana temporaria* found around the Oxford district is as follows, but it should be understood that just as the seasons are liable to variation, so are the stages in the cycle liable to be later or earlier :

Winter (January, February, March).—Eggs ready to lay and the germinal epithelium quiescent. Frog hibernating (Text-fig. 2).

Late March or early April spawning takes place.

Spring (April, May, June).—Germinal epithelium becomes by stages very active, many new ova being formed in it. Many eggs degenerate with the appearance of pigment, and the ovary assumes a characteristic speckled appearance (Text-fig. 3).

Summer (July, August, September).—Most of the new germ cells have reached the oocyte stage by August, and a gradual stoppage of activity commences.

Autumn (October, November, December).—The oldest and largest ova are now almost prepared for the spawning of next year, and the activity in the germinal epithelium ceases. Hibernation begins according to the state of the weather (Text-fig. 2).

Bufo spawns later in April.

THE REASONS FOR BELIEVING THAT THE GERM THICKENINGS
ARISE FROM PERITONEAL CELLS ARE AS FOLLOWS :

(1) No thickening containing any nuclei except peritoneal ones are to be found in winter. Very few are found in September, fewer in October, and hardly any in November. They are scarcely ever to be discovered in December ovaries.

(2) During early spring the differentiation of the future germ cell island can be clearly followed in all stages.

(3) Thickenings always arise at about the same months (March or April, more rarely February), are commonest in early summer, and begin to disappear towards autumn.

(4) From a count it is clear that the young frog has not enough eggs to supply it through life, and an anatomical and histological examination leaves only the peritoneum from which these new germ cells can arise.

(5) Germ cells may be seen to arise in the follicle sac left by the extruded egg, and must be derived from somatic cells (follicle or thecal) (Pl. 21, fig. 3, X X, and fig. 6, X X, etc.)

(6) Germ cells may be seen to arise from cells with a typical peritoneal cell outline (Pl. 21, fig. 9).

With regard to the first reason it should be said that frogs

hibernating in greenhouses, or during a mild winter, may have small inactive germ pockets. These are so few and the cases are so rare that the above statement is not affected by them. The large majority of winter ovaries have no germ thickenings of any kind, while every spring ovary has many. No exception to the latter statement has been found.

DISCUSSION.

Miss Helen Dean King (4) does not mention the germinal thickening in her paper of the "Oogenesis of *B. lentiginosus*," and I can only infer from this that she has not found them, and, therefore, that she has not needed to explain their presence in terms favourable to the view that at no period in the history of the gonad are germ cells produced by transition from somatic cells. In the tadpole Bouin and Kuschakewitsch both describe the resolution of peritoneal or retro-peritoneal cells into germ cells. Miss King stands alone in her view. I cannot count B. M. Allen (5), since his work resembles Beard's in treating of only a short period of the animal's life. Were Allen to search he would probably find other important periods in the history of the sex cells of *Rana pipiens*. Such evidence for Nussbaum's school is so incomplete as to be useless in a wide review of germ cell production in any one animal.

Beard (3) studied the germ cells of *Raja*, but in his paper on the "Germ Cells" I can find that in no case is any of his evidence derived from adult ovaries. He says regarding his material: "The present researches have been carried out on material of all sorts of phases, from the close of segmentation to embryos of 42 mm., and they do not extend sufficiently far to permit of the giving of any revised or confirmatory account of the later history, including the details of the formation of the secondary germ cells. But as Rabl has already said in his own work, so also it is true of mine, that it overlaps the older researches of Semper and Balfour." The italics are mine. From this statement it is very clear that Beard's work on *Raja* is too incomplete to enable him to advance it as

evidence that a study of the germ cells of Raja supports Nussbaum's point of view. Beard shows that according to his own opinion there are no transition stages in embryos up to 42 mm., but he does not, as his discussion, towards the end of his work, would suggest, entirely upset, at one fell swoop, all the edifice built around the germinal epithelium theory. It is extraordinary that Beard should dogmatise on germ cells in general when, by his own showing, his study was not complete enough to "permit of the giving of any revised or confirmatory account of the later history . . . of the germ cells." Even granting that his evidence for this short period in the germ cell history of Raja is correct (which I cannot say), is he justified in condemning Waldeyer until he has examined stages from 42 mm. upwards to maturity, and mature ovaries for every month of the year?

Beard also mentions that a study of the germ cells of the chick supports his views. Compare this with the results since arrived at by Jean Firket (12): "Il y a lieu d'admettre une dualité d'origine des gonocytes chez les oiseaux; les gonocytes primaires apparaissent très tôt au cours de l'ontogenèse, avant la formation de l'ébunche genital définitive; les gonocytes secondaires se différencient aux dépens des cellules de l'épithélium coelomique qui de ce fait mérite le nom d'épithélium germinatif."

Miss King (4), in her paper on the "Oogenesis of *Bufo lentiginosus*," figures in Pl. 1, fig. 4, a germ-cell marked Y, situated outside the gonad. Concerning this she says: "In early stages of development the germ cells are not always confined to the genital ridge," and "such germ cells must eventually come into the germinal area or degenerate, since cells of this character are never found outside of the genital ridge in later stages of development." This germ cell, which is figured outside the genital ridge, is, in my opinion, derived from the retro-peritoneal tissue. One can hardly assume otherwise from the position of the cell.

Though it is impossible to speak for certain in such cases as this, it is most unlikely that these germ cells should have

migrated out of the gonad into the position in which Miss King figures them. It seems more natural to suppose that they are developed in situ from retro-peritoneal cells. It is quite likely that some peritoneal cells near the gonad do sporadically become germ cells, and I have found such germ cells outside the gonad in older frogs long after migration had ceased.

Miss King does not explain how the germ cells in her figures came to be in that position. In her fig. 5 of Pl. 1, she depicts other germ cells outside the germinal ridge. Her figures of these stages give no support to the view that the peritoneum does not produce germ cells. In fig. 16 of Pl. 1 she has drawn a young ovary showing the beginning of the formation of the central cavity, and the invasion of retro-peritoneal cells described also by Kuschakewitsch (9).

The latter claims quite rightly, as I believe, that these cells become germ cells. Miss King, whilst admitting that the invasion of retro-peritoneal cells occurs, prefers to believe that the cells, when arrived in the gonad, form ovarian membrane cells and not germ cells.

During this stage the ovary grows very considerably, not only in size, but in the number of germ cells. Miss King writes: "Although mitotic figures are comparatively rare during the early stages of development they are found very abundantly when the tadpole approaches metamorphosis, and in a single section of the ovary of a toad killed at this time one may find several cells that are preparing to divide (Pl. 1, fig. 17)." In figs. 16 and 17 of Miss King's paper the ovaries depicted are already very large, and no mitotic figures are shown.

Most of the germ cells drawn in fig. 17 in Miss King's paper are not preparing to divide. Though such nuclei as that of fig. 17, *P.*, are present in *B. vulgaris* and *R. temporaria* I would not interpret them as "preparing to divide." I have found in the Amphibians above mentioned that the nucleus of the oogonium, when preparing to divide, becomes spherical (not irregular), and the chromatic network resolves

itself into a spireme, which then breaks up into chromosomes. Miss King herself was struck by the absence of mitoses during early stages in the growth of the ovary, be it marked, long after the migration of germ cells has stopped; how, then, does she explain the rapid growth of the ovary up to the stage just before metamorphosis, when numerous mitoses begin to appear?

The ovary at this stage is a large organ, and since she denies that amitosis takes place in the germ cells, it is obvious that the rapid growth of the embryonic ovary is due to some cause other than the mitotic division of the primordial germ cells.

Beard mentions the curious absence of the mitoses in the embryonic ovary of *Raja*. Can it be possible that my remark about *Rana* applies equally well to *Raja*?

From my own observations, which, I may say, coincide quite closely at almost all points with those of Kuschakewitsch (9) and Bouin (7) for the tadpole, I would suggest that the history of germ cell formation in the Anuran is as follows:

In the very young tadpole a few of the yolk sac endoderm cells become transferred to the gonadic region by migrating up a very short path along the mesentery. That this migration does take place in every tadpole I am not sure. It is less easy to be certain of migration in the tadpole than in the Elasmobranch. The number of migratory endoderm cells is very few in almost every case, and the early gonad has hardly ever more than four or five germ cells in transverse section in its broadest part. This remains true for a time, during which migration does no longer take place, since the gut soon becomes quite differentiated. About this time the mesentery and retro-peritoneal cells in the original gonad form the peritoneal epithelium of the ovary, and when the latter constitutes a true continuous epithelium it begins to proliferate germ cells. The invasion of retro-peritoneal cells also sets in, and the gonad grows quickly in size without many mitoses being present to otherwise account for the rapid increase in the number of germ cells. Soon, however, many mitoses

appear and many germ cells begin to differentiate. The ovarian cavity forms, and the ovary begins to assume the characteristic shape shown in Text-fig. 5, which is much older than the period to which I am now referring.

By autumn the tadpole born in March has an ovary containing about half the number of ova that, as an adult, it can spawn in one season, and not only this, but proliferation of germ cells from the peritoneum stops about the same time as given in the table for the adult frog (p. 290). We then find that the proliferation of germ cells in the tadpole lasts from about March to October or November (according to the weather), just as it does in the adult. The little metamorphosed frog hibernates, and if in its second year the gonad is examined in spring, a renewal of proliferation of germ cells is detected as in the adult. From birth up to the time of its first spawning, and ever afterwards during life between spring and autumn, the frog has new germ cells formed in its peritoneum. The tadpole ovary, therefore, is formed of:

(1) Germ cells of peritoneal origin.

(2) Germ cells of retro-peritoneal origin.

(3) Germ cells of endoderm (yolk sac) origin. The germ cells derived from the yolk sac may possibly all be laid during the first spawning. The first two origins are mesodermal.

The first germ cells which migrate from the endoderm may be regarded as precocious and relatively unimportant. Through the peritoneum the mesoderm supplies by far the greatest number of germ cells. Endodermal ova, being probably all spawned at the first egg-laying, this spawning consists of a mixed batch of eggs, endodermal and mesodermal in origin. The peritoneal cell always remains in a sufficiently undifferentiated condition to metamorphose, if needed, into a germ cell. The changes undergone in the differentiation of an oogonium into an oocyte are more important and extensive, as far as the nucleus is concerned, than those of the peritoneal cell when becoming a germ cell. We meet with no cataclysmic changes such as the prophases

of the heterotypic division, but simply with a loss of staining power, the appearance of a more granular cytoplasm, and the polymorphism of the nucleus.

There is no continuity of germ cells in the Amphibia I have studied—any peritoneal cell apparently can become a germ cell—and why not?

SUMMARY.

(1) Germ-thickenings in the peritoneal epithelium surrounding the ovary do not appear in winter.

(2) In early spring differentiating areas appear in the peritoneal epithelium. Followed out, these thickenings are found to become ovariform germ pockets containing newly formed germ cells in all stages.

(3) The intermediate stages between peritoneal cell and germ cell consist primarily in the elimination of a greater part of the chromatic matter of the nucleus, in the appearance of a granular zone in the cytoplasm which becomes totally granular and stains more heavily than before, and finally in the appearance of several nucleoli subsequent upon a loss of the regular shape of the nucleus.

(4) Intermediate stages have been looked for and found in other Amphibian ovaries.

(5) Thickenings containing many thousands of germ cells and larger than the entire ovary of a lately metamorphosed frog have been found, especially in May.

(6) Reasons are given why the cells composing the germ thickenings in the ovary are not to be considered stored-up germ cells.

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EXPLANATION OF PLATES 21 AND 22.

Illustrating Mr. J. Bronté Gatenby’s paper on “The Transition of Peritoneal Epithelial Cells into Germ Cells in some Amphibia Anura, especially in *Rana temporaria*.”

EXPLANATION OF LETTERS USED IN FIGURES.

[*a, b, c, d, e* refer to stages in transition of peritoneal cell into germ cell as drawn in Pl. 21, fig. 16.]

B. V. Blood-vessel. *C. Y.* Cytoplasm. *F. N.* Follicle nuclei. *F. S.* Follicle sac. *G. A.* Granular area. *G. E.* Germinal epithelium. *L. P.* Leptotene stage. *M. O.* Mesovarium. *N.* Nucleus. *O. M.* Oogonial mitosis. *P. C.* Peritoneal cell. *P. g.* Pigment mass. *T. S.* Transition stage between peritoneal cell and germ cell. *X. X.* Germ pocket. *Y. N.* Yolk nucleus. *Y. O.* Young oocyte.

PLATE 21.

[Outline of all figures, except those in Pl. 22, drawn with camera lucida. The arrow points towards the cœlom. Figures reduced by one half.]

Fig. 1.—A lately differentiated germ island in April containing germ cells with mulberry shaped nuclei. Zeiss E. 4. O., oil imm., 2 mm.

Fig. 2.—A germinal thickening a little later in April. There were fifty or more germ cells in this thickening. In the section some leptotene stages are cut across *L. P.* *P. g.* is pigment which is often found near or in thickenings of the peritoneum. Zeiss E. 4. Oil imm., 2 mm.

Fig. 3.—About three weeks after spawning. The follicle sacs here cut across contain in two places little germ pockets *XX*. Zeiss E. 4. O. A. A.

Fig. 5.—A thickening containing three lately metamorphosed oocytes. Zeiss E. 4. Oil imm., 2 mm.

Fig. 6.—The follicle sac of a young degenerate oocyte containing at *XX* a newly formed nest of cells derived from the original follicle or theca. The black mass is due to the degenerating yolk of the disintegrating oocyte. E. 4. Oil imm., 2 mm.

Fig. 7.—The lower part of Text-fig. 1, *R*, drawn to a much larger scale (objective D. D., eyepiece 4); *a*, *b*, *c*, *d*, and *e* stages in transformation of somatic cells into germ cells. E. 4. O. D. D.

Fig. 8.—A part of the mesovarium in which many cells were actively differentiating. E. 4. O. D. D.

Fig. 9.—Surface view of a peritoneal cell. Silver nitrate impregnation. The granular zone, *G. A.*, has begun to appear around the nucleus heralding a differentiation into a germ cell (May). E. 8. Oil imm., 2 mm.

Fig. 10.—A collapsed and partially disguised follicle sac which is beginning to form germ cells in the centre, especially near *X. X*. E. 4. Oil imm., 2 mm.

Fig. 11.—A thickening at a later stage. Granular zone to be seen around the cells marked *T. S*. This thickening is characteristic for April ovaries. E. 4. Oil imm., 2 mm.

Fig. 12.—A thickening in which the germ cells are already beginning to form nests. Late April. E. 4. Oil imm., 2 mm.

Fig. 13.—Lower part of a large thickening showing all stages in the differentiation of a germ cell from a soma cell. The cells opposite the letters *P. M.* are at stage *e* of Fig. 16. E. 4. Oil imm., 2 mm.

Fig. 14. — A nest of germ cells and peritoneal cells in various stages of differentiation. Stage *e* is the furthest, stage *a* the least differentiated.

Fig. 15. — An undifferentiated ovarian island common in the peritoneal or germinal epithelium in early spring. E. 4. Oil imm., 2 mm.

Fig. 16. A, B, C, D, E. — Stages in transition of peritoneal cells into germ cells; stained in Ehrlich's hæmatoxylin and eosin in order to show loss of staining power of chromatin. E. 8. Oil imm., 2 mm.

PLATE 22.

(Stained in iron hæmatoxylin.)

Figs. 17, 19, 20, 21, 22, 25.—Stages in resolution of a peritoneal nucleus into germ nucleus. At stage 20 a granular zone had appeared around the nucleus, but it is not shown in the figure.

Fig. 18.—A normal peritoneal nucleus from the same preparation staining almost blackly.

Fig. 23.—A peritoneal nucleus in a stage of losing its staining power.

Fig. 24.—One of the nuclei of Pl. 21, fig. 7. This nucleus has a very fine reticulum, and is in all probability a stage after the formation of the mulberry shaped germ cell (Pl. 22, fig. 25).

Fig. 26.—An oogonial mitosis from one of the germinal thickenings.

On the Embryology of *Stratiodrillus*
(*Histriobdellidæ*).

By

W. A. Haswell, M.A., D.Sc., F.R.S.,
Challis Professor of Zoology, University of Sydney.

With Plate 23 and 4 Text-figures.

THE development of the *Histriobdellidæ* is practically unknown. In fact, so far as I am aware, the only statements regarding it which have been published are contained in P. J. van Beneden's original description of *Histriobdella homari* (1). These are very brief and superficial, and the figures given, except in so far as they show a process of complete segmentation and the absence of any metamorphosis, do not throw much light on the essentials of the development.

The eggs of *Histriobdella homari* were found by van Beneden attached separately to the membranous bands which unite together the eggs of the lobster. It is likely, however, that this is not the only situation in which they occur, but that they are also deposited in the branchial cavities.

In *Stratiodrillus* they occur mainly, if not exclusively, in the latter situation, and in *S. novæ-hollandiæ*, which I have chiefly studied, though now and then an odd one may be found attached to the base of a gill, the great bulk of them occur in the narrow exhalant passage leading forwards from the branchial cavity to open externally at the side of the mouth. In this position, attached to the inner surface of

the narrow pointed extension of the branchial region of the carapace which bounds the passage in question externally, there are to be found in most specimens of *Astacopsis serratus* groups of the eggs, sometimes only a few, sometimes as many as a hundred, nearly always with a number of empty shells beside them. The eggs of *Histriobdella* are attached by one pole; those of *Stratiodrillus* by one side. The cementing material, in the latter case, is similar in appearance to the substance of the egg-shell, but reacts more readily to solvents such as hypochlorites.

The egg is about .14 mm. in long, and .10 mm. in short diameter. It is white, porcellanous, and very opaque, so that it is impossible to see much of the structure in the fresh condition. It is thus entirely out of the question to attempt to watch the development of the living embryo. Moreover, the shell, though not very thick, is extremely resistant, so much so that the making of preparations, whether of entire eggs or of sections, has proved a matter of exceptional difficulty. No satisfactory results could be obtained without the use of chitin-softening agents. Of these Henning's solution and soap-alcohol, after several trials, proved complete failures, and the only method by which results of any value were obtained was the following :

The area of cuticle bearing the batch of eggs was cut out and was subjected for some minutes to the action of hot (but not boiling) sublimate-acetic solution followed by distilled water. It was then placed in a weak solution of hypochlorite of soda ("liquor sodæ chlorinatæ" of the British Pharmacopœia 1 : water 25). The action of this had to be watched carefully. The rapidity of the effect is influenced in a marked degree by the temperature. The cementing material becomes softened first, so that the eggs may become detached by shaking or by touching with a camel's hair brush. When the softening process is judged to have gone on long enough, distilled water is substituted for the hypochlorite solution, and after a thorough washing is itself replaced by alcohol.

Since it is impossible to see anything of the structure of

the egg except in the very earliest stages, definite orientation is impossible. I have found it of advantage, however, to have all the eggs cut in the same general direction; all may be cut longitudinally by their being allowed to settle by gravitation during the process of saturation with celloidin, and collected into a small area by a rotatory movement. On account of the minuteness of the objects thin sections are required and double-embedding is essential.

The ova are probably internally fertilised, but segmentation was never observed to begin until the egg had been laid.¹ The process of segmentation is complete but irregular. The first division (Pl. 23, fig. 1) is usually not quite median, one of the two cells formed being somewhat larger than the other. The extent of this difference is variable, and in one specimen of which I have sections the two cells are of equal size. One of the two cells, probably the smaller, when, as is the rule, they are unequal, divides into two by a longitudinal fissure, the other remaining undivided (Pl. 23, fig. 2). In the following divisions the latter cell does not take part, or takes part only to a slight extent; it retains the character of a single median cell, which, though it decreases in size and may have small segments cut off from it, yet greatly exceeds any of the rest in size, and occupies a large area at one of the poles. The pole in question may be regarded as the vegetal pole, and the large cell may be distinguished as the vegetal cell.

The divisions which follow upon the three-celled stage result in the attainment first of the four-celled (Pl. 23, figs. 3 and 4), then of the five-celled stage, which was met with several times (Pl. 23, figs. 5 and 6). The embryo now consists of the large plano-convex vegetal cell (*v.c.*) and four others, two median, unpaired, and two lateral. The latter are not symmetrical. One of them undergoes a special modification; it loses its nucleus and takes no further part in cell

¹ The first polar body is probably thrown off before fertilization, and thus before the shell is formed. Two rounded bodies which seem to represent the second occur along the first furrow. Later one is found about the animal pole.

division; its finely granular substance afterwards spreads out as a thin layer on the surface. This non-nucleated material or secondary yolk, as it may be termed, persists till a late period (see Pl. 23, figs. 13-18, *yk.*).

Further divisions follow, the vegetal cell still retaining its dominant position until when about fourteen cells have been formed (Pl. 23, fig. 9) the vegetal cell begins to sink inwards, the neighbouring smaller cells coming to encroach on its margin. About the same time it sends off a thick, rounded process towards the centre of the egg, and this becomes cut off by a fissure to form a separate, centrally-situated cell.

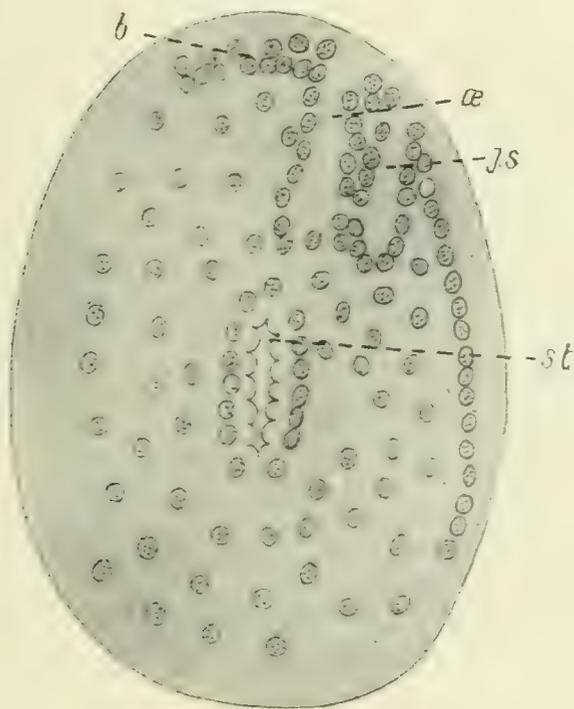
Meanwhile a change has begun which precludes the further following out of the history of the various elements.

The cells resulting from the segmentation process have up to this point been separated from one another by definite clean-cut fissures. But, at about the time when the sinking inwards of the vegetal cell takes place, this definite cell-division ceases, and complete fusion takes place among all the cells. The embryo now assumes the character of a quite regular, solid, oval mass with a few nuclei scattered through it without any trace of definite arrangement. Sections of this stage might readily be taken for sections of the unsegmented ovum, so complete is the homogeneity, were it not for the presence at irregular intervals of the small nuclei in various phases. Cell-division after this consists, so far as can be seen, simply of division of nuclei, and distinction between elements, on the ground of differences in their derivation, comes to be impossible.

In this undifferentiated body the first indications of certain structures are afforded by a mustering, multiplication, and arrangement of nuclei. In this way become established (Text-fig. 1) the first rudiments of the brain and nerve-cord, the œsophagus, pharynx, and stomach. Towards one end, the anterior, nuclei collect just below the surface and multiply to form what is at first an indefinite strand, but becomes a thick, dense plate of closely packed nuclei, the apical plate or

rudiment of the brain (*b*). Along the opposite side also, just below the surface, extends a string of nuclei, which multiply to form ultimately a thick cord of densely aggregated nuclei similar to those forming the brain rudiment. The anterior end of this is separated by only a short interval from the apical plate, and it grows backwards round the blastoderm so

TEXT-FIG. 1.



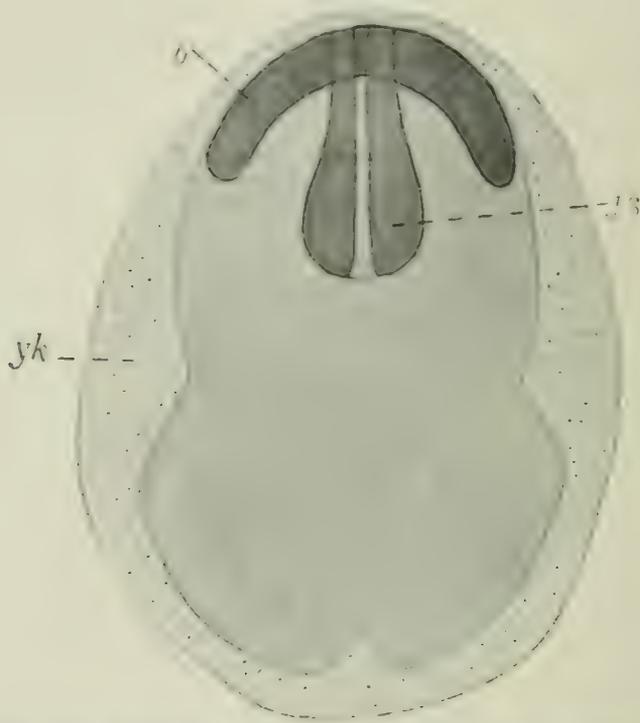
Sagittal section of stage in which the first rudiments of the brain, pharynx, and stomach have appeared. $\times 500$. *b*. Brain. *j.s.* Jaw-sac. *æ*. Oesophagus. *st*. Stomach.

that its posterior end comes to lie almost in contact with the anterior end of the apical plate. It is to be noted that the rudiments of the apical plate and ventral nerve cord are formed at a stage when an ectoderm is not represented in any way, even by an arrangement of nuclei.

About the same time as the rudiments of the nervous those of the enteric system are developed, and as in the former case this involves at first nothing more than processes of multiplication and marshalling of nuclei. In the interspace

between the rudiment of the apical plate and the anterior end of that of the nerve cord nuclei become arranged so as to circumscribe two spaces free from nuclei, though solidly filled with protoplasmic material. One of these represents (*w*) the rudimentary œsophagus; the other (*j.s.*) the pharynx, or

TEXT-FIG. 2.



Early constricted stage viewed as a transparent object. $\times 600$.
Letters as in Text-fig. 1; in addition, *jk*. secondary yolk.

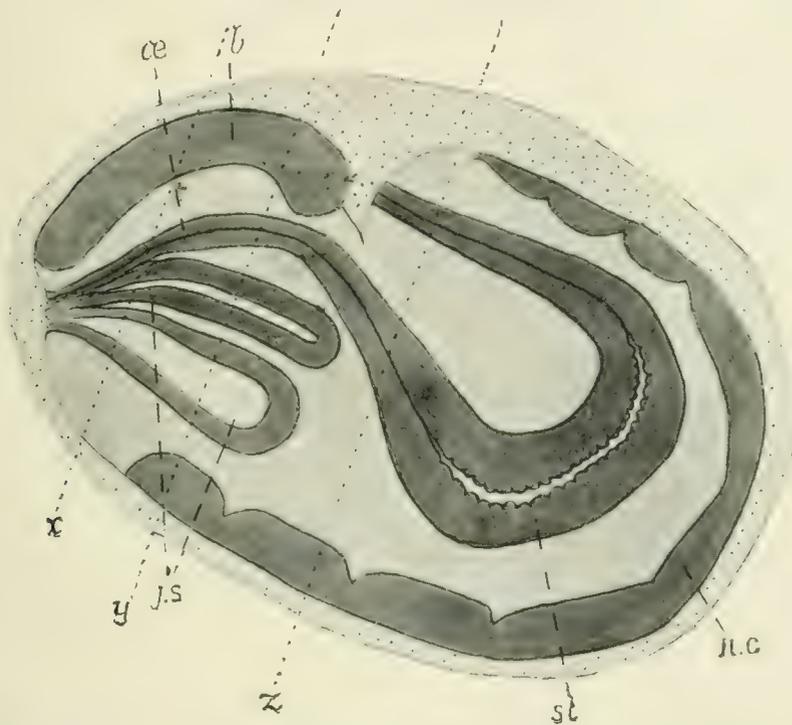
sac in which the jaws are destined to be developed; the former is nearer the brain rudiment, the latter near that of the nerve cord. At first they appear to be independent; later they unite externally so that, ultimately, they open by a common aperture, the mouth. Neither develops a lumen till a later stage.

Towards the middle of the embryo another enteric rudiment (*st.*), destined to give rise to the stomach-intestine, first appears during this stage before the constriction and curvature presently to be described have begun to alter the

original oval shape. But in this case a lumen appears very soon, and the nucleated layer which surrounds it takes on an epithelial character from a very early stage.

Up to this point the embryo has retained the original elliptical form of the ovum. After the appearance of the rudiments of the nervous and enteric systems, certain changes

TEXT-FIG. 3.



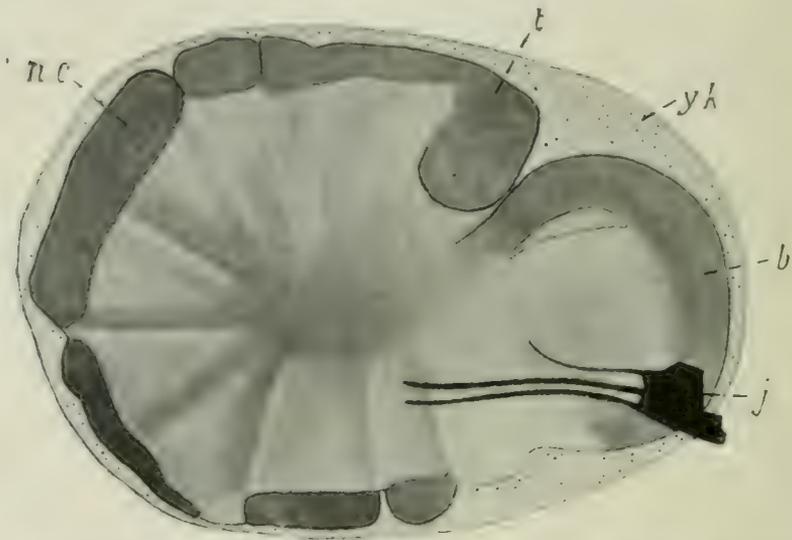
Sagittal section (semidiagrammatic) of stage just before the appearance of the jaws. $\times 600$. Letters as in preceding figures; in addition, *n.c.* nerve cord. The dotted lines *x, y, z*, indicate the approximate planes of the sections represented in Plate 23, figs. 16, 17, 18.

of importance in the external configuration take place. The oval body undergoes a process of constriction at about the middle of its length resulting in its superficial separation into two parts which correspond respectively to the head and body-regions of later stages (Text-fig. 2). The former encloses the apical plate and the œsophageal and pharyngeal rudiments, the latter contains the ventral nerve cord and the stomach-intestine.

At the same time the embryo undergoes a process of dorso-

ventral flexure with the concavity on the dorsal side and the convexity on the ventral (Text-fig. 3). The deep bay on the dorsal side formed as a result of this process is occupied by the main bulk of the secondary yolk. The developing brain becomes bent over more definitely towards the dorsal side. As the embryo grows in length the curvature increases, and the head in its dorsal region comes into close apposition with the tail. Further growth results in the overlapping of the

TEXT-FIG. 4.



Lateral view of stage after completion of jaws, viewed as a transparent object. $\times 600$. Letters as in preceding figures; in addition, *t*, tail.

regions, so that in the embryo ready to leave the egg the relations of the parts become complicated by a double process of folding, both head and tail being flexed on the trunk. Traces of segmentation appear as superficial transverse constrictions of the middle region soon after the primary dorsal ventral curvature has become well pronounced.

Around the developing intestinal epithelium is formed a thin layer with flattened nuclei. This thin layer, which is syncytial from the outset, is distinctly recognisable at a stage when no rudiment of the jaws has yet become visible. It becomes closely applied to the intestinal epithelium as the

latter assumes its definite character, and is converted into the splanchnic layer of the cœlenchyme. The parietal layer of the latter with the muscular layer to which it gives rise are differentiated considerably later. Up to the time of hatching there is no cavity between these two layers; the cœlom must develop as a result of their separation at about the time of escape from the egg.

The stomach-intestine is at first straight, but, when the flexure occurs which leads to the differentiation of the dorsal surface, it becomes bent on itself in the form of a loop (Text-fig. 3). The anterior limb of this comes into connection with the œsophagus and opens into it; the posterior extends along the axis of the developing tail region and opens on the exterior at the posterior end. The apical plate or primitive brain becomes a thick mass of nuclei by active division. Later the neuropile is formed below this. The nerve cord is also at first represented only by a thick strand of nuclei. The ganglia are not distinctly marked off till a late stage. But the bilateral character of the cord is well marked long before any trace of the jaws has appeared.

The above-recorded observations on the embryonic development do not lead to any very definite conclusion as to the relationships of the *Histriobdellidæ*. Widely divergent opinions have been expressed on this subject, but the general consensus seems to admit, in the first place, that there is a fairly close connection between the *Histriobdellidæ* and the *Dinophilidæ* (Harmer, 4, Pierantoni, 7, Haswell, 5, Shearer, 11, Goodrich, 3); and, in the second, that both these families present primitive, or degenerate, annulate characters.

Less widely maintained is the view (Haswell, 5, Shearer, 11) that there is a relationship of a fairly near character between the *Histriobdellidæ*, with *Dinophilus*, and the *Rotifera*.¹ With regard to the last question the observations above recorded, though tending somewhat in favour of this

¹ Schimkewitsch (10) admits this as a possibility in the case of *Dinophilus*.

conclusion, since there is quite striking similarity in the segmentation and gastrulation between the two groups, are disappointing as leading to the result that, owing to the syncytial condition which supervenes, the mesoderm cannot be traced to a derivation from any special cells.

With regard to the adult resemblances to Rotifers I may take the opportunity of pointing out two important difficulties to which attention has not yet been directed. It is easy to trace, as I have done (6) a correspondence between the parts of the mastax of the Rotifera and those of the jaws of the *Histriobdellida*; but such a comparison loses much of its force when we consider that the unpaired element or fulcrum is ventrally placed in the former case and dorsally placed in the latter. The second of the two points of difference which I now have in mind is that while in the male of the Rotifer the penis has a dorsal position, in the male of the *Histriobdellid* it is placed well forward on the ventral surface. But these differences, though they certainly add to the difficulty of co-ordinating the parts in the two groups, do not, I think, rule out the theory of a near connection of the *Histriobdellida* with the Rotifera, since, as regards the jaws, the proof of a precise correspondence of the chitinous pieces is not vital to the argument, and as regards the penis, in a form without enteric canal, a shifting from an original dorsal to a ventral position would not appear to be a change of a very radical character.

SUMMARY.

- (1) There is no metamorphosis.
- (2) Segmentation is complete but unequal, and resembles closely the corresponding process in the Rotifera.
- (3) At an early stage in segmentation one of the cells ceases to take part in the process of division and becomes converted into a mass of non-nucleated, finely granular material (secondary yolk), which remains distinct till a late stage.

(4) A large vegetal cell at one pole becomes immersed among the neighbouring cells, and the cells to which it gives rise in the interior of the embryo probably represent, in part at least, an endodermal layer.

(5) Complete coalescence subsequently takes place between all the cells of the embryo, this resulting in the formation of a syncytium in which the rudiments of organs first appear as a marshalling and multiplication of nuclei.

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EXPLANATION OF PLATE 23,

Illustrating Mr. W. A. Haswell's paper “On the Embryology
 of *Stratiodrillus* (*Histriobdellidæ*).”

PLATE 23.

- Fig. 1.—2-celled stage. × 600.
 Fig. 2.—3-celled stage.
 Fig. 3.—4-celled stage. From a mounted specimen.
 Fig. 4.—4-celled stage. From an alcohol specimen.
 Fig. 5.—5-celled stage.
 Fig. 6.—Lateral view of the same stage.
 Fig. 7.—6-celled stage.
 Fig. 8.—Section (approximately saggital) of the same stage.
 Fig. 9.—Stage of about 14 cells in which the large vegetal cell is beginning to become invaginated.
 Figs. 10 to 12.—Sections through an embryo in which the invagination has proceeded further than in that represented in Fig. 9, the vegetal cell being now enclosed and having given off a daughter cell (*e*). Fig. 11 represents a section separated by two (not figured) from that represented in Fig. 10.
 Figs. 13 and 14.—Two successive horizontal sections of an embryo at about the stage represented in Text-fig. 2; 13 more dorsal.
 Fig. 15.—Section from a horizontal series of a stage somewhat later than that represented in Figs. 13 and 14.
 Figs. 16-18.—Three sections of an embryo at about the stage represented in Text-fig. 3, in which the dotted lines *x*, *y*, and *z* indicate approximately the planes of section.

**Note on Intra-uterine Eggs of Heterodontus
(Cestracion) Phillipi.**

By

Prof. W. A. Haswell, M.A., D.Sc., F.R.S.,
Sydney.

With 2 Text-figures.

1. THE SEGMENTATION.

IN 1901 Bashford Dean published in the 'Annotations Zoologicæ Japonenses' a paper, entitled "Reminiscence of Holoblastic Cleavage in the Egg of the Shark, *Heterodontus (Cestracion) japonicus* Macleay," in which he described on the surface of the egg of that fish a system of lines (furrows) supposed by him to be indications or "reminiscences" of complete segmentation. In a postscript, he adds :

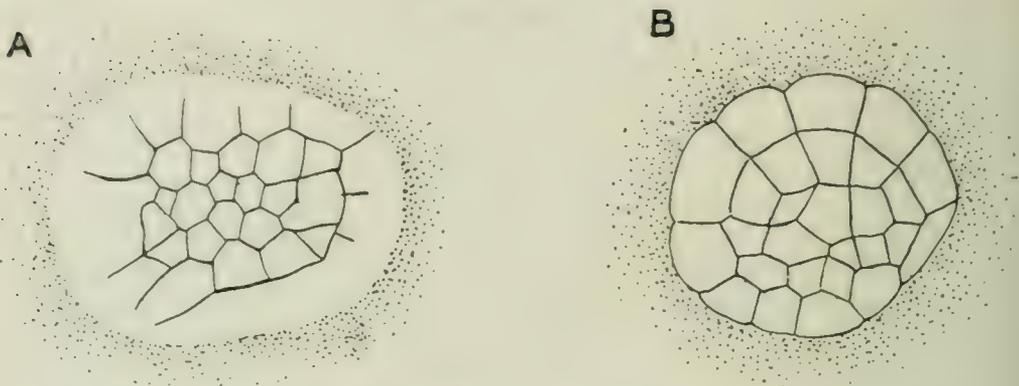
"I have recently taken several eggs (early blastula) from the oviduct of *Cestracion*, and there can now be little doubt that the lines represent cleavages. In one specimen the entire germ disc was successfully removed and viewed as a transparent object, and one could then detect cellular outlines bridging the space between the germ disc and the yolk furrows." I had previously published¹ an account of the early development of the common Australian species (*H. phillipi*) of the same genus. In none of the eggs then examined had I seen any indication of such lines. But all

¹ "On the Development of *Heterodontus (Cestracion) Phillipi*," Part 1, 'Proc. Linn. Soc., New South Wales,' vol. xxii, pp. 96-103, pls. iv, v (1898).

these were eggs which had already been laid, and the earliest stage represented was that of a blastoderm in which an ectodermal layer had already become differentiated from the underlying irregular mass of lower-laying cells.

Recently I have obtained successful preparations of several uterine eggs of *Heterodontus Phillipi*. Two of these show very similar stages of late segmentation, one a little further advanced than the other. In both the lines of cleavage are entirely confined to the area of the orange spot, and do not show any trace of a tendency to become extended

TEXT-FIG. 1.



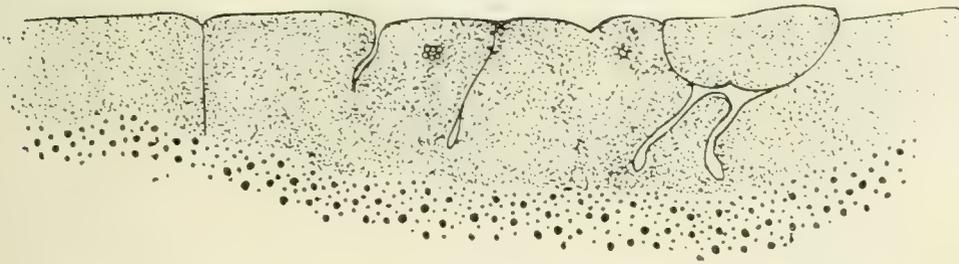
Surface views of two blastoderms of *Cestracion Phillipi*.

beyond its limits. In the less advanced stage (Text-fig. 1) (A) the peripheral part of the blastoderm has not yet become completely divided. In the other (B) the division is complete, the blastoderm is of sub-elliptical outline, and presents the appearance on a surface view of an irregular ring of larger cells separated from one another by fissures having a radial arrangement and surrounding an area of smaller cells of great irregularity in size and shape.

Two other eggs, taken from the uteri some weeks later, show much more advanced stages. In one the blastoderm, without having greatly increased in diameter, has grown very considerably in thickness. Beneath it, about its posterior limit, there is a narrow space bounded below by the fine-grained yolk—the early beginnings of the segmentation

cavity. This is a stage not far removed from one which is represented in Fig. 1 of my 1898 paper. The other is still further advanced. The segmentation cavity has become a large space below the posterior end of the blastoderm, with a thin roof through which the cavity shows itself as a dark area in the living egg. This is an earlier blastoderm than the one represented in Fig. 3 of the paper referred to, but shows essentially the same condition.

TEXT-FIG. 2.



Vertical section of the blastoderm of *Cestracion Phillipi* represented in Text-fig. 1, A.

2. THE "ORANGE SPOT."

Throughout the stages of segmentation and blastoderm formation a peculiar granular material is traceable which is not affected by the staining agents that colour the yolk granules and the protoplasm. In the earliest phases of segmentation it is very abundant and conspicuous, occurring in small irregularly scattered masses below the level to which the earlier segmentation fissures descend. The granules vary in shape and size, but are always smaller than the smallest granules of the parablast. In sections strongly stained with hæmatoxylin they appear bright and yellowish in colour. In later stages they all become enclosed in the cells of the blastoderm, and in some series they show a tendency to become massed together in the lower part of the cell, an arrangement which may be explicable on the view that the granules consist of a relatively heavy substance, which, originally diffused throughout the cell, becomes precipitated

when the blastoderm is fixed. However this may be, it appears to be almost certain that we have here the pigment to which the red colour of the early stages is due. The constant occurrence of this red colouring matter (producing the familiar "orange spot") in all families of Elasmobranchs would appear to indicate for it considerable functional importance, and I venture to suggest that it may play the part of a respiratory pigment aiding in the oxidation of the massive blastoderm and the underlying parablast.

The Development of the Sperm Duct, Oviduct,
and Spermatheca in *Tubifex rivulorum*.

By

J. Bronté Gatenby,
Exhibitioner of Jesus College, Oxford.

With Plate 24, and a Text-figure.

INTRODUCTION.

SOME months ago Mr. E. S. Goodrich kindly suggested that I should investigate the developmental history of the sperm duct in *Tubifex*. In this paper I have also described the formation of the oviduct and spermatheca. There is no modern description of the organogeny of the genital ducts of any Oligochæte of the Tubificid type. In 1886 Bergh (1) described, by means of the serial section method, the development of the genitalia of some Lumbricid worms; but histological detail of the kind, made possible by modern instruments and technique, is naturally lacking. Nevertheless, Bergh's results are, in the main, quite in agreement with my own. When Bergh wrote his paper comparative anatomists believed that the genital ducts of Annelids were always, in some way or other, connected phylogenetically with the nephridia. Even as late as 1895, F. E. Beddard (2), in his admirable monograph on the Oligochæta, wrote: "The generative ducts of the Oligochæta have for a long time been believed to have some connection with the nephridia, but the precise nature of this relation has only quite recently been cleared up." The belief in the phylogenetic relation between nephridium and gonoduct was at that time one of

the important doctrines of the comparative anatomist, being applied to the Hirudinea and Polychæta as well; but was soon to be completely upset by Mr. E. S. Goodrich (3 and 3a) in a series of important papers published in the 'Quarterly Journal of Microscopical Science.' The main results derived from a study of the genital duct and nephridium in the Polychæta are summed up in a paper published in 1900 (3b). Though Bergh nearly thirty years ago contributed evidence for the modern view of the nature and phylogenetic significance of the gonoduct and nephridium of Oligochæta, a view applying to all Annelida, and showed how completely independent were these structures in Lumbricus, investigators preferred almost to ignore his work, and to search in other types of Oligochæta for evidence of the supposed phylogenetic relation of genital duct and nephridium. Thus, though the modern view of the relation of these organs has been worked out first in the Polychæta, it will be seen that some evidence for Mr. Goodrich's views had already been accumulated in Oligochæta.

It is my pleasant duty to acknowledge my indebtedness to Mr. Goodrich both for valuable preliminary advice and for his kind interest and criticism throughout the work.

MATERIAL AND METHODS.

The worms were washed clear from their tubes, and then placed in a dish of running water, where they were left for twelve to twenty-four hours, till the gut became clear of all grit. After anæsthetising with a few drops of cocaine solution, the worms were killed in the following manner, which I devised after several other methods were tried: An anæsthetised worm was seized by the tail end and laid out straight on a piece of glass plate. Two narrow slips of glass were now apposed one at each side of the worm, so close as just to touch its sides, but not close enough to compress the animal in any way. A drop of the fixing fluid was placed at one end of the groove in which the worm was confined, and soon was

drawn over and around the specimen. Several such plates and strips of glass were used, so that while one worm was being killed others were being prepared. After the fluid had penetrated sufficiently to preclude further torsion, the worm was removed from the slip of glass and thrown into a capsule of fixative until it was properly preserved. I tried several fixatives; but the most satisfactory and uniform results were derived by using a mixture of picro-nitric and corrosive-acetic in equal parts. This mixture was recommended by Mr. Goodrich. One precaution alone should be taken—it is to thoroughly wash the worms in several changes of 70 per cent. alcohol in order to remove as much of the nitric acid as possible. If this is not done, one has a difficulty in inducing the sections to take up picric acid plasma stains and orange G, though eosin is easily used if the sections are soaked in it long enough. Picro-formol-acetic I did not find good. The usual carmine and hæmatoxylin stains were used; but some of my sections were stained in methyl-blue eosin, which was found very useful for distinguishing between and “picking out” the various tissues.

THE ORDER OF DEVELOPMENT OF THE GENITAL ORGANS IN TUBIFEX.

The gonads are developed very early in Tubifex, and in the smallest worms I have sectioned they are present; but long after they are formed from the cœlomic epithelium of the septa 9, 10 and 10, 11, the worm grows without any further addition to its genitalia. It is not till the Tubifex is nearly half grown that the other parts of the genital organs begin to appear, and as will be shown below, it is not till some of the genital products are almost ripe that the ducts are fully formed. Bergh also found in Lumbricus that the ducts did not appear till long after the embryonic period.

The funnel of the sperm duct is the first part of the genital ducts to appear. The duct itself next begins to differentiate while the funnel is undergoing further development. When

the sperm duct is still incomplete and very rudimentary, the spermatheca begins to appear in segment ten. Last of all, the oviduct is developed. The more highly differentiated structures begin to develop first, while the oviduct, which is very simple, appears quite late.

THE DEVELOPMENT OF THE SPERM DUCT.

As is well known, the funnel is attached to septum 10/11, and projects into segment ten. The genital opening is situated just above the ventral bundle of setæ of segment eleven.

In *Tubifex* the septa are thin membranes, consisting of a middle layer formed of connective tissue and muscle fibres, and on each side of this layer a covering of cœlomic epithelial cells (Pl. 24, fig. 9, s.). Even in very young worms the septa upon which the ovary and testis are attached are somewhat thicker, and the cœlomic cells more numerous than on other septa.

When the funnel of the male duct is beginning its differentiation, the first sign is found in multiplication of the cœlomic cells on the front of septum 10/11, in a position ventral to a horizontal line drawn through the middle of a transverse section of the gut, but a little above the nerve chord to the right and the left on each side of the body. The ovary in *Tubifex* is attached near the nerve chord, and hangs upwards in the segment eleven, as is shown in Pl. 24, fig. 9, which is longitudinal. In Text-fig. 1, I have drawn a diagram to illustrate the position and method of attachment of the gonad. The points o.a. mark the ventrally placed stalk of attachment of the ovary quite near the nerve chord n.c. From the points o.a. the stalk slopes rapidly towards the body wall (i.m.), being in reality pushed out in this way by the gut. The main part of the ovary (o.) projects upwards on each side of the gut, as is shown in Pl. 24, fig. 9, which is a section through the points o-o in Text-fig. 1. In Pl. 24 fig. 16 is drawn a slightly oblique transverse section,

which illustrates the truth of these remarks. The bottom part of the ovary is seen to be attached at *y* quite near the nerve chord (N.C.). In this figure the funnel thickening, which has reached a stage like that of Pl. 24, fig. 1, lies between the nerve chord (N.C.) and the bottom chloragogen cell (CH.), as an examination of the next two sections further forward showed. In Text-fig. 1 the gut occupies a position along the letters M.M., while the chloragogen cells fill some of the remaining parts of the coelom. It will now be clear that the funnel thickening on septum 10/11 lies almost beneath the gut in a ventral position, and in close connection with the stalk of attachment of the ovary.

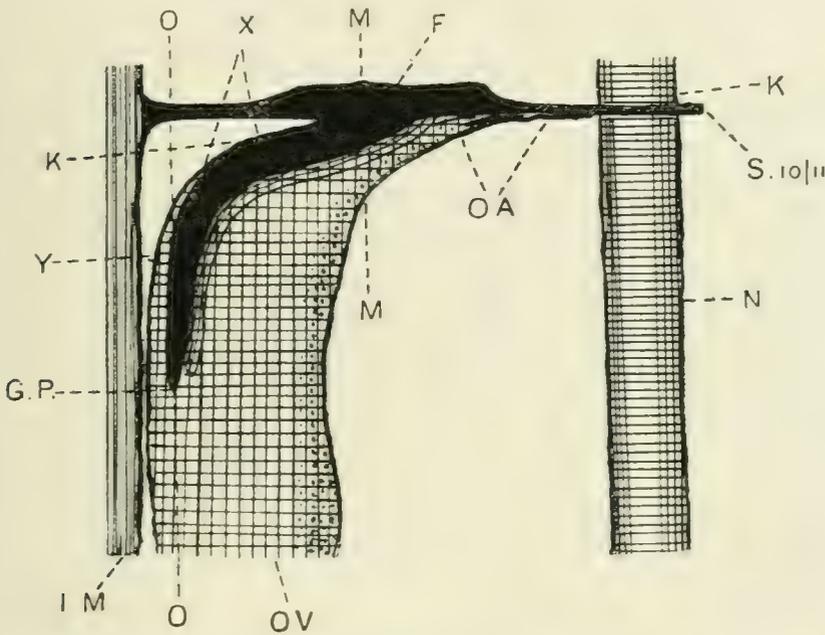
At the same time as the front coelomic layer of septum 10/11 is thickening, a number of cells, also from the front coelomic epithelium, grow back through the middle layer of the septum into segment eleven (Pl. 24, fig. 1). This backgrowth (B.S.D.) of the coelomic cells of segment ten appears at the most ventral edge of the funnel thickening, as is shown in Pl. 24, fig. 1, and in fig. 16. An examination of a good series of sections shows that this backgrowing chord pierces the septum 10/11 in different regions in different specimens. In Pl. 24, fig. 1, the backgrowth has appeared on a level just above the stalk of attachment of the ovary (OV.), while in Pl. 24, fig. 16, the backgrowth (B.S.D.) pierces the septum (S.P.) beneath the ovary (OV.). No definite statement can be given as to the region of septum 10/11, through which the early sperm-duct backgrowth will pierce; but it can be said that the latter always appears on the ventral edge of the circular funnel thickening of the septum (Pl. 24, fig. 1). This is rather remarkable, for one might possibly expect the sperm duct to appear in the middle of the early funnel. The stalk of the ovary is always found near the backgrowth, and it is often difficult to be sure that the two are different structures (Pl. 24, fig. 16). Quite close up to the place of origin of both the ovary stalk and the sperm-duct rudiment, it is difficult to distinguish between septal cells, cells of the stalk of the ovary, and backgrowth cells; and very often

in transverse section it is almost impossible to follow the duct through the septum to the funnel thickening in front. This difficulty is never so apparent in longitudinal sections. Invariably the early sperm duct is at first attached to the stalk of the ovary, as is shown in Pl. 24, figs. 1, 16, and diagrammatically in Text-fig. 1. The ovary, then, always provides the first support of the duct. From this position, marked by *x* and *y* in Pl. 24, fig. 16, the backgrowth may become directly attached to the body wall, or it may continue growing up the side of the ovary. In Text-fig. 1 the path of an early sperm duct is shown semi-diagrammatically. At the letter *x* the duct leaves the funnel (F.), runs along the stalk of the ovary at *x* till it comes into contact with the body wall. It remains partly in contact with the body wall and partly with the ovary at the region *x*. It then temporarily leaves the body wall, creeping instead up the ovary, which slopes upwards. This part of such a duct is cut in Pl. 24, fig. 9. The duct (s.B.G.) is seen to be mounting upwards, using the ovary as a support. The growing point (*xy*) is, in reality, on its path towards the body wall, as I have shown in Text-fig. 1. When the duct has reached a position about on a level with a line drawn horizontally through a transverse section of the gut—i. e., about half way up the body wall—the growing point continues on its path backwards parallel to the nerve chord (Text-fig. 1). The reason for this seems to be that the backgrowth appears on the ventral edge of the circular funnel thickening of septum 10/11, because the stalk of the ovary is situated there, and is necessary for the support of the early sperm-duct growth on its path backwards. Were the ovary to be attached higher up, one would expect that the duct would pierce the septum in the new position. In fact, it is the slightly varying position of attachment of the ovary which causes the locality of piercing of the backgrowth to vary. It is only after the backgrowth has left the stalk of the ovary that it begins to mount upwards.

It never seems to grow back *per se*, unattached to any other organ in the coelom, but always uses either the ovary or

the body wall as a support. Even where in sections the backgrowth seems to be unconnected to any body, closer examination will show that protoplasmic bridges serve to place the cord in communication with other organs. Pl. 24, fig. 13, shows the rudimentary sperm duct (SP.D.) and its connections with ovary (ov.) and body wall. The growing end of the

TEXT-FIG. 1.



Semi-diagrammatic plan of the early sperm-duct backgrowth viewed from above. At the point *x* the backgrowth was in contact with the up-curving body wall. At *y* the growth has no connection with the cœlomic wall, but the growing point (G.P.) is approaching the side of the body wall. Pl. 24, fig. 9, is drawn from a section between the points *o-o*. The attachment of the ovary (O.A.) is near the nerve chord (N.C.). This may be compared with Pl. 24, figs. 1 and 16. Pl. 24, fig. 1, is a section through *m-m* at about this stage. The point *x* in this Text-figure is identical with the same point *x* in Pl. 24, fig. 16, where the backgrowth first meets the cœlomic epithelium. This present figure was drawn from a specimen where the duct grows above the ovarian stalk (O.A.). In Pl. 24, fig. 16, the duct pierced the septum 10/11 below the ovary (ov.). (Drawn from partial reconstructions.)

sperm duct is pointed, and consists of a flattened, elongated cell, as is drawn in Pl. 24, fig. 9.

After the duct reaches the cœlomic epithelium of the body

wall it is very difficult to distinguish between those cells forming the duct and the cœlomic cells on the walls of segment eleven. It is sometimes possible to notice that the nuclei of the backgrowth stain a little more heavily than the cœlomic nuclei, but I have been unable to make certain whether any cells along the body wall contribute to the duct. From the mode of origin and its early behaviour it seems very likely that the duct is quite independent of the cells of the cœlomic epithelium of segment eleven. Nevertheless, the passing backwards of the growing end of the duct seems to exert some influence on other cells on the body wall, for along the course of the duct the cœlomic nuclei are more numerous than in other regions. This sympathetic activity is specially noticeable towards the distal end of the duct. The backgrowing cord penetrates backwards until it reaches a position right above the ventral bundle of chætæ of segment eleven. It now grows down sharply at right angles to its previous path, parallel to the transverse axis of the animal, and penetrates the muscle layers, as is shown in Pl. 24, fig. 14. In fig. 15 the communication between cord and epidermis is complete. Both these figures are drawn from the left side of the section.

Pl. 24, fig. 13, is drawn from the same individual as Pl. 24, fig. 14, the ovary (ov.) in each drawing being in nearly the same position. Fig. 13 was six sections further forward than fig. 14.

In the latter figure it will be noticed that the epidermis has synchronously thickened just where the future male pore will be situated, while the outgrowth of the sperm chord has yet to penetrate the circular muscle layer. It is a curious fact that the sperm duct should grow down from the middle of the body wall at right angles to its previous path. This is shown in a later stage in Pl. 24, fig. 4, on the right side. At the time the sperm duct has connected with the epidermis (Pl. 24, fig. 15), there is no lumen in any part of the duct. The further differentiation of the sperm funnel between the stage of Pl. 24, fig. 1, and the stage when the duct is

continuous with the epidermis, will now be described. At an early period the funnel is merely a circular area on the septum, consisting of a group of nuclei (Pl. 24, fig. 1). Not long after the stage when the epidermis and the sperm chord have met, the funnel has reached a stage such as that of Pl. 24, fig. 2, the points *x, x* of Pl. 24, fig. 1, have grown out to form an edge, while the more centrally-placed nuclei have become arranged with their long axes forwards; the whole cell structure becomes more columnar, especially towards the centre of the funnel. By the time stage drawn in Pl. 24, fig. 2, is reached, some cells from the testis have broken free and are developing in the cœlom (sp.z.). As has already been pointed out, the duct in Pl. 24, fig. 1, is very short and incomplete, but in fig. 2 it has reached the epidermis, and the distal end has acquired a lumen for a short distance.

At s.d. the sperm duct in Pl. 24, fig. 2, has been cut across on its path towards the body wall (l.m.), and consists in section of about three cells. At the bottom right-hand side of the duct is drawn a small cell, which is flattened upon the duct. The origin of such cells as this will be dealt with later. Pl. 24, fig. 1, also shows that the funnel is essentially derived from the front of septum 10/11, for the middle connective tissue and muscle layer still lies between the two cœlomic epithelial layers, and is only interrupted at the place where the backgrowth has pierced the septum. Pl. 24, fig. 3, shows that the duct (b.s.d.) now is attached to the middle of the funnel—contrast with fig. 2, where the duct has still the same relation to the funnel as drawn in fig. 1. This change of position seems to be brought about partly by the thickening of the duct and the consequent parting of, cells which bind the duct to the ventral edge of the funnel. In this way all other attaching cells become suppressed except near the middle of the funnel. In Pl. 24, fig. 3, the edges *xx* are still growing outwards, while the inside of the funnel is now profusely ciliated. A short, ingrowing lumen has appeared in the centre of the funnel, but the proximal end of the duct itself is still solid (Pl. 24, fig. 17, B). In Pl. 24,

fig. 3, the stalk of attachment of the ovary is shown, the duct appearing to the left of the latter. The subsequent development of the funnel is not different from what has already been described. The edges *xx* in fig. 3 grow outwards till the funnel is formed, and the whole structure becomes provided with long cilia, especially on the edges. In the adult organ the cells are not so columnar as is shown in fig. 3, nor does the funnel of the adult worm stain so heavily as does the developing organ of a stage such as Pl. 24, fig. 2.

The differentiation of the male duct was described up to a stage when it is merely a solid chord connecting to the epidermis (Pl. 24, figs. 13 and 15). Soon after the connection between the duct and the latter is established, a lumen appears at the most distal end (incipient in Pl. 24, fig. 15), and the rapid growth and lengthening of the organ causes it to break away at places and to begin to form folds in the cœlom. As this process goes on, the whole duct becomes coiled, and, as we know from the adult, soon forces back even into the segment behind. Long before this happens, however, much differentiation has occurred in the duct. In Pl. 24, fig. 17, I have drawn stages in the formation of the proximal part of the sperm duct. In Pl. 24, fig. 13, the latter (s.p.d) is seen to consist of about three cells in a transverse section. Now this primitive duct is found in later stages to become provided with a partial covering of cells (darkened in Pl. 24, fig. 17, Δ), which in the adult organ forms the epithelial covering of the sperm duct. The origin of these cells is undoubtedly difficult to make out, but I feel convinced that they arise from the cœlomic epithelium of segment eleven. They can first be found on the distal and proximal ends of the duct (see the darkened nucleus on the duct i.s.d. in Pl. 24, fig. 2). Moreover, they appear latest on the middle of the duct. In Pl. 24, fig. 4, these cells have already flattened themselves upon the sperm duct (c.e., c.e.), and it will be noticed that they do not cover that part of the duct which penetrates the muscle layers. The covering cœlomic layer is

quite continuous with the coelomic epithelium of the body wall near the outgrowing duct and at the proximal end near the funnel, and is, I believe, derived from those regions. I do not believe that the median region in the length of the duct receives its covering by proliferation of the cells already forming the chord; but it is quite possible that some cells forming the coelomic covering of the mid-region of the duct are derived from that part of the coelomic epithelium of the body wall to which the duct is at first attached. Probably what really occurs in most cases is that the coelomic cells on the back of septum 10, 11 grow back along the surface, and the coelomic cells in the region near where the duct meets the epidermis grow forward along the surface of the simple duct (Pl. 24, fig. 13) until they meet about mid-way, and the covering is completed. This would account for the fact that the mid-region of the duct is, more often than not, the last to become provided with the coelomic epithelium.

It has been mentioned already that the lumen first appears in the duct at the extreme distal end. Though the distal end develops latest of all the other parts of the duct, its lumen appears almost immediately after it meets the epidermis. It is broadly true that the lumen of the duct appears from behind forwards, though the early funnel soon acquires a small cavity (Pl. 24, fig. 3).

The stages in the formation of the lumen in the proximal end of the duct are given in Pl. 24, fig. 17. In Stage A the rudimentary duct has already been provided with its covering cells (darkened).

By Stage B the inner core of cells has undergone an important step in differentiation, the cells having become arranged regularly, with their longer axes meeting together in the centre. The coelomic epithelial covering has also become more definite.

In Stage C the multiplication of all the cells has caused the nuclei to become smaller, and a narrow lumen has appeared. Almost directly the lumen is formed cilia appear in it, and by Stage D the cilia are profuse. This stage shows

the incipient striation of the cells forming the duct; this striation is a peculiarity of the adult duct, and has been commented upon by Benham (4) in another Tubificid.

In Stage E, which is almost complete, the nuclei are now small, and a striation of the whole duct is pronounced, especially around the lumen. The cœlomic epithelium is much drawn out, and the nuclei are very small. The adult duct is not very much further differentiated from what is drawn in Stage E, and a description of further stages would be unnecessary. The development of the distal end of the sperm duct is necessarily complicated by the later appearance in that region of the cement gland and atrium. Vejdosky (5) has shown that the cement gland develops from the lining epithelium of the distal end of the sperm duct.

The atrium is formed by a thinning out of the distal end of the duct, so as to make a thin-walled sac of larger size than the rest of the duct.

In Pl. 24, fig. 15, the cœlomic cells which grow outwards to meet the epidermis, and those which later form the cœlomic epithelial covering of the duct are not to be distinguished from one another. In Pl. 24, fig. 4, this distinction is quite clear. After this stage one may consult Vejdosky for the formation of the spermiducal gland and for the atrium.

Before leaving the sperm duct, it might be mentioned that the muscle layer of the lower region of the male duct—*i.e.* that of the atrium—is not found in early stages (Text-fig. 4). It is only some time afterwards, but before the prostate and atrium have appeared, that the muscle layer appears. The origin of this layer is apparently due to the cœlomic epithelium, and it must be the same epithelium that forms the few muscle fibres along the duct itself. These are difficult to see in the adult worm; but in the young the muscle layer of the bottom region of the sperm duct is very thick, and only later becomes thinned out as the atrium is formed.

THE DEVELOPMENT OF THE OVIDUCT.

I have already mentioned that the oviduct is the last organ of the whole genitalia to appear. As is well known, this duct is simply a small funnel opening internally into segment eleven, and externally just where the segments eleven and twelve meet (Pl. 24, fig. 6, x). A little after the spermiduct funnel has reached a stage such as that of Pl. 24, fig. 3, and when the duct is provided with a perfectly formed lumen towards and at its distal end, the cœlomic epithelial cells on the ventral edge of septum 11/12, and those on the floor of the cœlom of segment eleven nearest the place where the adult oviduct is found, multiply so as to form a close plug of nuclei. Pl. 24, fig. 5, c.p., though drawn from a little later stage, gives a correct impression of the appearance of this plug in early stages. In Pl. 24, fig. 7, the plug is shown in transverse section just after it has begun to grow outwards towards the epidermis; the longitudinal muscle layer has already been pierced. It will be noticed that, at this time, the oviducal rudiment has a very close resemblance to the early stage in the formation of the spermatheca drawn in Pl. 24, fig. 10.

The nuclei of the cœlomic cells at the time the oviduct begins to form are a little smaller than when the sperm duct first appeared. This is made quite apparent by examining and comparing Pl. 24, figs. 7 and 8, with Pl. 24, figs. 1 or 13. Even at the early stages drawn in figs. 7 and 8, the upper cells of the plug have a partly detached and ragged appearance (y). In both figs. 7 and 8 the epidermis is still normal, but not long afterwards the clitellum begins to develop; but the oviduct still remains a solid chord, with neither lumen nor funnel. In stages drawn in Pl. 24, figs. 5 and 6, clitellum is well advanced, and yet no cavity has appeared in the outgrowth.

The true funnel and lumen of the duct apparently only appear a short time before, or when the eggs are ripe; but the solid connection between epidermis and cœlom, of course,

is present long before any eggs mature. Even in adult worms one cannot discover a lumen in the oviduct, and one concludes, therefore, that any cavity in the duct is of temporary appearance, and caused by the outpushing of the eggs. The duct is really only a region of the body wall which has been prepared beforehand by the thinning out of the clitellum, and by the internal collection of cells near where the eggs pass through the wall of the body. When one speaks of the "funnel" of the oviduct, one uses a term which gives a wrong impression. I have never found cilia on the oviduct, nor do I think that the ragged inner edge of the outgrowth of cœlomic cells which constitute the oviduct is quite aptly described by the term "funnel." Provided that one knows where to look for the oviducal rudiments, one has no difficulty in identifying the earliest stages in the formation of this organ. No muscle is found in connection with the oviduct of *Tubifex*.

DEVELOPMENT OF THE SPERMATHECA OR RECEPTACULUM SEMINIS.

Bergh (1), in his paper on the development of the genital organs in Lumbricids, describes the spermatheca as an epidermal invagination which pierces both muscle layers and protrudes into the cœlom. His details of the early stages are so meagre that I cannot compare the formation of this organ in *Lumbricus* with what occurs in *Tubifex*.

A very short time after the sperm-duct rudiment begins to grow back from septum 10/11 an examination of the mid-ventral region of segment ten will show two thickenings on either side of the body wall. This plug-like aggregation of cells grows out towards the epidermis, as is shown in Pl. 24, fig. 10. In the latter figure the ventralmost nuclei have reached the circular muscle layer. A comparison of this figure with the one drawn in Pl. 24, fig. 7, will show that the early stages in the development of the oviduct and spermatheca are nearly identical, except for the position and

time at which they begin differentiation; both begin as outgrowths of cœlomic epithelium. About the time the plug reaches the circular muscle layer the epidermis immediately below thickens synchronously (fig. 10). The cœlomic plug never pierces the circular muscle, but the epidermis now begins to invaginate, carrying the circular muscle layer with it (Pl. 24, fig. 11). The cœlomic cells are pushed aside, and the ingrowth becomes situated in the substance of the plug, which forms a cap over it.

As the invagination grows, it carries the cœlomic cells in with it, as is shown in fig. 12; while the bulk of the early cœlomic thickening forms a region encircling the mid-part of the spermatheca. Little by little, as the spermatheca becomes larger, the cœlomic cells at the point *x* become fed out till they form a single layer on the surface of the invagination (fig. 12, s.l.). That part of the spermatheca near the pore becomes, in later stages, drawn out to form a duct, and the whole organ then appears clavate. In the adult, cilia are to be found in the duct leading from the pore to the cavity of the spermatheca. These appear at a stage when the duct is constricted to form a narrow tube. The lower end of the duct never seems to have cilia, only that part of it leading immediately into the swollen terminal portion being so provided, and they are so short as to be easily overlooked. Vejdovsky has described the later stages in the formation of the spermatheca after my fig. 12, but he has overlooked the stages in figs. 10 and 11. Towards the end of differentiation there is another muscle layer added to the circular one which has been derived, as I have already shown, from the circular muscle layer of the body wall. This second and outer layer seems to be formed from the cœlomic epithelium of the spermatheca, which becomes modified in the regions where the muscle is best developed. The fibres run longitudinally.

I would venture to suggest that the cœlomic epithelial outgrowth figured in Pl. 24, fig. 10, has more significance than if it were only a preparatory stage merely aimed at separating the thicker muscle layer of the body wall before

invagination commences. If this cœlomic cell plug were to break through the circular muscle layer to the epidermis, it would resemble, in almost every particular except in its position, the oviduct. The stage drawn in Pl. 24, fig. 7, may be compared with Pl. 24, fig. 10. The suggestion naturally occurs that this outgrowth represents the last remains of the genital duct of segment ten, being, in fact, the distal extremity of the original duct. The invagination of the spermatheca may possibly represent the same region near the epidermis of the genital duct of the male segment, the region which in the male duct gives rise to the penis.

A somewhat awkward fact which is difficult to explain if we embrace the view that the spermatheca of the Oligochæta is the remains of the genital duct, is that many spermathecæ may occur in one segment (fourteen in *Kynotus madagascariensis*), and, moreover, that spermatheca quite often occur in the same segments as the male or female genital duct (see Beddard's monograph). Bergh believed all spermathecæ to be new structures. In the case of *Tubifex*, one might suppose that the spermatheca was first developed in connection with the orifice of the genital duct, and that the cœlomic epithelial plug is the remains of the old structure, while the epidermal ingrowth is the new. It is almost certain that the numerous spermathecæ in *Kynotus* are new structures, but it is quite possible that the Tubificid spermatheca is not homologous, but only analogous with the *Kynotus* spermatheca. It must, however, be admitted that such suggestions as I have brought forward are only within the bounds of pure hypothesis, and that no satisfactory conclusion can be drawn from the scanty evidence we have to work on.

ADDENDUM.

Just as this paper was being finished Miss G. C. Dixon's 'Monograph of *Tubifex*' (6) came into my hands. Miss Dixon states that she has found spermatozoa of two kinds in the sperm sac. I have examined my sections of adult worms

to see if I could find any dimorphism, and I could come to no satisfactory conclusion from sections either of the sperm sac or of the spermatophore. Smears were then made of the sperm sacs, and these were found to be more useful. I have not been able to make sufficient preparations, and I do not wish my words to be taken as my final opinion as regards the dimorphism of the spermatozoa of *Tubifex rivulorum* but I feel sure that neither of the spermatozoa drawn in Miss Dixon's monograph are mature. The heads of the Tubificid spermatozoa I have found, when drawn at the magnifications given in Miss Dixon's paper, are much longer than her supposedly ripe sperms, and, moreover, much thinner. I have found plenty of spermatozoa of the two sizes drawn by Miss Dixon, but they are not ripe, and intermediate stages are found in plenty.

Miss Dixon says: "If, however, in a mature worm one seeks for the testes, one will not be able to find them"; and in another part "that the latter [testes] soon disappear." In all my preparations of adults, containing ripe eggs and spermatozoa, I have been able to find testes, though they are slightly smaller than when the worm is half grown. Another of Miss Dixon's statements with which I am sorry to disagree is that the testes cannot be found in the adult because "they have been completely enclosed in the sperm sac." As a matter of fact, the sperm sac is not formed from that part of the septum near the nerve chord, alongside which the testes are attached. The position of the latter is not affected in any way by the formation of the sperm sac.

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EXPLANATION OF PLATE 24.

Illustrating J. Bronté Gatenby’s paper on ““The Development of the Sperm Duct, Oviduct, and Spermatheca in *Tubifex rivulorum*.””

LETTERING.

B.S.D. Backgrowing sperm duct. B.V. Blood-vessel. C.E. Cœlomic epithelium. C.H. Chloragogen cells. C.H.T. Chæta. C.L. Clitellum. C.M. Circular muscle layer. C.P. Cœlomic cell-plug. E.P. Epidermis. G. Gut. L.M. Longitudinal muscle layer. M. Muscle layer. M.P. Male pore. N.C. Nerve chord. OV. Ovary. S.P. Septum. S.D. Sperm duct. S.P.Z. Developing spermatozoa. T. Testis. X, Y, and SL refer to special parts of the figures mentioned in the text.

All figures except Fig. 9 have been drawn by camera lucida with a Zeiss 2 mm. oil immersion, and eye-piece 4. Fig. 9 was drawn with the same oil immersion and compensating eye-piece 8. In reproduction the figures have been reduced by one half.

Fig. 1.—Longitudinal section of a very early stage in the formation of the sperm funnel and duct. The sperm-duct backgrowth (B.S.D.) is seen to leave the septum 10/11 at the bottom of the sperm funnel thickening. This figure is drawn from such a section as that through the points M-M in Text-fig. 1. At XX the cells on the front of the septum 10/11 are multiplying rapidly to form the funnel.

Fig. 2.—Horizontal section of septum 10/11 at a later stage. The funnel is now a noticeable structure, and the points XX are still growing; at these edges of the funnel the nuclei are dividing rapidly, are smaller than those in the centre of the funnel, and are not arranged in a definite manner. The cells forming the more centrally placed part of the funnel have small cilia, and their nuclei have their long axes

pointing forwards. The sperm duct by this time has reached the exterior, and is at a stage somewhat like that drawn in fig. 15. At s.D. the duct is cut transversely, and at B.S.D. the outline of the rest of the duct is dotted in. At s.P.Z. some developing spermatocytes are seen free in the cœlom of segment ten.

Fig. 3.—Horizontal section of the septum 10/11 at a much later stage. The points xx are still growing outwards, but the centre of the funnel is now profusely ciliated. A short, narrow lumen has appeared in the centre of the funnel, but the rest of the proximal end of the duct is still blind, though the more distal end has reached a stage such as that drawn in Fig. 4. The ovarian stalk is cut across to the right of the duct (B.S.D.).

Fig. 4.—Transverse section of male opening (M.P.) and distal extremity of male duct before formation of atrium and penis, and after the appearance of the lumen. To follow after figs. 14 and 15.

Figs. 5 and 6 are consecutive longitudinal sections of the oviducal region of septum 11/12 a good time after the connection between the cœlomic plug (C.P.) and the epidermis has taken place. The clitellum is almost fully formed, but the oviduct is still incomplete. At x in fig. 6 the future oviducal pore will appear.

Fig. 7.—A very early stage in the formation of the oviduct. Transverse section just in front of septum 11/12. The plug of outgrowing cœlomic cells has penetrated the longitudinal muscle layer, but has still to pass the circular layer. The upper end of the plug has the characteristic ragged appearance of the early forming oviduct.

Fig. 8.—A little later stage after the junction of the epidermis (E.P.) with the cœlomic nuclei. The upper end of the young oviduct has the same ragged appearance (y) as is shown in fig. 7.

Fig. 9.—A highly magnified longitudinal section through the growing point of the early sperm duct. This figure is drawn from such a section as that through the points o-o in Text-fig. 1. The growing point (XY) is seen to consist of a single, much flattened cell. The early duct is mounting upwards, using the ovary as a support. At s. the septum 10/11 is cut, though the thickening of the funnel is more towards the nerve cord. Fig. 1 is drawn from a stage of about the same age as fig. 9.

Fig. 10.—A very early stage in the formation of the spermatheca. A cœlomic cell plug (C.P.) is growing outwards, parting the longitudinal muscle layer. At x the epidermis has synchronously thickened. T= the testis.

Fig. 11.—A later stage in the formation of the spermatheca. The epidermis has begun to invaginate, pushing into the cœlomic cell plug, which forms a sort of cap over the early spermatheca. The circular

muscle layer is still intact, and becomes carried in by the invaginating epidermis, forming one of the muscle layers of the adult spermatheca.

Fig. 12.—A still later stage to show how the remains of the cœlomic cell plug (YY) becomes fed out, eventually forming the single cœlomic epithelial layer of the spermatheca. At s.l. these cells are already one layer in thickness. As the neck of the spermatheca becomes longer, the cells at Y become stretched out to form the covering.

Fig. 13.—Transverse section of the early sperm duct just after it has reached the exterior (fig. 14). The sperm duct is connected to the ovary (ov.) and body wall by elongated cells.

Fig. 14 was drawn from the same specimen as fig. 13, only six sections further back. This shows that the sperm duct grows vertically downwards from its position near the ovary (fig. 13) in order to grow outwards to meet the epidermis. In the transverse section of this region of segment eleven a fairly long part of the sperm duct is cut in the same section (figs. 4, 14, and 15). In fig. 14 the outgrowing cœlomic epithelial cells have yet to pass through the circular muscle layer. At c.H.T. the ventral bundle of chatæ of segment eleven is partly cut across.

Fig. 15.—The cœlomic cells have penetrated the circular muscle layer and have reached the epidermis. A cavity has begun to appear in the scattered cells forming the outgrowth. All the region near this downgrowing sperm duct was much affected by the presence of the latter, for the cœlomic cells had multiplied much more than at any other part of the cœlom of segment eleven.

Fig. 16.—Obliquely transverse section through the septum 10/11 to show early sperm-duct (B.S.D) attachment of ovary to septum and surrounding structures. This figure is drawn from such a section as that through the points κ-κ in Text-fig. 1. The sperm-duct back-growth has pierced the septum beneath the place of attachment of the ovary (compare with figs. 1, 9, and Text-fig. 1). The cells marked c.H. are chloragogen cells belonging to segment eleven, but on the right-hand side of the figure the section cuts through the septum 10/11.

Fig. 17.—A B C D and E. Stages in the formation of the proximal end of the duct.

The nuclei marked more darkly in A will form the cœlomic layer of the sperm duct. At stage C small cilia have appeared. By stage E some muscle fibres (M.) can be found on the outer wall of the duct, and the cells forming the latter have become peculiarly striated.

Dendrocometes paradoxus (Stein).**Part II.—Reproduction (Bud-formation).**

By

Geoffrey Lapage, M.Sc.,Late Assistant Lecturer in Zoology, Victoria University
of Manchester,

And

J. T. Wadsworth,

Research Assistant in Zoology, Victoria University of Manchester.

With Plates 25 and 26, and 16 Text-figures.

CONTENTS.

	PAGE
I. INTRODUCTION	338
II. MATERIAL AND METHODS	338
III. HISTORY OF THE GENUS AND ANATOMY OF THE "ADULT"	341
IV. THE BUD	343
(1) The Morphology and Habits of the Free Bud	343
(2) The Formation of the Bud	347
(3) Early Stages of Bud-Formation	348
(4) The Position of the Bud in the Parent	355
(5) The Birth of the Bud	357
(6) The Fate of the Bud	360
(7) The Relation of Bud-Formation to the Size and Maturity of the Parent	3
V. THE NUCLEI	364
(1) The Meganucleus	364
(2) Division of the Meganucleus	368
(3) The Micronucleus	373
(4) Division of the Micronucleus	375
VI. SUMMARY	378
VII. LITERATURE	379

INTRODUCTION.

THE following account of reproduction by internal budding in the Acinetarian species *Dendrocometes paradoxus* (Stein) embodies the results of observations made from time to time in the zoological laboratories of the University of Manchester during the two years 1913-1914. In 1902 Hickson and Wadsworth (5) published in this journal an account of the anatomy of the species, together with a full description of the process of syngamy (conjugation). The present paper is a continuation of that work. It was undertaken at the suggestion of Professor Hickson, who pointed out that a careful study of the nuclei of the Protozoa by modern methods of technique cannot fail to produce useful results. *Dendrocometes* is a very favourable species for studies of this kind, since the meganucleus and micronuclei are comparatively large, and the animal itself is easily obtained and handled. Professor Hickson has very kindly placed at our disposal all the material that had been collected during the preparation of the paper referred to above. While the process of syngamy was being studied many observations were also made upon bud-formation, and we have been able to revise all the evidence so gained and to supplement it by further work upon fresh specimens. Throughout the work we have been much indebted to Professor Hickson for his constant interest and help, and we are very glad to have this opportunity of thanking him for criticisms which have materially aided us in bringing this paper to a successful conclusion.

MATERIAL AND METHODS.

Dendrocometes paradoxus lives epizoically upon the gill plates of the Amphipod *Gammarus pulex*, and is of very common occurrence in that situation. Most of our material was taken from specimens of *Gammarus pulex* collected from ditches and ponds around Northenden, near Manchester; but we also used specimens from the ditches in

Delamere Forest, and from streams at Nantwich in Cheshire. One batch of *Gammarus*, whose gills were particularly well infected, was very kindly collected for us by Mr. Raymond Williamson, B.Sc., from the neighbourhood of Whaley Bridge in Derbyshire, and we have received material from other sources. *Gammarus* is not difficult to obtain, and a supply of specimens of *Dendrocometes* is, therefore, easily secured. The genus is widely distributed, having been recorded from all parts of this country, from Europe, and from North America (Hickson and Wadsworth, *loc. cit.*). Owing to this wide distribution, and to the ease with which it is obtained and handled, since it is attached to such relatively large objects as the gill plates, it is a very favourable object for study, and may be recommended as a suitable Acinetarian for class work.

We have found that *Gammarus* may be kept in a healthy condition for some months in ordinary earthenware pie dishes, provided that not too many of the animals are put into one dish. Our method has been to accommodate some fourteen or fifteen specimens in each dish, together with some of the sand and water from its native haunts, a little fresh aquarium water being also added. The dishes were then kept in a cool room, ordinary laboratory temperature being usually unsuitable, and periodically the water was oxygenated by the addition of more fresh aquarium water. In this way we have been able to keep *Gammarus* in a healthy state for long periods.

In most of the specimens which were examined, at least a few *Dendrocometes* were found on each gill. Especially are they abundant on the two smallest gill plates. They may even occur here when all the other gills are free from infection. Further, we have generally been able to observe some cases of bud-formation in each batch of gills examined, at any rate during the summer months. In the winter months, buds are not so frequently seen. Whether this variation is seasonal, or related rather to the variations in the state of the food-supply, we cannot yet say. We are inclined to believe

that the latter interpretation is the correct one. Often, when the Gammarus were freshly collected, no buds were found in any of the Dendrocometes examined; but, after the Gammarus had lived in the dishes for a few days, buds began to appear. The reason for this is probably that the Dendrocometes required a few days in which to accommodate themselves to the new conditions of life. The multiplication of the other organisms in the water, and the consequent increase of food-supply probably also operated as an additional stimulus to bud-formation.

The method we have employed for the preparation of living individuals for observation has been to place the host Gammarus in a watch-glass with a little pond-water, and to destroy the head with forceps. The legs are then detached from the body, and the gill plates, which are attached to the thoracic legs, come away easily. They are generally set free at the same time as the legs, and either float freely in the water or sink to the bottom. They can then be taken up with a wide-mouthed pipette and transferred to a slide.

While low powers only are used it is wise to avoid the use of a cover-glass. If this can be avoided altogether so much the better. After the gill has been detached, Dendrocometes is very sensitive to local conditions, and will quickly die unless it is well supplied with oxygen and with water from its native pond. The slide, when not in use, should, therefore, be kept in a moist chamber, and fresh pond-water should be frequently added to it. In spite of elaborate precautions and many experiments with apparatus designed to keep up a constant, slow current through the water on the slide, we have never been able to keep the animals in a healthy state for longer than two days. Frequently they died earlier than this. The reason is probably to be found in the fact that the gill itself very quickly shows signs of death and degeneration of its tissues. Obviously it is impossible to keep the gill in a natural healthy state when it has been detached from the host. As we shall point out later (p. 363), Dendrocometes is peculiarly sensitive to any

change in the gill to which it is attached, and seems to react directly and very quickly to any alteration in the health of its "host."

For observations of the formation of buds in the living state we usually covered the gill with a cover-glass, and found the Zeiss Apoch. water immersion lens of great value, provided that efficient and critical illumination was employed. All the main features of the process can be seen, however, with the Zeiss D., the long working distance of which enables one to use it without the intervention of a cover-slip.

All our observations on the living animal have been checked and supplemented by the study of stained preparations. We have studied both whole mounts, prepared by immersing the whole gill in corrosive-acetic or Schaudinn's fluid and subsequently staining with brazilin, hæmatoxylin, borax carmine, or alum carmine. The best results were obtained by the two first-named stains. In addition to these we have prepared serial sections by embedding the gill in the usual way. The best stain for such sections is undoubtedly iron hæmatoxylin, but brazilin gives exceedingly good results. It is an advantage to stain the gills with borax-carmine or hæmatoxylin before embedding.

Preparations of the free swimming bud are easily obtained with a little care. Being large enough to be seen under the ordinary powers of a binocular microscope, they can be easily followed and caught in a fine pipette. They are then transferred to an albuminised slide, fixed, and very carefully passed through the stain and mounted. Extreme caution is necessary after fixation, both to avoid damaging these very delicate objects and to prevent their being carried away during the frequent washings. We found that they could be fixed well by Hermann's fluid or by corrosive-acetic and stained with brazilin or iron hæmatoxylin.

HISTORY OF THE GENUS AND ANATOMY OF THE "ADULT."

The history of the genus and the anatomy of the "adult" stage have been fully and accurately described by Hickson

and Wadsworth (*loc. cit.*) and in the works of earlier authors. We have nothing to add to these observations; but in order to facilitate a clear understanding of the process to be described, it is necessary to give here a brief outline of the general structure and to consider the orientation of the animal in relation to its surroundings.

The body of *Dendrocometes* is plano-convex in shape, the flat side being applied to the gill of the host. The whole body is enclosed in a firm and well-defined cuticle, which is continued along the arms; but there is no theca or protective envelope of any kind other than the cuticle. The absence of a theca is correlated with the absence of a stalk or peduncle, such as exists, for example, in the genus *Acineta* and in many other *Acinetaria*. The body of *Dendrocometes* is, therefore, directly applied to the surface of the gill. A basal plate or attaching disc of cement substance is secreted by the flat surface, and by means of this the animal is firmly fixed in position.

From the sides of the convexity of the body—that is to say, from the sides of the dorsal surface (*cf.* p. 343) three or four, or even five arms project. Each arm is provided with tentacles, and the whole apparatus serves to capture, kill, and absorb the protoplasm of the prey, which consists of free-swimming organisms present in the surrounding water. There is a single, large meganucleus and typically three micronuclei embedded in the finely granular cytoplasm. In addition, there are usually numerous large food-bodies and other granules of various kinds.

The question of the orientation of the animal is a difficult one, since none of the organs are present by which such a question can be decided. Further, the animal is sedentary, and therefore no evidence can be obtained from its behaviour while in motion. The matter cannot be properly discussed until the anatomy and orientation of the bud have been considered; but we may refer here to the views of Collin (3) on the subject. Collin, after a comparative study of the whole class *Acinetaria*, comes to the conclusion that the whole of

the convex surface of the "adult" *Dendrocometes* is dorsal, while the flat surface, which is applied to the gill, is ventral. The grounds for this view are adequately discussed by him (*loc. cit.*), and we do not propose to consider them here. Suffice it to say that our observations on the parent and on the movements and anatomy of the bud, together with a study of its method of settling down on the gill, all tend to confirm his view.

Adopting the view that the convex surface is dorsal and the plane surface ventral, it is possible to distinguish two main axes (*v. Text-fig. 1*). The first is dorso-ventral in direction, passing vertically through the middle of the dorsal surface and through the ventral basal plate of attachment. This is the true morphological axis (*Text-fig. 1, A.B.*). The second is perpendicular to this, and, therefore, roughly parallel to the ventral plane surface and to the surface of the gill. It corresponds to the physiological axis of the bud (*Text-fig. 1, C.D.*) (*cf. below, p. 344*).

Reproduction in *Dendrocometes* is effected solely by gemmation. The absence of simple binary fission is not remarkable, since it is well known that this process is extremely rare in *Acinetaria*. Fission in *Acinetaria* takes the form of gemmation in the vast majority of cases. While, however, gemmation may be either internal or external in other genera, it is always internal in *Dendrocometes*. Further, the number of buds formed is never multiple. Always a single, approximately plano-convex bud is formed inside the body of each individual parent. This subsequently escapes to the exterior, and swims about actively for a time by means of the cilia with which it is provided. Ultimately, however, it settles down upon a gill and, developing arms, assumes the form and habits characteristic of the parent.

We propose first to describe the anatomy of this bud and subsequently to consider the method by which it is formed.

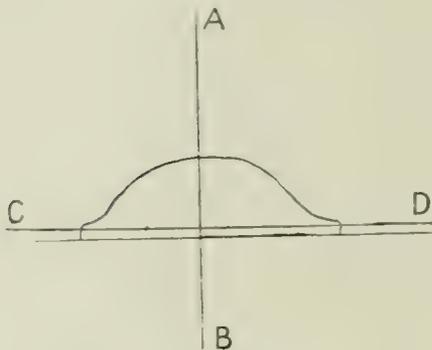
THE MORPHOLOGY AND HABITS OF THE FREE BUD.

When it is seen under low magnifications only, the general

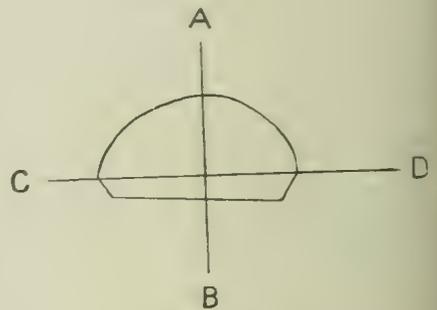
shape of the bud may be compared with that of an oval plano-convex lens. In other words, it possesses a broad, relatively flat surface upon which four rows of cilia are inserted; and an opposite surface, which is markedly convex, even in contour, and entirely free from cilia.¹ The dimensions of the bud are 0.06×0.04 mm.

When it is released from the parent the bud either swims freely in the surrounding water or "creeps" over the surface of a gill by means of its cilia, very much in the manner of a hypotrichous ciliate. In the latter case a well-marked bilateral symmetry is evident. One end is constantly anterior

TEXT-FIG. 1.



TEXT-FIG. 2.



in progression, the other posterior; the convex surface is always uppermost, the flat surface, on which the cilia are inserted, being applied to the gill. On the analogy of any bilaterally symmetrical animal moving forward along a substratum, the convex surface may therefore be described as being physiologically dorsal, the flat surface physiologically ventral. Two physiological axes are thus distinguishable. First, an antero-posterior axis passing through the anterior and posterior poles in a plane parallel to the flat ventral surface (Text-fig. 2, C.D.), and, secondly, a dorso-ventral axis perpendicular to this (Text-fig. 2, A.B.).

¹ The bud may be described as being hypotrichous in the sense that its cilia are entirely confined to the ventral surface. The use of the term in this connection can only be misleading and is better restricted to descriptions of ciliate morphology.

Since the bud subsequently settles down with its flat ventral surface applied to the gill, the convex surface of the adult is to be regarded as dorsal.

Although by their position in relation to forward movement the anterior and posterior end of the bud are readily distinguishable, there is very little difference in structure between the two. In a bud which has just emerged the posterior pole may be recognised by the presence on its dorsal convex surface of a small papilla (Text-fig. 15), which is the remnant of the stalk by which the bud was attached to the parent at the moment of birth (cf. p. 359). Plate (8) and Collin (*loc. cit.*) have both recorded this papilla, and the latter also mentions an interruption of the cilia at the posterior pole. We have never been able to see any such gap in the continuity of the ciliated bands. The presence of the papilla at the posterior pole proves, however, that the pole which leaves the parent last is subsequently posterior in progression. In other words the embryo is born, so to speak, head first.

The dorsal surface is always entirely free from cilia. The tuft of longer cilia, which constitutes the so-called "adoral zone" of some other Acinetarian embryos (e.g. *Tokophrya cyclopus*) is never present here. The dorsal surface is always uniformly even in contour, and may be described as extending almost as far as the ciliated bands (i.e. the line c.d., Text-fig. 2). At this point a more or less prominent ridge marks the ventral limit of the dorsal surface (Pl. 26, fig. 12). Turning round this we are, so to speak, on the ventral side of the body.

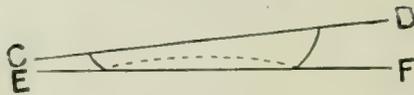
When the ventral surface is carefully examined, it is at once obvious that it is quite incorrect to speak of it as being a plane surface. The most that can be said is that it is much flatter than the dorsal surface.

After studying it from various points of view we have come to the conclusion that it presents two surfaces of different contours, namely, an outer area on which the ciliated bands are inserted and which is slightly convex ventralwards,

and a central area which is concave ventralwards. The whole bud is therefore better described as being like a biconvex lens from which the greater part of the convexity on one side has been obliquely cut away and a concavity substituted. Reference to Text-fig. 3, where the dotted line represents the central concave area, will elucidate this.

Taking first the outer portion, namely, the area upon which the ciliated bands lie, we find that its convexity is never so marked as the convexity of the dorsal surface. Its outline forms part of the circumference of a much larger circle, and it is partly due to this fact that the ventral surface as a whole looks flat in comparison with the dorsal. The presence of the ridge between them (referred to on p. 345) also accentuates this appearance.

TEXT-FIG. 3.



In addition to this, the surface extent of the convex area is greater behind than in front. In sagittal section, therefore, the ventral surface has the appearance of an inverted dome the summit of which has been cut off obliquely, along a line which is not parallel to the antero-posterior axis of the bud, but at an acute angle to it (v. Text-fig. 3 E.F.). Furthermore, the ciliated bands themselves lie parallel to this line also (E.F.), the innermost one marking the internal limit of this convex area of the ventral surface. It follows, therefore, that they are not parallel to the antero-posterior axis either, but are set obliquely to it.

Turning now to the central part of the ventral surface, namely, that part which is surrounded by and enclosed inside the ciliated ridges, it is, as we have already pointed out, slightly concave, the concavity being directed ventralwards. If a bud is viewed, therefore, while lying on its dorsal convex surface so that the ventral surface is seen en face, by focussing downwards one sees first the innermost ciliated

ridge, and subsequently the other ciliated ridges and the bottom of the concavity in the centre.

The ciliated ridges themselves are quite simple in structure. Each projects slightly from the surface of the body, presenting a double outline when carefully focussed. The ridges are always four in number—we have never seen more or fewer than this—and the cilia which they bear are inserted along the free edges. As we have explained above, the ridges lie obliquely to the plane of the antero-posterior axis, owing to the conformation of the convex area of the ventral surface on which they lie. The outermost ridge, which lies very near to the dorsal limit of the ventral surface, is, however, more nearly parallel to this plane than are the others.

THE FORMATION OF THE BUD.

The method by which the bud is formed will be better understood, perhaps, if a brief summary is given before the process is considered in detail.

Broadly speaking, it may be said the bud is, so to speak, cut out of the cytoplasm of the parent. At one point, nearer the dorsal side, the cytoplasm of the parent loses its normal consistency. This change shows itself by the appearance of a sharply outlined, refractive line, which extends across the body and subsequently turns ventralwards at each end, so as to assume the outline of a semicircle. This narrow, refractive line is the so-called brood-chamber of previous authors. It acquires an extension towards the surface of the body, meeting the cuticle of the parent at a point where the bud will subsequently emerge. No open communication, however, ever exists between the brood-chamber and the exterior, except at the moment of birth (cf. Text-fig. 5).

The outline of the brood-chamber encloses a dome-shaped mass of protoplasm, which is later organised into the bud itself. On this dome-shaped mass four ciliated ridges appear, and a contractile vacuole appears in it. The micronuclei of the parent, which are situated usually in its ventral region,

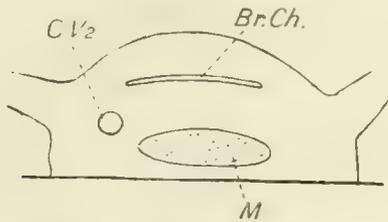
divide by a primitive kind of mitosis, three of the daughter micronuclei passing into the bud, three remaining in the parent. The meganucleus, also situated in the ventral region of the parent, divides, however, by amitosis, a very marked dumb-bell stage being seen, during which one head of the dumb-bell lies in the ventral region of the parent next to the gill, the other head in the bud. The stalk connecting these two heads gradually elongates, becoming narrower at the same time, and finally is severed while the bud is still within the parent. When all the organellæ of the bud are completely formed, it is expelled very rapidly to the exterior through a pore which appears at the point where the brood-chamber meets the cuticle of the parent.

EARLY STAGES OF BUD-FORMATION.

We have shown in Text-fig. 4 the earliest stage which we have been able to recognise in the living animals. The identification of a bud in its earliest stages is naturally very difficult, since the diagnosis must depend, so far as living observations are concerned, upon the presence or absence of either active cilia, or of a brood-chamber, or of two contractile vacuoles. The presence of any one of these features is sufficient to arouse suspicion, and they are given in the order of their relative reliability (but not in the order in which they appear). In the case figured, neither active cilia, nor a duplication of the contractile vacuole were present, but the individual showed a faint, refractive line running transversely across the body, perpendicular to the dorsoventral axis (Text-fig. 4, BR. CH.). This line was kept under observation for some hours, and eventually our patience was rewarded by its further development. The line extended further across the body, and subsequently turned downwards towards the ventral side of the animal, ending about half way between the dorsal and ventral surfaces. At this stage, therefore, the animal showed a refractive line, now quite well marked, which had become semicircular in outline, the convexity of

the curve being directed away from the gill—i.e., towards the dorsal surface of the animal. Within it was marked off a dome-shaped mass of cytoplasm, which was destined subsequently to give rise to the bud itself. A little later on a

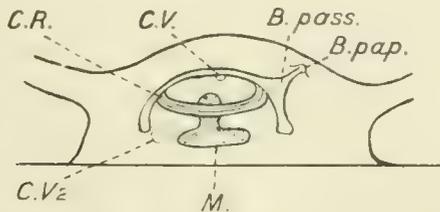
TEXT-FIG. 4.



*C.V.*₂ Contractile vacuole. *Br. Ch.* Brood-chamber. *M.* Meganucleus.

further development of this refractive area occurred. At one side an extension of it, which we shall call the birth passage (Text-fig. 5, *B. PASS.*), passed out towards the dorsal surface of the animal, meeting the cuticle of the parent at a point where the bud was subsequently expelled. At this

TEXT-FIG. 5.



C.R. Ciliated ridges. *C.V.* Contractile vacuole of bud. *C.V.*₂ Ditto of parent. *B. pass.* Birth passage. *B. pap.* Birth papilla. *M.* Meganucleus.

point a small papilla appears, which we shall call the birth papilla (Text-fig. 5, *B. PAP.*, and see also p. 351, et seq.).

The explanation of the nature of this refractive line is a matter to which we have given careful attention. By all previous authors it has been regarded as the expression of a definite cavity, the so-called brood-chamber (Bruthöhle, Bütschli: *cavité embryonnaire*, Collin), inside which

the bud develops, and the extension of it to the surface at the side has been regarded as a definite canal, of the nature of a vagina, opening to the exterior by a definite pore. For the sake of convenience we shall continue to refer to it as the "brood-chamber"; but to regard it as a well-defined cavity is, we believe, somewhat misleading. Possibly such an interpretation has arisen from an erroneous idea as to the way in which it is formed. Bütschli (1) and Plate (*loc. cit.*) have both stated that the brood-chamber begins in *Dendrocometes*, as it apparently does in *Podophrya quadripartita* and some other *Acinetaria*, as an invagination at the surface at the point where the bud is ultimately expelled. The birth-passage, in this view, is formed first, and the "cavity" inside which the bud develops is regarded as a later extension of this. In our opinion, however, this view is incorrect. The brood-chamber, in all the cases observed by us, began as a transverse refractive area across the body of the parent, which extended as described above, and only when fully formed, reached the surface of the animal. We believe, therefore, that the birth-passage is formed last, not first.

In this connection the views of Stein (10) are interesting. Describing the meganucleus of an individual in which bud-formation was in progress, this author states that it was "surrounded by a light area which resulted from a dissolution of the granules . . . around it." This "light area" corresponds with the refractive line described above. Although Stein does not seem to have grasped the significance of his "light area," his observation yet supports our contention that the brood-chamber begins in the parental cytoplasm near the meganucleus, and does not grow in from the surface. Moreover, the words "dissolution of the granules" give a much more accurate idea of what is actually seen than does the description of a definite cavity. We cannot agree that a definite cavity exists. We have never been able to demonstrate it, either in the living animal or in serial sections, and certainly if it were present, one would expect to find it in permanent preparations. The so-called "brood-chamber" is

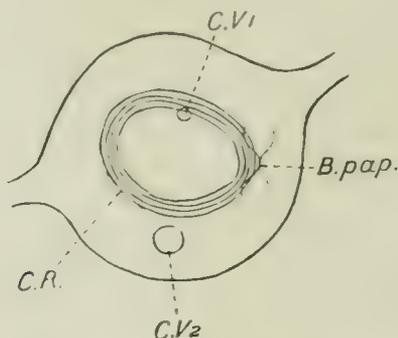
better described as an area over which the normal granular structure and consistency of the cytoplasm are lost, and which acquires an extension up to the cuticle of the parent. The bud, therefore, does not lie in a space which possesses definite walls, but is merely separated from the normal cytoplasm of the parent by a thin film, so to speak, of a more fluid nature, sufficiently fluid, in fact, to enable cilia to move in it (cf. p. 352).

A further point which throws some doubt on Bütschli's and Plate's interpretation of the chamber as a definite cavity is the structure of the birth papilla—namely, the point at which the brood-chamber meets the cuticle of the parent. Both Bütschli and Plate describe an opening here. Indeed, if the chamber begins as an invagination, as they believed, an opening must be present at the commencement of the process. Plate stated that this opening closed up while the bud was being formed, and that a new pore was formed at the moment of birth to allow the bud to escape. Bütschli (2), however, threw some doubt upon this, and stated that the original opening persisted throughout. Neither of these views is, in our opinion, correct, since they both depend upon the conception that the brood-chamber is a definite cavity, formed by invagination. The true nature of the birth papilla can only be properly made out in individuals which are situated, not upon the edge of the gill, but on the surface of the gill internal to this. If such an individual is selected, it is possible to look directly down upon its dorsal surface, and to see the birth papilla from various points of view. If this is done, it is seen that the papilla bears not a definite pore, but a closed slit, very minute in size, situated between two prominent lips. The papilla itself is formed, in fact, chiefly by the projection of these lips above the surface. It is by the detection of this prominence that the position of the slit can be made out when it is seen obliquely (Text-figs. 7 and 8), or from above (Text-fig. 6). We do not believe that it is present at all at the very beginning of the process; but it is formed at an early stage, as soon, in fact, as the brood-

chamber itself reaches the cuticle of the parent. Moreover, we have never been able to show that it is ever actually open—i.e., the brood-chamber does not ever openly communicate with the exterior, except at the moment of birth. When birth occurs, as we shall describe later, the two lips are readily seen, as they are forced apart by the passage of the bud between them.

Turning now to the dome-shaped mass of cytoplasm enclosed within the brood-chamber, indications may be seen that it is being organised to form the bud itself. The first

TEXT-FIG. 6.



C.R. Ciliated ridges. *C.V₁*. Contractile vacuole of bud. *C.V₂*. Ditto of parent. *B. pap.* Birth papilla.

structures to appear are the ciliated ridges, one only appearing first, the other three being added later. As a rule, the ciliated ridges are completely marked off before the nuclei have begun to divide. On them the cilia are easily seen, and appear, when the bud is seen externally, as small, brush-like tufts actively beating about in the brood-chamber at the sides (Text-fig. 5). In some individuals the cilia may even stretch right across the chamber, and, coming into contact with the firm resistance of the normal parental cytoplasm on the opposite side, they may beat against it and momentarily alter its shape by their movements.

The presence of active cilia is generally diagnostic of the presence of a bud. However, it is well known that they are also seen in individuals which are about to leave the gill, and

which are not cases of bud-formation at all (cf. p. 363). By their activity or sluggishness the state of health of the bud, and thus indirectly of the parent also, may be judged. When they stop altogether, it may be concluded that something is wrong in the preparation, and a fresh example should be sought (cf., however, p. 358).

With regard to the other organellæ of the bud, we have been able in certain instances to distinguish the outline of the meganucleus in the broad stalk which connects the developing bud with the parent. It is comparatively difficult to see, however, owing to the density of the cytoplasm and the abundance of food material. We have never been able to see the micronuclei in the living animals.

Contractile vacuoles are always very easily seen. In fact, the presence of two such structures is generally an indication that bud-formation is in progress, especially when one of them is larger than the other. The contractile vacuole of the bud appears early—soon after the ciliated ridges are formed. It may be recognised by the fact that it is always smaller than that of the parent, and it pulsates more rapidly, systole occurring, on an average, every half-minute, or more often still than that. Its position in the bud is more or less constant. It is usually found immediately dorsal to the ciliated ridges to one side of the middle line (Text-figs. 5 and 6, c.v₁, and Pl. 26, fig. 11). According to Bütschli (1) it is always found on that side of the bud which is furthest from the parental contractile vacuole. This author also describes a definite outletting canal which opens into one of the grooves between the ciliated ridges on the ventral surface of the bud. We have not, however, been able to see this structure.

The position of the contractile vacuole of the parent is not so constant. Generally it lies towards the ventral side of the parent in relation to the dorsal region of the bud (Text-fig. 5, c.v₂). This position is interesting in view of the fact that in some other Acinetaria (Collin, p. 169—Tokophrya cyclopum, Choanophrya infundibulifera, etc.) the parental contractile vacuole actually communicates with the

brood-chamber and expels its contents into it. In these forms the brood-chamber has the form of a definite cavity, which dilates with systole when the vacuole discharges its contents and contracts during diastole. We have never seen any such communication in *Dendrocometes*, where, it will be remembered, the brood-chamber is not a definite cavity.

In addition to the above well-defined organellæ, there is one other constant feature of the cytoplasm of the developing bud which is worthy of note. In a bud that has almost completed its development an area is observable in which the cytoplasm presents a different appearance than that of the remaining cell-substance (Pl. 25, fig. 8, sp. *CYT.*). The texture, if one may so express it, of this area is denser and more homogeneous in character than the rest of the cytoplasm, and in addition it exhibits a very finely granular structure. The absence of deeply staining bodies, such as particles of food, etc., from this area is also characteristic. Its position is constant, on the antero-posterior axis of the bud, and adjacent to the nucleus. The micronuclei of the bud are generally near this area, and in some cases they have been observed within it. Its staining reactions appear to be similar to those of the remaining cytoplasm.

It is first distinctly recognisable during the late dumb-bell stage of the division of the meganucleus and when the chromatic spindles of the micronuclei are undergoing absorption. The same appearance is visible in stained preparations of those *Dendrocometes* which are preparing to leave the gills.

We are unable to state definitely what this appearance truly represents; but we venture to put forward the suggestion that possibly a fluid substance, "a nuclear sap," is exuded from the nucleus at this stage, and under the influence of fixatives and other reagents this substance becomes coagulated, and gives rise to the appearance that we have described above.

THE POSITION OF THE BUD IN THE PARENT.

The position of the bud inside the brood-chamber is shown in the diagrammatic Text-fig. 5, which represents a lateral view of a bud which is almost ready to emerge. All its organellæ are formed, and its orientation is for that reason the more easily understood. The first point to notice, and one which is all-important, is the position of the ciliated ridges. They lie on the ventral surface of the free bud, and their position therefore at once localises that surface. From the figure it will be seen that while the bud is in the brood-cavity the ciliated ridges are always uppermost—that is to say, on that surface of the bud which is turned away from the gill and towards the dorsal side of the parent. The ventral surface of the bud is therefore turned towards the dorsal surface of the parent, and it is attached to the floor of the brood cavity by its dorsal surface, which is connected with the parent by a broad stalk. In other words, the orientation of the bud while inside the brood-chamber is the exact opposite of that of the parent and of the position which it will itself occupy when free. It is, so to speak, upside down while inside the parent. We have taken special care to establish these facts with certainty, because Bütschli has both described and figured (1) the bud in exactly the reverse position—namely, with its dorsal convex surface turned away from the gill and its ciliated ridges (ventral surface) at the bottom of the brood-chamber. According to Bütschli, therefore, the bud lies in the brood-chamber on its ventral surface, the same way up, so to speak, as the parent. That such a view is incorrect we are convinced by the study of living buds and of the actual process of birth. The latter observations are important in view of Bütschli's confession that he saw birth only once, and we shall have occasion to show that even that instance was probably atypical. Had he seen one typical birth, we are sure his views as to the orientation of the bud must have been different. Finally, the study of serial sections places the matter quite beyond doubt. It is

possible, in an individual cut at right angles to its dorso-ventral axis, to work down from the dorsal surface of the parent to the gill below. When this is done, the ciliated ridges are always met with before the meganucleus. Since in the free bud the meganucleus is dorsal to the ciliated bands, it follows that the dorsal surface of the bud while it lies inside the parent is that part which lies nearest the gill.

Further study of the bud reveals, moreover, another point: namely, that the bud lies somewhat obliquely in its brood-chamber. The dorso-ventral axis which would pass through the centre of the parent body would not, therefore, pass through the centre of the bud. Similarly the plane of its ventral surface is not exactly perpendicular to the dorso-ventral axis of the parent. The result of this is that a section which would divide the parent sagittally into two equal halves would divide the bud very unequally, considerably more of the ventral surface and ciliated ridges being cut off to one side than to the other.

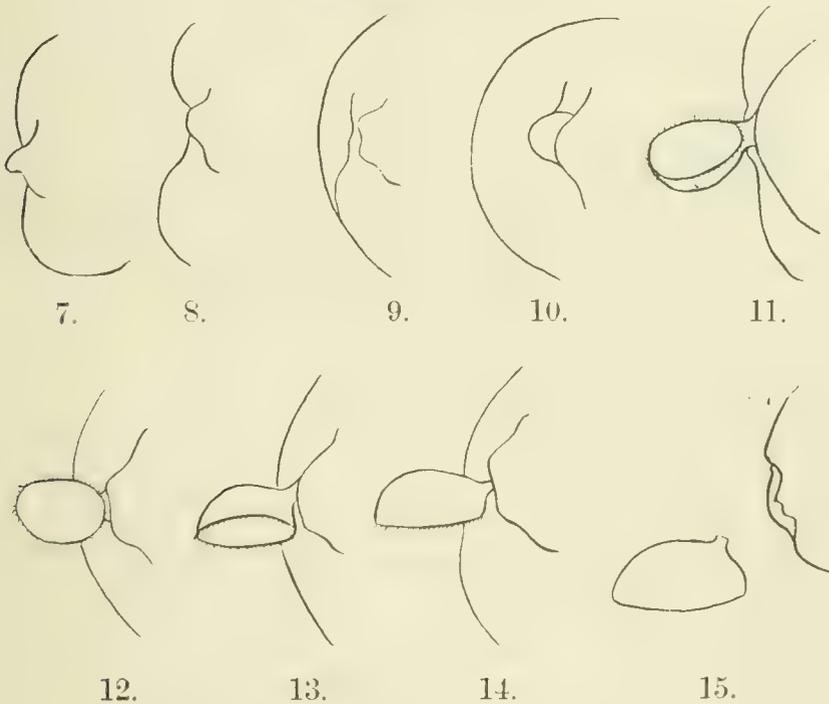
When the bud is almost ready to emerge, the broad stalk by which it is still attached to the parent is to a certain extent reduced in width by an extension of the brood-chamber into its base. This undermining, as one may call it, is always more noticeable on the side on which the birth papilla is situated. The bud thus has the appearance of "yearning" towards the slit through which it is destined to be born.

It must not be supposed, however, that the stalk ever becomes narrow; not, at any rate, while the bud is still with the parent. Further, we can confirm Bütschli's observation that the bud is never completely cut off from the parent inside the brood-chamber. Whatever may be the case in this respect in other Acinetaria, the bud of *Dendrocometes* never lies free in the brood-chamber. Stein (loc. cit.) stated that it moved back and forth in the chamber by means of its cilia. This is certainly wrong, as Bütschli has already pointed out. Stein was probably misled by the motion of the cilia themselves, which very easily give the impression that the bud itself is moving.

THE BIRTH OF THE BUD.

It is characteristic of the actual process of birth that it occurs with great rapidity and commences suddenly. For this reason we did not for some time observe the actual expulsion, though we had no lack of individuals in which buds were being formed. Bütschli evidently experienced the same difficulty, since he states (1) that he only saw the actual

TEXT-FIGS. 7-15.



birth of the bud in one solitary instance. To the best of our knowledge the process has never been described in detail, and since for reasons adduced below, we are convinced that the case seen by Bütschli was not typical, we shall describe fully what we have seen. All the steps in the process are illustrated in Text-figs. 7-15, which are diagrams made from rapid sketches taken while birth was being watched. It is usually difficult to determine when a bud is about to emerge. In all its essentials, such a bud is in an advanced state of maturity. By keeping such individuals under constant

observation, working turn by turn, we were able to see a number of cases of birth.

In all these cases we noticed one very constant indication that the time for delivery was at hand. For a few seconds immediately before birth the cilia of the bud become decidedly less active and may cease to move altogether. This may be mistaken for death of the individual, and, indeed, was so mistaken by us in several cases: but it is nevertheless a fairly reliable indication of what is to follow.

A second or two after the cessation of ciliary action the bud suddenly moves out towards the birth-pore. The process may best be described as a herniation of the bud, and is very rapid. Whether it is due to a contraction of the mother or to some other agency we cannot say. But in some 3-5 minutes from the first appearance of the bud at the birth-pore it is outside, free from the parent, and actively swimming about in the vicinity.

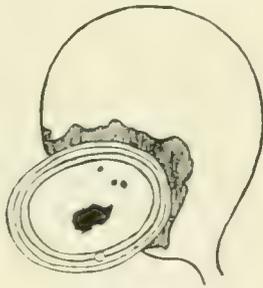
The actual herniation may be described thus. The bud is moved rapidly to the birth papilla. In surface view it is seen that the two lips of the birth papilla are pushed apart, and between them appears a ciliated mass of bud protoplasm (Text-fig. 10). The process continues until, viewed laterally, the parent shows a decided bulge at this point, formed by the bud itself. On this extruded portion of the bud a horizontal line of cilia, representing the ciliated ridges, is seen for a moment. Immediately afterwards this ciliated line has apparently migrated lower down on the bud (Text-fig. 11), and later still takes up a position at its lower margin. At this moment cilia come into view along the upper margin also, so that the bud bears a fringe of cilia all round its circumference (Text-fig. 12). Immediately after this the ciliated ridges again appear to be near the upper margin only. They move to the middle again (Text-fig. 13), and finally are seen at the lower border only (Text-fig. 14).

The shifting of the ciliated ridges is only apparent, of course, and is due to a twisting of the whole bud around its antero-posterior axis as it comes out. The lateral expulsion

is accompanied by a screw-like motion, so that, while at the very commencement the ventral surface is uppermost and the ciliated ridges are seen in profile along the upper border, the bud subsequently twists until, when half-way out, the ventral surface and ciliated ridges are seen en face. The twist continues further, and finally, when the bud is completely extruded, the ventral surface becomes lowermost, directed towards the gill, and the ciliated ridges are seen in profile again, but now along the lower border.

When the bud is definitely outside it remains attached for a time to the parent by a rather broad pedicle (Text-fig. 14). This rapidly becomes thinner and longer owing to the efforts

TEXT-FIG. 16.



of the bud to escape. The final severance of the connection is effected partly by a vigorous tugging movement executed by the bud itself, but also by another method. The bud may very rapidly rotate itself round the pedicle and twist itself off, so to speak, exactly as an apple might be twisted from its stalk. This gyratory movement of the bud round its stalk is quite remarkable.

Finally, when the stalk gives way, the bud swims away freely. A short peg-like remnant of the pedicle is always left on the dorsal, convex side of the bud at the end which is posterior in forward movement (Text-fig. 15).

A similar stump may be seen on the parent also for a short time. It is often difficult to make it out here, however, since the wall of the parent remains for some time markedly crumpled and shrunken (Text-figs. 15 and 16). Ultimately,

after the lapse of half an hour to an hour or so, the normal, even contour is regained.

One point, also discussed in the section dealing with the nuclear phenomena, may be briefly referred to here. Bütschli (1) has stated that the final division of the meganucleus does not take place until the bud is outside the parent. It is divided, in other words, at the time when the stalk connecting bud and parent is severed. We cannot agree with this view. Not only does the study of serial sections definitely disprove it, but also we were unable to detect any sign of meganuclear structure in the connecting pedicle during our observations on the living animals. This does not, however, necessarily mean that Bütschli's observations were inaccurate. The condition of affairs described by him is not impossible, for it is well known that cases of what may be termed "pathological birth" do occur in *Acinetaria* (cf. Collin, *loc. cit.*). In these cases the amitosis of the meganucleus may be very much delayed, and may, indeed, never occur at all, the bud remaining attached to the parent and ultimately dying. Such a case, we believe, was observed by Bütschli, and mistaken by him for the normal and typical process described above. Since it was the only case he saw, he had no opportunity of comparing it with other instances. In active, healthy *Dendrocometes*, very little variation of the process occurs, and any such marked difference as that under discussion must have been due to the unhealthy condition of the individual, or to the lack of proper precautions in preserving it in a healthy state while under observation.

THE FATE OF THE BUD.

The aim and normal destiny of the bud is, of course, to seek out a favourable area on the gill and to settle down there, becoming firmly fixed by its ventral surface, and growing eventually into the ordinary "adult" stage. The behaviour of the bud immediately after liberation varies somewhat. In the first place, it may remain in the neighbourhood of the

parent for some time, swimming about freely ; or, in other cases, it may swim away from the parent gill at once and seek out another gill altogether.

These phenomena are the rule in moist chamber preparations. In a state of nature it may well be the rule that a bud shall settle down at once on the same gill. We think it doubtful whether the bud would be able, powerful swimmer as it is, to overcome the strong currents of the swift-running streams in which *Gammarus* typically occurs. In the pond-inhabiting *Gammarus* no doubt these currents would be considerably weaker, but the risk which the bud would run of never finding a resting-place if it left the vicinity of the parent *Gammarus* would be considerable, because *Gammarus* is a very powerful and rapid swimmer. Moreover, the active swimming movements and the respiratory currents created by the host would be quite strong enough to sweep the bud away, even in a still pond. It is very probable, therefore, that in a state of Nature the bud generally settles either on the gill to which the parent is already attached, or to one of the other gills on the same *Gammarus*.

During its free life the bud exhibits at least two types of movement. The first type is characteristic of the time when the bud is swimming freely in the water in the neighbourhood of a gill. Its progress is rapid, and generally in a straight line. It does not, however, orientate itself with the dorsal surface upwards and the ventral surface downwards, as might be expected ; but swims, as it were, on one side. In other words, the dorsal surface is turned generally to the left, the ventral surface to the right, so that the right-hand margin is directed upwards. Moreover, the forward movement is accompanied by a very marked swaying round its antero-posterior axis, so that the observer sees now the dorsal surface uppermost, now the right-hand margin and now the ventral surface. An apt imitation of this phenomenon might be secured if it were possible to sling an oval plano-convex lens upon a string passing through its long axis. If it were now propelled along the string, and at the same time gently

tapped so as to make it sway slowly and rhythmically from side to side about the string, the movement of the bud would be very closely reproduced.

The second type of movement is characteristic of the time when a bud, having arrived at a gill, comes down upon it and begins to move over its surface. It moves now very much in the same way as a hypotrichous ciliate would move, the cilia on the ventral surface being in contact with the gill and acting after the manner of legs. In this way the bud "creeps," so to speak, carefully over the surface, picking its way among the Epistylids, Dendrocometes, and other fauna already established there. Its behaviour during these "creeping" movements strongly recalls that of a hypotrichous ciliate in search of food, and it may easily be mistaken for such by the careless observer. We have no evidence, however, to show that the bud ever feeds at all during its active, free-swimming stage. No mouth is present, and we must conclude that the "creeping" movements are mainly exploratory, and have for their object the "selection" of a suitable place for attachment.

As regards the positions on the gill most often chosen by the bud, we have not found that any absolute choice is ever exercised. Upon well-populated gills the Dendrocometes are scattered indiscriminately all over the surface. On sparsely-populated gills the animals are most often found towards, or actually on, the edge, often being folded over the edge so that they are attached to both surfaces of the gill plate. The rest of the gill surface is left quite free. From this it is reasonable to conclude that the edge is the position most preferred. Doubtless it is the situation most favourable for the capture of food.

We have also noticed that the animals also seem to prefer the proximal end of the gill when the edges are fully occupied, a preference also shown, curiously enough, by the Spirochonas and Epistylids, when these are present.

Whatever the position chosen, however, the bud loses no time in settling down. It becomes quite motionless at the

chosen spot, with the ventral surface applied to the gill, and secretes a basal plate of attachment, by which it is permanently fixed in position. The ciliated ridges disappear, and the bud now presents the appearance of a small *Dendrocometes* without arms. In preparations in which bud-formation is actively going on many such individuals may be found. Text-fig. 1 shows the appearance which they present. It will be seen that the ridge marking off the dorsal from the ventral side, referred to on p. 345, is particularly well marked at this stage.

It only remains now for the bud to put out arms and it becomes a typical "adult" *Dendrocometes*. From this stage onward it leads the normal life of that phase in the life-history, actively feeding and growing in size. Ultimately it may conjugate in the manner already described by Hickson and Wadsworth (*loc. cit.*), or may itself form buds.

THE RELATION OF BUD-FORMATION TO THE SIZE AND MATURITY OF THE PARENT.

With regard to this interesting question very little can be definitely said. Apparently there is nothing in the appearance of any given individual which will indicate whether it is capable of forming buds or not. Either well or sparsely fed individuals may form buds, and we have observed the process in forms of all sizes. In every case that we have seen, however, the parent possessed arms. It seems probable, therefore, that a young individual, but recently settled on a gill, does not normally form buds until it has developed arms, and has commenced to feed in the usual way.

In this connection we may refer to a fairly common phenomenon which is associated with the migration of the *Dendrocometes* from one gill to another. An individual, which has hitherto exhibited all the structural and physiological features characteristic of the species, will at certain periods draw in its arms altogether, taking on the appearance shown in Text-fig. 1. Very soon ciliated ridges appear, and

almost the whole substance of the animal parts from its base and swims away, leaving only the basal plate of attachment and a crumpled residuum of protoplasm, enclosed in the old cuticle, attached to the gill. The active individual so liberated subsequently settles on another gill, secretes a new basal plate of attachment, and, putting out arms again, resumes its normal mode of life. The process was observed and has been graphically described and figured by Bütschli (1), who regarded it as being essentially different from true bud-formation, and we entirely concur in this view. The mass which is left behind on the gill consists of nothing but a residuum of protoplasm incapable of physiological activity, and destined to disintegrate and die. The phenomenon is more of the nature of a migration, correlated with, and doubtless directly caused by some change in the gill itself. We have noticed that individuals exhibiting this phenomenon are attached to gills which are not quite normal. Sand (9) has pointed out that it always takes place upon gills which are about to moult. If this is so, it is probable that, when the host *Gammarus* is about to shed its skin, physiological changes commence in the gill which in some way influence the *Dendrocometes* living on it, so that they are stimulated to migrate in the manner described above. Bütschli, who first observed the phenomenon, regarded it as an excretory process. We have no evidence to support either view; but the phenomenon is so common that we have no doubt that it is to be reckoned with as a constant feature of the physiological relationships of the animal.

THE MEGANUCLEUS.

The meganucleus of *Dendrocometes* is a conspicuous structure of relatively large size, measuring from 0.02–0.034 mm. In shape it varies a good deal, according to the state of its activity. In the so-called “resting stage”—that is to say, when it is not taking part in any reproductive process or in syngamy—it is usually oval; but it may be more nearly spherical or elliptical and very much elongated.

It usually lies in the ventral region, over the attached base of the creature, and when it is oval its long axis is parallel to the plane of the ventral surface (Text-fig. 4). In the living animal it is usually seen without difficulty, and presents a finely-dotted appearance, due to the presence in it of fine chromatin granules.

The nucleus is a good example of the granular type of nucleus characteristic of the meganuclei of Heterokaryota in general. In permanent preparations it is often surrounded by a clear zone (Pl. 25, fig. 1), irregular in outline, which separates it from the cytoplasm. This zone is merely an artefact due to shrinkage during fixation, and does not represent any part of the nuclear apparatus. The nuclear matter itself is arranged in the form of a well-marked network with wide interspaces (Pl. 25, fig. 2); the strands of this network are composed of small granules aggregated together upon a linin framework. From the fact that they stain by all the ordinary nuclear stains, and from their subsequent behaviour during division of the nucleus, it is obvious that they are granules of chromatin, and represent the chromatinic elements of the nucleus. The interspaces of the network, on the other hand, invariably refuse to take up the stain, and in a healthy meganucleus are perfectly homogeneous in appearance.

We have never been able to demonstrate the presence of a true nuclear membrane. Previous workers, including Bütschli (1) and Plate (8), have definitely stated that this structure is well marked and easily seen. Collin also affirms its presence, and has figured what he believes to be a nuclear membrane in *Dendrocometes* (3) (Pl. 25, fig. 19). In that figure a very definite membrane is shown, lining the shrinkage space in which the meganucleus lies, and bearing chromatin grains adhering to its inner surface.

This latter fact, coupled with the fact that the structure figured stains deeply with iron hæmatoxylin, proves, in our opinion, its true nature. A true nuclear membrane is always achromatinic in nature, and only takes up nuclear stains with difficulty. The "membrane" described by Collin is therefore

much more likely to be merely a portion of the superficial layer of the chromatin grains, which, lying together, constitute what Minchin (6) has termed a "false" or "chromatinic" membrane. We have found portions of such a membrane in some of our preparations, especially in those which have been badly fixed and in which the meganucleus is distorted. We have never seen it in preparations which are well fixed and stained. Moreover, it is significant that, although Collin gives five figures of *Dendrocometes* (3) (Pl. 25, figs. 18, 19, 20, 21, and 22), in only one of these (fig. 19) is the so-called nuclear membrane shown. A further point of some importance is the fact that no trace of a nuclear membrane is ever seen during division of the meganucleus. It is not uncommon, of course, for the nuclear membrane to disappear during division—it does so frequently during mitotic division at any rate—but it is reasonable to assume that, were it present, some trace of it would be discoverable at some stage of the process.

Taking all these facts into consideration, we must conclude, therefore, that a true nuclear membrane does not exist in the meganucleus of *Dendrocometes*. A "false" or "chromatinic" membrane may, however, appear in the manner described above.

Kinetic elements, such as centrosomes, centrioles, etc., are also entirely absent from the meganucleus, and division is strictly amitotic. In this respect the meganucleus of *Dendrocometes* agrees with that of other *Acinetaria*.

Further, we have not found any nucleoli—i. e., pure lumps of plastin, although they are present in the meganuclei of other *Acinetaria*. Karyosomes, also, are not present as isolated lumps of chromatin apart from the general chromatin network.

The interpretation of the features mentioned above has given rise to conflicting views, and discussion has been mainly centred round the question as to whether the chromatin grains are supported upon a linin framework or not. Collin (3), and with him Bütschli (1) and others, denies abso-

lutely that any linin framework is present. He regards the meganucleus as a vesicle, enclosed in a definite membrane and filled with nuclear sap in which float quite freely the grains of chromatin (microsomes) and larger granules of a different nature (macrosomes). The conception is extended also to the structure of the cytoplasm, and is invoked further to explain the phenomena of the nuclear division and bud-formation. In support of it the author brings forward an extensive series of observations on the other Acinetaria, and seeks to supplement these with collateral evidence derived from other groups of Protozoa (Ciliata). In our opinion, however, this conception does not fit the facts as we have observed them in the case of *Dendrocometes*. The whole study of the meganucleus in the "resting state," as well as during division, goes to support the alternative conception of a number of chromatin grains borne upon and supported by a framework of linin threads.

We are not able definitely to affirm that we have seen this framework, at any rate while the meganucleus is in the "resting" phase. Even in the thinnest and most carefully stained sections no framework is visible there. But we do not feel justified in concluding from this fact alone that no framework is present. Minchin (*loc. cit.*) has clearly pointed out that the fact that no linin threads can be seen is no proof that they do not exist. Linin, he argues, is achromatinic in nature, and therefore difficult to render visible by the ordinary chromatinic stains. Further, he points out that were no framework present, it would be difficult to explain the arrangement of the chromatin in a very definite manner, in this case a network. We may add to this the fact that during division a very obvious framework appears (*cf.* pp. 369 *et seq.*, Pl. 25, *figs.* 7 and 8). If there is no linin framework during the "resting" stage, from what is it formed during division? Furthermore, it is pretty generally agreed that a linin framework supports the general cytoplasm, and is present there as a network similar to that which is found in many nuclei. Now, in *Dendrocometes* it is no

more easy to demonstrate the presence of a framework in the cytoplasm than it is in the meganucleus. Are we to conclude that, therefore, it is not present in either situation? We, at any rate, are not prepared to accept such a view.

The whole matter is, perhaps, only part of the much wider question of the structure of protoplasm in general. Collin's conceptions are no doubt the outcome of an acceptance of Bütschli's theories on this subject. In our view the presence of a linin framework is an essential point in a true conception of the structure of this meganucleus. It is composed, we believe, of a network of linin threads, bearing fine grains of chromatin which are approximately equal in size. There are never any centrosomes, centrioles, or other kinetic centres, and the presence of nucleoli is exceptional. The whole structure is bathed in a nuclear sap, which, when coagulated in permanent preparations, appears as a perfectly homogeneous substance filling up the interspaces of the network.

A word is necessary here in explanation of Pl. 25, fig. 1, because we believe that atypical appearances, such as this illustrates, have led to erroneous interpretations of the true structure. The figure shows a state of the meganucleus which we have met with fairly often. The interspaces of the network appear to be filled with solid bodies which stain rather more darkly (especially with brazilin) than the coagulated nuclear sap does as a rule.

We do not think that these apparently solid and separate bodies lying in the interspaces of the network are normal nuclear elements. We have come to the conclusion that they are local coagulations of nuclear sap which have stained in an irregular and abnormal manner. They are not present in the majority of our preparations.

DIVISION OF THE MEGANUCLEUS.

It has already been pointed out that the meganucleus of *Dendrocometes* does not possess a centriole, nor are there any centrosomes or other visible kinetic centres in relation to it. Division is always strictly amitotic.

The process of division consists simply of an elongation of the nucleus into a dumb-bell shape, and a subsequent separation of the two heads of the dumb-bell to form the two daughter-meganuclei. It will be remembered that the meganucleus lies in the ventral region of the parent. In division it elongates towards the dorsal surface into the region where the bud is being formed. At the dumb-bell stage one head of the dumb-bell lies in the dorsal region of the bud, the other in the ventral region of the parent. Subsequently the connecting strand divides before the bud is ready to be expelled, one half forming the meganucleus of the bud, the other half remaining as the meganucleus of the parent.

The structure of the meganucleus in the "resting" condition has already been described, and need not be gone into further. Soon after the commencement of the formation of the bud, the parent meganucleus begins to undergo change, and the first alteration in its structure is the disappearance of the regular meshwork arrangement of the chromatin grains. The nucleus now presents an even granular appearance, the chromatin being distributed more or less uniformly all over the nuclear area, no definite interspaces being visible (Pl. 25, fig. 3, contrast fig. 2). Close examination of the chromatin elements now reveals the fact that they are no longer granules, equal in size, but are beginning to be elongated into elliptical, or even short rod-shaped particles, some of which are noticeably larger than the others. We propose to call this phase the "granular stage" (Pl. 25, fig. 3).

In the next stage (Pl. 25, figs. 4, 5, and 6), the loss of the granular condition of the chromatin elements is complete, and the nucleus is now composed of rod-shaped lumps of chromatin, arranged along certain well-marked and obvious lines. All these figures show that at this stage the whole nucleus has the appearance of being involved in a kind of vortex movement which we shall refer to as the "whirl stage." Usually this phase is very well marked and is quite remarkable. The whole nuclear area seems to have been fixed in the act of undergoing a general "churning up," so

to speak, the lines of movement being always directed towards a definite focus, the position of which in the nucleus may vary. Fig. 4 shows this well. We have repeatedly looked for this movement in the living creature, but have never been able to see it. Collin (*loc. cit.*), however, has actually seen it in progress, and describes it as follows :

“Le prélude de la division observée *in vivo* consiste en mouvements de brassage d'abord extrêmement lents, incertains, comme hésitants, mais témoignant avec netteté d'une rupture d'équilibre; . . .”

Collin, in accordance with his conception of the meganucleus as a vesicle filled with fluid in which chromatin grains float freely, compares the phenomenon with that of an *Amœba* moving forward. Just as one can explain the movements of an *Amœba* as a simple effect produced by inequalities of surface tension on its different faces, so, he says, one can explain these movements in the meganucleus of *Dendrocometes* as being purely passive adaptations to a disturbance of the equilibrium of the cell. This disturbance, he believes, is directly caused by the development in the cell of the embryonic space (brood-chamber). This explanation is tempting and ingenious, but it depends entirely upon his own conception of the structure of the meganucleus. We have already stated that we cannot accept that conception, since it denies the existence of a linin network. In our view the whirl stage, above described, is much more truly compared to the condition which is seen during the spireme stage of mitosis in metazoan nuclei. It differs essentially from that, however, in that it is not governed and directed by any visible kinetic centre. It is very probable, however, that the two processes are analogous and have a similar significance.

The “whirl” stage coincides in time roughly with the stage of micronuclear division shown in Pl. 25, fig. 6. It is not confined to the meganucleus of *Dendrocometes* alone, but occurs also in the meganuclei of other *Acinetaria*. Probably it is to be regarded as a regular and constant feature of the amitosis of these nuclei.

Following on this phase, the meganucleus passes on at once to the actual process of division. It now has the very beautiful and characteristic shape shown in Pl. 25, figs. 7, 8. By this time the bud is well advanced, in some cases almost completely formed. Pl. 26, fig. 9, reconstructed from a series of sections, illustrates this point. The bud is complete, except for the final division of the meganucleus. Six micronuclei are present, three in the bud and three in the parent, the ciliated ridges are fully formed, and the connecting stem of the meganuclear dumb-bell is already thin and long-drawn out. A bud at this stage would, in the living state, present somewhat the appearance shown in Text-fig. 5.

The structure of the dumb-bell itself differs in different parts (v. Pl. 25, fig. 7). In the broad band which connects the two heads the linin framework is clearly seen. It consists of a system of fine threads connecting the two heads. On them lumps of chromatin, still rod-shaped in this region, are arranged in quite an irregular manner. Pl. 25, fig. 8, shows a dumb-bell at a rather later stage, when the connecting band bears little or no chromatin at all. In this stage all the chromatin has been accumulated in the two "heads." Pl. 26, fig. 9, shows a still more advanced condition in which the connecting band has become attenuated and will shortly be broken at its middle.

With regard to the structure of the "heads" of the dumb-bell, Pl. 26, fig. 9, is very instructive. Here the daughter meganucleus in the parent shows the chromatin elements still in the form of rods arranged along certain lines, as if streaming into that end of the dumb-bell. The daughter meganucleus in the bud, however, shows the chromatin elements once more in the form of grains, arranged in the manner characteristic of the "granular phase" with which the process of division began. The same condition is much more clearly shown in Pl. 26, fig. 10. In this case the chromatin in the two "heads" is again organised into approximately spherical grains, more or less equal in size and uniformly distributed over this part of the nucleus.

The impression given by the study of all these dumb-bells is, therefore, that the dumb-bell stage is to be regarded as the climax, so to speak, of a series of changes, commencing with the "granular phase" and passing through the "rod" and "whirl" stages subsequently. The aim of these preparations is to render the chromatin divisible between bud and parent. This is accomplished by the dumb-bell stage. When the act is completed, the daughter nuclei commence a series of similar changes, but in the reverse order, which finally lead back to the assumption by the daughter meganuclei of the meshwork structure characteristic of the "resting" meganucleus.

In considering this process as a mechanism for the division of chromatin between parent and bud, we must conclude that such division is only approximately equal. No doubt the assumption by the chromatin of the form of rods, their intimate mixing in the "whirl" stage, and their subsequent separation along certain definite lines in the dumb-bell stage, do all indicate an attempt, as it were, at equal division. But there is never any evidence of the existence of visible kinetic elements or of the elaborate mechanism which is characteristic of mitosis. The process is essentially amitotic; and the feature which decides, we think, its amitotic character is not so much the absence of kinetic elements, but the fact that at no stage are any structures found which could be interpreted as chromosomes. We would point out, however, that recent researches have tended to show that the dividing line between amitosis and the primitive forms of mitosis is by no means a sharp one. It appears necessary to recognise that amitosis, no less than mitosis, presents grades of differing complexity. We are dealing here, without any doubt, with a form of amitosis which is certainly elaborate and highly organised. It is not surprising, therefore, that it should approximate in unimportant features, to those forms of genuine mitosis which are very simple and primitive.

THE MICRONUCLEUS.

The first proof of the existence of a micronucleus in an Acinetarian was given by Bütschli (2) in 1876, when he described it in *Sphærophrya*. Since then its presence has been proved in all the Acinetaria which have been described, and it is now generally agreed that the presence of one or more micronuclei is characteristic of the group.

In the case of *Dendrocometes* itself, the first certain proof of the presence of micronuclei was definitely given by Hickson and Wadsworth (*loc. cit.*) when they described in detail their structure and their method of division during syngamy.

There are typically three micronuclei in each individual; the bud, therefore, normally possesses three also. This number may, however, vary. We have found in some cases as many as four or five, or even six in one instance, but never a greater number than this. In the vast majority of cases there are three, and this number must be regarded as typical. It is worthy of note that two of them usually lie close together, side by side, as it were, while the third is some distance away. We can offer no explanation of this fact, but have indicated it in the figures (*cf.* Pl. 26, figs. 9 and 10, and Pl. 25, fig. 7).

In the living animal it is rarely possible to demonstrate the presence of micronuclei. In stained preparations, however, they are very easily found. Under low magnifications (Zeiss D.) each micronucleus shows itself as an apparently solid granule of chromatinic matter lying freely in a clear zone, which separates it from the surrounding cytoplasm. It has the appearance, then, of the vesicular type of nucleus commonly seen in Flagellata, Amœbæ of the limax group, and some other Protista. But it is evident, when its structure is considered in detail, that it differs from these nuclei in important respects.

The structure of the resting micronucleus is shown in Pl. 26, fig. 13. There is a central chromatinic body sur-

rounded by a clear zone in which no structure of any kind can be detected. This clear zone is a very characteristic feature of the micronucleus, and serves as a valuable guide in distinguishing it from the other granules which are usually present in the cytoplasm. It is not an infallible guide, since very frequently, and particularly in well-fed individuals, food masses may occur in great abundance; and since they represent ingested organisms in various stages of digestion, these masses usually contain chromatinic material. Moreover, they are frequently surrounded by a clear zone similar in appearance to that which forms part of the micronucleus. They may, therefore, very closely resemble micronuclei, and it is often very difficult to establish their true nature. The only infallible test is their subsequent behaviour during bud-formation or syngamy.

With regard to the nature of the clear zone, which is so characteristic of the micronucleus, at least two alternatives are possible. Either it represents a purely artificial space produced by the action of reagents, and similar to that often found around the meganucleus in stained preparations (v. p. 365), or it is a part of the micronucleus itself. After careful consideration we have come to the conclusion that the latter interpretation is the correct one. In the first place, the circumference of the space is always regular in outline, an almost perfect sphere. It is never irregular, as, for example, is the space which often surrounds the meganucleus. Moreover, its constant presence points to some real relationship with the rest of the nucleus. Were it produced by shrinkage, we should naturally expect to find it around all other solid bodies of a shrinkable nature in the cytoplasm. We have already pointed out that such a space due to shrinkage is often present round the food masses, but when it is present, it is never so broad or so regular in outline.

Remembering all these points, we think it right to conclude that this clear zone does represent a part of the micronucleus. Against this view is the fact that we could never in any case

detect a membrane, or anything like one, separating the clear zone from the surrounding cytoplasm. This, however, does not necessarily invalidate our view. In many Protozoa, for example, *Amœbæ* of the limax group, simple, vesicular nuclei occur, which are composed of no more than a central grain of chromatin (karyosome) floating in a zone of clear, nuclear sap, and not enclosed in a membrane. Such a nucleus has been called a "protokaryon," and we believe that the micronuclei of *Dendrocometes* are of this type. The only differences between them and nuclei of the *Amœba* type are, first, the absence from the former of a centriole or any kind of visible kinetic centre; and, secondly, the fact that the chromatin of the central body is not present in the form of a compact, single lump, but is arranged in a network of strands, the whole having a structure which we may call spongy.

The structure of this central chromatinic body is shown in Pl. 26, fig. 13. In shape it is spherical, and it is composed of strands of chromatin arranged in a more or less regular network. Never in any "resting" micronucleus have we seen small grains of chromatin comparable to those characteristic of the "resting" meganucleus. The chromatin of the micronucleus is always laid down as a continuous strand on an achromatinic network, and this strand is never broken, nor overlaid with granules at any point. We shall see, however, that granules do appear during certain phases of division.

DIVISION OF THE MICRONUCLEUS.

The division of the micronucleus during conjugation has already been described by Hickson and Wadsworth (*loc. cit.*), and we have found that their description applies in broad outline to the process of division during bud-formation also. There are, however, one or two points which must be referred to.

The micronuclei of *Dendrocometes* divide by what must be described as a primitive form of mitosis, resembling, but

not identical with, the mitosis of the micronuclei of *Paramecium* described by Hertwig (4). The process also shows some general resemblance to the so-called "promitosis" described by Nägler (7) in the primitive *Amœbæ*. But there is one very important difference—namely, the complete absence of centrioles, polar plates, or any such kinetic centres from the micronuclei of *Dendrocometes*. We have repeatedly and very carefully searched for traces of such structures, since a priori one would expect them to be present; but we have never, in any case, been able to demonstrate them, and we do not believe that they are present.

The first feature which indicates that a micronucleus is commencing to divide is a noticeable increase in size of the central chromatinic body. This body swells up, occupying more of the nuclear area, and at the same time it loses its meshwork structure, and the chromatinic threads become arranged in the form of an irregular coarse skein. Subsequently the skein becomes finer, and may fill up almost the whole of the clear zone.

Following on this, the chromatinic body elongates, as is shown in Pl. 26, fig. 14. Now, for the first time, small granules of chromatin are seen irregularly scattered through the elliptical figure, lying on the longitudinal linin threads, which are themselves easily seen at this stage. We have come to the conclusion that these grains of chromatin represent the stage in more typical mitoses in which chromosomes are formed. We have never been able to find any more definite evidence of the formation of chromosomes than this, and we do not believe that definite rods are ever formed, such as have been described, for example, in the divisions during conjugation (Hickson and Wadsworth, loc. cit.). The figures of Hickson and Wadsworth in their paper are drawn from preparations made from the same material and by the same hand, so that our failure to find them in the division during bud-formation cannot be ascribed to bad technique.

Further, at a later stage (Pl. 25, fig. 3), the granules just mentioned are again seen, but situated now at the two poles of the figure. At this stage it will be seen that the linin framework is very well marked, and bears on it some chromatin in process of separation to the poles, in addition to the granules already in that situation.

Subsequent stages simply include the further elongation of the spindle. In Pl. 26, fig. 15, the chromatin is collecting at the poles, and this figure shows well the distinction between a lighter staining chromatin, forming caps at each end of the spindle, and the granules at the extreme poles, which represent the chromosomes. In Pl. 25, figs. 5 and 6, the elongation of the spindle is illustrated. Often this may proceed to such an extent that the figure may reach almost across the whole width of the body of the animal. The chromatinic elements gradually fuse, the granules disappearing again, until the pear-shaped masses shown in Pl. 25, fig. 6, are formed. Subsequently the spindle breaks down, and the "daughter nuclei" regain the meshwork structure characteristic of the resting stage. In Pl. 25, fig. 7, three micronuclei are shown in the bud, three in the parent, and between them traces of the disappearing spindle can be made out.

Considering this process in relation to mitosis in general, the most striking features are: (1) The complete absence of centrioles, centrosomes, or any visible kinetic centres; and (2) the absence of chromosomes in any defined form. With regard to the latter point, Hickson and Wadsworth, in their description of the mitosis of syngamy, figured and described definite chromosomes arranged in the form of an equatorial plate. In none of our preparations have we been able to find any definite rods such as those figured there. The chromatin is never more highly organised than as the grains shown in our figures. We must conclude, therefore, that the mitosis of bud-formation differs from that of conjugation in this respect.

The absence of visible kinetic centres is not in itself a very remarkable feature, since numerous instances are now on

record of mitosis which normally occur without such structures. Moreover, Hickson and Wadsworth were unable to find them in the mitoses of syngamy. The process must therefore be regarded as a mitosis of a primitive kind, resembling those primitive mitoses which occur generally in simple nuclei of the protokaryon type.

SUMMARY.

(1) This paper describes the reproductive process in the Acinetarian *Dendrocometes paradoxus* (Stein), epizoic on the gills of *Gammarus pulex*.

(2) Reproduction is effected by internal budding. Each individual produces a single bud at each reproductive act.

(3) The bud is roughly oval and plano-convex in shape, measuring 0.06×0.04 mm. The convex surface is dorsal and is devoid of cilia. The opposite ventral surface bears numerous long cilia, inserted along four ciliated ridges. This surface, hitherto regarded as flat, is in reality convex in the outer ciliated area, concave in the centre. The bud possesses, like the parent, three micronuclei.

(4) Bud-formation begins by a linear dissolution of the cytoplasm of the parent, which proceeds until a dome-shaped mass of cytoplasm is marked off. This mass is subsequently organised into the bud, acquiring a contractile vacuole and four ciliated ridges. The orientation of the bud while still within the parent is discussed.

(5) The area over which the above-mentioned dissolution of the parental cytoplasm occurs constitutes the so-called "brood-chamber." Unlike the similar structure in some other Acinetaria, it is not to be regarded as a definite space. It extends to the surface of the parent at one point at which the bud is subsequently born. There is, however, no definite opening to the exterior, except at the moment of birth.

(6) The development of the bud and the actual process of birth are described in detail.

(7) Division of the meganucleus of the parent is amitotic.

The meganucleus does not possess a true achromatinic nuclear membrane, but the surface layer of chromatin is so arranged as to form a false chromatinic membrane. Division of the meganucleus is always complete before the bud leaves the body of the parent.

(8) Division of the micronuclei is, on the other hand, by a primitive kind of mitosis. No chromosomes or visible kinetic centres are present.

(9) Reference is also made to the migration of the adult *Dendrocometes* from one gill to another. This process may simulate bud-formation, and is either of the nature of an excretory process or is associated with ecdysis in the host *Gammarus*.

(10) Attention is called to an area of special cytoplasm which is found in the bud during its formation. The significance of this structure is at present obscure.

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EXPLANATION OF PLATES 25 AND 26,

Illustrating Mr. Geoffrey Lapage's and Mr. J. T. Wadsworth's
paper on "Dendrocometes paradoxus." Part II.
—Reproduction (Bud-formation).

All the figures except figs. 7, 13, and 14 are drawn with the camera lucida, Comp. Oc. 6, Zeiss apo. 2 mm. Tube length 146, magnification $\times 900$ linear.

Fig. 7 is drawn with Ocular 2, and the same objective. Magnification $\times 630$ linear.

Figs. 13 and 14 are drawn with the Comp. Oc. 18, and the same objective. Magnification approximately 2,400 linear.

PLATE 25.

Fig. 1.—Section of individual with atypical resting meganucleus (v. p. 368).

Fig. 2.—Section of individual with typical resting meganucleus (*M*) and two micronuclei (*m*).

Fig. 3.—Figure constructed from two consecutive sections. Meganucleus in "granular" phase (*M*). Two micronuclei in early stage of division (*m.m.*).

Fig. 4.—Section of individual with meganucleus (*M*) in "whirl" phase of division. (*f.m.*) Food masses. (*g.*) Gill.

Fig. 5.—Section of individual with meganucleus (*M*) in "whirl" phase. Two micronuclei (*m*) dividing with spindles (*sp.*). (*C.R.*) Ciliated ridges of bud. The cytoplasm is heavily laden with food masses (*f.m.*). Contrast with fig. 6, where the cytoplasm is relatively clear.

Fig. 6.—Section showing a rather later stage. The meganucleus (*M*) is still in the "whirl" phase, but the micronuclei (*m*) are almost completely divided. (*C.R.*) Ciliated ridges of bud.

Fig. 7.—Whole mount showing the micronuclei (*m*) completely divided: three in the bud (*m*₁) and three in the parent (*m*₂). The remains of one spindle are indicated. (*C.R.*) Ciliated ridges of bud. Meganucleus in dumb-bell stage.

Fig. 8.—Section showing another meganucleus (*M*) in the dumb-bell stage. (*C.R.*) Ciliated ridges of bud. (*Sp. Cyt.*) Area of special cytoplasm (v. p. 345). Only one micronucleus (*m*) is present in the section. (*f.m.*) Food masses.

PLATE 26.

Fig. 9.—Figure constructed from a series of sections to show the bud almost completely formed. The meganucleus (*M*) is still in the dumb-bell stage, but the connecting strand is almost severed. Three micronuclei are present in the bud (*m*₁) and three in the parent (*m*₂), one being seen through the bud itself. (*C.R.*) Ciliated ridges of bud.

Fig. 10.—Figure constructed from a series of sections to show the meganucleus completely divided into a portion in the bud (*m*₁) and a parental portion (*m*₂). Three micronuclei are present in the bud (*m*₁) and three in the parent (*m*₂). (*C.R.*) Ciliated ridges of bud.

Fig. 11.—Diagrammatic drawing of free bud fixed with osmic acid and stained with brazilin. (*M*) Meganucleus. (*m*) The three micronuclei. (*c.v.*) Contractile vacuole. (*C.R.*) Ciliated ridges.

Fig. 12.—Lateral aspect of the bud, diagrammatic.

Fig. 13.—Resting micronucleus.

Fig. 14.—Two micronuclei in early stage of division. See description in text.

Fig. 15.—Three micronuclei in later stage of division. See description in text.

On the Development and Morphology of the Pharyngeal, Laryngeal, and Hypobranchial Muscles of Mammals.

By

F. H. Edgeworth, M.D.,

Professor of Medicine, University of Bristol.

With Plates 27—39.

THIS paper is a continuation of one published in this Journal (1914, vol. 59) on "The Development and Morphology of the Mandibular and Hyoid Muscles of Mammals." It deals with the structure of the pharyngeal, laryngeal, and hypobranchial muscles of *Ornithorhynchus* and *Echidna*, with the development of these muscles in *Dasyurus viverrinus* and some other Marsupials, in the pig and rabbit. The last few pages contain a summary of the similarities and differences between the cranial muscles of Monotremes, Marsupials, and Eutheria.

PLIÆ PALATO-PHARYNGÆ.

Göppert stated that palato-pharyngeal folds are absent in adult Monotremes, and did not describe them in developmental stages of *Echidna*. In a 25 mm. specimen of *Echidna*, however, they are present (Pl. 27, figs. 1-4)—continuous anteriorly with the soft palate and extending backwards on the lateral wall of the pharynx as far as the antero-posterior level of first branchial bars; they contain the pharyngo-palatinus muscles.

The palato-pharyngeal folds of *Dasyurus* are developed in

stage iv; in the pharyngeal region they do not extend further back than the first branchial segment, where they form slightly marked projections into the lumen of the pharynx (Pl. 29, fig. 13). In stage A (just born) they are much more marked, have extended back in the pharynx, and end by meeting together in a dorsal median fold at the junction of the pharynx and œsophagus (Pls. 29, 30, figs. 15-23). It is not until stage H that the pharyngo-palatinus muscle begins to lie in the fold.

In the pig the palato-pharyngeal folds first appear in embryos of 17 mm. crown-rump length, and do not extend backwards beyond the first branchial segment (Pl. 37, figs. 64 and 65). At the same time a median dorsal pouch develops in the roof of the pharynx in the second branchial segment (Pl. 37, fig. 68) and projects backwards as a flat, hollow pocket of epithelium (Pl. 38, fig. 69). In 20 and 21 mm. embryos the palato-pharyngeal folds have extended a little backwards, but fall short of the median pharyngeal pouch. The soft palate becomes fully formed in 38 mm. embryos.

The palato-pharyngeal folds in the rabbit form continuous structures extending from the palate in front along the sides of the pharynx, and meeting in a median dorsal fold at the junction of the pharynx and œsophagus.

The primary condition of the palato-pharyngeal folds is probably that present in *Echidna*, where they do not extend further back than the first branchial segment. In the pig they extend backwards slightly further. In *Dasyurus*, *Didelphys*, *Phascolarctus*, and rabbit they reach to the hind end of the pharynx, where they meet each other in a median dorsal fold. They become more developed in Marsupials than in the other animals investigated, and form thin reduplications of the mucous membrane.

Rückert, who described the median pharyngeal pouch in the adult pig, was of opinion that it was formed by the hind ends of the palato-pharyngeal folds. As a matter of fact, however, the pouch is formed posterior to the hind ends of the folds and has no direct relationship to them.

EPIGLOTTIS.

The epiglottis of Monotremes has been described by Göppert. The epiglottis of *Dasyurus* is developed in stage iv as a fold of mucous membrane in the floor of the pharynx, in the first and second branchial segments, just in front of the aditus laryngis (Pl. 29, fig. 14). In stage A (just born) the soft palate is formed, and the epiglottis projects upwards into the nasopharynx, with its anterior surface close to the posterior edge of the soft palate; its lateral edges are embraced by the palato-pharyngeal folds, whilst below these folds there is, on each side, a wider space between the lateral edges of the epiglottis and the wall of the pharynx—the “fauces” of Gegenbaur (Pls. 29, 30, figs. 16 and 24). This condition remains up to the stage J—the latest investigated.

The formation of the epiglottis in the pig has been described by Kallius. It begins to develop in 8 mm. embryos.

PLICÆ ARY-EPIGLOTTICÆ.

In stages A to D of *Dasyurus* (stage A, Pls. 29, 30, figs. 16–20; stage D, Pl. 31, figs. 30 and 31) the lateral edges of the epiglottis are continuous, posteriorly, with the ary-epiglottic folds bounding the aditus laryngis. In stage E (Pl. 32, fig. 38) small prominences appear over the anterior ends of the arytenoid cartilages, projecting into the lumen of the larynx, on the inner sides of the ary-epiglottic folds, a little distance below their free edges. Slight grooves separate the prominences over the arytenoid cartilages from the dorsal edges of the original ary-epiglottic folds. In front of the prominences the original ary-epiglottic folds are continuous, as in former stages, with the lateral edges of the epiglottis (Pl. 32, figs. 35–37).

The prominences over the arytenoid cartilages become more marked, but they do not extend forward to the epiglottis. The original ary-epiglottic folds increase in height, and form thin, inward-arching folds whose free edges bound the aditus

(Pl. 33, fig. 45). The original ary-epiglottic folds thus become those called *Plicæ laterales* by Symington and *Partes laterales epiglotticæ* by Göppert. In the figures I have employed the former term. The folds subsequently developed over the arytenoid cartilages may be called secondary arytenoid folds.

In the pig the method of formation of the secondary arytenoid folds is different from that in *Dasyurus*. In a 11 mm. embryo (Pl. 36, figs. 60 and 61) the ary-epiglottic folds on either side of the *aditus laryngis* are continuous anteriorly with the lateral parts of the slightly developed epiglottis. In a 17 mm. embryo the lateral boundaries of the *aditus* are formed, posteriorly, by the ary-epiglottic folds (Pl. 37, fig. 67); a little further forwards (Pl. 37, fig. 66) grooves are visible on the dorso-lateral sides of the ary-epiglottic folds, separating median arytenoid folds from the lateral *plicæ laterales*, at first partially, and still further forwards completely (Pl. 37, fig. 65). The *plicæ laterales* are the original ary-epiglottic folds, as shown by the fact that they are continuous, anteriorly, with the epiglottis, as were the ary-epiglottic folds in the 11 mm. embryo. The secondary arytenoid folds have free forward projecting extremities on the floor of the pharynx a little behind the epiglottis (Pl. 37, fig. 64). The *plicæ laterales* increase in height, and in a 32 mm. embryo (Pl. 39, figs. 76 and 77) form the lateral boundaries of the *aditus laryngis*, whilst the secondary arytenoid folds are prominences on the inner surface of the *plicæ laterales* below the *aditus*. The secondary arytenoid folds have free anterior extremities just behind the posterior surface of the epiglottis in both this and the 38 mm. stage, but in the adult are continuous with the epiglottis forming secondary ary-epiglottic folds (Némai).

The developmental phenomena in the rabbit are similar to those occurring in the pig.

Gegenbaur (1892), Symington (1899), and Göppert (1902) have discussed the nature of the lateral boundaries of the larynx in Marsupials. Gegenbaur stated that the lateral edges of the epiglottis form *plicæ laterales*, which pass

backwards and form a tube into which project the arytenoids. Symington stated that in the majority of Marsupials there are no ary-epiglottic folds, and that the lateral boundaries of the epiglottis, turning back, form plicæ laterales, separated from the arytenoids by sulci. In some of the smaller Marsupials, where the arytenoids are not so prominent, the plicæ laterales may join the upper borders of the arytenoids to form ary-epiglottic folds. Göppert stated that in Marsupials, Rodents (Leporidae and Muridae), and Lemurs the partes laterales epiglotticæ become developed, so that the entrance to the larynx is raised into a tube projecting into the pharynx. In these cases the plicæ ary-epiglotticæ lose in importance, but are visible inside the epiglottis tube.

The observations described above show that the primitive condition is one in which the aditus laryngis is bounded laterally by ary-epiglottic folds, which are continuous in front with the epiglottis. The arytenoid cartilages are developed in these ary-epiglottic folds. This is the condition in Monotremes, as described by Göppert.

In *Dasyurus*, pig, and rabbit this primitive condition is succeeded by one in which secondary arytenoid folds develop on the inner sides of the ary-epiglottic folds. The latter extend in height, and form plicæ laterales bounding a new aditus laryngis. This condition becomes the permanent one in Marsupials.

The initial stages of formation of the secondary arytenoid folds are not identical in *Dasyurus*, pig, and rabbit. In *Dasyurus*, where they develop in extra-uterine life and subsequent to the formation of the arytenoid cartilages, they are formed on the inner side of the ary-epiglottic folds. In the pig and rabbit, where they develop in intra-uterine life and previous to the formation of the arytenoid cartilages, they are at first situated more dorsally than in *Dasyurus* and for a time bound the aditus laryngis, and only subsequently assume the position they have from the first in *Dasyurus*. This difference is apparently due to the relative

lateness in upgrowth of the original ary-epiglottic folds to form plicæ laterales in the pig and rabbit.

In the pig and rabbit the secondary arytenoid folds gain an attachment to the posterior surface of the epiglottis and form secondary ary-epiglottic folds,¹ ventro-internal to the original ary-epiglottic folds s. plicæ laterales.

It is probable that there is a similar development in the other classes of Mammals in which, according to Göppert, plicæ laterales occur, i.e. in Carnivora other than Canidæ and Ursidæ, Insectivora, Prosimiæ, Platyrrhina, and Catarhina other than Anthropomorphæ.

In stage A of *Dasyurus* (Pls. 29, 30, figs. 17 and 18) a solid outgrowth of the epithelium lining the cavity of the larynx is formed, which projects forwards between the two anterior cornua of the thyroid cartilage. There is no further development of this outgrowth until stage E, when it begins to enlarge and to become hollowed out, and forms a cavity opening posteriorly into the ventral part of the larynx (Pl. 32, figs. 36 and 37). A similar recess was described by Gegenbaur in *Phalangista vulpina*, by Albrecht in *Cuscus*, and by Symington in almost all the cases he examined.

HYOBRANCHIAL CARTILAGES.

In stage A of *Dasyurus* (Pl. 29, figs. 16 and 17) the cartilaginous ventral ends of the hyoid and first branchial bars are continuous with the median cartilaginous basi-branchial. The upper, precartilaginous, end of the first branchial bar passes backward and is continuous with the upper edge of the thyroid ala (Pl. 30, figs. 18 and 19). It becomes cartilaginous in stage C.

The development of the hyobranchial cartilages in the pig has been described by Kallius.

¹ Plicæ ary-epiglotticæ (Göppert), Plicæ inferiores (Albrecht), Plicæ ary-epiglottidæ s. Plicæ inferiores (Némai).

THYROID CARTILAGE.

The morphology of the thyroid cartilage of Mammals has been the subject of many investigations. Dubois (1886) showed that the thyroid cartilage of Monotremes consists of a median copula and two bars on either side, which are homologous with the second and third branchial (fourth and fifth visceral) bars of lower Vertebrates. This opinion was confirmed by the embryological investigations of Göppert. Dubois also stated that the thyroid cartilage of Marsupials—consisting of a broad plate with marked anterior and posterior horns—is homologous with that of Monotremes, and probably due to fusion of two bars and a copula.¹ He was also of opinion that the thyroid cartilage of Eutheria, though its posterior horns are less marked, has a similar derivation.

Investigations into the development of the thyroid cartilage in Eutheria have not yielded concordant results. His (1885), as stated by Göppert, found that the thyroid cartilage of man is developed in the second branchial arch.

Nicholas (1894), whose researches began with 22 mm. human embryos, showed that the thyroid cartilage develops from two independent lateral halves which fuse ventrally, at first in front and then behind. An unpaired median cartilaginous nodule subsequently develops in the cellular band connecting the alæ in the intermediate region and fuses with them. This nodule is to be distinguished from the intermediate piece of cartilage of the adult larynx, which is a secondary formation, and “*resulte du remainement dans une région limitée d’une lame cartilagineuse homogène.*” He doubted any homology of the primary median nodule with the basithyroid copula of Monotremes owing to its filling a part only of the interval between the alæ.

According to Kallius (1897), each half of the thyroid cartilage develops in human embryos of thirty-nine to forty

¹ A difference of opinion between Dubois and Göppert in regard to the morphology of the Monotreme laryngeal cartilages is discussed later on (pp. 414, 415) in connection with the interthyroideus muscle.

days, as a plate of dense connective tissue, chondrified at its cranial and caudal borders, and with a central foramen thyroideum. Chondrification spreads, and a cartilaginous plate is formed which extends ventrally in a dense connective tissue. A median thyroid copula, homologous with that of Monotremes, is developed in this tissue. The thyroid cartilage is thus formed by the fusion of elements homologous with the second and third branchial (fourth and fifth visceral) bars and an intermediate copula.

Soulié and Bardier (1907) investigated the development of the thyroid cartilage in man. They did not regard the presence of a foramen in the thyroid cartilage and of a notch in its border as certain indications of a formation from two pieces, but remarked that it was not possible to be absolutely sure of the morphology of the thyroid alæ in man, inasmuch as the gill-clefts have disappeared in 14 mm. embryos, whilst cartilage is first developed in 19 mm. embryos. They concluded that "la partie médiane du quatrième arc est utilisée pour la formation de l'épiglotte, et le squelette de ses parties latérales donnera les lames latérales du thyroïde, dont les grandes cornes se constitueront aux dépens du squelette des portions latérales du troisième arc. Le cinquième arc, rudimentaire, ne fournit aucun dérivé." The vocal or intermediate nodule first appears in 37 mm. embryos, and they denied its equivalence to a thyroid copula, owing to this lateness in development.

Frazer (1910) stated that "the thyroid cartilage of man is primarily a fourth arch derivative, and if it has any fifth arch element this is a later addition, and its line of junction is not indicated by the occasional persistence of the foramen in the ala." The cartilage first appears at the end of the first month. In a 35 mm. embryo two small nodules of cartilaginous structure are interposed between the alæ, probably representing the cartilages of Nicholas.

Grosser (1912), in the course of a description of the gill-clefts of man, stated that the skeletal portion of the fifth visceral (third branchial) segment "which is included in the

thyroid cartilage is, however, of considerable size." Soulié and Bardier, however (*vide supra*) had stated that the gill-clefts of man lose their connection with the pharynx before the thyroid cartilage begins to develop, so that determination of the segment or segments of origin of the thyroid cartilage is not possible in man. The same thing was shown by Soulié in the mole.

The development of the thyroid cartilage could not be followed in *Dasyurus* owing to the absence of the necessary stages. In stage iii β it is not yet developed; in stage iv it is present, and does not differ from that at birth (stage A), except in that chondrification has not yet taken place. In *Trichosurus vulpecula*, however, the process of development could be followed. In stage viii β (Pl. 34, figs. 50-52) the gill-clefts are still continuous with the pharynx. The thyroid cartilage consists of two primordia, the anterior between the third and fourth gill-clefts in the fourth visceral (second branchial) segment, and the posterior behind the fourth gill-cleft in the fifth visceral (third branchial) segment. In stage ix, *a* and *b* (Pl. 35, figs. 53-55), these primordia are more clearly defined. Their outer ends (Pl. 35, figs. 53-55) are united together, whilst a little nearer the middle line (Pl. 35, fig. 54) they are still separate. The upper end of the first branchial bar is continuous with the 1st thyroid primordium. The hind end of the second thyroid primordium abuts against the ill-defined primordium of the cricoid cartilage.

In stage A of *Dasyurus* (Pls. 29, 30, figs. 17-22 and 24-25) the thyroid alæ form slightly curved cartilaginous bars, the anterior ends of which are connected by precartilaginous tissue. Their ventral edges are connected by cells which form a membrane. The hind ends of the first branchial bars are continuous with the upper edges of the alæ. There is no thyroid copula.

In stage B the lower edges of the thyroid alæ have extended ventrally in the membrane connecting them. In stage C the precartilaginous tissue connecting the anterior ends chondrifies and a copula is developed in the ventral

membrane. Behind the copula the projecting wedge-shaped ventral part of the cricoid, hitherto separate, has become continuous with the thyroid alæ. In stage D the copula has chondrified (Pl. 31, fig. 31), and become continuous laterally with the alæ.

In 17 mm. pig embryos no definite primordium of the thyroid cartilage is present (Pl. 37, figs. 66-68). In 18 mm. embryos (Pl. 38, fig. 69) it is developed, on each side, lateral and ventro-lateral to the pharynx. The ductus pharyngo-branchialis of the fourth gill-cleft passes outwards from the pharynx behind the primordium, which is consequently developed in the fourth visceral (second branchial) segment. In 21 mm. embryos (Pl. 38, figs. 71, 72) the primordium of the thyroid cartilage has extended ventrally, and the thyroid copula has appeared in the middle line. The fourth gill-cleft is no longer continuous with the pharynx. The epithelial body of the fourth gill-cleft lies just external to the hind end of the primordium of the thyroid cartilage. In 24 mm. embryos chondrification has occurred both in the thyroid alæ and copula. In 32 mm. embryos the alæ and copula form a continuous whole.

In the pig each thyroid ala is thus developed in one segment only—the fourth visceral (second branchial).

The morphology of the thyroid cartilage of Mammals would thus appear to be as follows: In Monotremes and Marsupials each lateral half is developed from two primordia developed in the fourth and fifth visceral (second and third branchial) segments, and homologous with the second and third branchial bars of Amphibia. In Monotremes the bars remain separate; in Marsupials they fuse and form the thyroid alæ. In Eutheria it appears probable that the thyroid ala is formed, as in the pig, from one primordium only, developed in the fourth visceral (second branchial) segment. In Eutheria the fifth visceral (third branchial) segment, marked in early stages by an aortic arch and segmental branch from the vagus, appears to have only an ephemeral existence.

This disappearance in the phylogeny of Eutheria of a

third branchial constituent of the thyroid cartilage offers an explanation of the observation of Dubois that the recurrent laryngeal nerve passes into the larynx ventral to the articulation of the thyroid and cricoid cartilages in Monotremes and Marsupials, dorsal to it in Eutheria. The articulations are not homologous.

A median thyroid copula was described by Dubois in Monotremes, and its existence was confirmed by Symington, Miss Walker, and Göppert.

A similar copula is developed in *Dasyurus* and in the pig. In man a median cartilage between the ventral edges of the thyroid alæ was described by Nicholas, and subsequently by Kallius, Soulié and Bardier, and Frazer. Its homology with the thyroid copula of Monotremes was affirmed by Kallius and Göppert, but denied by Nicholas, who called it the "cartilage vocal," and by Soulié and Bardier mainly on the ground of its very late appearance. Thus Soulié and Bardier state that the alæ develop in 19 mm. embryos, and the median cartilage only in 37 mm. embryos.

It appears doubtful if this relative lateness in development is a sufficient ground for denial of homology, as this is a constant feature of the copulæ of the visceral bars.

EPIGLOTTIC CARTILAGE.

Dubois (1886) stated that the epiglottic cartilage represents a chondrification of the submucous tissue of the transverse glosso-laryngeal fold. Its intimate relations to the thyroid cartilage are to be regarded as secondary.

Gegenbaur (1892) put forward the theory that the epiglottic cartilage is derived from the fourth branchial (sixth visceral) bars. This view was based on the asserted hyaline condition of the cartilage in Monotremes and on the frequent paired condition of the base of the cartilage.

Symington (1900), however, showed that the epiglottic cartilage of Monotremes consists of elastic cartilage, and rejected Gegenbaur's theory.

Göppert (1894 and 1900) investigated a large number of Mammals, and showed that the condition of the base of the cartilage is variable—in some cases paired, in others not—and adhered to the view that the paired condition is the more primitive.

Schaffer (1907) advanced the following arguments against Gegenbaur's theory. The epiglottic cartilage has not been shown to have a hyaline primordium in any animal, as would be the case in a typical skeletal cartilage. It bears the character of a secondary chondrification, in agreement with its late formation and its frequent replacement by other connective tissues. Its first primordium has a close relation to the thyroid cartilage, varying from one of contiguity to cartilaginous continuity, and also to the mucous membrane of the epiglottis in its later development. He concluded that the epiglottic cartilage is not derived from a pair of branchial bars, but is a secondary formation. "Der Grund für diese Bildung wird ungezwungen darin zu suchen sein dass die glosso-laryngeale Schleimhautfalte durch eine neugewonnene Funktion einer festeren Stütze bedurfe."

Soulié (1909), in his paper on the development of the larynx of *Talpa*, expressed the opinion that the epiglottic cartilage was not derived from the branchial skeleton, and was a special formation, on the nature of which we cannot pronounce.

Dasyurus is born with a fully developed epiglottis (Pls. 29, 30, figs. 16 and 24), but the epiglottic cartilage is not developed until later. It is first visible in stage E (Pl. 32, fig. 35) as an aggregate of cells dorsal to the united anterior ends of the thyroid cartilage. It increases in size, and its lateral edges extend a little backward along the upper edge of the thyroid cartilage (Pl. 33, figs. 43 and 44). By stage H it consists of elastic cartilage. There is no intervening stage in which it consists of hyaline cartilage, nor any in which it shows indications of being formed by coalescence of two lateral halves.

The epiglottic cartilage of the pig is not yet developed in

24 mm. embryos, but is present in 32 mm. embryos (Pl. 39, fig. 76) as an aggregate of cells beneath the epithelium of the epiglottic fold.

The evidence suggests that the theory of Gegenbaur and Göppert may be rejected in favour of that of Dubois, Symington, and Schaffer. The epiglottis is developed in the floor of the pharynx just in front of the opening of the larynx, and its cartilage is subsequently formed in it as a supporting structure. Both are exclusively Mammalian structures and have been developed in association with the mammary function.

The segment of origin of the epiglottis appears to vary a little. In *Echidna*, *Dasyurus*, and pig it is the first and second branchial (third and fourth visceral) segments. In *Talpa* (Soulié) it is the second branchial (fourth visceral) segment. In man, according to Soulié and Bardier, it is the second branchial (fourth visceral) segment, whilst, according to Frazer, the epiglottis is derived from a central mass which has a first branchial (third visceral) element on its oral and upper aspect.

CRICOID AND ARYTENOID CARTILAGES.

In stage iii β of *Dasyurus* there are aggregated cells round the lower part of the larynx and upper part of the trachea, but no definite primordium of skeletal structures is visible. In stage iv these cells form a continuous precartilaginous primordium of the arytenoid-cricoid-tracheal cartilages—in the arytenoid region Ω -shaped with a connecting bridge dorsal to the lumen of the larynx, and behind that surrounding the posterior part of the larynx and anterior part of the trachea; the cricoid is slightly marked off from the tracheal skeleton.

In stages A to C (Pl. 30, figs. 19–22, 24–25) the arytenoid-cricoid forms a continuous structure. The cricoid is a cartilaginous ring, complete dorsally and ventrally. The two arytenoid cartilages project forward from the upper part of its anterior edge. These are distinct structures anteriorly, but continuous with each other and with the cricoid posteriorly.

The cartilaginous continuity of cricoid and arytenoid is not complete laterally, where the intercellular matrix is scarcely present. In stage D (Pl. 31, figs. 32, 33) the arytenoids are fully separated from the cricoid, but are united dorsally by a bridge which has lost its hyaline cartilage appearance and forms the primordium of the interarytenoid cartilage. The interarytenoid cartilage moves dorsally, and by stage J (Pl. 34, fig. 46) lies dorsal to the upper edges of the arytenoid cartilages. It is formed of elastic cartilage. The anterior parts of the arytenoid cartilages are rounded in stages A to C (Pl. 30, figs. 19, 20); the processus muscularis and ventral process begin to develop in stage D (Pl. 31, figs. 32, 33) and subsequently become still more marked (Pl. 34, fig. 46).

The position of the interarytenoid cartilage varies in Marsupials. Symington found it lying between the two arytenoids and articulating with their internal processes, and this position was also described by Henkel in *Macropus rufus*. Körner described and figured it, in *Halmaturus giganteus*, lying dorsal to the arytenoid cartilages. This position was also described by Henkel in *Phascolomys platiceps* and *Petrogale lateralis*, and it exists in late pouch stages of *Dasyurus* and *Didelphys aurea*, and also in adult *Monotremes* (Göppert).

The interarytenoid cartilage primarily has an interarytenoid position in *Echidna* (Göppert) and in *Dasyurus*, and subsequently a dorsal one. The condition found by Symington in many Marsupials and by Henkel in *Macropus rufus* is thus probably due to persistence of a developmental stage.

The cartilage was termed "interarytenoid" by v. Luschka and Symington, "cartilago sesamoidea sive papilionacea" by Körner and Henkel, "procricoid" by Göppert. The developmental phenomena suggest the use of the first name in preference to either of the other two.

In the pig there is no definite primordium of the cricoid and arytenoid cartilages until the stage of 18 mm. Previous to this—from the stage of 6 mm. to that of 17 mm.—there is a continuous layer of aggregated mesoblast cells round the tracheal

and laryngeal epithelium (Pls. 35, 36, 37, figs. 57-59, 61-63, 66-68). They extend from behind the sixth aortic arch into the second branchial segment. In the 18 mm. stage the primordium of the cricoid and arytenoids is visible, dorsal and lateral to the hinder portion of the larynx (Pl. 38, figs. 69, 70).

It is more marked and may be spoken of as precartilage in 21 mm. embryos (Pl. 38, figs. 71, 72). In 24 mm. embryos chondrification has taken place lateral to the laryngeal lumen, whilst dorsal and ventral to it the structures are still precartilaginous; and, anteriorly, what will become the arytenoids are precartilaginous. In 32 mm. embryos the arytenoids are separated from the cricoid; they are cartilaginous and are connected by a bridge, dorsal to the laryngeal lumen, of precartilaginous structure (Pl. 38, fig. 71); the cricoid is a cartilaginous ring, and patches of cartilage have appeared round the trachea. In 38 mm. embryos the interarytenoid cartilage has separated from the laterally lying arytenoid cartilages.

LARYNGEAL MUSCLES.

The development of the laryngeal muscles has been the subject of many investigations. Strazza (1888) found the first indication of the musculature of the larynx in human embryos of 12 and 13 mm. in intimate connection with that of the tongue.

Nicholas (1894) stated that in the first stages of development the laryngeal sphincter of man forms a complete ring, and subsequently becomes subdivided into three groups of muscles. He did not describe the derivation of this sphincter.

Kallius (1897) did not describe the development of the laryngeal muscles.

Göppert (1902) found the primordium of the laryngeal muscles of *Echidna* in stage 42 as a triangular mass of aggregated cells lying lateral to the mesodermal cells immediately surrounding the epithelium of the larynx. "Man kann sie an der Seite des Kehlkopfes caudalwärts verfolgen, bis in die Gegend des Beginnes der Trachea. Sie nimmit dabei an

Umfang noch etwas zu." In stage 44 the cells had developed into embryonic muscle-cells, easily recognisable when cut longitudinally; the muscles of this primordium already had the same arrangement as in the adult larynx.

Soulié and Bardier (1907) were not able to confirm Strazza's observation on man. They did not see any indication of intrinsic laryngeal muscles in 14 mm. embryos; in 19 mm. embryos, however, four muscle-groups were recognisable, corresponding to the interaryténoïdien, crico-aryténoïdien-postérieur, crico-thyroïdien, and thyro-crico-aryténoïdien. No statement was made as to the origin of these primordia.

Soulié (1909) found the first indication of the laryngeal musculature of the mole (*Talpa europæa*) in 10 mm. embryos formed "par les muscles crico-aryténoïdiens postérieure et par quelques rares faisceaux musculaires interposés aux divers précartilages." He did not state the origin of these muscles.

Fraser (1910) stated that the intrinsic laryngeal musculature of man is developed from two planes, inner and outer, which are separated from one another by mesoblast of the fourth arch. The inner plane or constrictor, developing first, in 5 mm. embryos, is derived from the fifth visceral (third branchial) arch; the outer plane or constrictor, developing a little later, is derived from the fourth visceral (second branchial) arch, which becomes antero-external to the fifth arch. "The inner constrictor appears to be subsequently split up into the internal intrinsic muscles in its laryngeal part, and dorsally forms part of the pharyngeal musculature, whilst the outer constrictor becomes dorsally a part of this musculature, and in its laryngeal area gets a secondary attachment to the cricoid and thyroid, and seems to form the crico-thyroid muscle in consequence of the downgrowth of the inferior thyroid cornu into it."

The early development of the laryngeal muscles could not be followed in *Dasyurus*, owing to want of the necessary stages. In stages i and iii of *Trichosurus vulpecula* the larynx and anterior part of the œsophagus and trachea are

surrounded by mesoblast cells in which no differentiation is visible. In stage v the primordia of the constrictor of the œsophagus and laryngeal muscles is found (Pl. 34, figs. 47-49). The latter lie lateral to the anterior part of the trachea, extending from behind the sixth aortic arch to the fourth gill-cleft. There is no connection between them and the œsophageal constrictor. The recurrent laryngeal nerve passes from the vagus directly inwards to the hind end of the laryngeal muscle primordium. In stage β viii (Pl. 34, figs. 50-52) the laryngeal muscle primordium has shifted forward—its hind end lies opposite the fourth aortic arch, and it extends into the second branchial segment.

In stage ix (Pl. 35, figs. 54, 55) it forms a continuous mass, in which the dilatator laryngis and laryngeus ventralis are distinguishable; the laryngeus dorsalis is not yet formed.

The intrinsic laryngeal muscles of *Dasyurus* in stage A consist of dilatator, laryngeus dorsalis, and laryngeus ventralis. The dilatator muscle (Pl. 30, figs. 20-22, 24) arises from the posterior cornu of the thyroid and dorsal surface of the cricoid, and is inserted into the dorsal and lateral surfaces of the arytenoid cartilage, the processus muscularis not being yet developed. The greater number of the fibres have a longitudinal direction, but a few fibres can be seen extending down from the dorsal portion of the muscle next to the cricoid cartilage and medial to the main mass of the muscle. By stage C (Pl. 31, figs. 26, 27) these descending fibres are much more marked. In stage H (Pl. 33, fig. 41) the original dilatator muscle is fully separated into a ventro-lateral portion—the kerato-crico-arytenoideus, and a dorso-medial portion—the crico-arytenoideus posticus internus. The latter arises from the cricoid cartilage dorso-medial to the kerato-crico-arytenoideus, and also by fibres which pass upwards and inwards internal to the kerato-crico-arytenoideus. The developmental phenomena described are due to the occurrence of a lateral extension of the origin of the crico-arytenoideus posticus internus on the cricoid before separation of the dilatator into the two muscles it forms.

The laryngeus dorsalis forms the interarytenoideus. In stages A (Pl. 27, fig. 6) to C (Pls. 30, 31, figs. 20, 28) it is represented by a very few muscle-cells only, and these lie dorsal to the upper surface of the arytenoid cartilage. It is not until stage D (Pl. 31, fig. 32), when the arytenoids have separated from the cricoid, that the muscle-cells increase in numbers and form a transverse muscle. On separation of the interarytenoid cartilage, in stage E, some of the fibres are inserted into it (Pl. 34, fig. 46 of stage J), whilst in front of the cartilage the fibres cross from side to side.

The laryngeus ventralis in stage A (Pl. 30, figs. 19, 20) of *Dasyurus* arises from the lateral surface of the wedge-shaped anterior projection of the ventral edge of the cricoid ring, and is inserted into the lateral surface of the arytenoid cartilage. In stage B its origin has spread uninterruptedly forwards so that it also arises from the membrane connecting the ventral edges of the thyroid alæ. In stage D, when the thyroid alæ have extended down, the anterior part of the muscle arises from the thyroid ala (Pl. 31, fig. 32). It thus forms the crico-thyro-arytenoideus (Pls. 33, 34, figs. 44-46).

Two theories have been advanced in regard to the morphology of the crico-arytenoideus posticus internus of Marsupials.

Symington stated that this muscle and the kerato-crico-arytenoideus "represent the crico-arytenoideus posticus of the majority of Mammals, which has become split into two parts, and its attachments modified owing to the large size of the inferior cornu of the thyroid cartilage, the slight vertical extent of the posterior part of cricoid cartilage, the development of a large internal process to the arytenoid, and the existence of an interarytenoid cartilage."

Göppert, on the other hand, stated that the crico-arytenoideus posticus internus (which he called "crico-procricoideus") of Marsupials, is a portion of the laryngeus dorsalis which has divided into the interarytenoideus (which he called "ary-procricoideus") and this muscle. This opinion was founded on the homology of the laryngeal muscles with those of Monotremes and on the observation that in *Echidna* the

crico-procricoideus is formed by extension of the laryngeus dorsalis to the cricoid cartilage.

The above recorded observations on *Dasyurus* appear to prove the theory of Symington as regards the crico-arytenoideus posticus internus of Marsupials. In stage A of *Dasyurus*, when the muscle is beginning to be formed, there is a distinct gap between the anterior end of the dilatator muscle and the primordium of the laryngeus dorsalis (cp. Pl. 30, figs. 20 and 21). This suggests that the development of the muscle in the Monotreme larynx is worth reinvestigation.

The form of the crico-arytenoideus posticus internus in *Dasyurus* is not the most primitive present in Marsupials. It has been shown above that the origin of the medial portion of the original dilatator muscle of *Dasyurus* (which will form the crico-arytenoideus posticus internus) begins to spread laterally on the cricoid beneath the outer portion (which will form the kerato-crico-arytenoideus) even before the two muscles become fully separated from one another.

In *Phascolarctus* (Pl. 35, fig. 56), and also in *Petrogale lateralis* (Henkel), such a lateral extension of the crico-arytenoideus posticus internus does not take place, and it lies wholly medial to the kerato-crico-arytenoideus.

But in many Marsupials the origin of the muscle spreads laterally, and the lateral portion may remain in continuity with the medial portion, e. g. in *Dasyurus*, *Didelphys aurita*, and also in *Macropus rufus* and *Phascolomys platiceps* (Henkel). Or the lateral portion may become a separate muscle, lying under the kerato-crico-arytenoideus; this is the case in *Halmaturus giganteus* and *Billardieri* (Körner), *Macropus robustus* and *cervinus*, and *Onychogalea frenata* (Henkel).

Körner, who described the condition in *Halmaturus giganteus* and *Billardieri* (where the lateral portion is a separate muscle), gave the name crico-sesamo-arytenoideus to the medial portion and that of crico-arytenoideus profundus to the lateral portion of the crico-arytenoideus posticus

internus. Henkel described the crico-arytenoideus profundus as being absent in the cases where it did not form a separate muscle—both in those, e.g. *Petrogale lateralis*, where the crico-arytenoideus posticus internus remains medial to the kerato-crico-arytenoideus, and in those, e.g. *Macropus rufus*, where it spreads laterally on the cricoid. Comparison, however, of the various conditions shows that they are due to the non-occurrence or occurrence of a lateral spread, and in the latter case to the non-separation or separation of the lateral portion.

Möller stated that he had found the crico-arytenoideus profundus of Marsupials closely bound to the crico-thyreo-arytenoideus, and was of the opinion that the former muscle was homologous with the crico-arytenoideus lateralis of higher Mammals. In *Dasyurus*, however, the lateral edge of the crico-arytenoideus posticus internus (which, as stated above, does not separate into two muscles) is at some little distance from the posterior edge of the crico-thyreo-arytenoideus in stage A, and only in stage C do the two muscles come into contact. The same thing is true of *Didelphys aurita*—in 10 mm. specimens there is a gap between the two muscles, which is gone in 20 mm. specimens. The opinion of Möller, consequently, does not appear to be tenable.

Various names, as stated above, have been given to the laryngeal muscles of Marsupials. They are, in part, associated with those of the cartilage here called "interarytenoid." The arrangement in the following tabular statement differs from that of Göppert in regard to the derivation of the crico-arytenoideus posticus internus. In it and the above discussion I have (1) employed the term "crico-thyro-arytenoideus" in preference to that of "thyro-crico-arytenoideus," used by Symington and Göppert, as in the development of *Dasyurus* the first attachment of the muscle is to the cricoid cartilage, from which it spreads forward to the thyroid cartilage: (2) used the name "interarytenoideus"; and (3) have followed Symington in the nomenclature of the subdivisions of the *M. dilatator*.

	Symington (1899).	Goppert (1901).	Körner (1894).	Henkel (1909).
<i>M. dilatator</i> .	{ Kerato-crico-arytenoideus Crico-arytenoideus posterior internus	Kerato-crico-arytenoideus Crico-procricoideus	{ Kerato-crico-arytenoideus (medial) Cricos sesamo-arytenoideus (lateral) Crico-arytenoideus profundus	{ Kerato-crico-arytenoideus (medial or whole muscle) Cricos sesamo-arytenoideus. (lateral) Crico-arytenoideus profundus.
<i>M. laryngeus dorsalis</i>	Arytenoideus	Ary-procricoideus	Hinder segment of sphincter laryngeus internus, or interarytenoideus	Interarytenoideus.
<i>M. laryngeus ventralis</i>	Thyro-crico-arytenoideus	Thyreocrico-arytenoideus	Anterior segment of sphincter laryngeus internus	Sphincter laryngeus internus.

In the pig the primordium of the laryngeal muscles is first visible in 8 mm. embryos, as a mass of cells continuous with, and apparently proliferated from, the primordium of the constrictor of the œsophagus, posterior to the sixth aortic arch. It extends forwards as far as, but not anteriorly to, the sixth aortic arch. The vagus nerve passes backwards and downwards lateral to the primordium of the laryngeal muscles (Pl. 35, figs. 57-59).

In 11 mm. embryos the laryngeal muscle-primordium has extended forwards and a little in front of the fourth gill-cleft, i. e. into the fourth visceral (second branchial) segment, though it is still continuous with the primordium of the constrictor of the œsophagus, posterior to the sixth aortic arch (Pl. 36, figs. 60-63). The recurrent laryngeal nerve which has now developed passes inwards and slightly forwards from the vagus to the hind end of the muscle-primordium, behind the sixth aortic arch.

In 14 mm. embryos the recurrent laryngeal nerve has been carried backwards by the "descent" of the sixth aortic arch, so that it passes into the laryngeal muscle-primordium from behind.

In 17 mm. embryos (Pl. 37, fig. 68) the anterior part of the laryngeal muscle-primordium has extended a little ventrally, and it lies lateral to the aggregated cells surrounding the posterior part of the larynx.

In 18 mm. embryos (Pl. 38, figs. 69, 70) the hind end of the laryngeal muscle-primordium is separated from the anterior end of the constrictor of the œsophagus, lying ventral to this and dorsal to the primordium of the cricoid cartilage.

The separation of the laryngeal muscle-primordium into *M. dilatator* (crico-arytenoideus posticus), *laryngeus ventralis*, and *laryngeus dorsalis* (lateral half of interarytenoideus) begins in 21 mm. embryos (Pl. 38, fig. 71), and is complete in 24 mm. embryos. In 32 mm. embryos the *laryngeus ventralis* has separated into *thyro-arytenoideus* and *crico-arytenoideus lateralis*.

The above-described phenomena show that in *Trichosurus* and pig the primordia of the laryngeal muscles are formed behind the sixth aortic arch, i. e. behind the branchial region. In the pig the primordia are at first continuous with, probably proliferated from, the primordium of the constrictor muscle of the œsophagus; in *Trichosurus* they are developed at the same stage as, though not in continuity with, the œsophageal constrictor.¹ The primordia grow forward into the branchial region, losing in the pig their continuity with the œsophageal constrictor, and develop into the *dilatator laryngis*, *laryngeus ventralis*, and *laryngeus dorsalis* on each side.

The primordia of the laryngeal muscles are thus post-branchial in origin, and are probably developed from the constrictor of the œsophagus.

These phenomena harmonise with the first development of the larynx as a post-branchial structure and its subsequent forward migration into the meso-branchial region. Thus Grosser showed that, in man, the primordium of the larynx

¹ Possibly the laryngeal muscle-primordium in stage iv of *Trichosurus* is continuous with the œsophageal constrictor, but this stage was not available.

appears caudal to the pharyngeal pouches as a ventral groove, developing simultaneously with the last pharyngeal pouches. The laryngeal portion of the groove encroaches on the meso-branchial region until it lies between the medial ends of the fourth and, later, between those of the third visceral¹ arches. Similarly, I found that in a *Dasyurus* embryo of stage ii. α (of Hill) the opening of the larynx lies caudal to the fourth (the last) gill-cleft (Pl. 29, figs. 11, 12), and that in stage iii. β it has migrated forwards into the branchial region. In a 6 mm. pig embryo (the youngest investigated) the hind end of the aditus laryngis lies 230 μ caudal to the opening of the fourth gill-cleft; this distance gradually lessens until, in 14 mm. embryos, it lies on the same antero-posterior level.

The relationship of the aditus laryngis and intrinsic laryngeal musculature to the branchial region and thyroid cartilages is thus secondary and due to forward migration during development.

It has yet to be determined whether in the cases, e. g., man, mole, where the intrinsic laryngeal musculature has been described as originating in the branchial region, this is really so, or whether further investigation will show that the muscle-primordia can be traced back behind the sixth aortic arch to the œsophageal region. As quoted above, Göppert stated that the primordia of the laryngeal muscles in *Echidna*, at their first appearance in stage 42, could be followed backward to the beginning of the trachea, and he gave a figure which shows that they extended at least to the sixth aortic arch. But this is the only account, in previous investigations, of phenomena similar to those described above.

The primitive condition is probably that existing in Dipnoi (Protopterus), where the larynx opens behind the branchial region and its musculature other than the *transversus ventralis v.* is a direct derivative of the œsophageal constrictor. The phylogenetic history of the Mammalian larynx and its musculature from such a condition is clearly shown in its

¹ The term used by Grosser is "branchial," but it is clear from the context that he uses it in the sense of "visceral" or "post-oral" and not in the restricted way employed in this paper.

ontogenetic development. The separation of this musculature into dilatator, laryngeus dorsalis, and laryngeus ventralis occurs also in Amphibia and Sauropsida.

PHARYNGEAL AND PALATAL MUSCLES.

Kostanecki described the pharyngo-palatinus muscle of *Ornithorhynchus*, but the stylopharyngeus and pharyngeal constrictor have not been described in either *Monotreme* nor the pharyngo-palatinus in *Echidna*.

In a 25 mm. specimen of *Echidna* (Pl. 27, figs. 1-4) the stylo-pharyngeus arises from the inner surface of the stylohyale and passes inwards, and then inward and upward in a dorsally convex curve over the pharynx and nasopharynx to meet its fellow in the mid-dorsal line. It thus forms an anterior pharyngeal constrictor. It has no fibres passing directly towards the lateral wall of the pharynx. The posterior pharyngeal constrictor forms a sheet of fibres arching over the pharynx (Pl. 27, figs. 3-8). It is attached, from before backwards, to the stylohyale (Pl. 27, figs. 3, 4), to both medial and lateral surfaces of the first branchial bar (Pl. 27, figs. 5, 6) and thyroid ala, to the first branchial and first thyroid cornua¹ of the thyroid ala (Pl. 27, fig. 7), and to the cricoid cartilage. It has no attachments to the second thyroid bar. No superior pharyngeal constrictor is present. The pharyngo-palatinus arises from the anterior part of the posterior pharyngeal constrictor (Pl. 27, figs. 5, 4), passes downwards and forwards into the palato-pharyngeal fold (Pl. 27, fig. 3), then forwards in it, ventral to the stylo-pharyngeus, to the soft palate (Pl. 27, fig. 2). In the soft palate many fibres spread forwards and inwards towards the median raphé; the most lateral fibres are attached to the hinder wall of the medial end of the Eustachian tube (Pl. 27, fig. 1). No levator veli palatini is developed from the pharyngo-palatinus.

In stage A (Pl. 29, fig. 17) of *Dasyurus* a stylo-pharyngeus

¹ I adopt the interpretation of Dubois in regard to the morphology of the cornua of the thyroid ala (vide p. 414, 415).

muscle is present; it is attached laterally to the stylohyale and spreads round the pharynx to the dorsal middle line, some of the fibres pass towards the epithelium of the lateral wall of the pharynx. Behind the stylopharyngeus is a sheet of mesoblast cells (Pl. 30, figs. 18-21), not yet muscle-cells, with long axes in the transverse plane, arching over the pharynx and attached laterally to the first branchial cornu and upper edge of the thyroid cartilage—this is the primordium of the posterior pharyngeal constrictor and palato-palatinus. It develops into muscle-cells in stage B. In stage C (Pl. 30, fig. 25) muscle fibres, with longitudinal long-axis, can be seen extending forwards from the primordium of the posterior pharyngeal constrictor and pharyngo-palatinus, past the stylopharyngeus, towards the soft palate, but not reaching it in either this stage or stage D (Pl. 32, figs. 28-31). These longitudinal fibres are the pharyngo-palatinus, and transverse sections show that, behind the stylo-pharyngeus, they lie internal to the transverse fibres of the posterior constrictor.

In stage D embryonic muscle-cells can be seen surrounding the œsophagus.

In stage E the front end of the pharyngo-palatinus muscle has reached the soft palate (Pl. 32, fig. 34); some of its fibres diverge laterally towards the floor of the Eustachian tube, forming a pars salpingia. The hind end of the muscle has extended a little further back.

In stage H (Pl. 33, fig. 41) there is a well-marked posterior pharyngeal constrictor round the hinder part of the pharynx; it is attached laterally to the posterior cornu of the thyroid cartilage, and is continuous posteriorly with the constrictor of the œsophagus. It forms a continuous sheet up to the stylopharyngeus muscle, with the constrictor fibres of which it mingles. The pharyngo-palatinus muscle lies between the wall of the pharynx and the posterior constrictor; some of its fibres penetrate the palato-pharyngeal fold; it ends posteriorly between the wall of the pharynx and the pharyngeal constrictor; it extends forwards to the soft palate and Eustachian tube (Pl. 33, fig. 40). No superior pharyngeal

constrictor is formed by forward extension of the posterior pharyngeal constrictor, nor is a levator veli palatini separated from the longitudinal pharyngo-palatal sheet. Longitudinal fibres are developed from the anterior part of the œsophageal constrictor and are attached anteriorly to the cricoid (Pl. 33, fig. 42).

In stage J some of the fibres of the posterior pharyngeal constrictor become separated from the others and form a middle constrictor, whilst the main mass forms the inferior constrictor. The hind end of the pharyngo-palatinus does not extend quite so far posteriorly as the median fold in which the palato-pharyngeal folds end.

The primordium of the pharyngeal constrictors, crico-thyroid, pharyngo-palatinus, and levator veli palatini of the pig is first visible in $12\frac{1}{2}$ mm. embryos as a band of aggregated mesoblast cells latero-dorsal to the pharynx immediately outside the epithelium, in the second branchial segment. In 14 mm. embryos a similar primordium—that of the stylo-pharyngeus—appears in the first branchial segment.¹ In 17 mm. embryos the primordium developed in the second branchial segment has spread forwards past the primordium of the stylo-pharyngeus into the hyoid segment, forming the longitudinal pharyngo-palatinus, whilst the part behind is the posterior pharyngeal constrictor (Pl. 37, figs. 64–68). The latter, in 18 mm. embryos, is attached laterally to the first branchial bar and thyroid cartilage, and has grown ventrally outside the posterior extremity of the thyroid cartilage (Pl. 38, fig. 69). This downgrowth has extended further down in 21 mm. embryos and forms the crico-thyroid muscle (Pl. 38, fig. 72). The stylo-pharyngeus gains an attachment to the stylohyale in 24 mm. embryos and passes towards the lateral wall of the pharynx, but does not develop any constrictor fibres over the pharynx (Pl. 39, fig. 76). The pharyngo-palatinus extends in 21 mm. embryos as far forwards as the soft palate and medial end

¹ I do not give any figures of the stages, as they are similar to those of the rabbit which I have previously described.

of the Eustachian tube, and it is penetrated by fibres of the stylo-pharyngeus passing towards the lateral wall of the pharynx (Pls. 38, 39, figs. 74-76). In 24 mm. embryos the hind end of the pharyngo-palatinus has extended backwards underneath the posterior pharyngeal constrictor, and ends over the median dorsal pouch (Pl. 39, fig. 77). In 32 mm. embryos the tensor veli palatini separates from the dorsal surface of the anterior end of the pharyngo-palatinus (Pl. 38, fig. 74). In 38 mm. embryos the anterior edge of the posterior pharyngeal constrictor, which hitherto had not extended further forwards than the stylo-pharyngeus, extends uninterruptedly forwards, covering in the longitudinal fibres of the pharyngo-palatinus, and forming the superior pharyngeal constrictor. The part of the posterior pharyngeal constrictor behind the stylo-pharyngeus separates into the constrictor medius arising from the first branchial bar and the constrictor inferior arising from the thyroid and cricoid cartilages.

The above-described phenomena show that the primitive condition of the stylo-pharyngeus is that of an anterior constrictor of the pharynx, developed in the first branchial segment and having a lateral attachment to the stylohyale. From this origin the fibres spread over the anterior part of the pharynx and naso-pharynx. This is present in *Echidna*.

In Marsupials additional fibres—dilatator fibres—passing towards the lateral wall of the pharynx are developed.

In the pig and rabbit, and in *Eutheria* generally (vide description by Rückert), the stylo-pharyngeus has no constrictor fibres, only those passing towards the lateral wall of the pharynx are developed.

The constrictor fibres of the stylo-pharyngeus—in *Monotremes* and Marsupials—are dorsal to the pharyngo-palatinus; those passing towards the lateral wall of the pharynx—in Marsupials and *Eutheria*—penetrate the pharyngo-palatinus.

The primordium of the posterior pharyngeal constrictor is developed in the mesoblast immediately surrounding the pharyngeal epithelium, behind the stylo-pharyngeus. Its

primitive condition is that of a sheet of transverse fibres arching over the pharynx, with lateral attachments to the upper edge of the thyroid cartilage and first branchial bar, and in *Eutheria* also to the stylohyale.

The pharyngo-palatinus is developed from the posterior pharyngeal constrictor as a direct forward extension. This longitudinal sheet extends forwards, and also backwards internal to the pharyngeal constrictor. In *Echidna* its hind end extends only a little distance behind the anterior edge of the posterior constrictor; in Marsupials (*Dasyurus*, *Didelphys*, and *Phascolarctus*), pig, and rabbit to a greater extent. In the pig it ends on the dorsal surface of the dorsal pharyngeal pouch.

The front end of the pharyngo-palatinus grows forward past the stylo-pharyngeus to the soft palate and medial end of the Eustachian tube.

A levator veli palatini is not formed in *Echidna*, *Dasyurus viverrinus*, *Didelphys aurita*, and *Phascolarctus*. Nor did Kostanecki describe this muscle in *Didelphys cancrivora*, *D. azarae*, *D. virginiana*, *Dasyurus macrurus*, *D. ursinus*, *Perameles*, *Phalangista vulpina*, *Macropus spec.* On the other hand, Symington stated that there is one in *Macropus bennettii*.

In the pig and rabbit the levator veli palatini is developed from the anterior end of the pharyngo-palatinus.

The relationship of the pharyngo-palatinus muscle to the palato-pharyngeal fold is not constant. In *Echidna* both muscle and fold are short, the muscle lies in the fold, and extends a little way behind its hind end (Pl. 27, figs. 3-5). In *Dasyurus* the fold is well marked at birth, but the muscle does not extend into it until stage H, and even in stage J does not penetrate to its free edge. This is generally true of Marsupials, as described by Symington. The folds extend the whole length of the pharynx and meet in a mid-dorsal fold, but the muscle does not extend so far back as does the fold. In the rabbit and pig the folds are not nearly so marked as in *Dasyurus*. In the rabbit the folds extend the

whole length of the pharynx and meet in a mid-dorsal fold; the muscle lies in the fold, but does not extend back to its hind end. In the pig, as in *Echidna*, the fold does not extend further back than the first branchial segment, the muscle lies in it and extends back beyond, ending over the median dorsal pouch.

Of these conditions, that of *Echidna* appears to be the primitive one; those found in other Mammals investigated are secondary developments.

In *Echidna*, *Dasyurus*, *Didelphys*, and *Phascolarctus*, the posterior pharyngeal constrictor retains its position relative to the constrictor fibres of the stylo-pharyngeus. In the pig and rabbit—where no constrictor fibres of the stylo-pharyngeus are formed—the anterior edge of the posterior pharyngeal constrictor extends forwards in front of the stylo-pharyngeus at a late stage of development, and forms the superior constrictor. The part behind the stylo-pharyngeus separates into the middle and inferior constrictors.

Konstanecki, who investigated adult forms only, came to the conclusion that the levator veli palatini and pars palato-salpingo-pharyngea are "Abkömmlinge des *M. palato-pharyngeus* und, dar dieser selbst ein Derivat des Constrictor superior ist, würden sie sich mittelbar von dem letzteren, also von der Ringmusculatur des Pharynx herleiten lassen."

The embryological phenomena as stated above suggest some modification of this opinion. The primitive condition appears to have been an anterior constrictor s. stylo-pharyngeus, and a posterior constrictor. From the posterior constrictor was developed a longitudinal stratum—the pharyngo-palatinus—which extended forwards to the soft palate. In *Macropus bennettii* (Symington) and in *Eutheria* the levator veli palatini was developed from the anterior end of the pharyngo-palatinus. In Marsupials dilatator fibres, passing towards the lateral wall of the pharynx, were additionally developed in the anterior constrictor s. stylo-pharyngeus. In *Eutheria* no constrictor fibres, but only dilatator fibres, were developed in the stylo-pharyngeus,

and in relation to this, the posterior constrictor extended forwards forming anteriorly a superior constrictor—which takes the place of, but is not homologous with, the constrictor fibres of the stylo-pharyngeus of Echidna and Marsupials.

These phenomena are at variance with the statements of Futamura that in man the *M. levator veli palatini* and *M. uvulæ* are developed from a “Muskelblastengewebe” which “deutlichen Zusammenhang mit dem tiefen Teil der Platysmaanlage erkennen lässt,” and that in the pig they are derived from “Gewebe des Platysma colli das von der vorderen Seite des Oberkieferfortztes nach seiner medialen Seite zieht.” On the other hand they offer an explanation of the experiments of Beevor and Horsley, who found that in *Macacus sinicus* movements of the palate occurred in intracranial stimulation of the vago-accessorius, and did not occur on intracranial stimulation of the *N. facialis*. Dr. Elizabeth Cords has traced a nerve from the pharyngeal plexus to the levator veli palatini, in man; and the motor impulses probably pass by this route.

CRICO-THYROID MUSCLE.

Miss Walker described a crico-thyroideus muscle in both Monotremes, innervated in Echidna by a branch of the recurrent laryngeal nerve. This was confirmed by Göppert, who termed the muscle “thyreo-cricoideus,” and denied its homology with the crico-thyroideus muscle of Eutheria. He stated that it belongs to the group of the inner (intrinsic) laryngeal muscles. The evidence given in favour of this derivation does not appear very convincing.

No cricoid-thyroid muscle is developed in *Dasyurus*, nor is one present in 10 mm. specimens of *Didelphys aurita*, or 15 mm. specimens of *Phascolarctus*. Symington found a small crico-thyroideus posticus in pouch specimens of *Macropus*, but not in any adult Marsupials. Henkel stated that the muscle is absent in *Macropus rufus*, *robustus*, and *cervinus*, *Phascolomys platiceps*, *Petrogale*

lateralis, and *Onychogalea frenata*. The non-development or atrophy of the muscle in Marsupials is related to the fusion of the cricoid with the thyroid cartilage.

In the pig the crico-thyroid is formed by a downgrowth of the posterior pharyngeal constrictor lateral to the hind end of the thyroid cartilage.

HYPBRANCHIAL CRANIAL MUSCLES.

In *Dasyurus* only one hypobranchial cranial muscle—the branchio-hyoideus¹—is developed (Pl. 33, fig. 43). It is also present in *Echidna* (Fewkes) (Pl. 27, figs. 4, 5), *Ornithorhynchus* (Dubois), *Didelphys*, *Trichosurus* (Pl. 34, fig. 50), *Phascolarctus*, rabbit, pig, and many other Eutheria. It passes from the first branchial bar downwards and forwards to the ventral end of the stylohyal, is innervated by the ninth nerve, and in the pig is developed from the ventral portion of the first branchial muscle-plate.²

Fewkes also described in *Echidna*, but did not figure, a second muscle passing from the first branchial bar forwards and outwards to the stylohyale, under the name *stylothyroideus*. It was not described by Coues, Dubois, Miss Walker, Fräulein Westling, or Göppert, but is present in both Monotremes (vide Pl. 27, figs. 3–5, and Pl. 28, figs. 9, 10 from a 25 mm. specimen of *Echidna*). In a 8.5 mm. specimen of *Ornithorhynchus* it lies just outside and is not fully separated from the branchio-hyoideus.³ I have called it the branchio-hyoideus dorsalis, as the name proposed by Fewkes is not very suitable.

Miss Walker described a muscle—the “thyro-hyoid”—in *Echidna* and *Ornithorhynchus*, passing between the first thyroid (second branchial) and the first branchial bar. In *Echidna* “its fibres are very short, and are to a great extent

¹ *S. cerato-hyoideus*, *hyothyroideus* (Fewkes), *interhyoideus* (Dubois).

² ‘Quart. Journ. Mic. Sci.’ vol. 56, part 2 (1911), p. 253, and Text-figures 98 and 99.

³ *Ibid.*, vol. 59, part 4 (1914), fig. 33. The muscle is not named in the figure as I did not then know what it was.

replaced by ligament," but in *Ornithorhynchus* is better developed. Göppert, who did not refer to Miss Walker's statements, said that the interval between these bars is occupied by dense connective tissue. I found this to be the case in a 25 mm. specimen of *Echidna* (vide Pl. 28, figs. 9 and 10), and in an adult specimen of *Ornithorhynchus*, and in a 8.5 mm. embryo of the latter did not see any muscle-primordium between the first thyroid (second branchial) and first branchial bars. The muscle is therefore variable and its homology doubtful.

Dubois described an interthyroideus muscle in both *Monotremes*, passing from the cornu laterale to the cornu posticum of the thyroid ala, innervated by the superior laryngeal nerve, and serially homologous with the "interhyoideus" s. branchiohyoideus between the hyoid and first branchial bars. The existence of this muscle was confirmed by Miss Walker and by Göppert (Pls. 27, 28, figs. 7, 9, 10).

The upper end of the first branchial bar in *Echidna* is continuous with the thyroid ala, which ends in two posterior cornua. The dorsal one enters the valve at the junction of the pharynx and œsophagus (Göppert), and the ventral one ends free (see figures by Dubois, Miss Walker, and Göppert, and drawings (Pl. 28, figs. 9, 10) of a model of a 25 mm. *Echidna*).

Dubois described the ventral cornu as the cornu laterale, and the second thyroid (third branchial) bar as the cornu posticum, and regarded the former as the posterior end of the first thyroid (second branchial) bar. Göppert, on the other hand, regarded the dorsal process of the thyroid ala as the upper end of the first thyroid (second branchial) bar, and its ventral process as a "processus muscularis," but did not offer any embryological evidence for this view.

In a 8.5 mm. specimen of *Ornithorhynchus*—in which the upper ends of the first branchial and first thyroid (second branchial) bars were unconnected—I found the primordium of the "interthyroideus" muscle passing between the first thyroid (second branchial) and second thyroid (third

branchial) bars. This proves the correctness of the theory of Dubois. The dorsal cornu of the thyroid ala is thus a backward prolongation of the upper end of the first branchial bar, the middle portion of which has fused with that of the first thyroid (second branchial) to form a thyroid ala, and the ventral cornu is the posterior end of the first thyroid (second branchial) bar. The "interthyroideus" muscle thus passes between the first thyroid (second branchial) and the second thyroid (third branchial) bars.

The view of Dubois also harmonises with the place of entry of the superior laryngeal nerve into the larynx—between the first branchial and first thyroid bars, i. e. serially homologous with the ninth nerve between the hyoid and first branchial bars. (In Marsupials and Eutheria—vide Dubois and Henkel—the place of entry of the superior laryngeal nerve is variable.)

The branch of the superior laryngeal nerve to the interthyroideus muscle is given off just after the nerve passes between the first branchial and first thyroid cornua of the thyroid ala, and passes downwards and backwards internal to the first thyroid cornu (vide figure of Göppert). This muscle, theoretically, should be innervated by a second thyroid (third branchial) branch of the vagus, and Dubois took this view for the whole of the superior laryngeal nerve. But Göppert showed that in *Echidna* embryos the main stem of the superior laryngeal nerve lies just posterior to the third gill-cleft and fourth arterial arch, and must therefore be regarded as the nerve of the first thyroid (second branchial) arch, and that no branch corresponding to the second thyroid (third branchial) arch, homologous with the atrophying branch found by Froriep in calf embryos, was given off by the vagus.

It may be concluded that the superior laryngeal is the nerve to the first thyroid (second branchial) arch; that its motor branch to the "interthyroideus" is a second thyroid (third branchial) element which has been taken up into the superior laryngeal nerve in place of being a separate branch of the vagus.

These hypo-branchial cranial muscles are homologous with the *Mm. interarcuales ventrales* of Amphibia. The foremost, *interarcualis ventralis i*, passes from the first branchial bar to the hyoid bar, is represented in Monotremes by the *branchio-hyoideus* and *branchio-hyoideus dorsalis*, in Marsupials and many Eutheria by the *branchio-hyoideus*. Possibly the variable *thyro-hyoideus* of Miss Walker represents *interarcualis ventralis ii*. *Interarcualis ventralis iii*, passes from the second thyroid (third branchial) to the first thyroid (second branchial) bar, and is present only in Monotremes. *Interarcualis ventralis i* is innervated by the ninth and *interarcualis ventralis iii* by a branch of the superior laryngeal nerve.

In Sauropsida only *interarcualis ventralis i* is present—the *branchio-hyoideus* (*Sphenodon*) or *branchio-mandibularis* (other Sauropsida)—and has a secondary innervation from the hypoglossal nerve.

HYPOBRANCHIAL SPINAL MUSCLES.

Monotremes. The hypobranchial spinal muscles of *Echidna* were described by Fewkes, Fürbringer, Leche, Toldt, and myself. Dissection of an adult *Ornithorhynchus* showed the following: *M. omo-hyoideus* (employing the usual nomenclatures, see below) arises just within the thorax from the outer end of the clavicle and adjacent part of the scapula. It separates into a superficial and a deep layer. The former¹ passes forwards and slightly inwards, ventral to the hypobranchial bars, to which it has no attachments, and is inserted into the longitudinal raphé a little in front of the hyoid bar. The median edges of the two muscles are in contact in the anterior third of their extent. The deep layer² is very thin and not so broad as the superficial; it passes forwards and is inserted into the basithyroid and median ends of the first and second branchial bars. The

¹ Coles did not give its origin, and stated that it is inserted to the *os hyoides*. I found the same insertion as Schulman.

² Not previously described.

*M. sterno-thyroideus*¹ arises from the dorsal surface of the manubrium sterni; it passes forwards and is inserted into the ventral surfaces of the third and distal extremity of the second branchial bars. The median edges are fused in the cervical part of their extent.

There are no tendinous intersections in the sterno-thyroid and omo-hyoid muscles. These muscles are innervated by the descending branch of the twelfth nerve, with which—as shown by Fürbringer—the first and second cervical nerves anastomose.

The *M. genio-hyoideus* consists of a median and a lateral portion. The former arises from the distal extremity of the second branchial bar and passes forwards and slightly inwards, and is inserted into the median raphé anterior to the superficial portion of the omo-hyoid muscle. The lateral portion blends posteriorly with the superficial fibres of the *M. laryngo-glossus*, from which it diverges inwards and is inserted into the median raphé just in front of the superficial portion. The ascending branch of the twelfth nerve passes into the tongue between the median and lateral portions.

There is no muscle taking origin from the symphysis of the jaws and passing into the tongue which could strictly be called a *M. genio-glossus*.² It is probably represented by a well-marked lingualis muscle which takes origin from the median raphé and passes vertically upwards into the tongue internal to the *M. laryngo-glossus*. It has no direct attachment to the hyoid bar. It is apparently the muscle described as the *M. genio-hyo-glossus* by Coues.³ It is homologous with the *M. genio-glossus* of *Echidna*.

¹ Described by Coues as an unseparated sterno-hyoid and sterno-thyroid. I did not see any fibres passing to the hyoid bone, or continuous with the laryngo-glossus, as described by him.

² According to Coues and Toldt the *M. genio-glossus* reaches the lower jaw. Schulman mentions the same insertion as I found.

³ Coues states that "The genio-hyo-glossus forms as usual a vertical plane in apposition with its fellow in the middle line; behind it has the ordinary attachment to the os hyoides and is considerably blended with the hyo-glossus; its anterior connections are rather with the genio-hyoid than with the jaw itself."

In a 8.5 mm. embryo of *Ornithorhynchus* the anterior end of the *M. genio-hyoideus* reaches Meckel's cartilage, so that there is a secondary loss of the attachments of the *Mm. genio-hyoideus* and *genio-glossus* to the jaw.

The *M. laryngo-glossus* takes origin from the distal extremity of the second branchial bar and passes forward; its origin is small, it attains its maximum size at the level of the hyoid bar, to which it has no attachment, and then tapers as its fibres pass into the tongue. Its anterior end joins its fellow and passes to the tip of the tongue.

Thyro-hyoid, stylo-glossus, and palato-glossus muscles are not developed. The genio-hyoid, laryngo-glossus, and lingualis muscles are innervated by the ascending branch of the twelfth nerve.

The homologies between the hypobranchial spinal muscles of *Echidna* and *Ornithorhynchus* with those of other Mammals are doubtful owing to ignorance of their development. In *Dasyurus*, however (vide p. 420), the posthyoid longitudinal strip separates into three muscles—from within outwards, the sterno-thyroid, sterno-hyoid, and omo-hyoid, and subsequently the anterior part of the sterno-hyoid shifts inwards and lies ventral to the sterno-thyroid. The sterno-glossus of *Echidna* is formed of two constituents—a posterior part, homologous with the lateral portion of the sterno-thyroid of *Ornithorhynchus*, and an anterior part, homologous with a separated portion of the laryngo-glossus. The "omo-hyoid" muscles of the *Ornithorhynchus* and *Echidna* are homologous; in each case the muscle arises lateral to the sterno-thyroid, passes forwards and inwards so as to be ventral to it, and separates into two portions, of which the deep is inserted into the laryngeal cartilages and the superficial passes forwards to the median longitudinal septum.

Fürbringer was of opinion that the posterior part of the sterno-glossus of *Echidna* was homologous with the sterno-hyoid of other Mammals, and, like Coues, he designated the muscles just described as "omo-hyoid." In this he was

followed by Schulman, Bijvoet, and Toldt. Comparison, however, with *Dasyurus* suggests that their "omo-hyoid" is the sterno-hyoid, that a true omo-hyoid is not formed, and that the separation of the posterior part of the sterno-glossus of *Echidna* from the sterno-thyroid is a peculiarity of that animal.

The genio-hyoid of *Echidna* arises from the first branchial and second branchial (first thyroid) cartilages, that of *Ornithorhynchus* from the second branchial (first thyroid) only. The laryngo-glossus arises from the second branchial (first thyroid) bar in both animals. As in 8.5 mm. embryo of *Ornithorhynchus* the adjacent ends of the anterior and posterior portions of the primordium of the hypobranchial spinal muscles lie at the level of the first branchial bar, the more posterior attachments of the genio-hyoid and laryngo-glossus in the adult are due to a subsequent backward growth. Owing to this and the forward growth of the superficial part of the sterno-thyroid muscles, there is a partial overlapping of the anterior and posterior portions of the hypobranchial spinal muscles.

The condition of the hypobranchial spinal muscles of *Dasyurus* in stage iv (a little before birth) differs very slightly from that in stage A (just born). In stage A (Pls. 29, 30, figs. 15-24) they consist of intrinsic lingual muscle-fibres, genio-glossus, genio-hyoid, stylo-glossus, hyo-glossus, sterno-thyroid, sterno-hyoid, and omo-hyoid muscles. The intrinsic lingual muscle-fibres consist of well-marked transversalis and verticalis fibres. The genio-hyoid and hyo-glossus are attached posteriorly to the first branchial bar, and the stylo-glossus to the stylohyale. The sterno-hyoid and sterno-thyroid muscles form a common flat band, which arises from the dorsal surface of the sternum, as low down as the second rib, and passes forwards, with its inner edge close to that of its fellow; the median fibres are attached to the membrane uniting the two thyroid alæ, whilst the more lateral fibres pass on and are attached to the first branchial bar. The omo-hyoid arises from the scapula and passes forward,

converging to the sterno-hyoid, and is inserted into the first branchial bar. There is no thyro-hyoid muscle.

In the immediately preceding stage iv the only difference is that the sternum is not yet formed, and (unseparated) sterno-thyroid and sterno-hyoid muscles are attached posteriorly to the sternal plate connecting the ventral ends of the first and second ribs.

In stage B the median united sterno-thyroid muscles have shifted a little upwards and lie dorsal to the medial edges of the sterno-hyoid muscles. In stage C (Pls. 30, 31, figs. 25, 28) the adjacent ends of the genio-hyoid, sterno-hyoid, and omo-hyoid muscles have lost their attachment to the first branchial bar and are united together by a short tendon. The sterno-thyroid muscles are inserted into the thyroid cartilage. In stage D (Pl. 31, figs. 32, 33) the thyro-hyoid is beginning to be proliferated from the medio-dorsal surface of the anterior part of the omo-hyoid. It becomes a separate muscle by stage F, though with smaller fibres than the omo-hyoid. It arises from the posterior cornu of the thyroid cartilage and is inserted into the common tendon of the genio-hyoid and sterno-hyoid. In stage E, and the process is still more marked by stage J (Pl. 33, figs. 43, 44), the outer fibres of the genio-hyoid extend a little more posteriorly than do the inner fibres, so that the inner fibres are connected with the sterno-hyoid and thyro-hyoid, and the outer fibres, which are connected with the omo-hyoid, overlap the anterior end of the sterno-hyoid.

The primordium of the hypobranchial spinal muscles posterior to the first branchial bar thus forms in *Dasyurus* a broad strip which separates into three muscles—from within outwards, the sterno-thyroid, sterno-hyoid, and omo-hyoid. The two sterno-thyroids subsequently lie dorsal to the sterno-hyoids. The thyro-hyoid is proliferated from the anterior portion of the omo-hyoid. The sterno-hyoid and omo-hyoid are at first attached to the first branchial bar, then lose this insertion, and become united to the posterior end of the genio-hyoid by a tendon to which also the thyro-hyoid becomes

attached. The union of the genio-hyoid with the sterno-hyoid, thyro-hyoid, and omo-hyoid is thus a secondary condition.

In *Didelphys aurita* (10 and 12 mm. pouch specimens) the sterno-hyoid and omo-hyoid muscles are inserted into a tendon from which arises the genio-hyoid. This tendon is situated dorsal to the transverse tendon connecting together the hyoid ventral constrictor s. posterior digastricus. The thyro-hyoid is inserted into the hinder edge of the lower end of the first branchial bar and the sterno-thyroid into the thyroid cartilage. The stylo-glossus arises from the stylohyale, and the hyo-glossus from the first branchial bar, by an extensive dorso-ventral origin. The posterior end of the anterior digastric is attached to the tendon of the hyoid ventral constrictor.

In *Phascolarctus* (15 and 25 mm. pouch specimens) the sterno-hyoid and omo-hyoid muscles are inserted into the transverse tendon of the hyoid-ventral constrictors. The thyro-hyoid is inserted into the basibranchial, and the sterno-thyroid into the thyroid cartilage. The stylo-glossus arises from the stylohyale; the hyo-glossus arises by two heads from the tendon of the hyoid ventral constrictor—an inner and an outer, the former of which, passing forwards and outwards to join the outer head, forms a band of muscle lying dorsal to the genio-hyoid, much as do the inner fibres of the hyo-glossus of *Didelphys*. The posterior end of the genio-hyoid is attached to the hyoid ventral constrictor. This account of *Phascolarctus* confirms that given by Young in most particulars.

The primary condition of the hypobranchial spinal muscles of Marsupials is thus one in which the adjacent ends of the genio-hyoid, sterno-hyoid, and omo-hyoid are attached to the first branchial bar. This may persist as e. g. in *Cheironectes*. Various conditions may ensue in which these attachments are lost and the muscles become connected together by tendon, but no uniform type of such secondary connection is present in these three Marsupials. In particular, no long retractor of the lower jaw, of constant elements, is formed.

The primordium of the hypobranchial spinal muscles in the pig forms in 11 and 12½ mm. stages an undivided strip of cells extending from the mandibular segment backwards in the hyoid and branchial segments into the neck. In 13 mm. embryos the primordia of the hyoid and first branchial bars are visible, but it is not until the stage of 17 mm. is reached that the muscle-primordium divides into anterior and posterior portions at the level of the first branchial bar. Previous to this, however, in the 14 mm. stage a longitudinal division begins at the hind end of the posterior portion; this extends forwards and in the 17 mm. stage separates the (lateral) omo-hyoid from the (median) primordium of the sterno-hyoid and sterno-thyroid; in the branchial region the muscle-strip is as yet undivided (Pl. 37, figs. 64-68). In 21 mm. embryos the thyro-hyoid is separated from the dorsal portion of the anterior part of the primordium of the sterno-hyoid and sterno-thyroid (Pl. 38, figs. 71, 72). In the 24 mm. stage the sterno-hyoid and sterno-thyroid have become separate.

The method of formation of the thyro-hyoid is thus not identical in *Dasyurus* and the pig—in the former it is developed from the anterior part of the omo-hyoid; in the latter from the anterior part of the primordium of the sterno-hyoid and sterno-thyroid, i.e. in the one case from the lateral, in the other from the medial of the two strips into which the primordium of the hypobranchial spinal muscles separates behind the first branchial bar.

The palato-glossus muscle of *Dasyurus* begins to develop in stage D by the upgrowth of fibres from the dorso-external edge of the stylo-glossus muscle into the soft palate (Pl. 31, fig. 29). It is better marked in stage F (Pl. 32, fig. 39) and stage H (Pl. 33, fig. 40), though still connected with the stylo-glossus. It has become separate in stage J.

The palato-glossus of the pig was mentioned by Kallius, but he did not describe its development. It is formed in 32 mm. embryos by an upgrowth into the soft palate from the dorso-external edge of the stylo-glossus (Pl. 38, fig. 73).

These phenomena show that in *Dasyurus* and the pig the palato-glossus is a derivative of the stylo-glossus, and not one of the pharyngeal group of muscles.

It is stated in "Quain" (Ed. Symington) and in "Cunningham" (Ed. Robinson) that the palato-glossus of man is innervated by the pharyngeal plexus. I found, however, in one subject that the muscle was innervated by a twig from the distal of the two branches of the hypoglossal nerve passing to the stylo-glossus. Further, my friend Dr. Fisher found in another subject that the stylo-glossus was supplied by four branches of the hypoglossal nerve, the two distal of which formed a loop from which twigs passed to the palato-glossus. The developmental phenomena and the innervation thus agree.

COMPARISON OF THE CRANIAL MUSCLES IN MAMMALS.

The embryological history and adult condition of the muscles of the head in Monotremes, Marsupials, and Eutheria suggest the following conclusions :

Adult Monotremes are more primitive than Marsupials and Eutheria in the following particulars :

(1) The presence of a pterygo-tympanicus (homologue of the tensor veli palatini) in *Ornithorhynchus* and in the embryo of *Echidna*—also in some Edentates. This condition is not fully developed during the pouch stage of *Dasyurus*.

(2) The existence of a depressor mandibulæ anterior (homologue of the anterior digastric), with no attachment to the hyoid ventral constrictor, so that no digastricus mandibulæ is formed. This condition is present in the adult stage of *Myrmecobius* and in the pouch stage of *Dasyurus*.

(3) The existence of a stapedius in a levator hyoidei form. This condition is present in the pouch stage of *Dasyurus*.

(4) The existence of a hyoid ventral constrictor arising from the stylohyale. This condition is present in the pouch stage of *Dasyurus* and in the adult stage of *Manis pentadactyla*.

(5) The existence of relatively undifferentiated facial muscles.

(6) The existence of an *Interthyroideus* s. *Interarcualis ventralis* iii. This is associated with the presence of a second thyroid (third branchial) bar, as a separate structure not fused with the first thyroid bar.

(7) The existence of a stylo-pharyngeus as an anterior constrictor of the pharynx.

(8) The absence of a stylo-glossus, palato-glossus, and thyro-hyoid.

On the other hand, some of the cranial muscles of *Monotremes* undergo modifications:

(1) The internal pterygoid atrophies.

(2) The pterygo-tympanicus atrophies in *Echidna*.

(3) The intermandibularis loses its attachments to the jaw in *Echidna*.

(4) The *detrahens mandibulæ* and *branchio-hyoideus dorsalis* are probably secondary formations. Neither of these muscles has any representative in non-Mammals, Marsupials, or *Eutheria*.

(5) According to Göppert the crico-thyroideus is absent and its place is taken by the thyreo-cricoideus, which is innervated by the recurrent laryngeal nerve, and is developed from the intrinsic laryngeal musculature. The embryological evidence in favour of this last statement is not, however, convincing.

(6) In both *Monotremes* there is a secondary overlapping of the adjacent ends of the anterior and posterior elements of the hypobranchial spinal muscles—in *Echidna* a *sternoglossus* is developed and in *Ornithorhynchus* the *genio-hyoid* and *genio-glossus* lose their attachment to the jaw.

In *Monotremes* and Marsupials the *dilatator laryngis* separates into the *kerato-crico-arytenoideus* and *crico-arytenoideus posticus internus*. This does not occur in *Eutheria*, and is, perhaps, a secondary feature of both *Monotremes* and Marsupials.

No one of the cranial muscles of Marsupials is more

primitive than in Monotremes if, as above suggested, the *detrahens mandibulæ* and *branchio-hyoideus dorsalis* are secondary formations.

Secondary changes occur in the attachments of the adjacent ends of the anterior and posterior elements of the hypobranchial spinal muscles in Monotremes and some Marsupials, but such changes are very different in character and have been separately acquired.

Marsupials are more primitive than Eutheria in the following particulars: (1) The presence of constrictor as well as dilator fibres in the stylo-pharyngeus (in Monotremes only constrictor fibres are present); (2) the absence of a superior pharyngeal constrictor derived from the posterior constrictor (also absent in Monotremes); (3) the absence of a levator veli palatini, except in *Macropus bennetti* (Symington), (also absent in Monotremes); (4) the presence of a second thyroid (third branchial) bar (also present in Monotremes in a more primitive condition).

In *Myrmecobius* the depressor mandibulæ anterior and hyoid ventral constrictor remain separate, as in Monotremes. In all other Marsupials the hind end (morphologically, the inner end) of the depressor mandibulæ anterior fuses with the hyoid ventral constrictor to form a digastricus.

In Eutheria, other than Edentates, the hyoid ventral constrictor divides into dorsal and ventral portions, which partially lose their primary relative positions owing to up-growth of the upper end of the ventral portion (*stylohyoideus*) so that overlapping results. The digastricus is formed by connection of the depressor mandibulæ anterior with the dorsal portion.

In Edentates, in general, the depressor mandibulæ anterior unites with (a portion or the whole of) the sternohyoideus to form a sternomandibularis. The hyoid ventral constrictor either remains single or divides into dorsal and ventral portions which retain their relative positions. The lower end of the undivided hyoid ventral constrictor, or of its lower portion, has various relations to the sternomandibularis. A

digastricus of either the Marsupial or the Eutherian type is not developed.

Certain developmental changes which, on comparison with Monotremes, appear to be of importance in the phylogenetic history of the cranial muscles of Marsupials and Eutheria occur in the pouch stage of *Dasyurus*, but are omitted or slurred over in the development of the pig and rabbit. The majority of these are mentioned above in dealing with Monotremes. In addition there are (1) the existence at birth of two masticatory muscles only, a medial and a lateral; (2) after separation of the medial muscle into internal pterygoid, pterygo-tympanicus s. tensor veli palatini, and tensor tympani, there is a temporary origin of the tensor tympani from the (Mammalian) pterygoid bone. Neither of these conditions is preserved to the adult stage in any living Mammal. They and the others mentioned above are referable to the fact that *Dasyurus* is born at a very early stage of development, whereas the parallel stages in the pig and rabbit occur during intra-uterine life.

The pterygo-tympanicus retains more primitive conditions in some Edentates than in Marsupials.

I have in conclusion to express my thanks to Professor J. P. Hill for the loan of sections of *Dasyurus* and *Trichosurus* and for the opportunity of examining sections of an embryo of *Ornithorhynchus*; to Dr. Assheton for the loan of specimens of *Echidna*; and to J. M. Gillespie, Esq., for an adult specimen of *Ornithorhynchus*.

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EXPLANATION OF PLATES 27—39,

Illustrating Dr. F. H. Edgeworth's paper “On the Development and Morphology of the Pharyngeal, Laryngeal, and Hypobranchial Muscles of Mammals.”

ABBREVIATIONS.

Aryten. cart. Arytenoid cartilage. *ary. epiglott. plica.* arytenoid-epiglottic fold. *basibr. cart.* basibranchial cartilage. *1st branch. bar.* First branchial bar. *1st branch. cornu.* First branchial cornu (of thyroid ala of *Echidna*). *branch. hy. m.* Branchio-hyoideus muscle. *branch. hy. dors. m.* Branchio-hyoideus dorsalis muscle. *bucc. cav.* Buccal cavity. *cons. œsoph. m.* Constrictor muscle of œsophagus. *cricoid cart.* Cricoid cartilage. *cricothy. ary. m.* crico-thyroid-arytenoideus muscle. *dil. lary. m.* Dilatator laryngis muscle. *dor. aorta.* Dorsal aorta. *dor. phary. pouch.* Dorsal pharyngeal pouch. *duct. branch. iii.* Ductus branchialis of third gill-cleft. *duct. ph. br. 4th gill-cleft.* Ductus pharyngo-branchialis of fourth gill-cleft. *epiglot. cart.* Epiglottic cartilage. *epithel. b. iv.* Epithelial body of fourth gill-cleft. *Eust. tube.* Eustachian tube. *fund. præcerv.* Fundus præcervicalis. *gen. gloss. m.* Genio-glossus muscle. *gen. hy. m.* Genio-hyoideus muscle. *hyo-epiglott. m.* Hyo-epiglotticus muscle. *hyo-gloss. m.* Hyo-glossus muscle. *hyoid vent. cons. m.* Hyoid ventral constrictor muscle. *interary. cart.* Interarytenoid cartilage. *interary. m.* Interarytenoid muscle. *intermand. m.* Intermandibularis muscle. *interthy. m.* Interthyroideus muscle. *kerato-cricothy. m.* Kerato-crico-arytenoideus muscle. *lary. vent. m.* Laryngeus ventralis muscle. *lev. pal. m.* Levator veli palatini muscle. *ling. br. ix.* Lingual branch of ix. *long. m. of œsoph.* Longitudinal muscle of œsophagus. *mylohy. m.* Mylohyoid nerve. *œsoph.* Œsophagus. *omohy. m.*

Omo-hyoideus muscle. *pal. gloss. m.* Palato-glossus muscle. *pal. phary. plica.* Palato-pharyngeal fold. *phary. pal. m.* Pharyngo-palatinus muscle. *post. branch. b.* Post-branchial body. *pri. of cric. cart.* Primordium of cricoid cartilage. *pri. of cons. œsoph. m.* Primordium of constrictor muscle of œsophagus. *pri. of hypobr. sp. m'.* Primordium of hypobranchial spinal muscles. *pri. of lary. m'.* Primordium of laryngeal muscles. *pri. of post. phary. cons. m.* Primordium of posterior pharyngeal constrictor muscle. *pri. of thy. cart.* Primordium of thyroid cartilage. *pri. of thyrohy. m.* Primordium of thyro-hyoideus muscle. *recurr. lary. n.* Recurrent laryngeal nerve. *sec. ary. plica.* Secondary arytenoid fold. *sternohy. m.* Sterno-hyoideus muscle. *sternomast. m.* sterno-mastoideus muscle. *sternothy. m.* Sterno-thyroideus muscle. *stylogloss. m.* Stylo-glossus muscle. *stylohy. m.* Stylo-hyoideus muscle. *stylophary. m.* Stylo-pharyngeus muscle. *sup. cons. m.* Superior constrictor muscle of pharynx. *sup. lary. n.* Superior laryngeal nerve. *symp. n.* Sympathetic nerve. *tend. gen. hy. m. to omohy. m.* tendon passing from genio-hyoideus to omo-hyoideus. *tend. gen. hy. m. to sternohy. m.* tendon passing from genio-hyoideus to sterno-hyoideus. *tend. hyoid. vent. cons. m.* Tendon connecting the two halves of the hyoid ventral constrictor muscle. *thy. gl.* Thyroid gland. *thyrœ. cric. m.* Thyreo-cricoid muscle (in Echidna). *thyroid cart.* Thyroid cartilage. *thyrohy. m.* Thyro-hyoideus muscle. *1st thyroid cornu.* First thyroid cornu of thyroid ala (in Echidna). *trach. ring.* Tracheal ring. *vent. aorta.* Ventral aorta. Roman numerals. Cranial nerves.

Echidna, figs. 1-10.

Greatest length 25 mm. (stage 50 of Semon); figs. 1-8, transverse sections; No. 1 is the most anterior; figs. 9 and 10, sketches of a model of the hyoid, first branchial, thyroid, and cricoid cartilages; fig. 9 shows the ventral aspect, fig. 10 the lateral.

Dasyurus, figs. 11-46.

Stage A (embryo C '01; greatest length 4.3 mm.): fig. 11, slide 1, row 5, number 15; fig. 12, s. 2, t. 1, n. 3; fig. 11 is the more dorsal. The right side of the sections is a little more dorsal than the left.

Stage iv ('01; greatest length 4.7 mm.): fig. 13, s. 2, t. 3, n. 8; the right side of the section is a little anterior to the left side, and the upper part is posterior to the lower. Fig. 14, sagittal section, s. 5, r. 1, n. 11.

Stage A (just born; greatest length 5.5 mm., head length 2.5 mm.): fig. 15, s. 1, r. 7, n. 21; fig. 16, s. 1, r. 8, n. 9; fig. 17, s. 1, r. 8, n. 18; fig. 18, s. 1, r. 9, n. 2; fig. 19, s. 1, r. 9, n. 7; fig. 20, s. 1, r. 9, n. 11; fig. 21, s. 1, r. 9, n. 17; fig. 22, s. 2, r. 1, n. 6; fig. 23, s. 2, r. 1, n. 21; fig. 24, s. 2, r. 1, n. 2.

Stage C (few hours old; greatest length 5.75–6 mm., head length 3 mm.); fig. 25, s. 2, r. 2, n. 8; fig. 26, s. 2, r. 2, n. 12; fig. 27, s. 2, r. 3, n. 2; fig. 28, s. 2, r. 3, n. 7.

Stage D (26 hours old; greatest length 6 mm., head length 3.25 mm.): fig. 29, s. 1, r. 2, n. 14; fig. 30, s. 2, r. 2, n. 13; fig. 31, s. 2, r. 3, n. 4; fig. 32, s. 2, r. 3, n. 16; fig. 33, s. 2, r. 4, n. 2.

Stage E (5–6 days old; greatest length 8 mm., head length 4.5 mm.): fig. 34, s. 2, r. 8, n. 5; fig. 35, s. 2, r. 8, n. 20; fig. 36, s. 3, r. 1, n. 6; fig. 37, s. 3, r. 1, n. 10; fig. 38, s. 3, r. 2, n. 1. In fig. 38 the lower end of the section is broken away.

Stage F (about 7 days old; greatest length 8.5–9 mm., head length 5–5.5 mm.): fig. 39, s. 3, r. 1, n. 13.

Stage H (about 14 days old; greatest length 13.5 mm., head length 8.8–5 mm.): fig. 40, s. 6; r. 3, n. 5; fig. 41, s. 8, r. 2, n. 2; fig. 42, s. 8, r. 5, n. 3. In figs. 41 and 42 the left side of the sections is a little posterior to the right side.

Stage J (25 days old; greatest length 20 mm., head length 12.5 mm.): the plane of the sections is such that the upper part of the sections is anterior to the lower. Fig. 43, s. 14, r. 2, n. 7; fig. 44, s. 14, r. 3, n. 8; fig. 45, s. 15, r. 1, n. 5; fig. 46, s. 15, r. 3, n. 4. In fig. 46 the lower part of the section is broken away.

Trichosurus vulpecula, figs. 47–55.

Figs. 47–49.—Stage v (greatest length 6 mm. XI '01): transverse sections; fig. 47 is the most anterior. Fig. 47, s. 4, r. 1, n. 3; fig. 48, s. 4, r. 2, n. 1; fig. 49, s. 4, r. 3, n. 2.

Figs. 50–52.—Stage viii *b* (greatest length 7.25 mm. XII '02): Fig. 50 is the most anterior. The plane of the sections is from above downwards, nearly horizontal to the primordia of the thyroid cartilages. Fig. 50, s. 5, r. 1, n. 7; fig. 51, s. 5, r. 2, n. 1; fig. 52, s. 4, r. 3, n. 6.

Figs. 53 and 54.—Stage ix *a* (greatest length 8.5 mm.): sagittal sections; fig. 53 is the more lateral. Fig. 53, s. 4, r. 4, n. 2; fig. 54 s. 4, r. 4, n. 5.

Fig. 55.—Stage ix *b* (greatest length 8.5 mm.): the plane of the the section is from above downwards and forwards, nearly horizontal to the primordia of the thyroid cartilages; s. 6, r. 4, n. 2.

Phascolarctus (greatest length 15 mm.) fig. 56.

Fig, figs. 57–78.

Figs. 57–59.—Transverse sections, embryo 8 mm. vertex-breech length; fig. 57 is the most anterior. The left side of the sections is a little anterior to the right side.

Figs. 60-63.—Transverse sections, embryo 11 mm. vertex-breech length; fig. 60 is the most anterior.

Figs. 64-68.—Transverse sections, embryo 17 mm. vertex-breech length; fig. 64 is the most anterior.

Figs. 69-70.—Transverse sections, embryo 18 mm. vertex-breech length; fig. 69 is the more anterior.

Figs. 71-72.—Transverse sections, embryo 21 mm. vertex-breech length; fig. 71 is the more anterior. The right side of the sections is a little anterior to the left side.

Figs. 73-77.—Transverse sections, embryo 32 mm. vertex-breech length; fig. 73 is the most anterior.

Fig. 78.—Transverse section, embryo 38 mm. vertex-breech length.

On the Corpora lutea and Interstitial Tissue
of the Ovary in the Marsupialia.

By

Chas. H. O'Donoghue, D.Sc., F.Z.S.,

Senior Assistant in the Zoological Department, University
College, London.

With Plate 40.

CONTENTS.

	PAGE
INTRODUCTION	433
MATERIAL	436
<i>Phascolarctos cinereus</i>	436
<i>Trichosurus vulpecula</i>	441
<i>Didelphys aurita</i>	446
Further species	449
THE CORPUS LUTEUM IN THE MARSUPIALIA	451
THE INTERSTITIAL TISSUE	455
SUMMARY	467
1) The Corpus luteum	467
2) The Interstitial Tissue	468
LIST OF REFERENCES	469
DESCRIPTION OF PLATES	472

INTRODUCTION.

It is becoming increasingly evident that the secretions of the ductless glands play a very important part in determining the functions and even the structure of an animal. Not least among these are the secretions of the sexual organs, and in particular the ovary. In the mammal interest is added by the fact that ovarian secretions appear to control two very characteristic mammalian activities, namely, the preparation of the uterine wall for the attachment of the embryo, and the growth of the mammary glands (28, 29, 30).

According to Fränkel and Cohn (11, p. 295), "die Aplacentalia (Monotremata and Marsupialia) und die übrigen Tiere, deren Eier ausserhalb des Uterus zur Entwicklung kommen, einen rudimentären oder gar keinen gelben Körper aufweisen."

From this quotation it will be seen that some misconceptions are still prevalent regarding the corpora lutea in the marsupials, to remove which further investigation seems desirable. Moreover, as suitable material from this group of animals is difficult to obtain, it is all the more necessary that advantage should be taken of such as is available.

While looking out material for a previous investigation into the structure and origin of the corpus luteum in certain of the Marsupialia (31), Prof. J. P. Hill directed my attention to the peculiar form assumed by this body in *Phascolarctos cinereus*, the Australian Native Bear. Circumstances prevented me from investigating the matter more fully at the time, and it had to be laid aside for a more suitable opportunity. In the course of that inquiry, also, a well-marked glandular-looking tissue was found to be present in the ovarian stroma of certain species, the origin of which was not clear from the material then at hand, and a more extended examination was to be desired. It has since been found that the mode of formation of the corpus in *Trichosurus vulpecula* differs slightly from that of any other marsupial examined. Lastly, since the above-mentioned investigation was carried out, Prof. Hill has collected additional marsupial material in South America, and it is now possible to study the corpus luteum in two species of the Didelphyidæ.

Much work has been done on the corpora lutea of the Eutheria, but it is not intended to review it in any detail here, although the work of certain authors must necessarily be referred to in connection with particular points. Very good records of the literature relating to the Eutheria are already available in contributions by Sobotta (45, 46, 47), Van der Stricht (48, 49), and Marshall (24, 25). It is now perhaps generally admitted that the corpus luteum in the

Eutherian mammal is a glandular structure whose cells are the greatly modified and hypertrophied cells of the membrana granulosa of the Graafian follicle, a view expressed by Bischoff in 1842 (6). This statement is equally accurate when applied to the corpus luteum of the Marsupialia.

Sandes (40), in 1903, was the first to investigate the corpus luteum (C. L. verum) in this sub-class of the Mammalia. The material he used was obtained from pregnant Native Cats, *Dasyurus viverrinus*, and having access to his slides I have been able to confirm his results. The corpus luteum of *D. viverrinus* in which ovulation had not been followed by pregnancy was examined by me in 1912 (32), and I found that "at no stage is it possible to distinguish between the corpus luteum of the non-pregnant female, i. e. corpus luteum spurium, and that of the pregnant female, i. e. corpus luteum verum."

Fraenkel, in 1905 (10, p. 465),¹ described an ovary of *Petrogale penicillata* in which two corpora lutea were present. He does not deal with them in detail, as he is mainly concerned with the interstitial tissue, but his description and figure agree closely with my own findings in this species. The condition of the more recent corpus corresponds with that I have found in animals with pouch young some time after birth, although Fraenkel does not record the presence of young in the pouch.

My own previous inquiry in 1914 (31) into the origin and structure of this body in certain species of the Marsupialia, already referred to above, was carried out on *Perameles obesula*, *P. nasuta*, *Macropus ruficollis*, *Petrogale penicillata*, and *Phascolomys mitchelli*. In the first, third, and last of these corpora lutea spuria were also examined. The general result, with which that of *Dasyurus* is in agreement, is that the corpus luteum in all these animals resembles in all essential points that of a Eutherian mammal. The corpus luteum is a very well-marked glandular structure

¹ This isolated reference to a corpus luteum in a marsupial was not known to me when my earlier papers were written.

containing two sorts of tissue, glandular and connective. The glandular lutein cells are derived from those of the membrana granulosa mainly by simple hypertrophy, but in two cases at any rate definite evidence of a multiplication of these cells by mitosis during the formative stages was forthcoming. The connective tissue is derived from both layers of the theca folliculi, the theca interna being almost completely used up. In all cases examined it was not possible to distinguish between corpora lutea vera and spuria.

I have to thank Prof. J. P. Hill for his generosity in giving me access to his valuable and extensive collection of Marsupial material and for his criticism and advice. My thanks are further due to Mr. F. Pittock, of this College, for assistance in the preparation of the photomicrographs on Plate 40.

MATERIAL.

The source of the material has already been indicated. The ovaries of *Didelphys marsupialis* were nearly all fixed in micro-nitro-osmic acid, those of the remaining species for the most part in micro-corrosive-acetic acid, but one or two in Fleming's fluid or corrosive-formol-acetic acid, all of which give very good fixation. Serial sections of the ovaries about 10μ thick were made and stained with Ehrlich's hæmatoxylin and counterstained with eosin. The sections cut for the previous investigations of Sandes (40) and myself (31, 32) were utilised for the purposes of comparison.

Phascolarctos cinereus.

Material employed.

Series.	Ovary.	Corpus luteum.	Remarks.
1	Part of ovary	1 small old corpus luteum	Some time after the birth of young and before the ripening of new follicles.
2	Sections already cut by Prof. J. P. Hill	1 large active corpus luteum	Nothing found in uteri. Probably not long after ovulation.

Phascolarctos cinereus—(continued).

Material employed—(continued).

Series.	Ovary.	Corpus luteum.	Remarks.
3	Part of ovary	1 large active corpus luteum, 9.25 mm. in diam.	Greatest length of embryo, 4.5 mm.
4	Ovary small, 11 × 16 mm.	1 very large corpus luteum at one end, 11 mm. in diam.	Greatest length of embryo, 7.5 mm.
5	Ovary irregular, 15.25 × 10.5 mm.	1 large corpus luteum at one end, 10 mm. in diam.	Greatest length of embryo, 9 mm.
6	Ovary, 13.5 × 10 mm.	1 large corpus luteum, 9.75 mm. in diam.	Greatest length of embryo, 11 mm.
7	Ovary, 12 × 8.5 mm.	1 active corpus luteum, 8.5 mm. in diam.	Greatest length of embryo, 12.25 mm.
8	Ovary, 13 × 10 mm.	1 large corpus luteum forming nearly whole of ovary, 10 mm. in diam.	Greatest length of embryo, 13 mm.
9	Ovary very large, 19.5 × 11.5 mm.	1 large corpus luteum at one end, 10 mm. in diam.	Moderately advanced uterine embryo, length not ascertained.
10	Ovary long, 15 × 7.5 mm.	1 active corpus luteum at one end, 7.5 mm. in diam.	Greatest length of embryo, 17 mm.
11	Ovary irregular, 16 × 9.5 mm.	1 active corpus luteum, not a large part of ovary, 8 mm. in diam.	Newly born young. Greatest length, 16.5 mm.
12	Ovary triangular, irregular, 17 × 14 × 13 mm.	1 active corpus luteum, not a large part of ovary, 8 mm. in diam.	Shortly after birth of young.

The ovary of *Phascolarctos* is quite a large body, in some cases reaching a length of more than 19 mm., and is more or less smooth. After ovulation it possesses a single active corpus luteum which forms a large part, sometimes almost the whole, of the ovary. In no case was more than one active corpus luteum found in the same ovary, so that only one follicle bursts at a time. In correlation with this it is to be noted that generally only one uterus becomes pregnant for in the records available there is no instance of a female

with both uteri pregnant. The corpus luteum is conspicuous throughout pregnancy, and the point of rupture of the follicle from which it was derived is visible for a long time. The ovary in a resting condition (series 1) still has present in it remains of a previously active corpus luteum, and in all of the remaining ovaries a similar structure is to be found. In spite of the fact that the corpus luteum in this animal probably persists for a long time, the presence of an old one at the same time as an active one seems to indicate beyond doubt that there is more than one ovulation period in the year. It is then unlike *Dasyurus* (16), and may be poly-estrous with several ovulations following one another, or, perhaps more probably, has two or more breeding periods in the year and the corpora lutea persist for a comparatively long time, an interpretation that is borne out by the fact that in some cases two old corpora are present. The same is supported by the finding of follicles beginning to ripen in the ovaries of Nos. 11 and 12 just after the birth of the young.

The Follicular Wall.—The structure of the Graafian follicle and its formation agree closely with that in *Dasyurus* and other marsupials. No quite ripe follicle was examined, although nearly ripe follicles are present in series 11 and 12. These are distinguishable not only by their size, but by the fact that the membrana granulosa is reduced to three or four cells deep and that no indication of mitoses is to be found in its cells, although mitotic figures are common in them during the earlier stages of the follicle. The ovum in its discus proligerus is situated to one side and the central cavity is filled with the coagulum of the liquor folliculi. The outer limit of the membrana granulosa is marked by the membrana propria, and this homogeneous basement membrane shows clearly at the points where the granulosa cells have shrunk away from it. The theca folliculi is well marked, and its cells readily distinguishable from those of the membrana granulosa. It is divisible into a theca interna of somewhat flattened polygonal cells and a theca externa of greatly

elongated cells which grades off into the surrounding ovarian stroma. Careful searching failed to reveal the inclusion of any interstitial cells in either layer of the theca.

The Formation of the Corpus Luteum.—Unfortunately no stage immediately after the rupture of the follicle was available, the earliest being No. 2 (Pl. 40, fig. 1) in which the corpus luteum is already definitely established. In this female the uteri were slightly enlarged, but nothing was found in them, a fact that may indicate that the animal was now pregnant or that the ova had been overlooked. It is young enough, however, to indicate clearly that the method of formation resembles that met with in *Perameles obesula*, *P. nasuta*, and in *Macropus ruficollis* (31). The connective tissue of the theca folliculi bursts through the membrana granulosa and quickly spreads out to form an internal layer surrounding the central cavity, the cavity itself being filled with a denser coagulum than that in the follicle (Pl. 40, fig. 2). This differs from the condition in *Dasyurus viverrinus* (40), where the connective tissue does not form an inner layer, but goes straight into the cavity and commences to form an aggregation in the centre.

Mitoses are present in this early corpus luteum, but in all cases they are distinctly in connective tissue cells and not in the lutein cells themselves. There is no evidence, therefore, that these cells multiply mitotically, although they may do so in an earlier stage. In the formation of the ingrowths both layers of the theca folliculi take part, and the inner one is almost entirely used up; it is possible that some of its cells may remain in their original position, but they are few in number. The theca cells are at all times totally unlike the cells of the membrana granulosa in situ or when they are transformed into lutein cells, and there is no indication that any of them are modified to form the latter. Accompanying the thecal irruptions, which sometimes run straight through to the internal connective tissue layer, are blood-vessels coming from those of the theca externa.

The lutein cells are derived entirely from the cells of the

membrana granulosa, which with their nuclei undergo enormous hypertrophy and soon begin to secrete actively.

The fully formed Corpus Luteum.—On the whole the stage of the corpus luteum corresponds to the stage of the embryo in the uterus, that is to say, the older corpora lutea are found in the animals with older embryos. No. 3 offers a notable exception to this, for, although the embryo is quite young, the corpus luteum is at a stage of development corresponding with that in an animal in which the young are born. This may indicate that ovulation is independent of copulation and consequent fertilisation, but the evidence is insufficient to enable one to assert that such is the case.

It has just been pointed out that in two species of *Perameles* and in *M. ruficollis* the corpus luteum is formed as a hollow structure, but it only remains so in the very early stages, and the central cavity entirely disappears soon after the embryo has acquired one or two somites. In *Phascolarctos cinereus* this hollow condition persists throughout the period of pregnancy, and, although the cavity gradually gets reduced in size, it is always present, so that a section of the corpus luteum presents a very striking appearance (Pl. 40, fig. 3). Even after the birth of the young, as, for example, in Nos. 11 and 12, the central cavity is still obvious enough, although the internal layer of connective tissue has become stellate instead of circular. Whether or not this hollow condition has any significance either functionally or phylogenetically it is not yet possible to say, but the fact remains that it produces a corpus luteum which, during the time that the embryo is in the uterus, is quite unlike the corpus in any other marsupial that has been examined. Indeed, as far as I am aware, its structure is unique, and has not been observed in any other mammal.

An interesting phenomenon calls for notice in connection with this form of the corpus luteum. Prof. J. P. Hill informs me that in all the genital tracts of marsupials that he has handled, some hundreds in number, it is only in *P. cinereus* that he has encountered cystic ovaries, and in this species

such a condition is by no means uncommon. Moreover, the cystic condition is not confined to the ovary; it may extend to the Fallopian tube and uterus. Whether the hollow corpora lutea favour the formation of cysts in the ovaries, or whether there is any relation between the two, is not clear, but the occurrence of cystic ovaries in this species far more frequently than in any other is certainly worthy of record.

An old corpus luteum in an ovary that also has an active one is readily distinguishable from the latter by the absence of the central cavity. In addition to this it is smaller in bulk, its lutein cells are smaller and far less active, and the theca folliculi is not so sharply marked off from the surrounding stroma. The material at my disposal does not show at what period the central cavity is lost, but it is certainly some time after the birth of the young.

There is also present in the ovary, besides the active corpus luteum and one or perhaps two old corpora, a fair amount of interstitial tissue scattered in moderate-sized masses throughout the stroma. This, however, will be more fully dealt with later, and here it is only necessary to call attention to its presence.

Trichosurus vulpecula.

Material employed.

Series.	Ovary.	Corpus luteum.	Remarks.
1	Ovary, 8 × 4.25 mm.	1 small corpus luteum, 3 × 2 mm.	These are from animals apparently not long after ovulation, that may or may not have been pregnant; but no records are available.
2	Large ovary, 10.5 × 6 mm.	1 small corpus luteum, 2.5 mm. in diam. Probably recently ruptured	
3	Large ovary, 9.5 × 6 mm.	1 corpus luteum, 4 × 3 mm.	
4	Ovary, 8 × 5 mm.	1 corpus luteum, 3 × 3 mm.	
5	Ovary, 9 × 6 mm.	1 corpus luteum, 4 × 3.5 mm.	
6	(a) Right ovary, 6 × 9 mm. (b) Left ovary, 9 × 5 mm.	1 old corpus luteum, 3 × 2.75 mm. 1 corpus luteum, 2.25 mm. in diam.	Left ovary not long after ovulation, but no record available.

Trichosurus vulpecula—(continued).

Material employed—(continued).

	Ovary.	Corpus luteum.	Remarks.
7	Ovary, 8 × 5 mm.	1 corpus luteum, 4 × 2.5 mm.	Early ovum.
8	Ovary, 7.5 × 4.75 mm.	1 corpus luteum, 4 × 2.25 mm.	Small blastodermic vesicle, 1.6 × 1.4 mm.
9	Small ovary, 6.75 × 6 mm.	1 corpus luteum, 3.25 × 3 mm.	Blastodermic vesicle, 1.8 mm. in diameter.
10	Ovary, 7 × 5 mm.	1 corpus luteum, 4.75 × 5.25 mm.	Blastodermic vesicle, 6 mm. diam.
11	Small ovary, 6 × 5 mm.	1 large corpus luteum, 6 × 4.5 mm.	Larger blastodermic vesicle.
12	Ovary, 7.25 × 6 mm.	1 large corpus luteum, 7 × 4 mm. Top widely expanded	Slightly older than 11 stage γ .
13	(a) Right ovary, 6.5 × 6 mm. (b) Left ovary, 8 × 7.5 mm.	1 large corpus luteum, 5.75 × 6.25 mm. 1 old corpus luteum, 2.75 diam.	} Greatest length of embryo, 11.5 mm.
14	Ovary, 7 × 4.5 mm.	1 corpus luteum, 4 × 2.25 mm.	

Trichosurus vulpecula is a smaller animal than *Phascolarctos* and the ovary is correspondingly smaller, the largest measuring 10.5 × 6 mm. In this, as in the previous animal, there was never more than one active corpus luteum in the ovary, showing that only one follicle bursts at a time. Also only one uterus was pregnant in each animal. Furthermore, where the two ovaries from the same female were examined, it was seen that, although each contained a corpus luteum, they were not of the same age. That on the side of the pregnant uterus was an active corpus, while that in the other was older. Often in the same ovary with the active yellow body was another still older than that of the opposite ovary. These facts seem to indicate clearly that the ovaries ovulate alternately and, further, that one ovulation period follows another after a moderately short interval. The female then has undoubtedly more than one œstral period in a year, a point that is further indicated by the

fact that the ovaries with older corpora lutea usually contain several follicles approaching maturity.

The Follicular Wall.—As before, no absolutely ripe follicle was examined, but in Nos. 6, 8, and 9 well-advanced follicles were obtained. The description already given for *Phascolarctos* will apply equally well to *Trichosurus*. The main and perhaps only noticeable difference between the two species is that of size, and the structure of the follicular wall and its component cells is the same in the two cases.

The Formation of the Corpus Luteum.—No example shows the follicle immediately after the expulsion of the ovum, though several exhibit very early stages in the formation of the corpora lutea. Nos. 1–5 are all early stages, i. e. soon after ovulation, as is shown by the small size of the corpora lutea and also by the fact that in none of them are the other follicles nearly ripe. Nothing was found in the slightly enlarged uteri of these animals, so that here again we are dealing with females that were either not pregnant, or, more probably, pregnant and the ova overlooked on account of their small size, a very likely occurrence when the fixation is done in the field.

In No. 2 (Pl. 40, fig. 4), the corpus luteum is quite young, as the transforming cells of the membrana granulosa are not much enlarged and are only five or six cells deep. It indicates that the rupture of the follicle is entirely different from that of all the marsupials previously described. In all the others the rupture takes place at a definite point, which is almost immediately closed by a plug of cells (Bouchon épithélial), so that immediately after the egg has been discharged there is still a large central cavity, though it is probably somewhat smaller than that of the ripe follicle. The connective tissue of the theca then bursts in at a number of places and runs in strands more or less radially towards the centre, afterwards forming a network through the lutein cells. The follicle in *Trichosurus vulpecula*, however, when it bursts, collapses altogether, and its walls are brought together so that the central cavity is entirely obliterated

(Pl. 40, fig. 5), save for isolated portions that may remain here and there closed in by folds of the *membrana granulosa*. Remains of the hollow shut in in this way are very well displayed in No. 11. At the same time the theca is, of course, drawn in by the collapsing follicular walls, and in consequence large irregular masses of connective tissue are to be found in between the forming luteal cells. In some places it appears to burst right through the *membrana granulosa* and help form a plug of connective tissue and *membrana cells*, which closes the actual point of rupture. The result is that an extremely irregular structure is formed (Pl. 40, fig. 6). Mitoses were not found in the young luteal cells in any of the early stages. The connective tissue ingrowths, as in other species, are derived from both layers of the theca folliculi, but the folding of the wall of the follicle consequent upon its collapse brings in some of the connective tissue of the ovarian stroma that would be considered outside the actual theca externa. In spite of this no evidence of the inclusion of interstitial cells was obtained.

A similar method of formation of the corpus luteum in which the follicle collapses on rupture is described by Sobotta (44) in the mouse, but does not appear to be common even in the Eutheria.

The fully formed Corpus Luteum.—The corpus luteum from the stage just described increases enormously in size and reaches its maximum shortly after the embryo has passed through the blastodermic vesicle stage, i. e. in Nos. 11 and 12, in the latter of which it measures 7×4 mm. The fully grown corpus luteum is much more irregular than in the other marsupials so far examined, and is further unlike them in not possessing a central plug of connective tissue (Pl. 40, figs. 5, 6, and 7). The very large bands of connective tissue cut the body up into a number of parts, and there is not the slightest suggestion of a radial arrangement of the main strands of the connective tissue. Although so irregular it is more or less bound together by a fibrous sheath to form one whole body. Owing to the way in

which the follicle bursts it sometimes happens, as in Nos. 6 (Pl. 40, fig. 6) and 12 (Pl. 40, fig. 7), that it is partly inverted and that the lutein cells become pushed out and a mushroom-shaped corpus luteum is produced. These cells, however, are quickly covered by a layer of connective tissue. Such cases are interesting from the light they throw on the origin of the lutein cells. Here, if anywhere, among the Marsupialia was an opportunity for shedding the membrana granulosa, but not only is this not done, but the membrana cells still remain adherent to the theca folliculi even in the inverted portion of the lips of the follicle. Some of these cells are very probably lost, but by far the larger number remain and give rise to lutein cells. The marsupial ovary offers marked advantages for the observation of these changes, for, as has already been pointed out, there is always a marked difference between the cells of the theca interna and those of the membrana granulosa. There is no doubt whatever that throughout this group the theca interna plays no part in the production of lutein cells, these being, on the contrary, all derived from the membrana granulosa. It is necessary to insist on this point, as several fairly recent writers, for example, Hegar (15), Pottet (35), and Delestre (9), in certain Eutherian mammals support the theory of von Baer (4), who maintained that the lutein cells originate solely from the theca interna, while the membrana granulosa cells were either discharged with the ovum or degenerated immediately afterwards. It does not seem probable that the corpus luteum, which is practically identical in the two groups, should arise in such different ways, and, moreover, a number of careful observers, including Sobotta (44-47), Van der Stricht (48, 49), Loeb (22), and Marshall (23), have maintained that in the Eutheria the lutein cells come from the membrana granulosa. The only feasible explanations seem to be that the accounts given by these supporters of von Baer rest upon faulty technique, an incomplete series of stages, or misinterpretation of the sections.

Didelphys aurita.

Material employed.

Serial No.	Ovary.	Corpus luteum.	Remarks.
1	Small, 7 × 6 mm.	Points of rupture still visible	Unsegmented eggs.
2	Small, 8 × 5 mm.	Corpora lutea small	Segmentation stage; 2- and 3-celled.
3	Small, 8 × 6 mm.	Corpora lutea small	Segmentation stage.
4	Small, 8 × 5 mm.	Corpora lutea small	Segmentation stage.
5	Larger, irregular, 10 × 6 mm.	Corpora lutea large	Eggs in a late segmentation stage, but abnormal.
6	Irregular, 9 × 8 mm.	Corpora lutea large	Blastodermic vesicles, .86-1.1 mm. in diam.
7	Irregular, 9 × 7 mm.	Corpora lutea large	Blastodermic vesicles, 1.3-1.5 mm. in diam.
8	Small, 7 × 5 mm.	Corpora lutea not visible externally	Pouch young.

This animal differs considerably from the foregoing two and agrees with *Dasyurus* in that a large number of eggs, 12-14 (in one case 19), may be discharged simultaneously from each ovary. Selenka (43, p. 104) states that normally the opossum *D. marsupialis* breeds only once in the year, but in certain cases may experience œstrus again at the latest at the beginning of June. In Brazil, however, Prof. J. P. Hill found that there were at least two breeding seasons in *D. aurita*, pregnant females being obtained in July and again in October, so that either this is a point of difference between the species or Selenka's statement may require modification.

D. aurita, therefore, may be polyœstrous, or, more probably, it may have several breeding seasons in the year. The corpora lutea persist throughout pregnancy, but not long afterwards, and in no case where active corpora lutea are present are corpora fibrosa to be found. In the example, with fairly well-grown pouch young, the corpora lutea have

already nearly disappeared. The ovary resembles very closely that of *Dasyurus* in appearance, and is of moderate size, the largest examined measuring 9×8 mm.

The Follicular Wall.—Fairly ripe follicles are present in No. 1, in which the structure of the walls can be readily made out. The basement membrane is well marked, and the theca folliculi differentiated into an external and internal layer. The theca interna is not so clearly marked off from the outer layer as in the macropods, and is similar to that in *Dasyurus viverrinus*, and both layers are readily distinguishable from the membrana granulosa cells. The latter form a layer three or four cells deep on the inner side of the basement membrane.

The Formation of the Corpus Luteum.—This series is fairly rich in early stages, for no less than four animals yielded eggs in various stages of segmentation before the formation of a blastodermic vesicle. The membrana granulosa is not thrown off when the follicle bursts, though doubtless a little of it is lost in the very superficially situated follicles, and the point of rupture is soon closed. This has already occurred in the earliest example (No. 1), where the membrana granulosa cells have started to enlarge and are arranged around the young corpus luteum 5–9 deep. On ovulation the basement membrane is broken through in a number of places by the thecal ingrowths and their accompanying blood-vessels. They do not burst through the membrana granulosa until later, but instead they push the transforming membrana in front of them, and consequently this appears plicated in section (Pl. 40, fig. 9). The central cavity thus assumes a stellate form, which, however, is not to be confused with the stellate plug of connective tissue that is to be found in other species, e.g. *Phascolomys mitchelli* (31). It is still the central cavity and contains only a coagulum, the remains of the liquor folliculi, and perhaps one or two blood-corpuscles and phagocytes let in as a result of the rupture. This cavity is still lined with the membrana granulosa cells in course of transformation

into lutein elements, and not by a layer of connective tissue as in the case of *Phaseolaretos cinereus*, *Perameles*, and certain macropods (31). No. 1 in the series is further interesting, as mitoses are to be found in the transforming membrana cells, thus indicating clearly that in the very early stages these cells multiply by mitosis. Evidence of a similar multiplication by karyokinesis has been brought forward in the case of *Perameles obesula* and *P. nasuta* (31). The later stages of *Trichosurus* do not show these mitotic figures in the lutein cells, and as they are never plentiful even in the early stages, it would appear that although hypertrophying young lutein cells do multiply, it is not to a very great extent.

The later formation stages to be found in Nos. 2, 3, and 4 show that the enlargement of the lutein cells gradually reduces the central cavity (Pl. 40, fig. 10), and it is not until it is almost obliterated that the connective tissue sprouts break through and form the central plug (Pl. 40, fig. 11). It is to be noted that where the follicles are situated right on the periphery of the ovary the rupture is followed by a complete collapse of the follicle, and consequently the central cavity disappears right from the beginning. This occurs normally in *T. vulpec*, as just described.

The fully formed Corpus Luteum.—The fully formed corpus luteum which is found in Nos. 5 (Pl. 40, fig. 12) and 6 calls for little comment, as it is typical in all respects, and it resembles most nearly those of *Dasyurus* (40) and *Perameles*. The connective tissue in it is very well developed and ramifies throughout the entire corpus, accompanied by small blood-vessels. The large number of follicles bursting at the same time leads to the formation of a corresponding number of corpora lutea, and when they are fully grown the ovary is mainly composed of these glandular bodies (Pl. 40, fig. 8), the stroma with the remaining young and primordial follicles being reduced to a minimum.

The old Corpus Luteum.—No. 8 had young some weeks after birth, and in the ovaries traces of old corpora

lutea were to be seen. They were much reduced in size, and the cytoplasm and nuclei of the individual cells were in an advanced stage of degeneration. Phagocytes are plentiful and appear to be concerned with the removal of the old lutein cells much in the same way as Sandes (40) has described in *D. viverrinus*. The connective tissue sheath of the corpus is very indistinct and hardly to be distinguished from the surrounding stroma. This animal was taken in the earlier breeding season of the year, and there is no doubt that the corpora lutea would have completely disappeared before the onset of the next. That this would not occur immediately is indicated by the development of the follicles which are not yet ripe.

Material from Further Species.

In connection with the question of interstitial tissue, to be dealt with shortly, a number of other ovaries of different species were cut and examined, but in no animal were early stages in the corpus luteum formation obtained. All the ovaries possessed corpora lutea vera, but as they do not offer any special points of interest they may be dealt with quite briefly.

Metachirus nudicaudatus.—The ovaries were small, squarish, and fairly smooth, measuring 6×5 mm. Early blastodermic vesicles were present in the uteri. The corpora lutea, of which there were several in each ovary, were fully formed, of small size, and their blood-vessels well distended with blood. In transverse section the ovary is very similar in appearance to that of *D. aurita* and *Dasyurus viverrinus*. No interstitial tissue was found.

Macropus ualabatus.—This female was in the later stages of pregnancy. The ovaries were large, 11.25×6 mm., and contained a single corpus luteum 6 mm. in diameter situated at one end and clearly marked off from the remainder of the ovary. In transverse section the ovary and corpus luteum were similar to that of macropods previously described,

and a fair amount of interstitial tissue is scattered in small islands throughout the stroma. The lutein cells were full of droplets of fatty secretion, dissolved out in preparing the sections, indicating that the gland was secreting actively.

Macropus dorsalis.—The animal was killed shortly after parturition. The ovary contains one large active corpus luteum and two older ones (Pl. 40, fig. 13). A fair amount of interstitial tissue is present, differing in appearance from both the active and old corpora lutea and situated in one part of the ovary, although split up into a number of smaller separate masses. The whole ovary is similar to that of the other macropods and measures 10×6.25 mm. The active corpus is situated towards one end and is 6.25 mm. in diameter.

Macropus thetididis.—The ovary of this pregnant female was smallish, 7×4.5 mm., and the major portion of it is occupied by two large fully grown corpora lutea and a small amount of interstitial tissue. The general appearance is that of a typical macropod ovary.

Macropus parma.—Here again we are dealing with the ovary of a typical macropod. It is irregular, 8.5×6.5 c.c., and its bulk is mainly taken up by a single prominent corpus luteum and a large amount of interstitial tissue. The uteri contained embryos in the blastocyst stage, but the corpus luteum appears to be fully hypertrophied.

Onychogale frenata. (The species is somewhat doubtful. It is undoubtedly a macropod and probably *O. frenata*.)—The ovary was large, smooth, and roundish, and measured 10×10 mm. It contained two corpora lutea, one apparently slightly younger than the other, but both fully formed. Although the embryos were in an early blastodermic vesicle stage, the corpora appear about fully grown. A little interstitial tissue is present in the form of small scattered groups of cells, and the transverse section of the whole ovary is very similar to that of *M. ualabatus*.

THE CORPUS LUTEUM IN THE MARSUPIALIA.

A certain number of questions have arisen in regard to the structure and histogenesis of the corpus luteum in the Eutheria: whether the lutein cells are derived from the cells of the membrana granulosa or of the theca interna wholly or in part; whether these cells increase by mitotic or amitotic division; what are the precise parts played by the theca interna and theca externa in the formation of the connective tissue network; whether any difference exists between the corpus luteum in the pregnant and non-pregnant animal, and so on. It is not my intention to enter into a discussion of the evidence that has been adduced on both sides in these matters, as that has already been dealt with briefly elsewhere (31), and subsequent investigations have only confirmed the conclusions set forth in that review. Now, however, when a fairly representative collection of ovaries has been examined, it does seem advisable to review briefly the main characteristics of the corpus luteum in the Marsupialia. This may be done the more readily as all the fifteen species so far described have been cut and examined by myself, and moreover, the sections cut by Sandes for his work on the pregnant *Dasyurus viverrinus* are in the possession of Prof. J. P. Hill, who has kindly handed them to me for examination. It is a distinct advantage for the purposes of comparison that almost without exception the material was very well fixed and preserved, and that a fairly uniform technique in cutting and staining has been adopted throughout. In all cases, too, serial sections have been employed. Perhaps the first point to which attention should be directed is the striking similarity between the corpus luteum of the Marsupial and the Eutherian. Indeed, I know of no criterion whereby the structures in the two classes can be distinguished from one another. The statement of Fraenkel and Cohn (11) given in the introduction, and repeated without criticism by Van der Stricht ('Archiv. de Biol.,' t. xxvii, 1912, p. 586), is therefore without foundation in fact, for the marsupial ovary, if examined at the right

time, always possesses a well-marked corpus luteum. Minor differences in appearance are to be found in various species as they are in the Eutheria, and although the corpus in *Phascolarctos cinereus* is different from that in any higher mammal yet described, it also differs in the same way from that in any other marsupial. Generally, then, the histological structure and appearance of the gland, and also its size relative to the follicle or ovary, as a whole is the same in the two groups.

The Graafian Follicle.—The follicle is very similar throughout, and when ripe or nearly ripe consists of a large central cavity filled with liquor folliculi and surrounded by the membrana granulosa, to one point of which is attached the ovum in a discus proligerus. The membrana granulosa is composed of small polygonal cells from three to five deep, with spherical nuclei and moderately clear cytoplasm, and in which mitotic figures are uniformly absent. Its outer limit is marked by a clear homogeneous membrane—the membrana propria—and the whole is similar to the same structures in the Eutheria. The theca folliculi is, on the whole, perhaps, not quite so distinct as in the higher mammals, but always divisible into internal and external layers. The theca interna varies slightly in its development in different species, and it is best marked in *Phascolomys wombat*, where it is composed of longish polygonal cells three or four deep lying immediately outside the membrana propria. Its development in this species most closely approaches that met with in the Eutheria, but it is relatively thinner and its cells more elongated. A very similar condition obtains in *Phascolarctos cinereus*. The macropods and *Trichosurus vulpecula* do not possess such a well-marked theca interna, and it is still less developed in the two species of *Perameles* in *Metachirus nudicaudatus* and *Didelphys aurita*. The extreme is met with in *Dasyurus maculatus* and *viverrinus*, where, although easily recognisable in a young follicle, this layer is almost indistinguishable from the theca externa in the ripe follicle. It is further important to notice

that even in those species where it reaches its highest state of development the cells of the theca interna are very different from those of the membrana granulosa, so that the subsequent history of the two layers can be followed with more certainty than in the Eutheria. No evidence of the inclusion of interstitial cells was forthcoming in any case. The theca externa calls for no comment, as its fibrous cells are similar in the different species and again in the higher mammals.

The Rupture of the Follicle.—With one exception, *Trichosurus vulpecula*, when the follicle bursts it discharges the ovum and almost immediately closes again, either by the formation of a sort of plug of connective tissue and lutein cells (Bouchon épithélial) or by the coming together of its walls. In *Trichosurus*, as just described, the follicle completely collapses, a phenomenon recorded also in the mouse. The very superficially situated follicles in *Didelphys aurita* collapse in a similar manner, but not so irregularly. The membrana granulosa is always retained at ovulation, although it is possible that a few of its cells may be lost at the actual point of rupture.

The Formation of the Corpus Luteum.—In most species, immediately after ovulation the theca folliculi, accompanied by blood-vessels, breaks through the membrana propria and then through the membrana granulosa. Both theca interna and externa take part in these irruptions, and the former is almost completely used up in the process. In *Perameles obesula* and *nasuta*, *Macropus ruficollis* and *P. cinereus*, the irruptions quickly spread out on the inner side of the membrana granulosa to make a sort of inner connective tissue sheath. This does not happen in *D. viverrinus*, *D. aurita*, and *T. vulpecula*. The connective tissue sprouts in the first of these break through and form a loose central plug of tissue; the same occurs in the second, though in this case the membrana granulosa remains intact for a longer time, its transforming cells are pushed inwards by the ingrowths. The collapse of the follicle in the third of these species is so complete that the irruptions break

through in a very irregular manner and are of such size that they may even carry some of the surrounding fibrous tissue with them. The cells of the thecal ingrowths multiply by mitosis in the early stages.

Shortly after the rupture, the cells of the membrana granulosa and their nuclei commence to hypertrophy and to undergo modification into lutein cells. Definite evidence of mitotic division of these cells has been obtained in *P. obesula* and *nasuta*, and also *D. aurita*. In the opinion of the observer the multiplication of the membrana granulosa cells is not of great extent and confined to the very early stages, and this accounts for the fact that mitotic figures have not been observed in other species.

The fully formed Corpus Luteum.—The corpus luteum is formed when the embryo is in the early blastodermic vesicle stage, and reaches its maximum size a little later. It is a solid body, except in *P. cinereus*, where it is hollow, composed of a mass of lutein cells surrounding a central plug of connective tissue. Between the cells ramifies a network of the same tissue accompanied by blood-vessels serving for nutriment and perhaps support. The central plug is absent in the corpus of *T. vulpecula*, which presents a much less regular appearance in section than any of the others. The whole structure is separated from the ovarian stroma by a fibrous sheath, the remains of the theca externa. The lutein cells are derived entirely from the cells of the membrana granulosa, and, although similar throughout, are slightly less compressed and have slightly clearer nuclei in *D. viverrinus*, *P. obesula* and *nasuta*, *D. aurita* and *M. nudicaudatus*, than in the remaining species. The connective tissue network is derived from both layers of the theca folliculi, and ramifies through the cells slightly more intimately in the just-mentioned species than in the others. The result is that the corpora lutea in these species resemble one another more closely than they do the corpora of the other species, but the difference is only small. It is interesting to note that the ovary and corpus luteum of the South American form *D. aurita* most

closely approach those of the Australian *D. viverrinus*, and thus confirm the near relationship between them that is indicated by other anatomical and developmental features.

The *Corpus Luteum Spurium*.—Ovaries of non-pregnant females containing corpora lutea have been examined in *D. viverrinus*, *P. obesula*, *P. mitchelli*, and *Petrogale penicillata*, and in each case they were found to be identical in size, structure, and appearance with corpora lutea vera. Further, in the first two species their mode of formation was also studied and found to be the same as in the pregnant female. The formation and structure of the corpus luteum then, is not influenced by the subsequent fate of the ovum, that is whether it is fertilised or not, and in the case of *D. viverrinus* the duration of the two bodies was found to be about the same (32).

THE INTERSTITIAL TISSUE.

In both *P. cinereus* and *T. vulpecula*, described above, there is present in the ovary a quantity of tissue embedded in the stroma that is not to be found in *D. aurita*. It is particularly plentiful in the first-mentioned species. Similar tissue was found in certain other marsupials previously examined, about some of which it was stated: "Die Ovarien . . . enthalten in ihrem Stroma eine mässig grosse oder auch grosse Menge eines Gewebes, welches dem Gewebe der interstitiellen Drüse bei den Ovarien einiger höherer Säugetiere gleicht" (31, p. 28). At that time it was not possible to say more, owing to insufficiency of material for comparison, and so it is proposed to enter a little more fully into the matter here.

These interstitial cells have received a great deal of attention from various authors, and good reviews of their work are to be found in the papers of v. d. Stricht (49) and Schaeffer (41). But, so far as I am aware, only four authors, Benthin (5), Fraenkel (10), V. d. Broek (7a), and Schaeffer (loc. cit.), have examined this tissue in the mar-

supial ovary, and their results will be discussed later. Interstitial tissue is not universally present in the mammalian ovary; a number of species do not possess any trace of it, but it is very widespread throughout the entire class, and is often present in large quantities. Various theories as to its origin have been put forward, but no general agreement has yet been arrived at.

Paladino (33) attributes an epithelial origin to these cells, and this was also maintained by certain earlier authors, Nussbaum (27), Harz (14), etc., but the evidence in support of this view is unsatisfactory. Lane-Clayton (19, 20) describes them as originating in the rabbit as sister cells of the ova, and, therefore, to be regarded as potential ova. This view has not met with very much support, and in the very young marsupial their distribution and histological appearance are entirely against such an interpretation. They lie more deeply than the superficially situated primordial ova, and do not in the least resemble them. Schrön (42) derives them from the lutein elements of old corpora lutea, but it will be readily seen (Pl. 40, fig. 13) that there is a well-marked difference between the cells of an old corpus luteum and interstitial cells in marsupials. Rabl (36) sees in them modified elements of the theca interna and of atresic follicles, but among the marsupials the theca interna cells are not so well developed as in higher mammals, and do not resemble interstitial cells. Benthin (5) thinks they are derived from the conjunctive cells of the ovary, but only after these elements have been differentiated into atresic follicles (*faux corps jaunes*); this is also the view of Cohn (8) and Limon (21).

A number of recent observers—Sainmont (39), Regaud et Policard (38), Athias (3), etc.—maintain that the interstitial cells originate by the transformation of conjunctive cells. Popoff (34) admits this in the dog, but in the mole and weasel thinks they come from the theca interna cells after these have formed an atresic follicle. Another very prevalent opinion is that held by Kingsbury (18) and Van der

Stricht (49), who regard the interstitial tissue as derived in the first place from stroma cells, since it is to be found before the formation of follicles, and consider that it is later added to by cells derived from atresic follicles. Limon (*loc. cit.*) considers that when once these cells are formed they do not undergo any alteration. Regaud and Dubreuil (37) point out that this would lead to an enormous increase of the tissue, and conclude that it is necessary for the old cells to be removed and consequently recognise stages in their degeneration.

Turning now to the marsupials, we find that only one follicle appears to burst at a time in *P. cinereus*, but it is possible to find two or sometimes even three corpora lutea in the ovary at the same time, one being active and the others old. It seems legitimate to assume from this that they represent the corpora formed at the one or two preceding ovulations. The corpus of the immediately preceding ovulation is often more eosinophile than the active gland, but the still older one is less so. The older ones, too, are not so sharply marked off from the surrounding stroma by a connective tissue theca. They appear to be invaded by the stroma and cut up into pieces. This is marked in *P. cinereus*, and care is necessary to distinguish a gland in this state from a collection of groups of interstitial cells, but there is always a difference between them. A somewhat similar condition obtains in *P. mitchelli*, but the old corpora lutea do not become so cut up, and always appear to retain a connective tissue sheath and to undergo complete degeneration therein. In the remaining species where old corpora lutea were examined, among them *T. vulpecula*, it is almost certain that the old lutein cells degenerate *in situ* and that their remains are removed by phagocytosis, as described by Sandes in *Dasyurus viverrinus* (40). The evidence, then, is strongly against the assumption that lutein cells are transformed into interstitial cells.

In the one or two cases in *P. cinereus*, *P. mitchelli*, and *T. vulpecula* where atresic follicles are present the

cells are hypertrophied, and to a certain extent resemble interstitial cells, but I have no undoubted indication of any modification from one to the other, and the two can always be distinguished. As pointed out above, Limon states that the interstitial cells, after they are formed, persist and undergo no noticeable alteration, and this certainly seems to be true in the marsupials. Certain objections to the acceptance of this fairly widely advocated theory of the transformation of the cells of the atresic follicle are obvious. In the first place, it would be extremely difficult to bring forward conclusive evidence of such a change; the evidence so far adduced is far from convincing, and my own observations lead me to think that it does not occur in the Marsupialia. Secondly, if the interstitial tissue were added to indefinitely in this way, there would be an enormous difference between the ovary of the young and old mature female, which is not the case, and a striking increase in the size of the ovary with age. Regaud and Dubreuil (*loc. cit.*) see this and say that therefore there must be degeneration in the interstitial cells. Sainmont (39) actually goes so far as to distinguish degeneration stages. But surely if there is interstitial tissue to start with, and the atresic follicle is a product of the degeneration of the Graafian follicle, it is more natural to suppose that these degeneration stages are simply the end of the breakdown of the follicle, and that the true interstitial cells are untouched. In other words, a more straightforward explanation of these phenomena seems to be that during atresia the cells of the follicle pass through a stage in which they resemble interstitial cells.

Sections of the ovaries of two specimens of pouch young of *T. vulpecula* were examined, and it was found that even in the young still attached to the teats interstitial cells were present. The ova and follicles were in very early stages of development, in many cases the follicle was not formed around the ovum, and in no case was the theca folliculi formed. A number of groups of polygonal interstitial cells in a non-active condition were found situated in

the inner part of the ovary. These could not possibly be regarded as coming from an old corpus luteum or an atresic follicle, for neither structures could yet have been formed, nor were they derived from a theca interna, as this had not yet been laid down.

A similar observation has been made by v. d. Broek (7a) on a pouch-young of *Sminthopsis crassicaudatus*, 25 mm. in length, in which interstitial tissue was present, and he concludes: "So meine ich, dass die Entwicklung dieser Drüse, bei Beutlern ohne jeden Zusammenhang mit dem Alter der Tiere ist, auch nichts mit der Bildung von Corpora lutea und atretischen Follikeln zu thun hat"; a conclusion in entire agreement with my own, although v. d. Broek's paper was not known to me when I arrived at it. A number of authors, among others Van der Stricht (49), Allen (2), and Aimé (1), describe these cells in the ovaries of embryos of various species of mammals.

The interstitial tissue, then, is undoubtedly present at such an early stage that it must have originated in the stroma of the ovary independently of the theca or its derivatives, whether corpora lutea or atresic follicles, and is to be regarded as a tissue sui generis, perhaps derived from the stroma cells. In the Marsupialia I have not found anything to indicate that it is added to by cells from the theca interna, degenerating corpora lutea, or atresic follicles; indeed, such evidence as is forthcoming is against any of these interpretations. The tissue persists without degenerating and undergoes slight hypertrophy during the periods of ovarian activity.

Interstitial cells are always easily distinguishable from ordinary stroma cells (Pl. 40, fig. 13). They are polygonal instead of elongated and fibrous, and possess a typical vesicular nucleus with a nucleolus. The chromatin is more scattered than in the long nuclei of the cells of the stroma. When stained their nuclei are much lighter in colour and their cytoplasm takes eosin more readily. If in small numbers they appear, under a low magnification, as pale reddish

islands scattered in the general body of the stroma, and when present in large quantities they form the bulk of the substance of the ovary through which the stroma cells pass in interlacing strands.

This interstitial tissue takes part in the general ovarian activity during pregnancy, or, rather, subsequent to ovulation. The individual cells and their nuclei undergo hypertrophy, although they never become so large as the cells of the corpora lutea. This enlargement is very similar to that described by Lane-Clayton (19) in the rabbit, and the masses of active interstitial cells in the marsupial ovary are very similar in appearance to those in the rabbit. I have been unable to obtain the slightest evidence to show that in any marsupial certain of these cells situated near the periphery pass through a form of ovogenesis and become true ova, as is stated to occur in the rabbit (Lane-Clayton, *loc. cit.*, p. 55). On the contrary, it is always perfectly easy to distinguish them from the oocytes at all stages, and careful searching has failed to reveal any cells that might be interpreted as intermediate stages.

When fully hypertrophied the interstitial masses present the appearance of typical epitheloid glandular tissue in a state of activity. Small chromagen granules are scattered through them, and their fatty or lipid globules are dissolved out by the xylol so that they resemble lutein cells in appearance. They are plentifully supplied with blood-capillaries, which ramify through them in all directions. It seems beyond dispute that they are to be regarded as masses of secretory tissue. Their maximum enlargement is reached about the time that the corpus luteum becomes fully formed. Although they somewhat resemble the latter in transverse section, the difference between the two tissues becomes obvious when a strip of interstitial tissue comes to lie quite close to an active corpus luteum. In a similar manner the difference between interstitial cells and those of a degenerating corpus may also be seen at a glance (Pl. 40, fig. 13).

It has been noted in the rabbit that interstitial cells appear

to remain active for a longer time than those of the corpus luteum, and are in a fully active condition long after the birth of the young, when the corpus luteum has begun to decline. This statement appears to hold good also for those marsupials in which the tissue is present.

A very remarkable fact, that had not previously been noticed, came to light when making a list of the marsupial ovaries from the point of view of the quality and quantity of interstitial tissue they contained. It was found that they could be divided into two groups, according to whether this tissue was present or absent, as follows:

Interstitial tissue present.	Interstitial tissue absent.
1. <i>Macropus ualabatus</i> .	1. <i>Perameles obesula</i> .
2. <i>Macropus ruficollis</i> .	2. <i>Perameles nasuta</i> .
3. <i>Macropus dorsalis</i> .	3. <i>Dasyurus maculatus</i> .
4. <i>Macropus thetididis</i> .	4. <i>Dasyurus viverrinus</i> .
5. <i>Macropus parma</i> .	5. <i>Didelphys aurita</i> .
6. <i>Petrogale penicillata</i> .	6. <i>Metachirus nudicaudatus</i> .
7. <i>Onychogale frenata</i> (?).	
8. <i>Trichosurus vulpecula</i> .	
9. <i>Phascolarctos cinereus</i> .	
10. <i>Phaseolomys mitchelli</i> .	

It will be seen at once that this grouping corresponds with the two main divisions of the Marsupialia, namely, the group Polyprotodontia and the group Diprotodontia. In the former the interstitial tissue is absent and in the latter it is present.

Four other authors have recently examined single ovaries from certain species of marsupials. Benthin (5) describes very briefly the ovaries of *Pseudochirus* (sp.?) and *Phalanger orientalis*. The latter ovary is admittedly badly preserved, and in neither case, apparently, was attention paid to the interstitial tissue, as the investigator was concerned almost entirely with follicular atresia.

v. d. Broek (7a), in a work on the development of the urogenital system of marsupials, states that he has found an "interstitial gland" in the ovaries of adult females of

Petrogale penicillata, which agrees with my own results, in a young female of *Halmaturus* (*Macropus*) *Derbianus*, and in a pouch-young of *Sminthopsis crassicaudatus*, 25 mm. long. All three species belong to the group *Diprotodontia*.

Schaeffer (41) investigated in particular the interstitial "gland" in a large series of fifty mammals, including the marsupial *Macropus melanops* (sic! *M. giganteus* var. *melanops*) and *Petrogale penicillata*. Of the last it is stated that it possesses "eine typische interstitielle Eierstocksdrüse," a finding quite in agreement with my observations. In regard to *M. melanops* it is stated that "Eine glande interstitielle ist demnach nicht vorhanden." This positive assertion appears to be open to grave doubt, for in all the other *Macropodidæ* examined, not only is interstitial tissue present, but a large quantity of it is to be found. Moreover, the author himself describes in this very ovary a tissue of which he writes: "Er besteht aus kleineren zellgruppen, die durch zahlreiche Kapillaren von einander getrennt werden. Die Zellen sind polygonal, messen $10\ \mu$ in Durchmesser, die Kerne sind $8\ \mu$ gross mit einem Kernkörperchen." This, however, is a sufficiently accurate description of the interstitial tissue in a non-active condition and, at any rate, does not apply to the ordinary stroma cells. The author admits that it is "drusenähnlich," but states that it cannot be considered as interstitial because it occurs near the hilus and is marked off from the tissue containing the egg-cells. This certainly does not appear to be a sufficient reason for not regarding it as interstitial tissue, which may occur in any part of the ovary, and I am of the opinion that it is better to regard it as interstitial tissue until it can be shown to be something different, and that apparently unique in the marsupial ovary. The ovary used was preserved in 5 per cent. formol, a fluid that gives very bad fixation of ovarian tissue.

Fraenkel (10) examined a series of forty-five mammals, six of which were marsupials. Three of these, i. e. *Halmaturus* (*Macropus*) *thetidis*, *Petrogale penicillata*, and

Onychogale frenata, I have also examined, and our results are in agreement: interstitial tissue is present in the ovary. Another species, *Phascologomys latifrons*, is closely allied to *P. mitchelli*, and in this too he reports the presence of interstitial tissue. In the remaining two species, namely, *Halmaturus (Macropus) giganteus* and *Macropus* sp., he states that such tissue is absent, but in both these cases his evidence is quite unsatisfactory. Of one, *H. giganteus*, he does not say how the material was preserved, and admits with regard to it, "Es muss also durch pathologische Veränderung oder Conservirungsfehler das Organ geschädigt worden sein," yet still uses it for histological purposes. Moreover, as we have seen above, Schaeffer describes in the ovary of the same species cells that are almost undoubtedly interstitial cells. The other, *Macropus* sp., was preserved in 3 per cent. formol, a bad fluid for histological work, and the sections employed were 40 μ thick!

It is to be regretted that in investigations of this kind better fixatives were not employed throughout, and this applies particularly to Schaeffer's work, in which formol was generally used. Also it is to be noted that no account was taken of the condition of the animal in regard to pregnancy or œstrus, and these are important points, as the tissue undergoes well-marked hypertrophy during these periods of functional activity. The failure to take into account the state of activity of the reproductive system, and to use suitable fixing fluids, detracts greatly from the value of both papers; and, indeed, in order to be certain of the absence of this tissue it is necessary to examine serial sections of one or preferably a series of pregnant animals.

With the possible exception of *Macropus* sp.?, which cannot be admitted until it has been more satisfactorily established, we may say of the marsupials up to the present examined that the Diprotodontia possess and the Polyprotodontia lack interstitial cells.¹

¹ It is interesting in this connection to call attention to the observations of Symington, confirmed more recently by Fraser and Hill (13),

The amount of tissue present in the ovary is not the same in all species of Diprotodontia. It is least marked in *Phascolumys mitchelli*, and, indeed, in a previous investigation (31) it was stated: "Das interstitielle Gewebe des Ovariums scheint bei *P. wombat* (i. e. *mitchelli*) ganz zu fehlen." Re-examination of the sections, however, shows that the tissue is present, but not in nearly such large masses as in the other species. Small clumps of interstitial cells are to be found here and there in the stroma. When the sections were examined previously the only ovaries available for comparison were those of *M. ruficollis* and *P. penicillata*, and these happen to be the two species in which the tissue is most abundantly present. It forms half or more of the entire ovary, and is easily recognisable in sections with the naked eye; in comparison with this the small amount in *P. mitchelli* was overlooked. With the exception of this last species all the Diprotodontia examined have a good deal of interstitial tissue which appears to reach its maximum in the Macropodidæ and culminates in *P. penicillata*, where it forms at least half the total bulk of the ovary, or if the corpus luteum be excluded, by far the greater part of the remaining ovarian tissue.

No trace of similar interstitial cells was found in the Polyprotodontia even in pregnant animals. The stroma here is composed almost entirely of ordinary elongated spindle-shaped stroma cells with their long-drawn-out nuclei. In it may be found a few very small groups of cells that are not so elongated as the remainder, but they are not in the least like the interstitial cells of the Diprotodontia. They are not so large, not polygonal, have not spherical nuclei, do not react

for, although they have no bearing on the main points of this paper, they instance an analogous difference in the distribution of glandular tissue in the two sub-groups of the Marsupialia. These authors have found that Diprotodonts possess a very large and highly developed cervical thymus which is entirely absent in Polyprotodonts. At present it does not seem possible to advance any satisfactory theory to explain such differences in the occurrence of the various glandular tissues.

in the same way to stains, and, most important of all, they do not undergo hypertrophy during pregnancy.

It is not within the scope of this paper to deal at any length with the physiological import of the interstitial tissue, since no physiological observations have been made on the animals examined, and physiological deductions from purely histological data are often of little value. [A fairly full review of the physiological aspect of this subject is given by Schaeffer (41)]. Conversely, however, histological and anatomical observations must not be disregarded when the functions of a structure are being considered.

The marked influence of the ovary upon the general metabolism of the body of the female has long been recognised, and of late years evidence has been accumulated to show that this influence is not of a nervous character but of the nature of an internal secretion or hormone. Part of this influence has been held to be due to the corpus luteum (30), and recently a certain amount of it has been ascribed to the interstitial cells. Marshall (26) has come to the conclusion that the maturation of the ovum and the phenomenon of pro-œstrum and œstrus are the result of a factor whose origin is to be sought in the interstitial cells. A few lines previously he mentions that "epitheloid interstitial cells" are numerous in the stroma, so that there can be no doubt he is referring to ordinary interstitial cells as dealt with above. It is obvious that this explanation is insufficient, or, at any rate, not of general application, for we cannot look to these cells for the stimulus in the animals where such cells do not exist, as, for example, in the Polyprotodontia.

Bouin and Ancel (7), partly from their own observations and partly from those of previous writers, suggest that mammals may be divided into two classes. Those which possess two kinds of corpus luteum, one periodic (i.e. C. L. périodique), and the other that of pregnancy (i.e. C. L. gestatif). The other group includes those animals which only ovulate after copulation, and consequently have only the corpus luteum of gestation. They state further that only the

second group possess an interstitial "gland," and deduce from this that the gland replaces functionally the periodic corpus luteum.

If this be so, why is it that the interstitial tissue appears in its most active condition when corpora lutea are present in the ovary? Further, it has been pointed out by Frank (12) that guinea-pigs, white rats and cats ovulate spontaneously and yet possess an interstitial "gland." In examples of *M. ruficollis* and *P. mitchelli*, probably non-pregnant, corpora lutea were well developed. These may constitute further examples of animals ovulating spontaneously and yet possessing interstitial tissue. Jardry (17) maintains that the secretion of this "gland" has a general trophic influence. He says: "Elle règle, accélère la nutrition intime des tissus, en agissant avec prédilection sur le système génital d'une part, et d'autre part sur l'assimilation des albuminoïdes et des phosphates au sein des tissus."

The general criticism made by Fraenkel (10) and later by Kingsbury (18) on all such théories as those mentioned above, seems to apply with even greater force when we consider the marsupials. This latter author says (p. 79) that we should expect "a gland existing for the specific purpose of forming substances of very distinct value to the organism as a whole would be constant in its presence and development." He points out that there is great variability in these matters, and Schaeffer (41) and Fraenkel (10) have further emphasised this variability by an examination of the ovaries of large series of different species of mammals. It has been pointed out above that within the limits of the group Diprotodontia there is marked variation in the number of interstitial cells present, it may be enormous, or, on the other hand, only a comparatively few small groups of such cells are to be found. In the Polyprotodontia, as we have seen, such cells are absent altogether.

At present there appears to be no satisfactory function assigned to these interstitial cells, and any theory put forward, in order to be completely satisfactory, must be able

to show why it is necessary for certain animals to possess the cells and yet not necessary in other animals that do not appear to have any compensating structure.

In spite of the fact that these cellular masses present such a typically glandular appearance, I have endeavoured throughout to avoid the use of the term gland because its use hardly seems justifiable. Used in a morphological sense, the word gland implies a certain definiteness and constancy, and these are not exhibited by the interstitial tissue. Physiologically, also, the word gland is currently used to denote a structure secreting a substance that plays some assignable rôle in the vital activity of the organism. The interstitial cells probably produce a secretion of a fatty or lipid nature, but whether this has any influence on the whole or any part of the female has yet to be shown. The term interstitial cells or tissue does not imply so much as gland, and is preferable in the present state of our knowledge.

SUMMARY.

The Corpus Luteum.

(a) Follicular Wall.—The membrana granulosa in the three species, *P. cinereus*, *T. vulpecula*, and *D. aurita*, is composed of typical polygonal cells arranged three or four cells deep around the ripe follicle. The theca folliculi also calls for no special comment in any case. It is composed of internal and external layers, does not contain any included interstitial cells, and its cells are always readily distinguishable from membrana granulosa cells.

(b) The Formation of the Corpus Luteum.—The corpus luteum in *P. cinereus* is formed by the irruption of both layers of the theca folliculi, which burst through the membrana granulosa and form a lining on its inner side. This method of formation is similar to that in *P. obesula*, *P. nasuta*, and *M. ruficollis*. The ripe follicle in *T. vulpecula* collapses when the ovum is extruded, and the

central cavity is at once obliterated. The theca folliculi is drawn in with the membrana granulosa, which it penetrates, and the connective tissue becomes irregularly distributed through the body. It is unlike the process in any other marsupial so far examined, but to a certain extent resembles that in the mouse.

In *D. aurita* the thecal irruptions do not at once go through the membrana granulosa, but push it before them until the central cavity is practically filled in, and then they break through and form the central plug of connective tissue. In one example, a very early stage, mitoses were found in the cells of the membrana granulosa, as was also the case in *P. obesula* and *P. nasuta*.

(c) The fully formed Corpus Luteum.—The corpus luteum in *P. cinereus* remains hollow even when fully grown, and the central cavity does not get filled in until some time after the birth of the young, apparently not until the gland has started to decline. This condition is apparently unique.

In *T. vulpecula* the corpus luteum is fairly typical when full grown, save that its connective tissue is much more irregularly arranged than in other marsupials.

The condition of the corpus in *D. aurita* is very similar to that in *D. viverrinus*.

In no case is the membrana granulosa shed, nor does the theca interna contribute to the lutein cells of the corpus luteum.

The Interstitial Tissue.

There is present in the ovary of certain species of marsupials a tissue which corresponds histologically to the interstitial tissue in the ovary of the higher mammals. The cells are always distinguishable from ordinary stroma cells, cells of the theca interna, old lutein cells, or the cells of an atresic follicle, and there is no evidence that any of the last three are at any time transformed into interstitial cells.

Such cells are present in the pouch young of *T. vul-*

pecula before they could have been derived from any of the sources suggested above.

Interstitial tissue is to be regarded as a tissue *sui generis*, although it is possible that it may originate from modified stroma cells at a very early stage.

The tissue is irregularly distributed in the various species of marsupials, and it is worthy of note that it is present in all the Diprotodontia and absent in the Polyprotodontia so far examined. It may be present only as a few small groups of cells or in such quantity as to form by far the largest part of the bulk of the ovary, excluding corpora lutea, as, for example, in *P. penicillata*.

The tissue has a typical glandular appearance, but no satisfactory account of its function has yet been put forward, and in view of this and its irregularity it is preferable not to call it a gland, but retain the term interstitial tissue or cells.

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EXPLANATION OF PLATE 40,

Illustrating Mr. Chas. H. O’Donoghue’s paper, “On the Corpora lutea and Interstitial Tissue of the Ovary in the Marsupialia.”

Fig. 1.—Photomicrograph (\times circa 6). Transverse section of the ovary of *Phascolarctos cinereus* (No. 2), showing an early stage in the formation of the corpus luteum. Atresic follicles are present and the islets of interstitial tissue are clearly visible in the ovarian stroma.

Fig. 2.—Photomicrograph (\times circa 6). Transverse section of the corpus luteum of *P. cinereus* (No. 5). The large size is clearly shown, and the characteristic hollow form is retained although the embryos are well developed and 9 mm. in length.

Fig. 3.—Photomicrograph (\times circa 6). Transverse section of the corpus luteum of *P. cinereus* (No. 9). The embryos are in a late uterine stage, shortly before birth, but the corpus is still hollow and about at its maximum size.

Fig. 4.—Photomicrograph (\times circa 12). Transverse section of the corpus luteum of *Trichosurus vulpecula* (No. 2). An early stage in the formation, showing the way in which the follicle collapses completely and even becomes partly inverted and draws the theca in with it.

Fig. 5.—Photomicrograph (\times circa 12). Transverse section of the corpus luteum of *T. vulpecula* (No. 5). A slightly later stage than fig. 4. The very irregular distribution of the connective and luteal tissue is clearly shown.

Fig. 6.—Photomicrograph (\times circa 12). Transverse section of the corpus luteum of *T. vulpecula* (No. 6). The fully formed corpus is irregular and protruding, and three fairly ripe follicles are shown.

Fig. 7.—Photomicrograph (\times circa 12). Transverse section of the corpus luteum of *T. vulpecula* (No. 12). The maximum size of the corpus accompanying late uterine embryos. The irregular form is shown, and also the manner in which the body projects from the ovary.

Fig. 8.—Photomicrograph (\times circa 8). Transverse section of the entire ovary of *Didelphys aurita* (No. 6). Parts of twelve fully formed, but not fully grown, corpora lutea are present in the section. Comparison with fig. 1 brings out the difference between the ovary of this species and of *P. cinereus*. Interstitial tissue is absent.

Fig. 9.—Photomicrograph (\times circa 40). Transverse section of an early stage in the formation of the corpus luteum in *D. aurita* (No. 2). The connective tissue ingrowths have pushed the membrana granulosa cells inwards, but not yet broken through it.

Fig. 10.—Photomicrograph (\times circa 40). Transverse section of a later stage in the formation of the corpus luteum in *D. aurita* (No. 4). The whole structure has enlarged and the connective tissue ingrowths have broken through into the central cavity.

Fig. 11.—Photomicrograph (\times circa 40). Transverse section of a still later stage in the formation of the corpus luteum in *D. aurita* (No. 3). The corpus is almost fully formed and the central cavity nearly obliterated.

Fig. 12.—Photomicrograph (\times circa 40). Transverse section of fully formed and grown corpus luteum in *D. aurita* (No. 5). The whole structure is very similar to a corpus luteum in *Dasyurus viverrinus*.

Fig. 13.—Photomicrograph (\times circa 40). Transverse section of a portion of the ovary of *Macropus dorsalis*. This shows at the same time, A, part of an active corpus luteum, C, part of an old corpus luteum, and B, active interstitial tissue in the ovarian stroma between them. The differences between the three kinds of tissue are obvious.

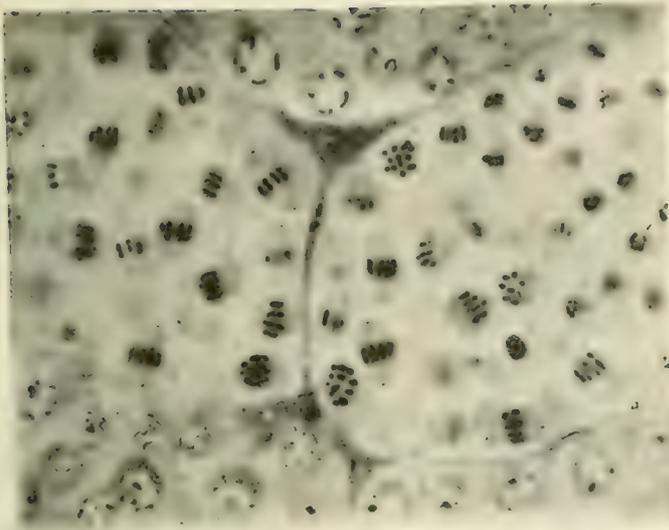
INDEX TO VOL. 61,

NEW SERIES.

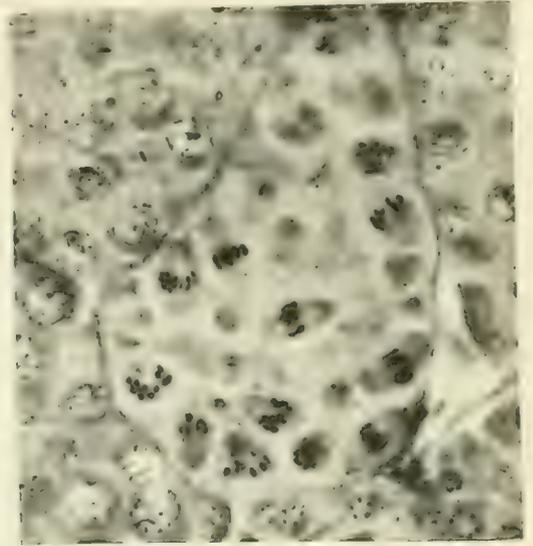
- Aphelinus, an insect parasite of
Lepidosaphes, by Dr. A. D. Imms,
217
- Asterias rubens, double hydro-
coele in the larva of, by J. F.
Gemmill, D.Sc., 51
- Cestracion (*Heterodontus*),
intra-uterine eggs of, by Prof.
Haswell, F.R.S., 313
- Chorda tympani, the, and middle ear
in Reptiles, Birds, and Mammals,
by Edwin S. Goodrich, 137
- Coccidæ, insect parasites of, by Dr.
A. D. Imms, 217
- Corpora lutea and interstitial tissue
of the ovary in Marsupialia, by
C. H. O'Donoghue, D.Sc., 433
- Dendrocometes paradoxus,
reproduction of, by G. Lapage and
J. T. Wadsworth, 337
- Didymorchis (a Turbellarian), by
Prof. Haswell, F.R.S., 161
- Edgeworth, Prof., on the develop-
ment and morphology of the
pharyngeal, laryngeal, and hypo-
branchial muscles of Mammalia,
383
- Egg of Monotremes, by Wilson and
Hill, 15
- Eggs, intra-uterine, of Port Jackson
Shark, by Prof. Haswell, F.R.S.,
313
- Electrolytes, relation of spermatozoa
to, by J. Gray, B.A., 119
- Embadomonas, by Doris Mac-
kinnon, 105
- Embryology, text-book of, Vol. I,
Invertebrata, by Prof. McBride,
F.R.S. (review), 201
- Fertilization, the problem of, by
J. Gray, B.A., 119
- Forficula, mitotic spindle in sper-
matocytes of, by Meek, 1
- Gatenby, J. Bronté, on the develop-
ment of the sperm-duct, oviduct,
and spermatheca of *Tubifex*
rivulorum, 317
- on the transition of peritoneal
epithelial cells into germ-cells in
some Amphibia, 275
- Gemmill, James F., on double hydro-
coele in the larva of *Asterias*
rubens, 51
- on the larva of the Starfish
Porania pulvillus, 27
- Germ-cells derived from peritoneal
epithelial cells in *Rana tempo-*
raria, by J. Bronté Gatenby, 275

- Glycera, Gregarines of, by Helen L. M. Pixell-Goodrich, B.Sc., 205
- Goodrich, Edwin S., F.R.S., on the chorda tympani and middle ear in Reptiles, Birds, and Mammals, 137
- Gregarines of Glycera, by Helen L. M. Pixell-Goodrich, B.Sc., 205
- Haswell, Prof., F.R.S., studies on the Turbellaria. Part III. Didymorchis, 161
- on intra-uterine eggs of *Cestracion Phillipi*, 313
- on the embryology of *Stratiodrillus*, 301
- Hett, Mary L., on a new species of Pentastomid from a N. African snake, 185
- Hill and Wilson, Professors, on the early Monotreme Egg, 15
- Hydrocele, double, in larva of *Asterias rubens*, by J. F. Gemmill, D.Sc., 51
- Imms, Dr. A. D., on the insect parasites of some Coccidæ. I. On *Aphelinus*, a parasite of *Lepidosaphes*, 217
- Interstitial tissue of the ovary of Marsupials, 433
- Jenkinson, J. W., M.A., D.Sc., on the Placenta of a Lemur, 171
- Klossiella muris*, by A. C. Stevenson, M.B., 127
- Lapage and Wadsworth on the reproduction of *Dendrocometes*, 337
- Lemur, placenta of a, by J. W. Jenkinson, D.Sc., 171
- Lepidosaphes ulmi*, the mussel scale, its insect parasite *Aphelinus*, by Dr. A. D. Imms, 217
- McBride, Professor, on the embryology of the Invertebrata (review), 201
- Mackinnon, Doris, D.Sc., on Parasitic Protozoa (*Embadomonas* and a *Trichomastigine*), 105
- Mammalia, development and morphology of their pharyngeal, laryngeal, and hypobranchial muscles, by Prof. Edgeworth, M.D., 383
- Marsupials, corpora lutea and interstitial tissue of their ovary, by C. H. O'Donoghue, 433
- Meek, C. F. U., on the mitotic spindle in spermatocytes of *Forficula*, 1
- Middle ear and chorda tympani, by Goodrich, 137
- Mitotic spindle in spermatocytes of *Forficula*, by C. F. U. Meek, 1
- Monotreme Egg, the early, by Wilson and Hill, 15
- Muscles (pharyngeal, laryngeal, and hypobranchial) of Mammalia, by Prof. Edgeworth, M.D., 383
- O'Donoghue, Chas. H., on the corpora lutea and interstitial tissue of the ovary in Marsupialia, 433
- Ovary and corpora lutea of Marsupials, 433
- Oviduct of *Tubifex rivulorum* 317
- Parasites of some Coccidæ, by Dr. A. D. Imms, 217
- Pentastomid, a new species of, by Mary L. Hett, B.Sc., 185
- Peritoneal epithelial cells, transition of, into germ-cells, by J. Bronté Gatenby, 275
- Pharyngeal and other muscles of Mammalia, development of, by Prof. Edgeworth, M.D., 383
- Pixell-Goodrich, Helen L. M., on the gregarines of *Glycera siphonostoma*, 205

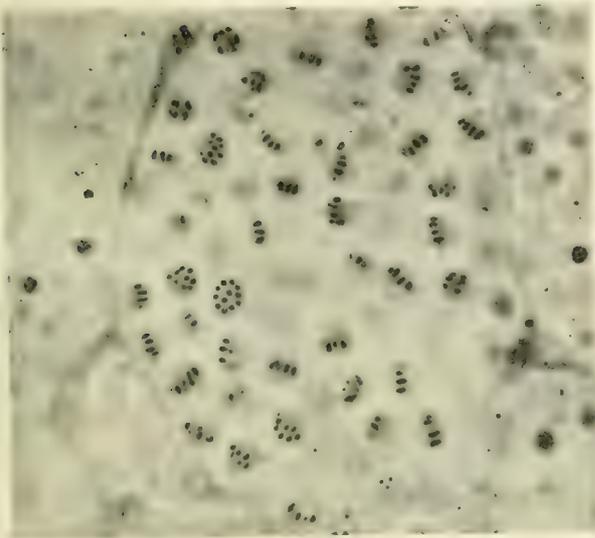
- Pixell-Goodrich, Helen L. M., on the sporozoa of Spatangoids and some allied forms, 81
- Placenta of a Lemur, by J. W. Jenkinson, M.A., D.Sc., 171
- Porania pulvillus, larva of, by Gemmill, 27
- Protozoa, parasitic, by Doris Mackinnon, 105
- Spatangoids, sporozoa of, by H. L. M. Pixell-Goodrich, 81
- Spermatheca of *Tubifex rivulorum*, 317
- Spermatocytes of Forficula, mitotic spindle in, by Meek, 1
- Spermatozoa, relation of, to Electrolytes, by J. Gray, B.A., 119
- Sperm-duct of *Tubifex rivulorum*, 317
- Sporozoa of Spatangoids, by Helen L. M. Pixell-Goodrich, 81
- Starfish Porania, larva of, by Gemmill, 27
- Stevenson, A. C., on *Klossiella muris*, 127
- Stratiodrillus, embryology of, by Prof. Haswell, 301
- Trichomastigine, on a, by Doris Mackinnon, 105
- Tubifex rivulorum*, the development of the sperm-duct, oviduct, and spermatheca in, by J. B. Gatenby, 317
- Turbellaria, studies on, by Prof. Haswell, F.R.S., 161
- Wadsworth and Lapage on the reproduction of *Dendrocometes*, 337
- Wilson and Hill, Professors, on the early Monotreme Egg, 15
- Zamenis, an African snake, Pentastomid from, by Mary L. Hett, B.Sc., 185



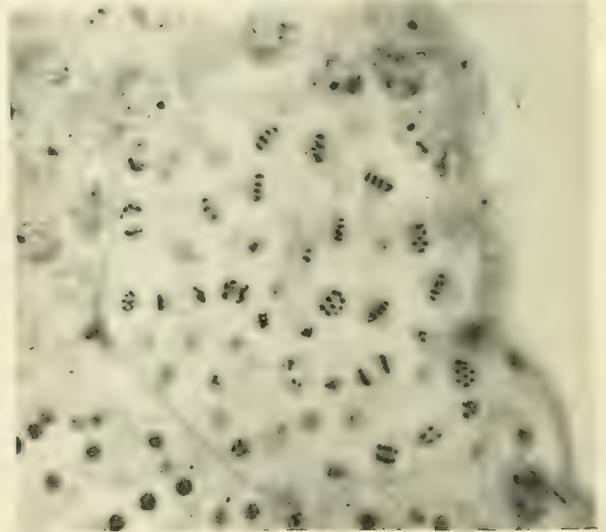
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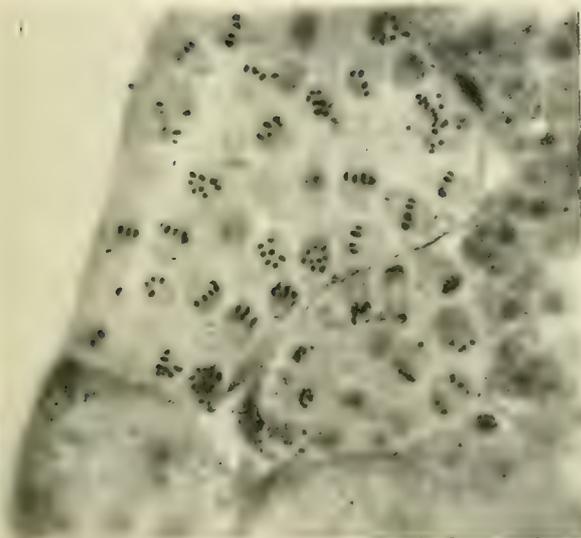
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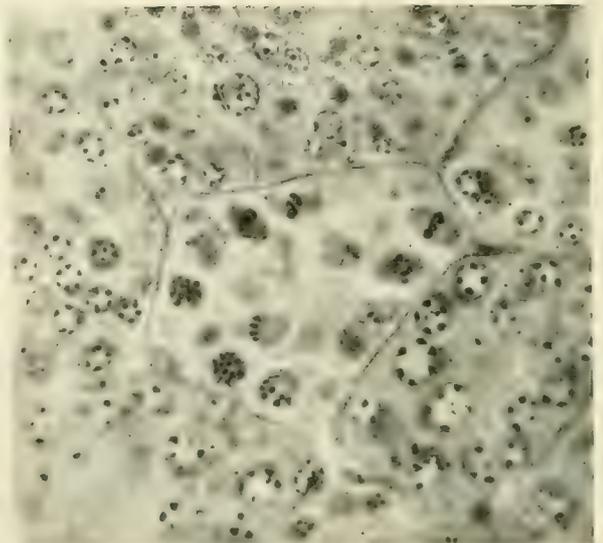
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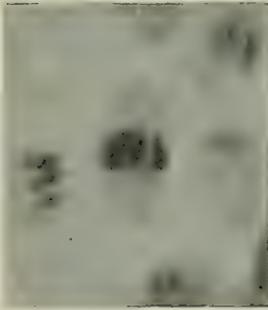
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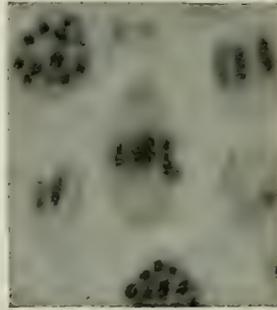
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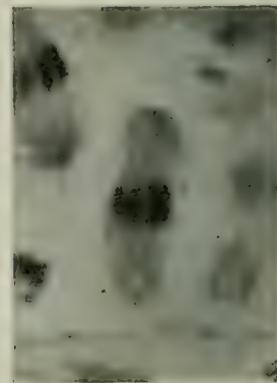
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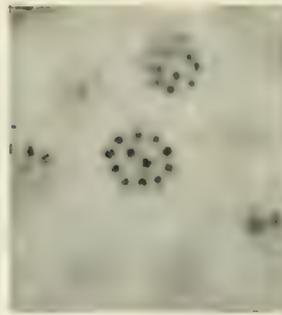
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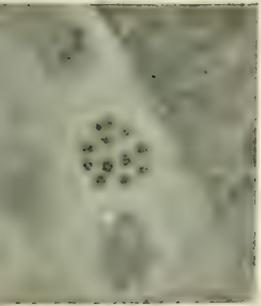
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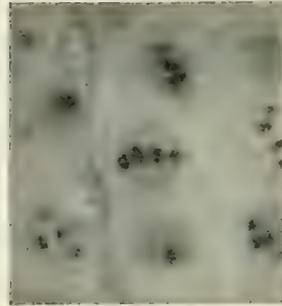
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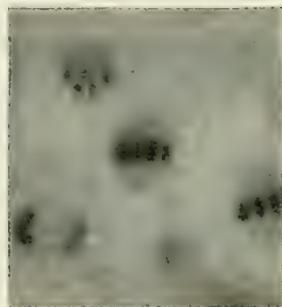
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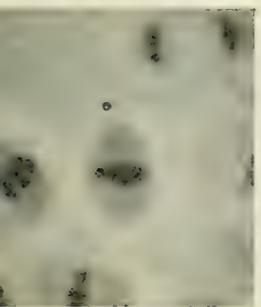
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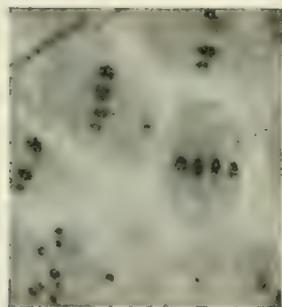
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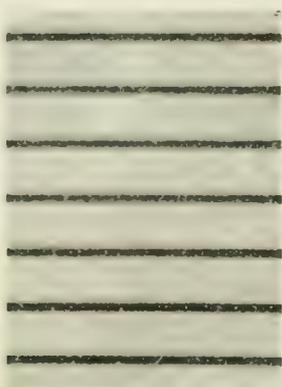
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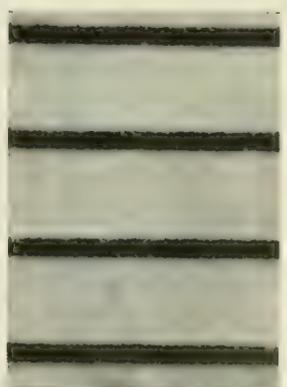
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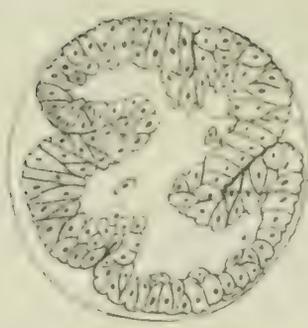


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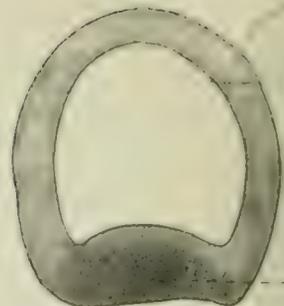




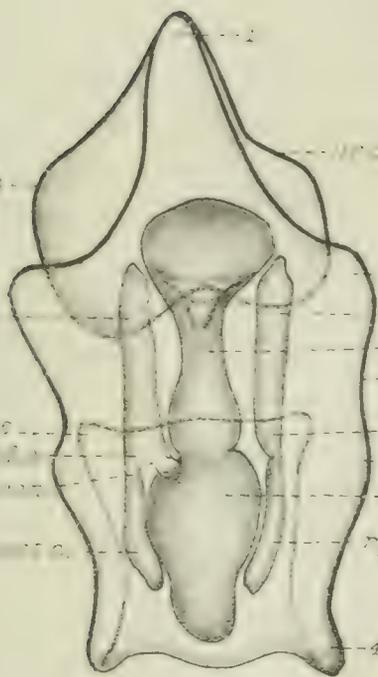
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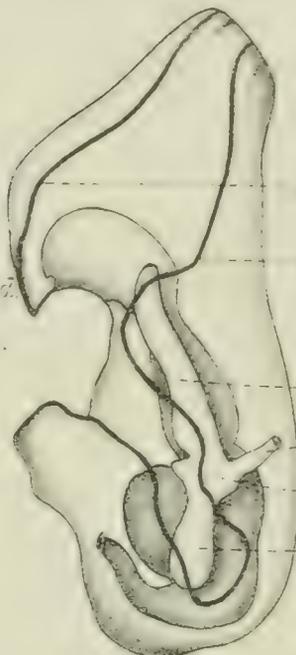
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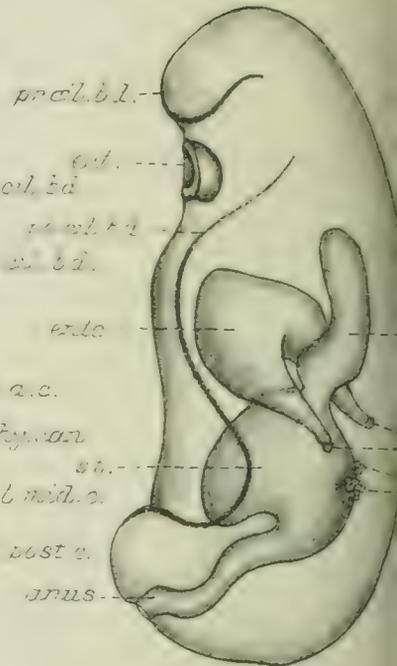
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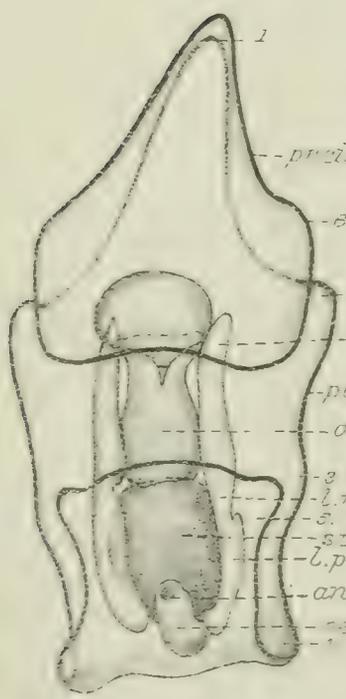
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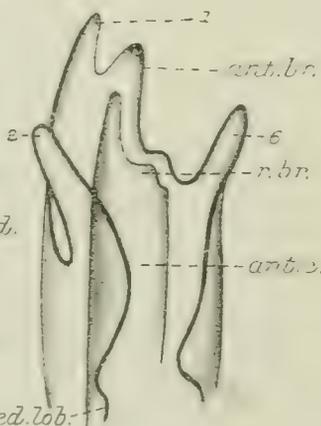
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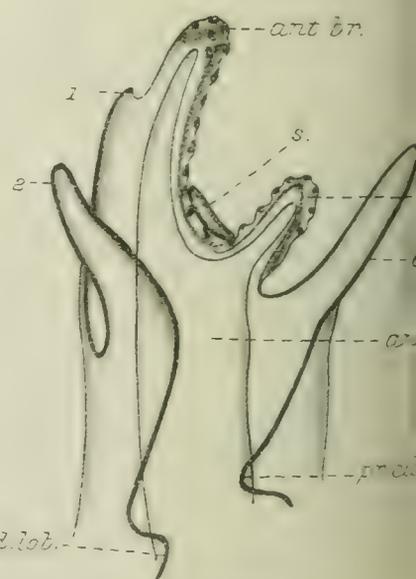
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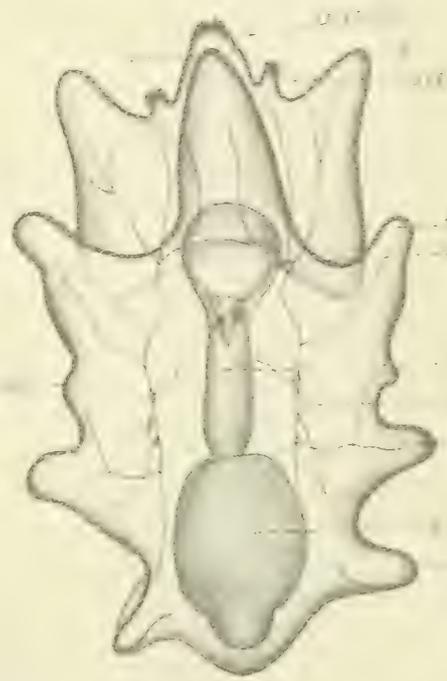
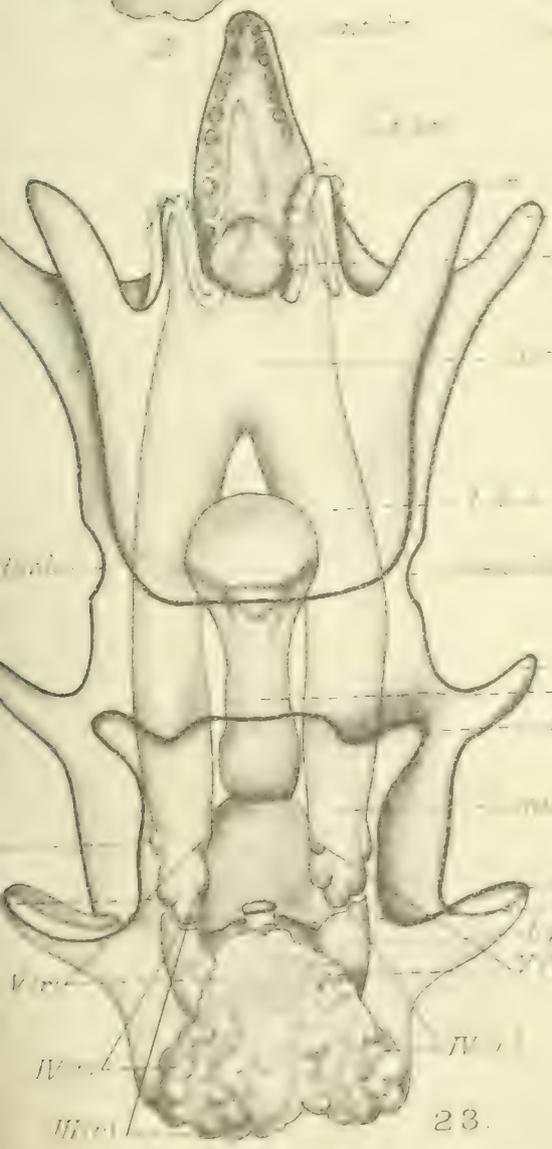
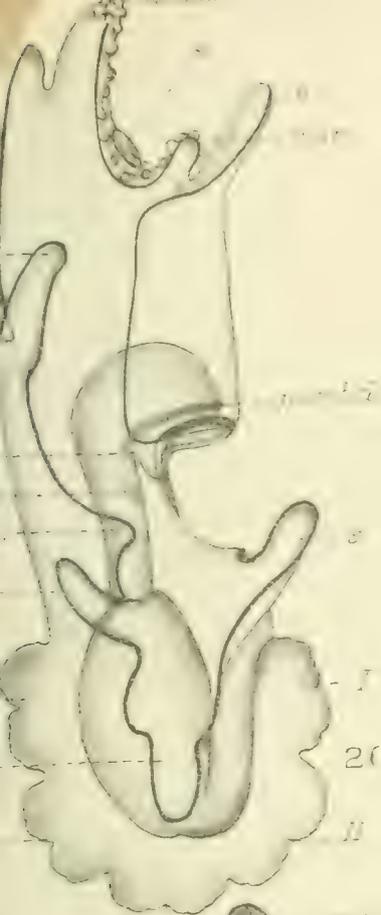
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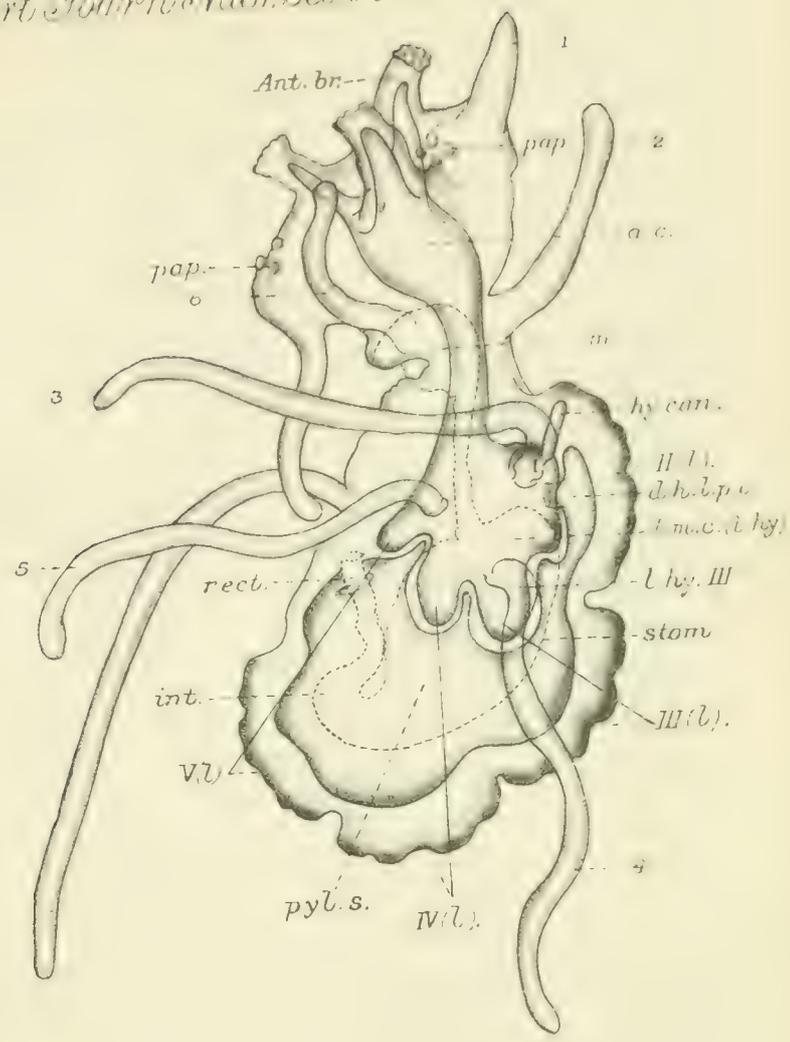
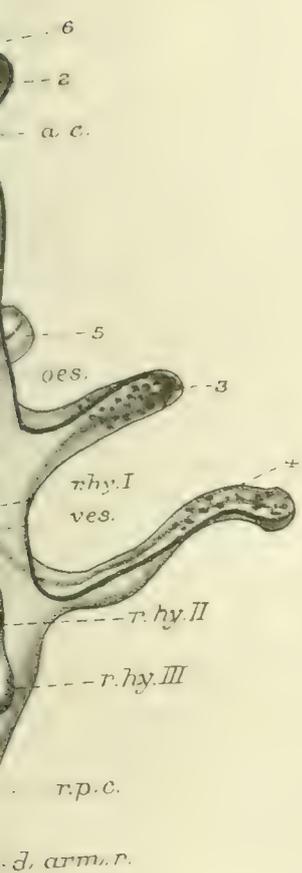
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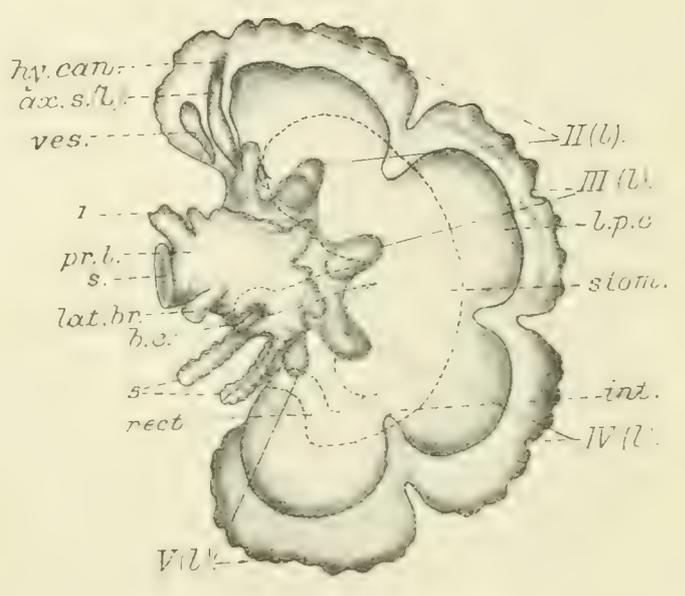
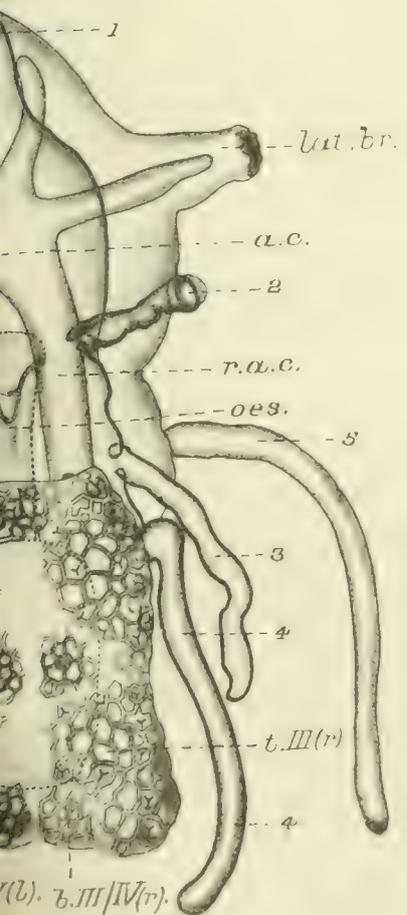
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Gemmill del.



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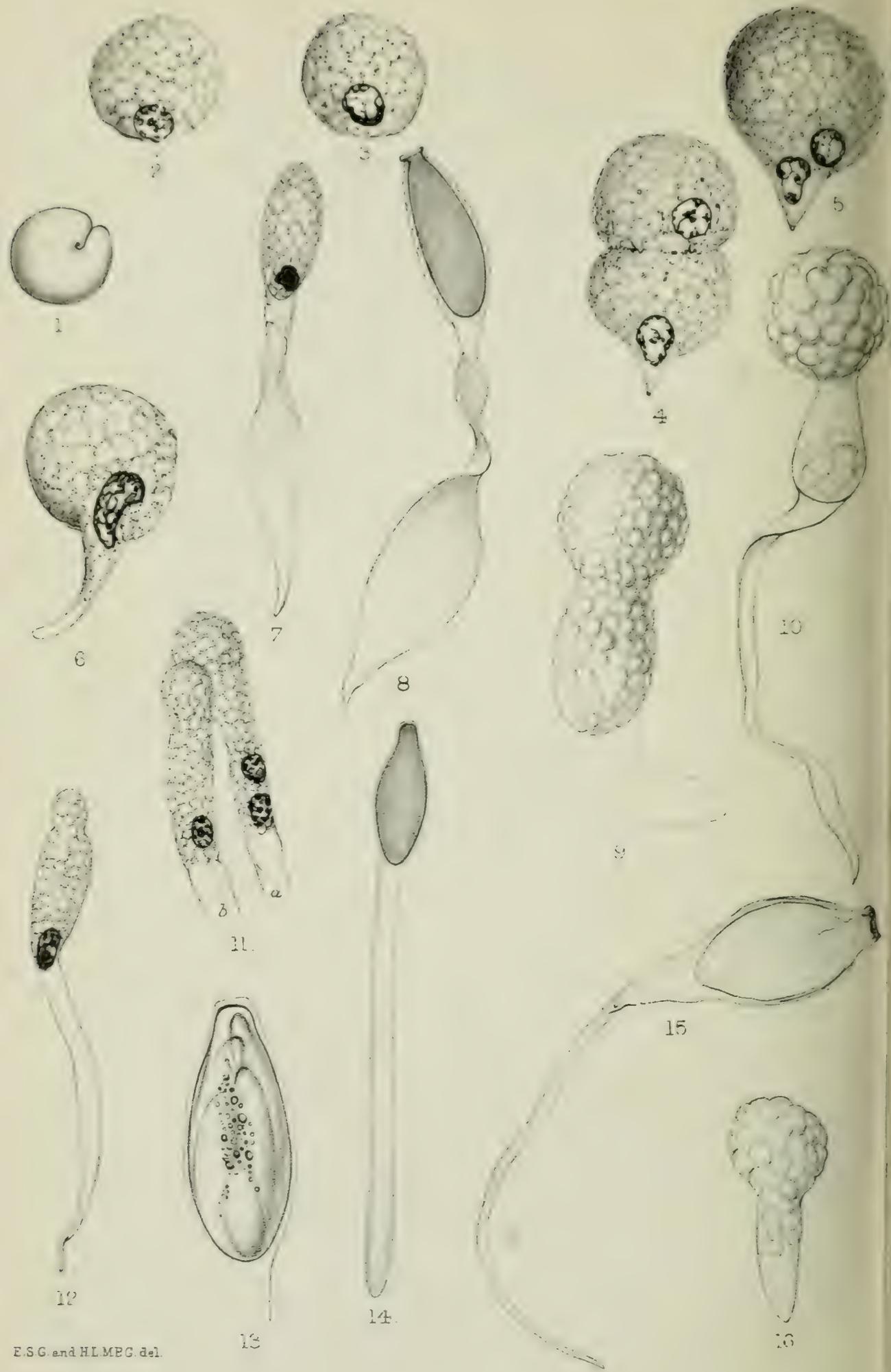


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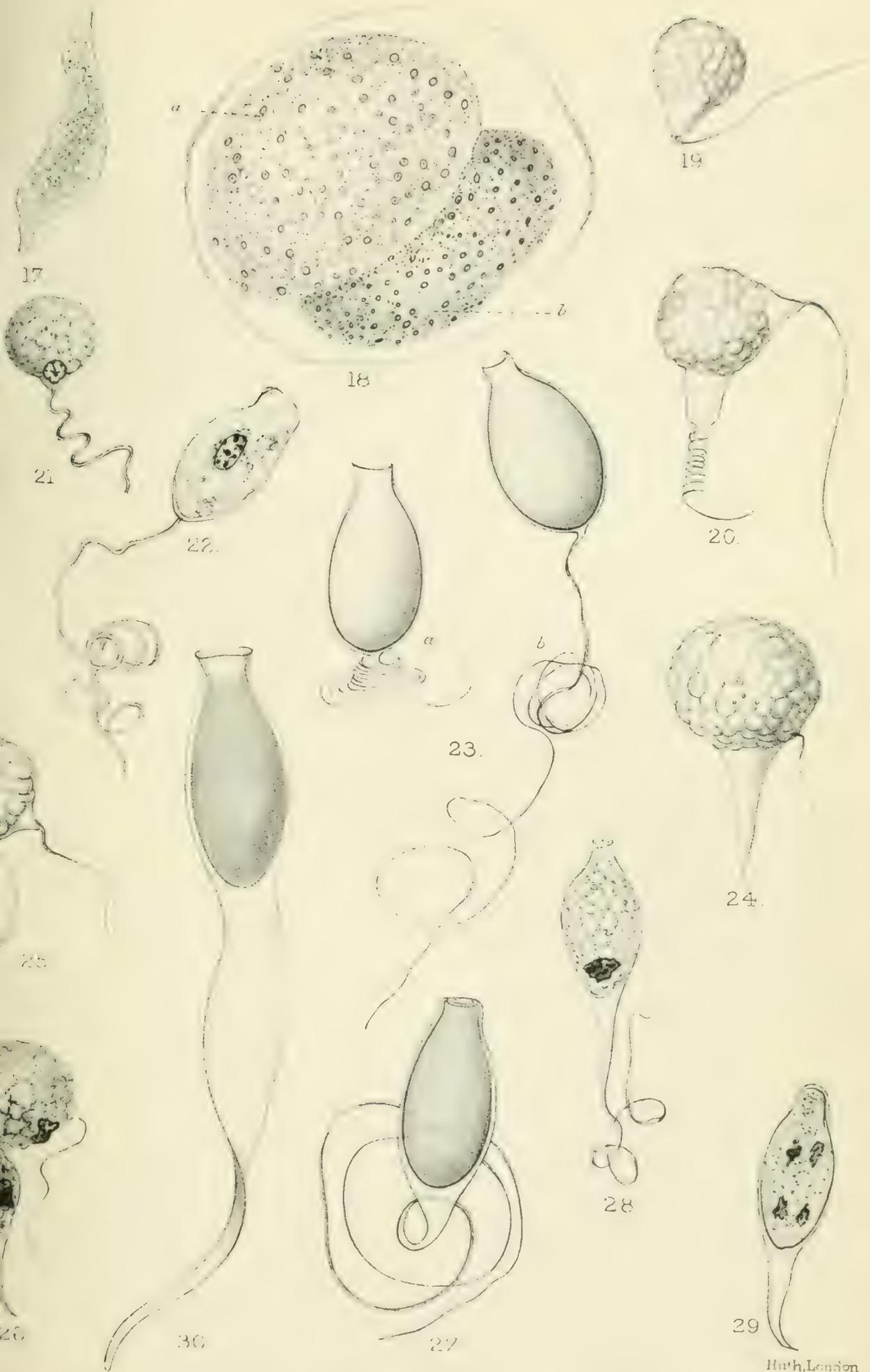
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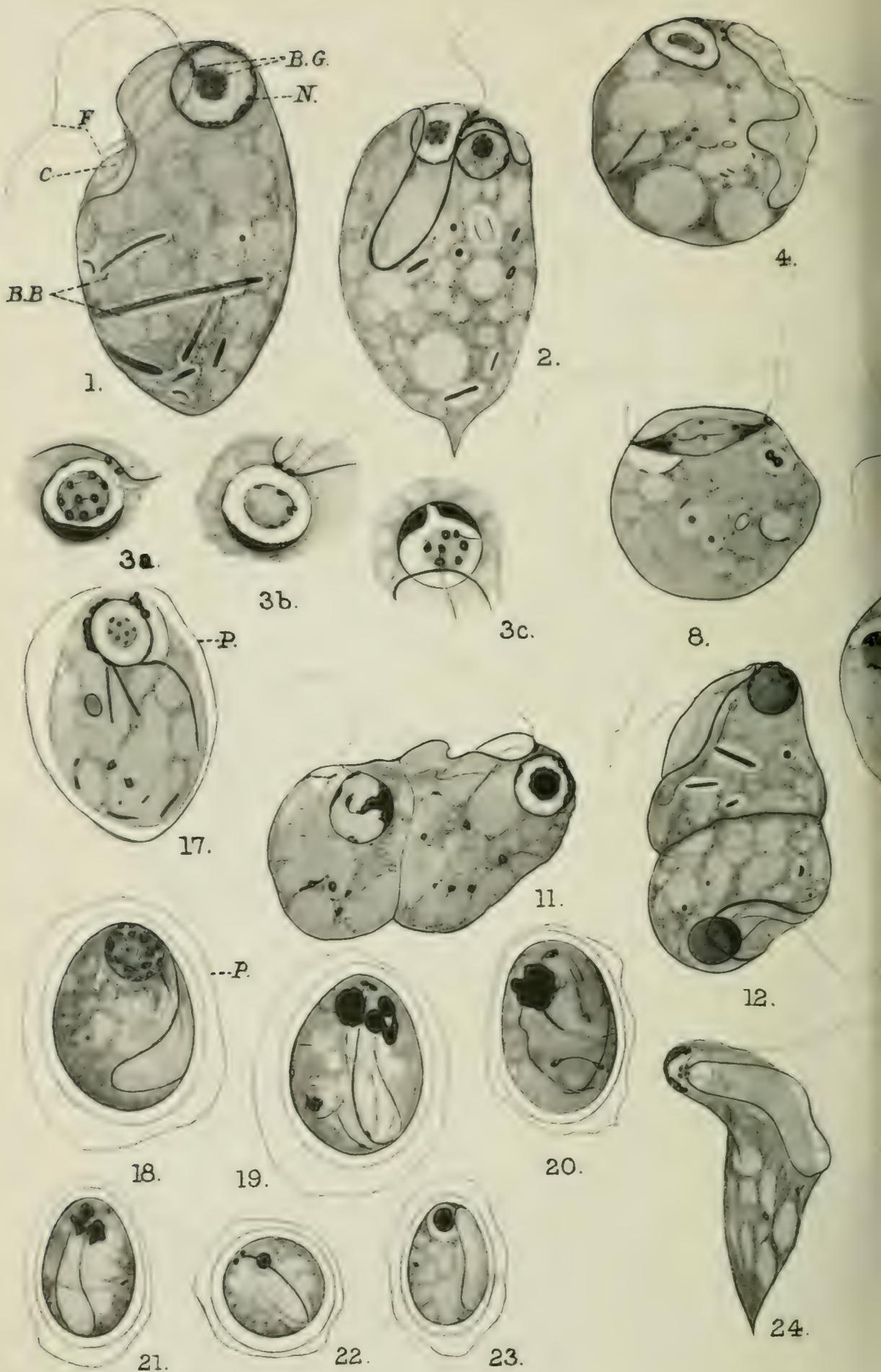
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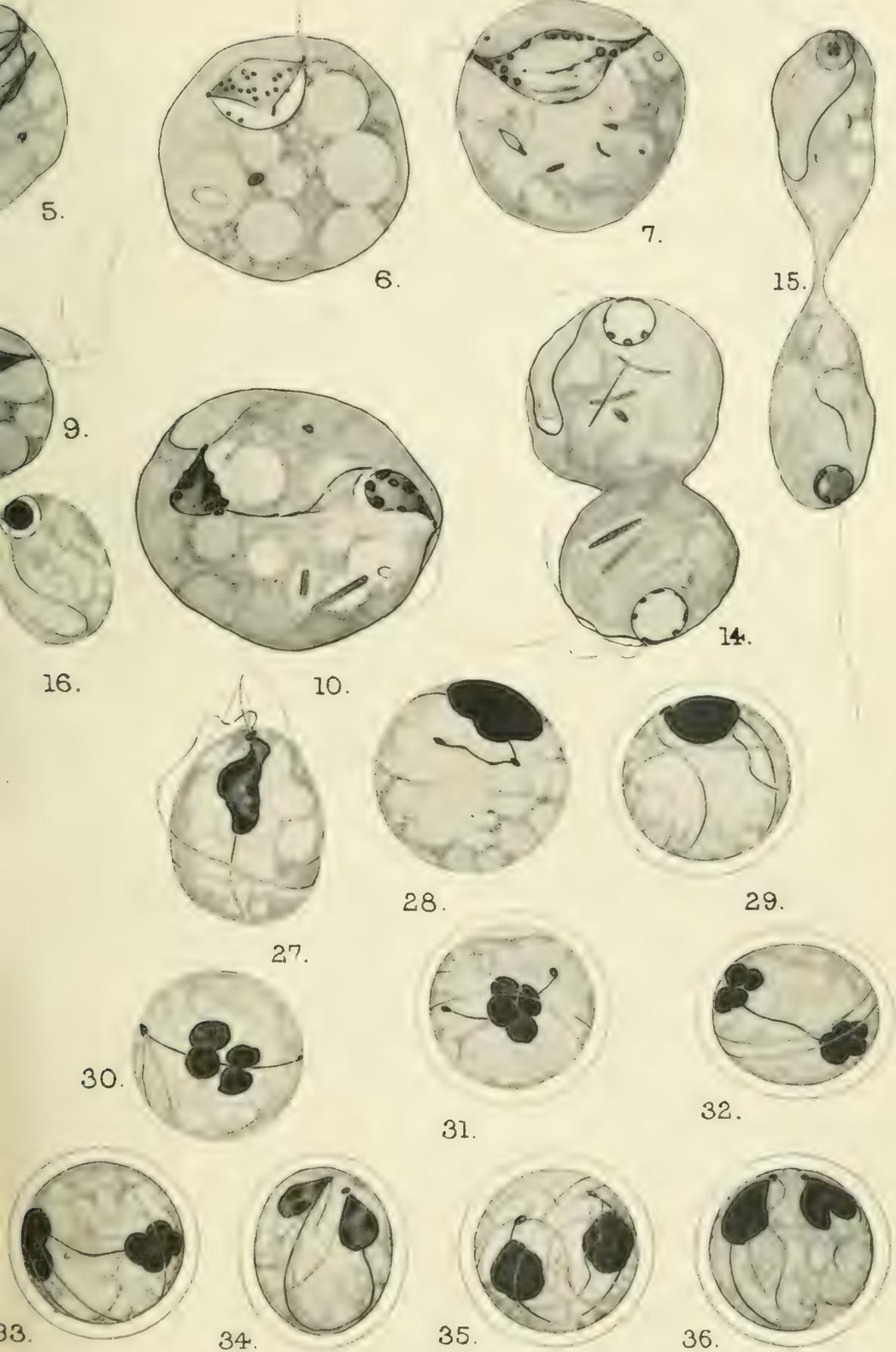
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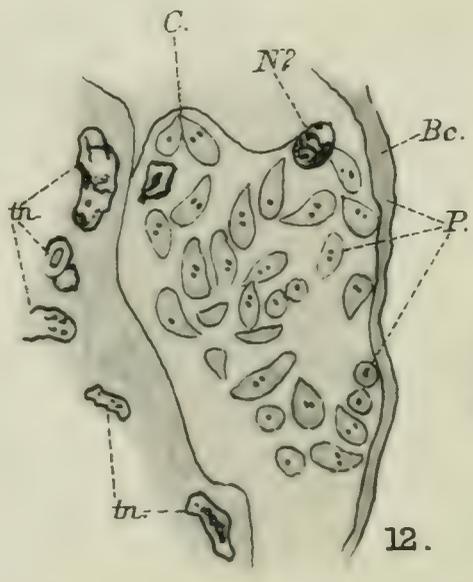
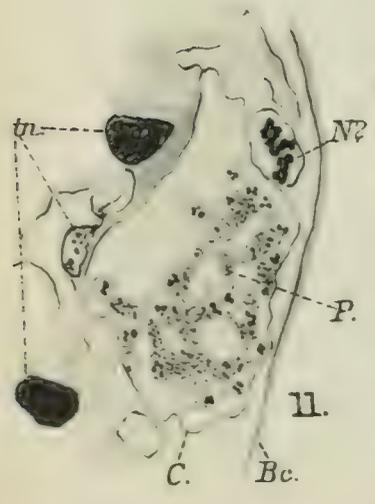
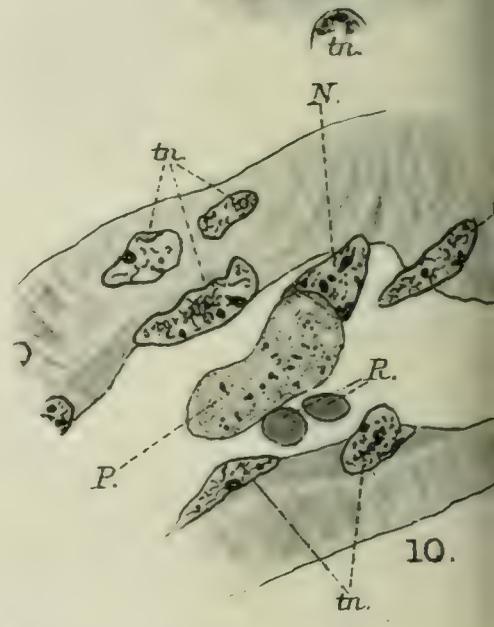
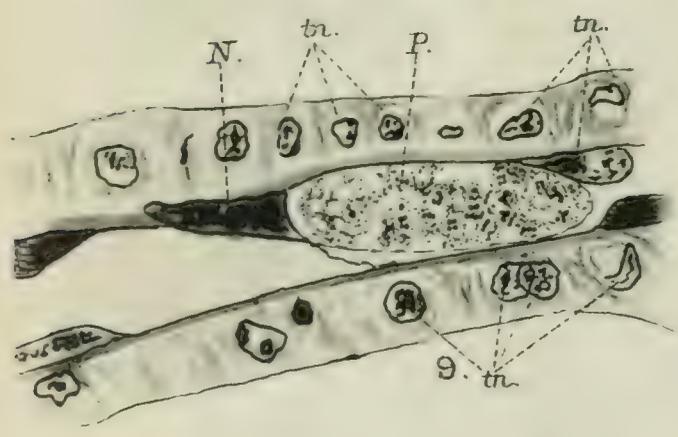
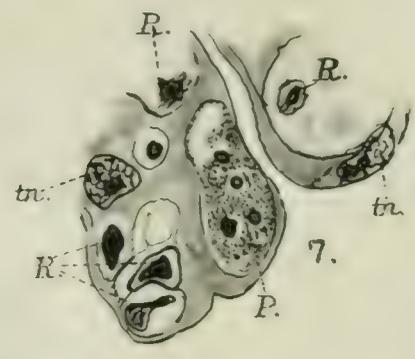
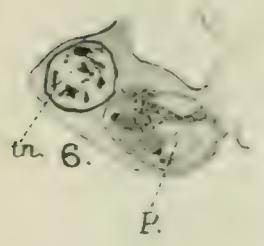
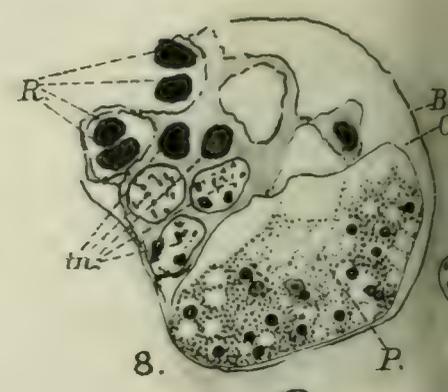
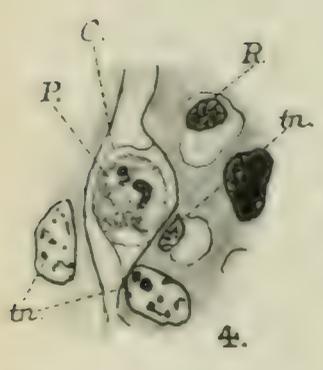
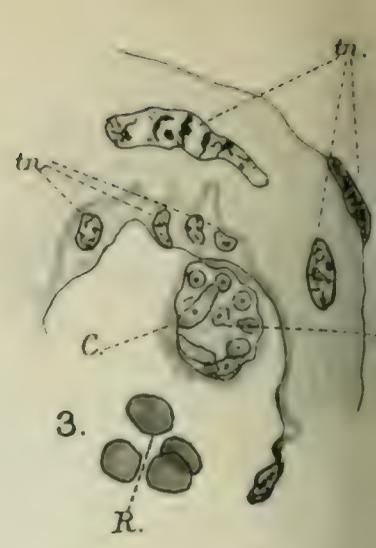
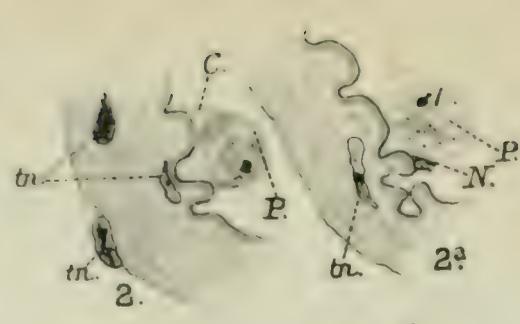
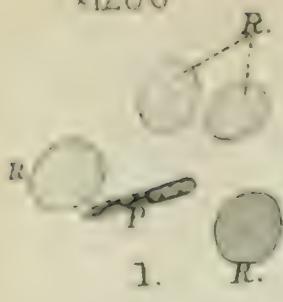
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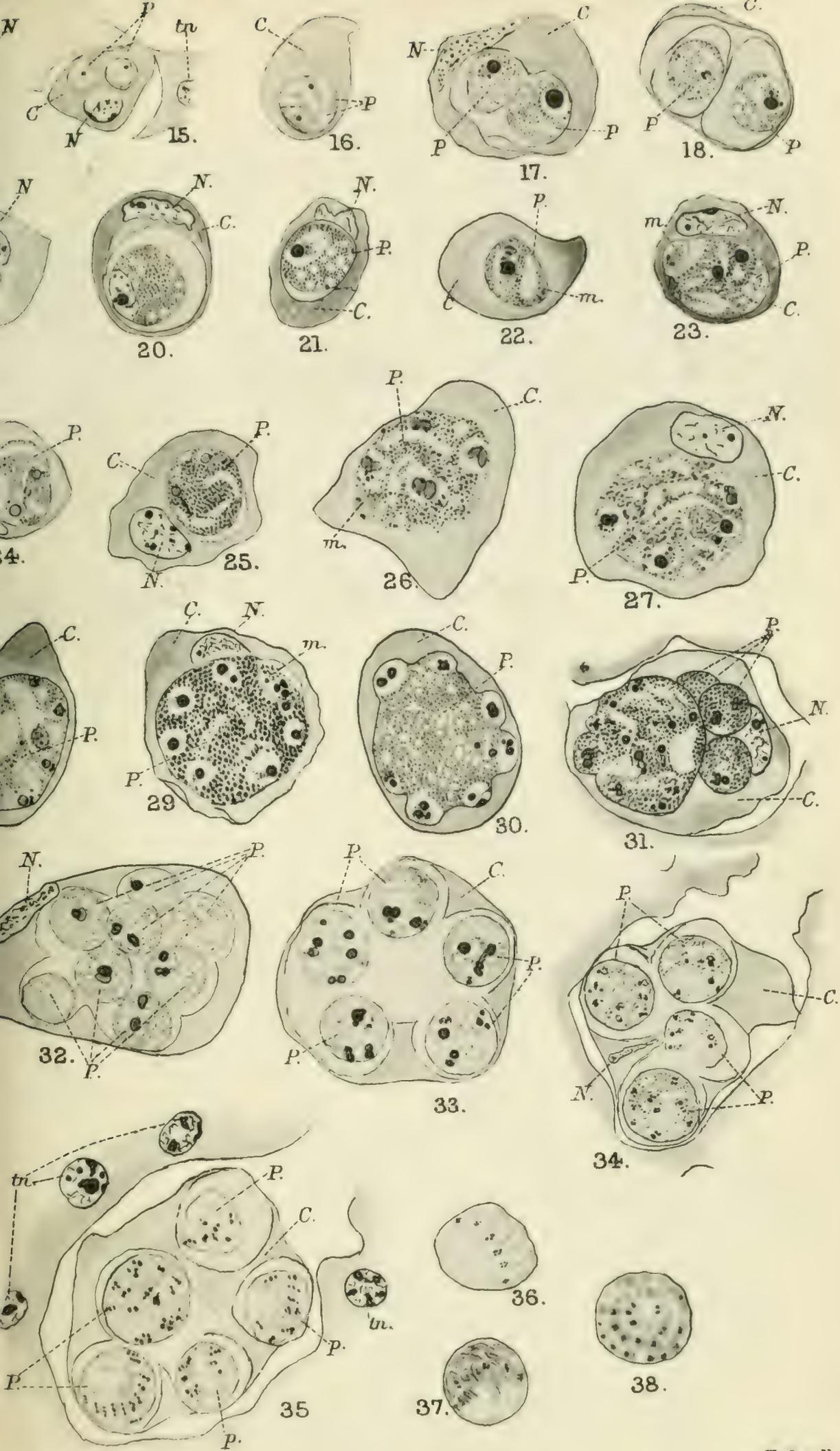
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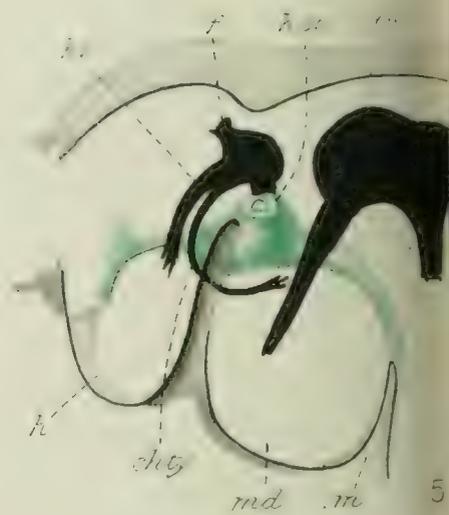
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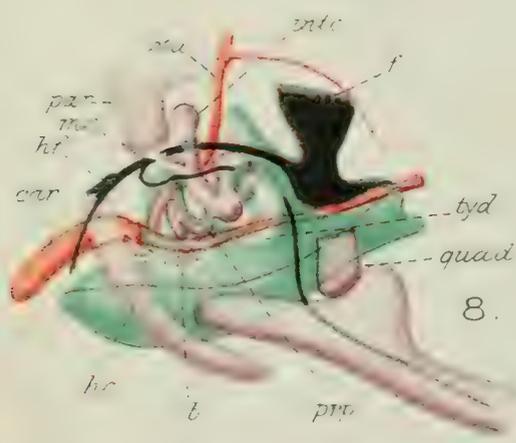
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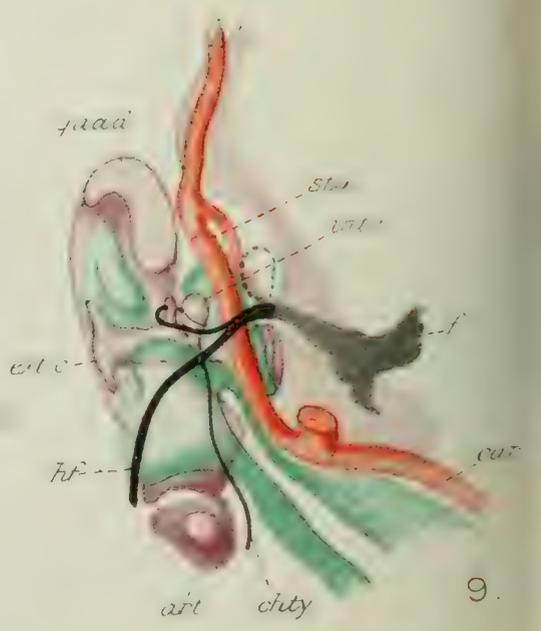
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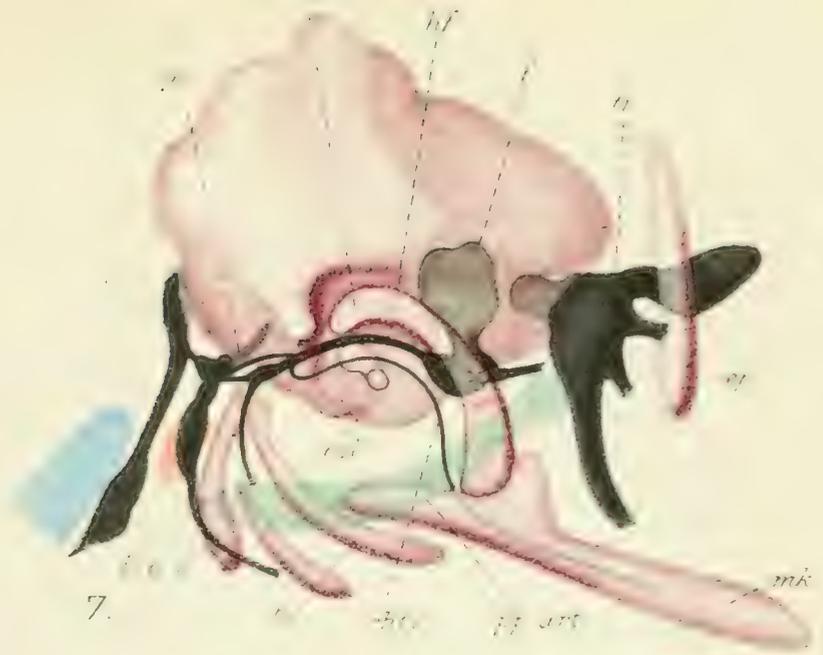
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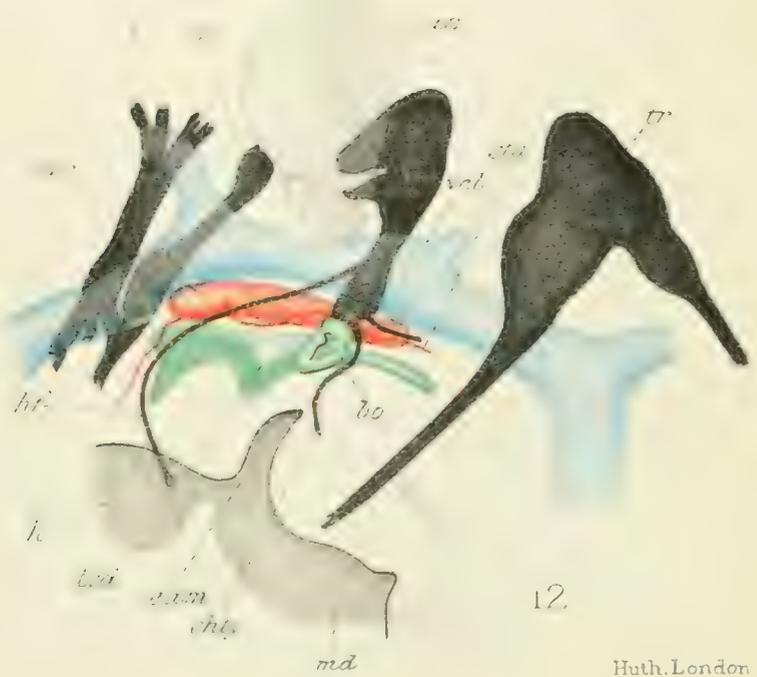
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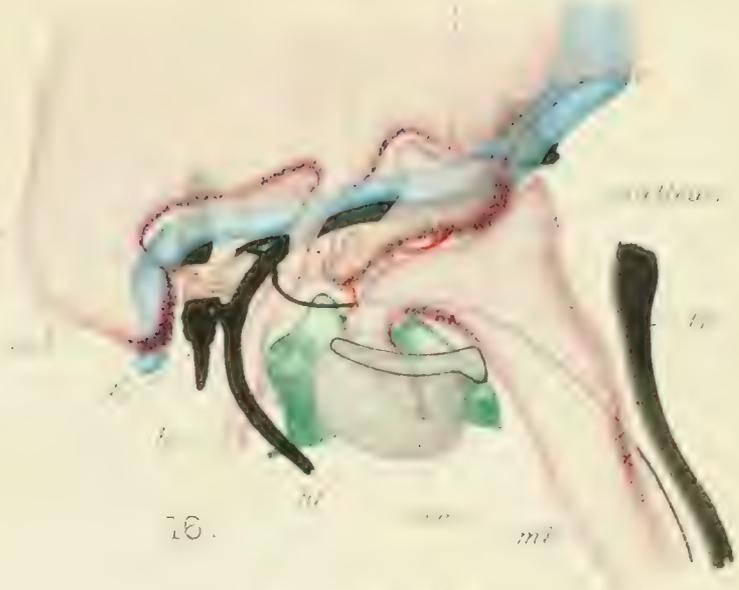


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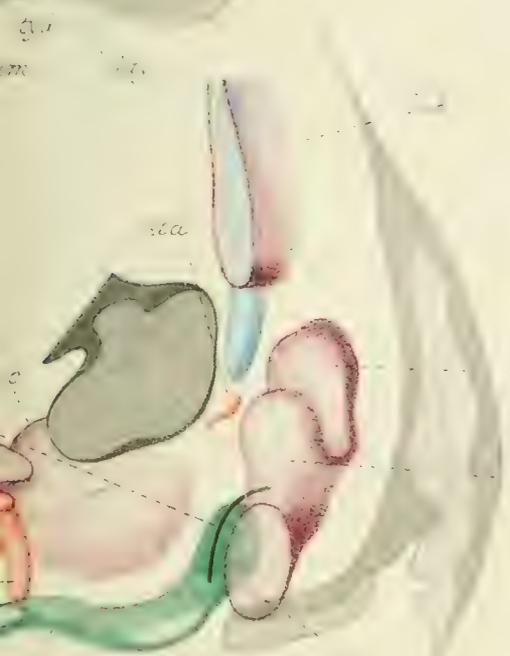


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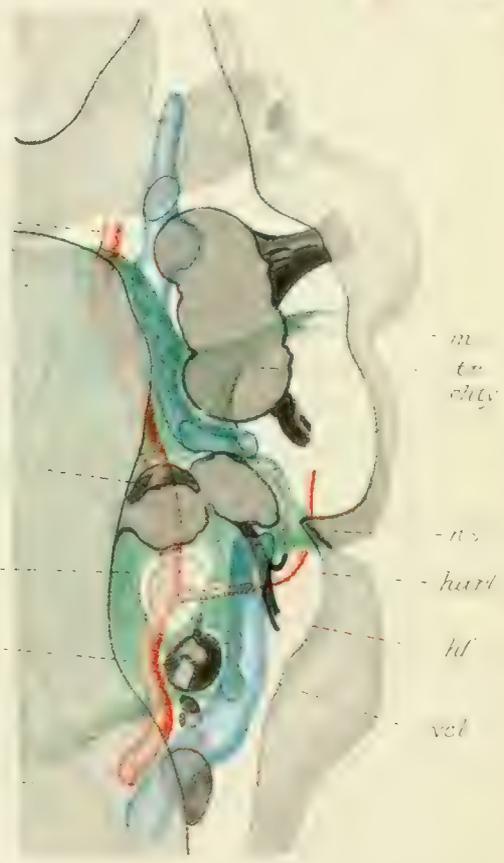
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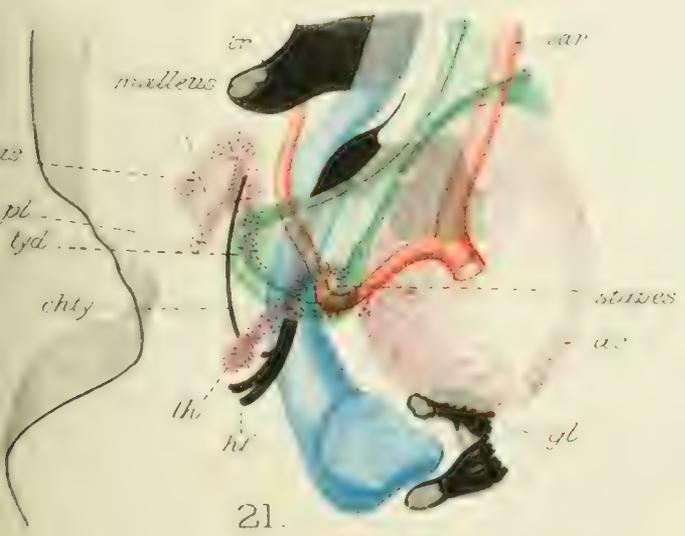
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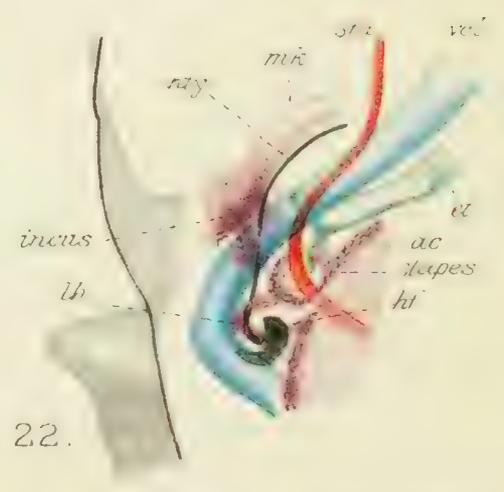
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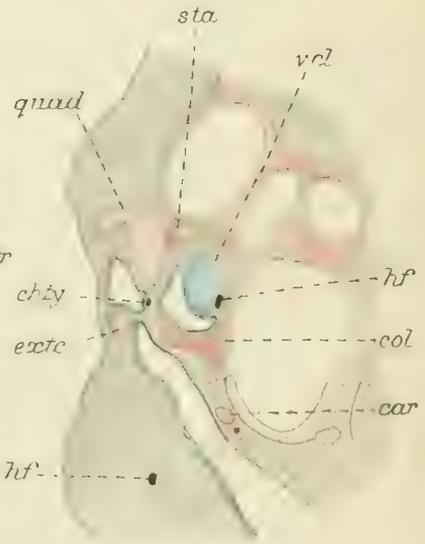
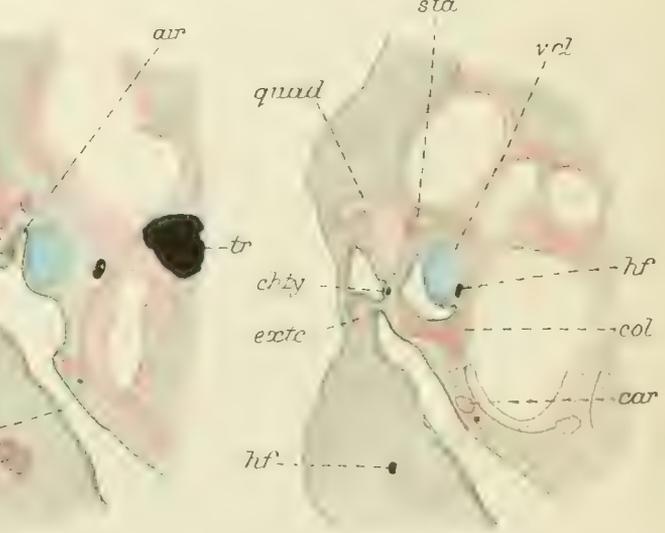
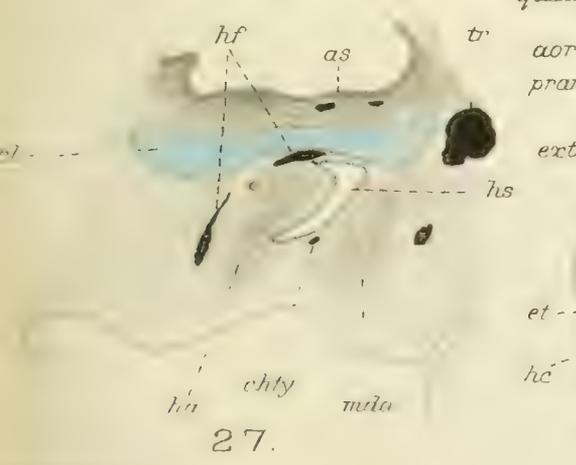
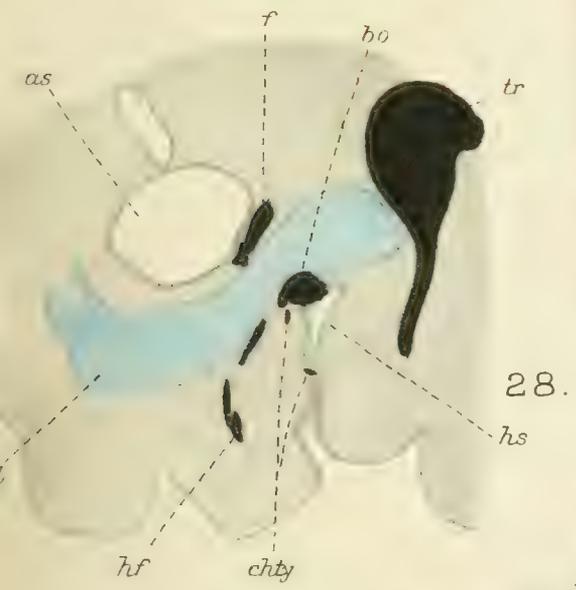
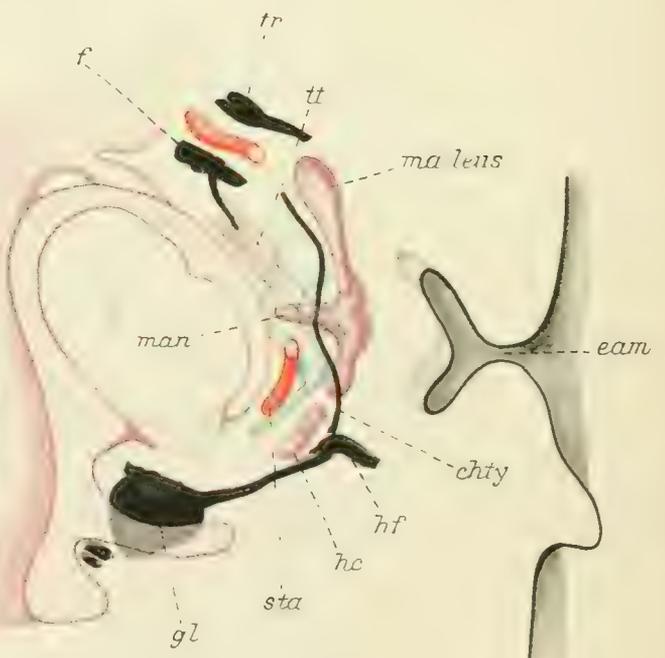
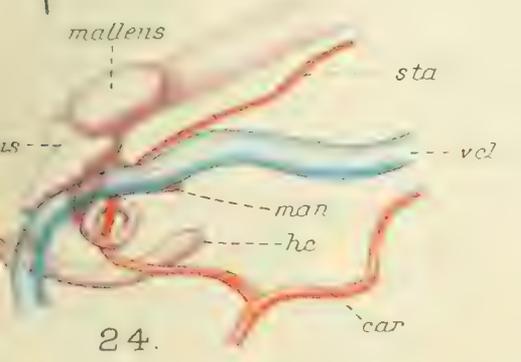
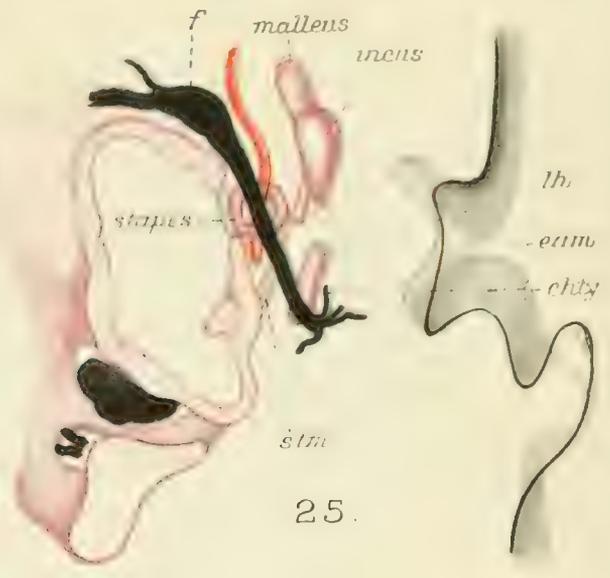


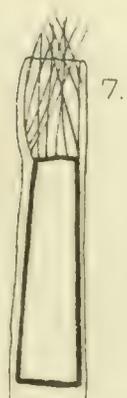
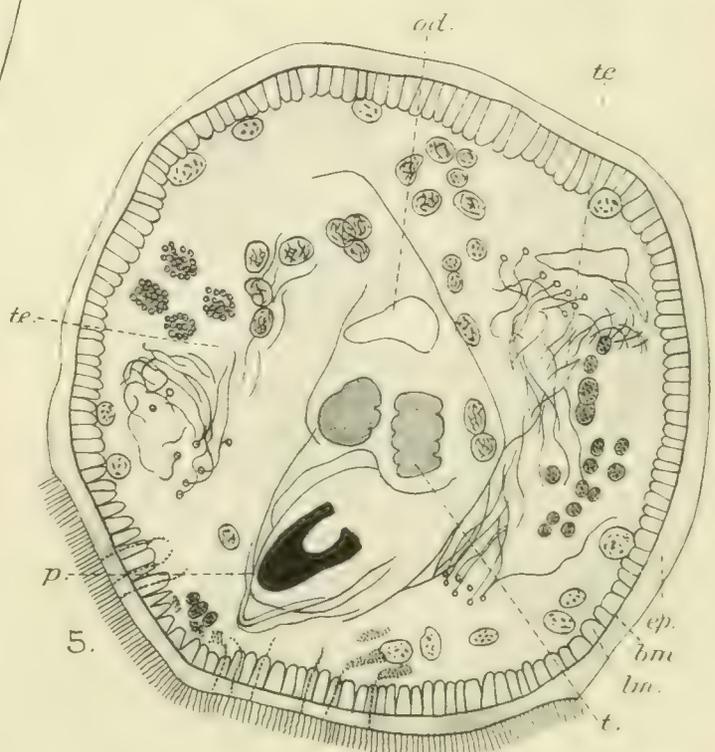
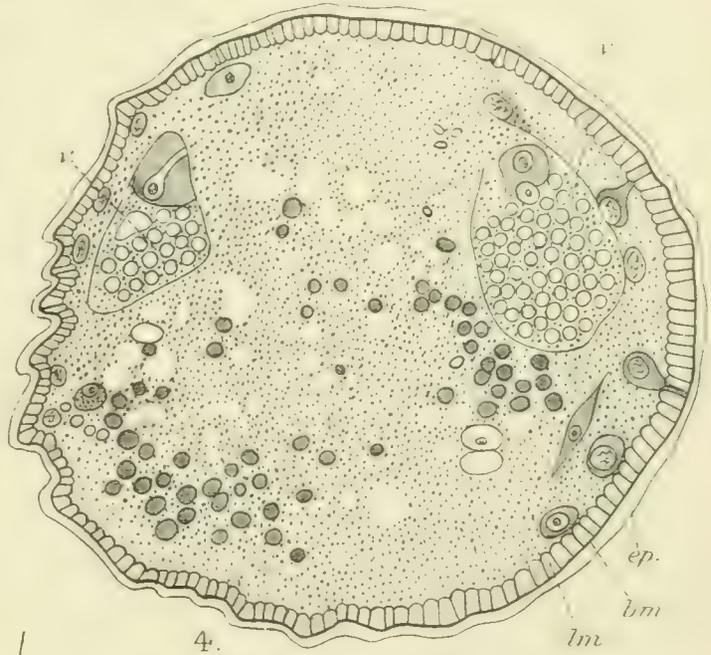
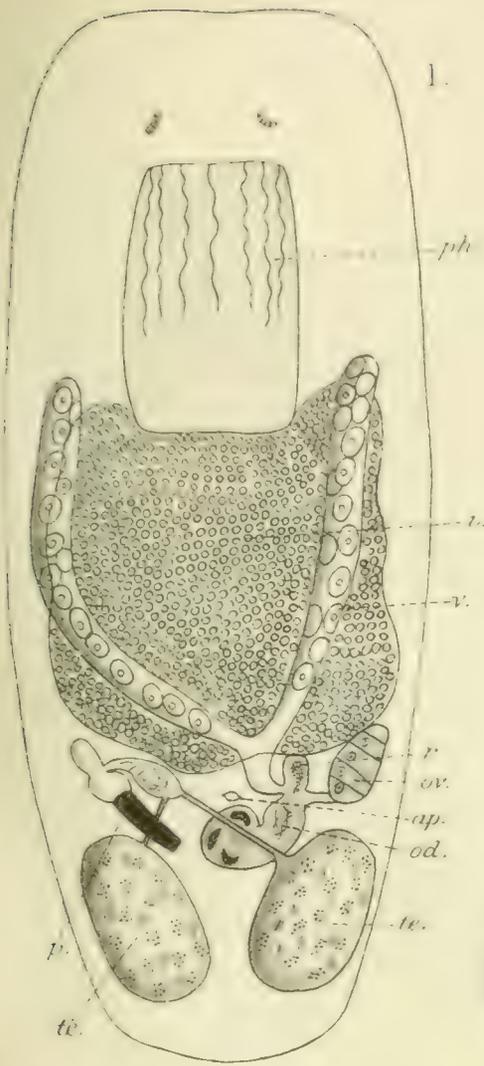
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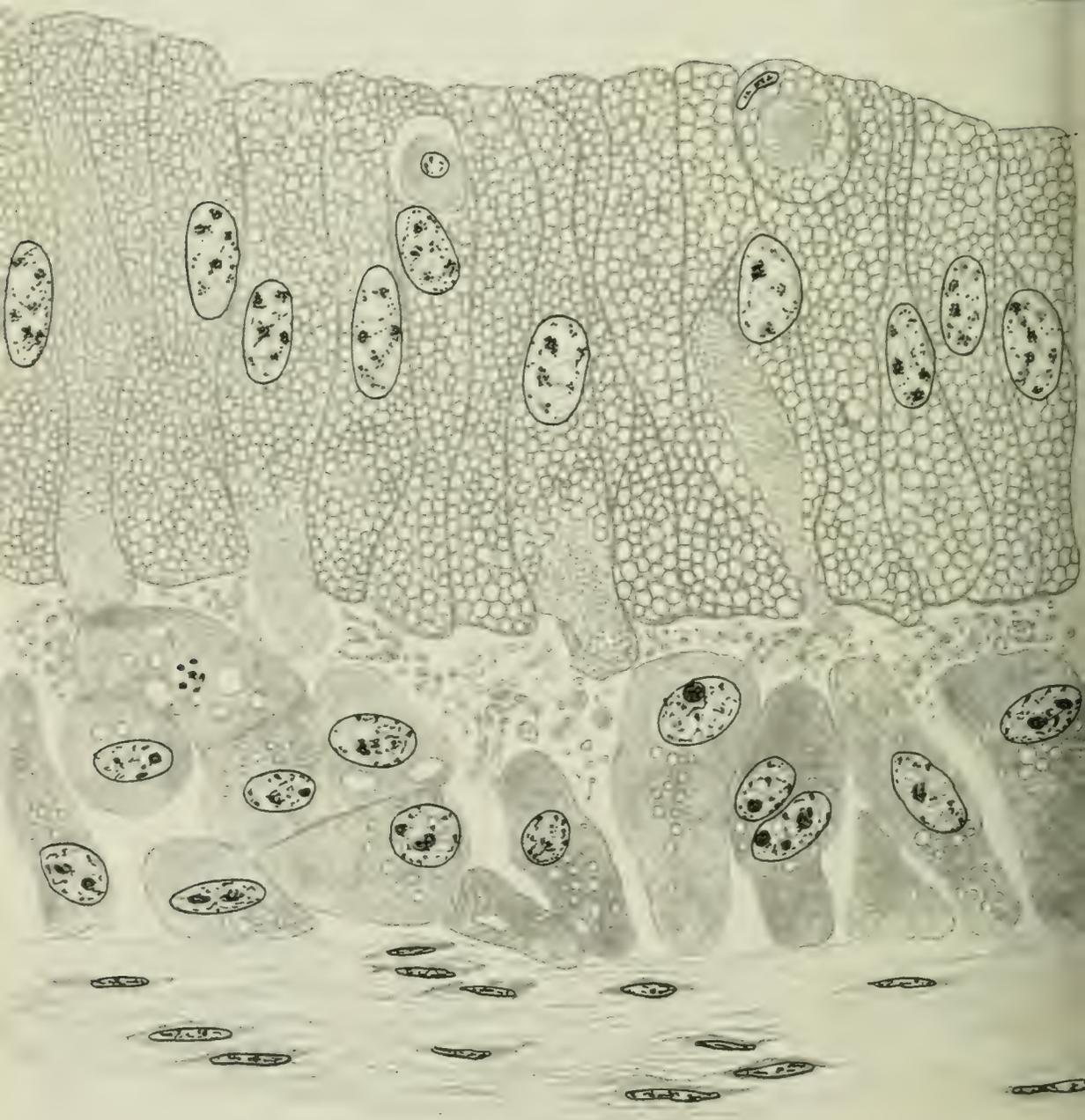
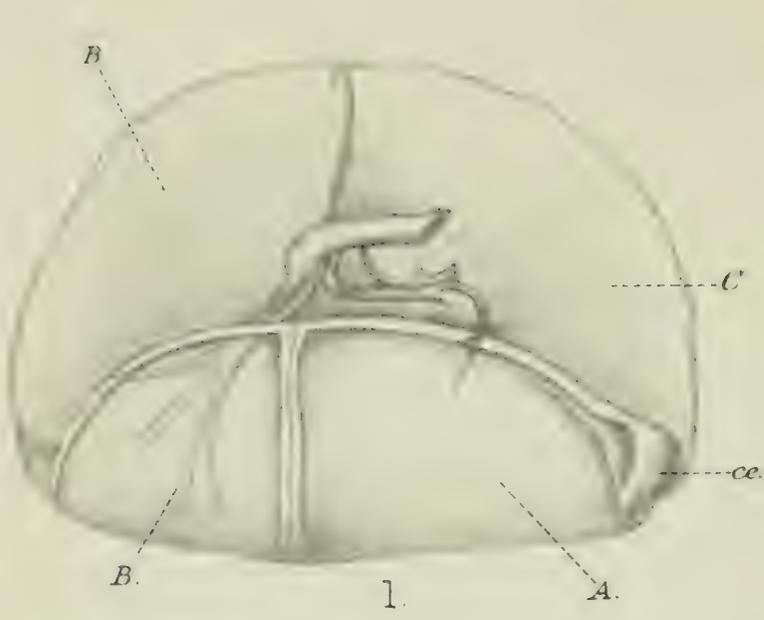
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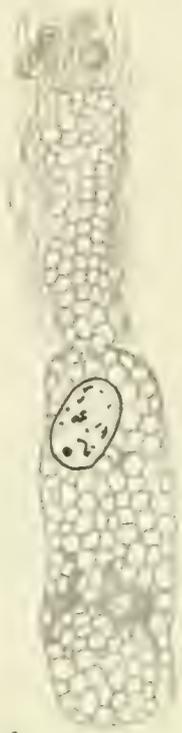
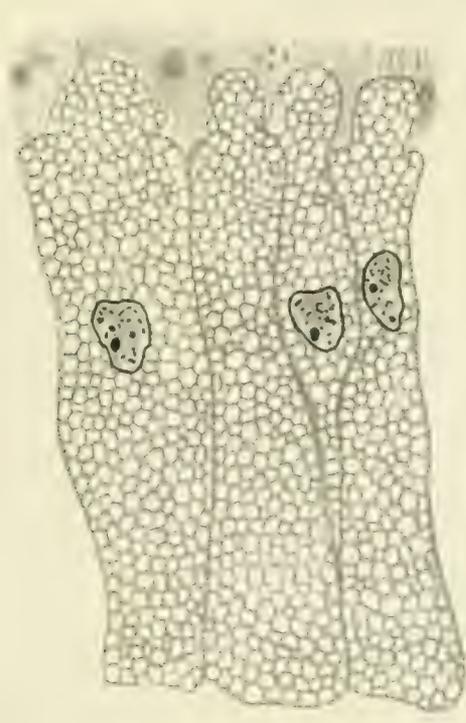




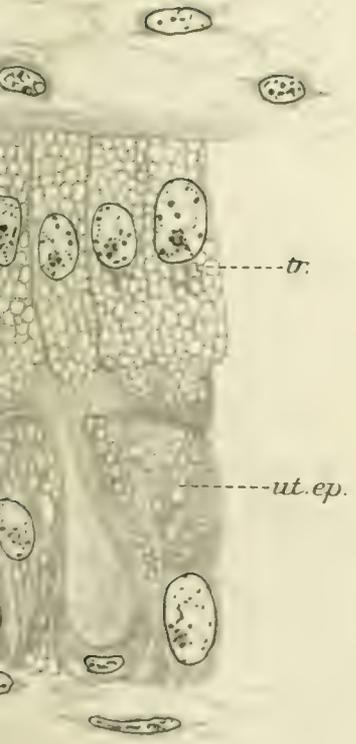
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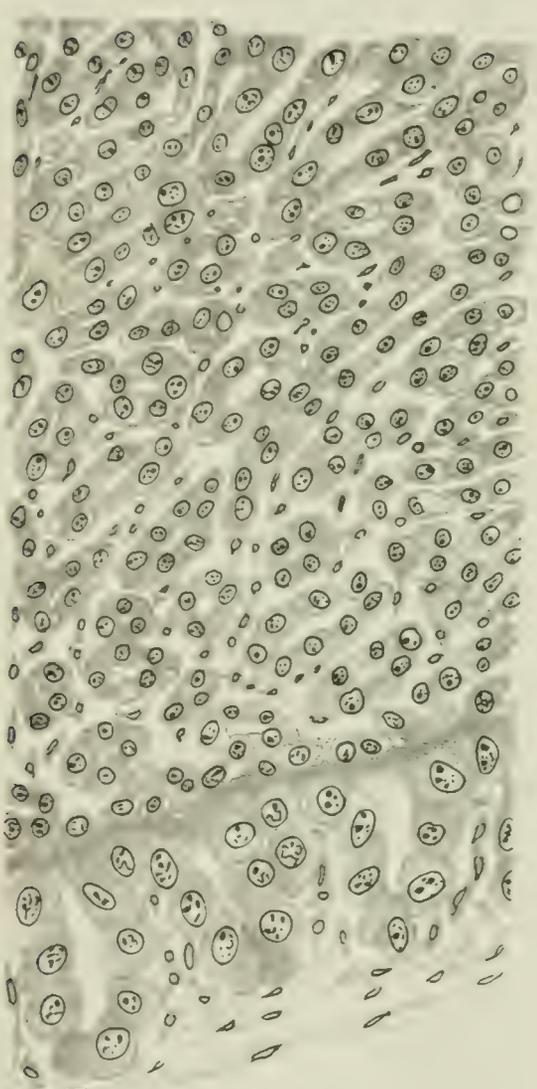
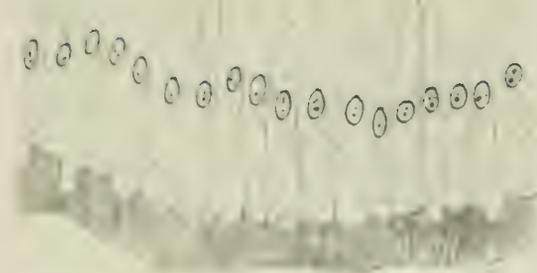
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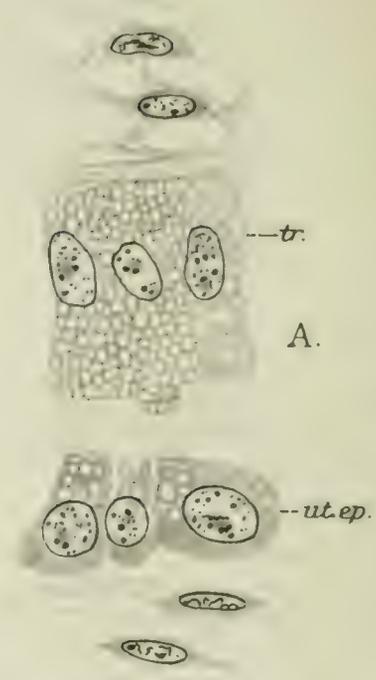


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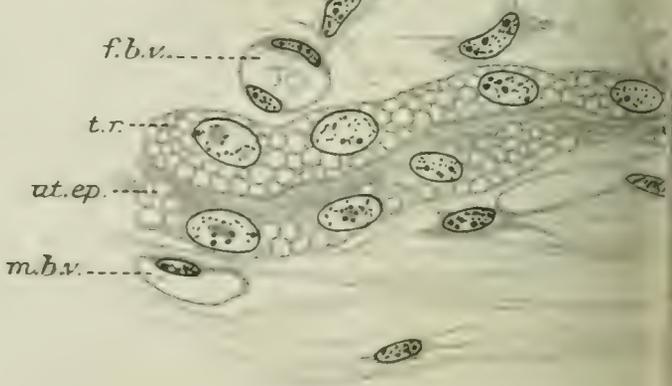
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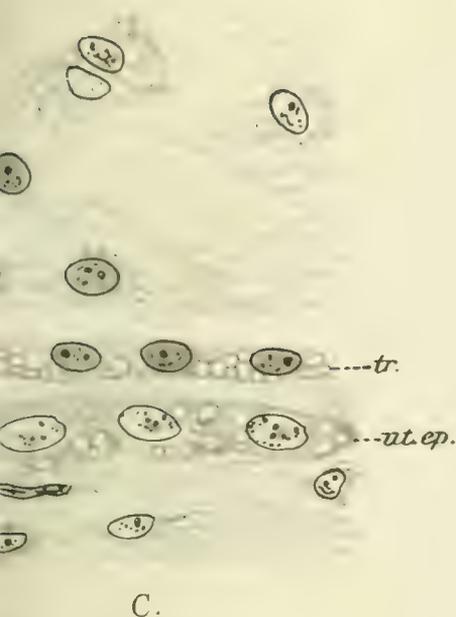


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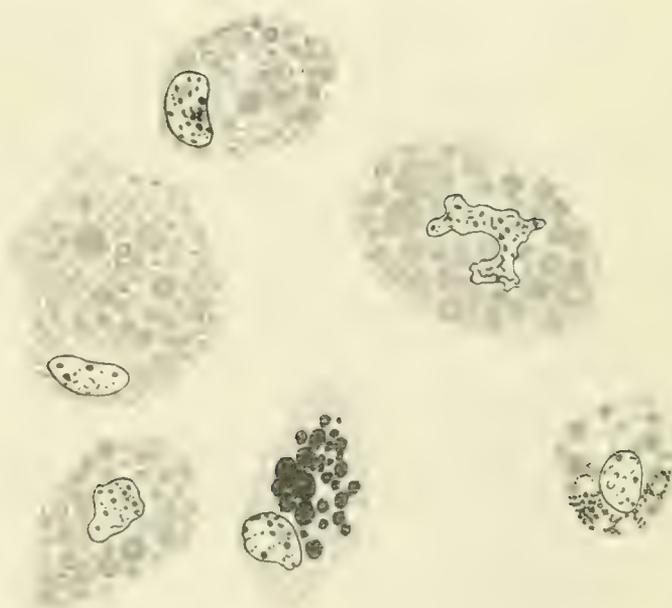


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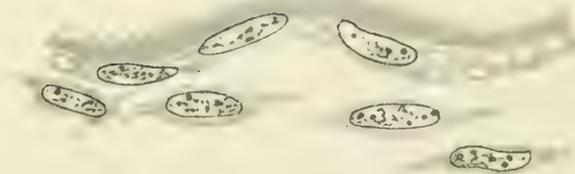




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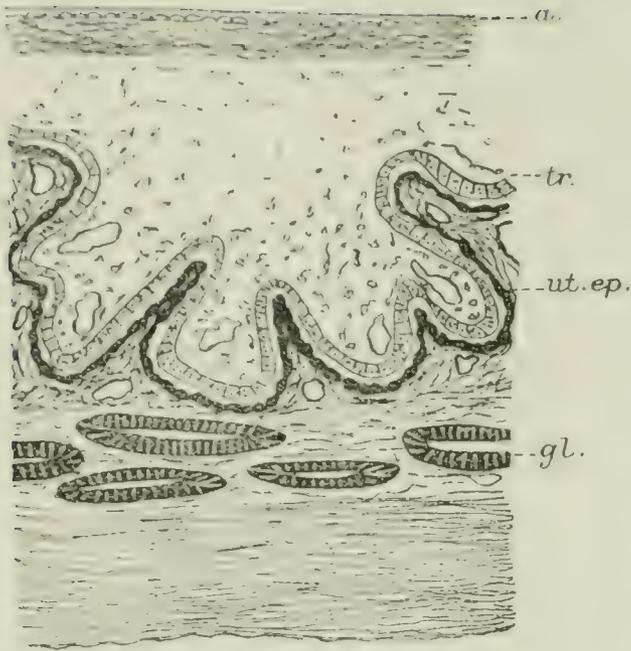
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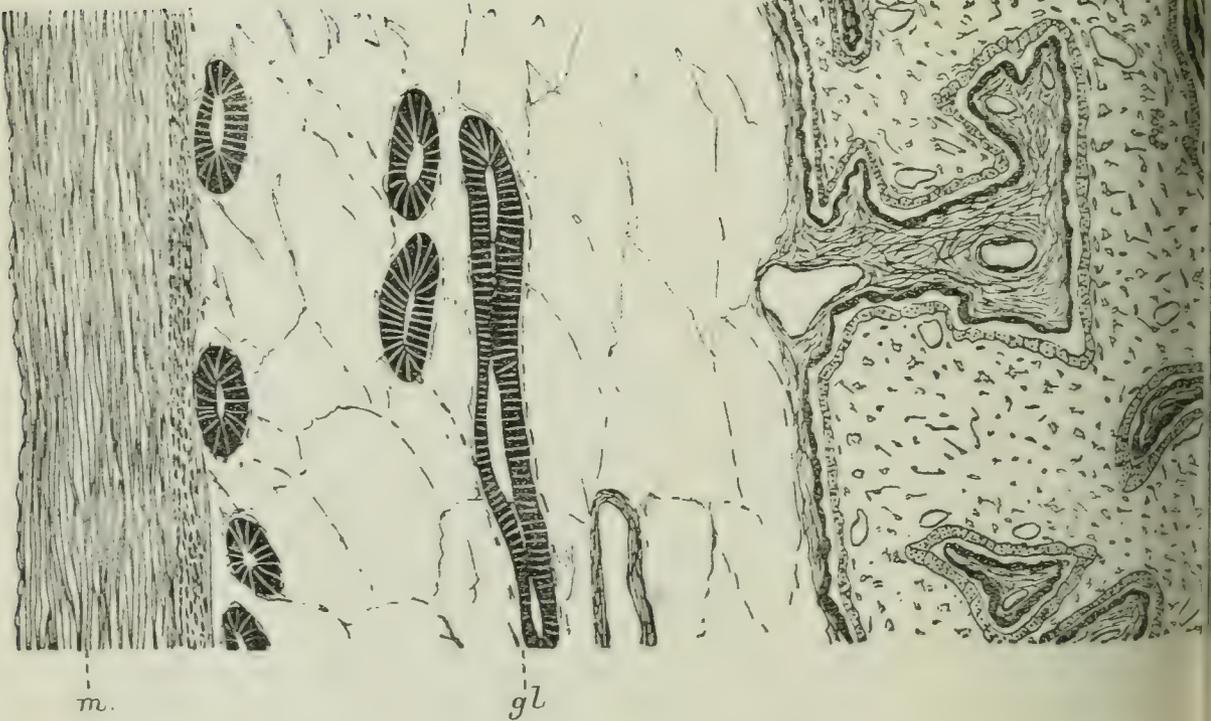
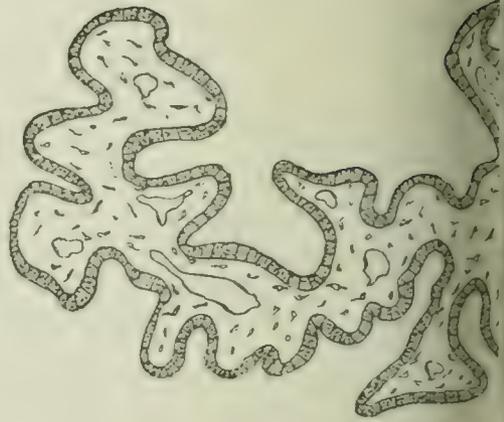
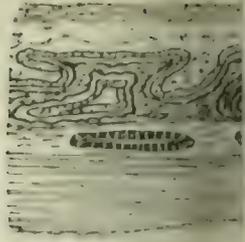
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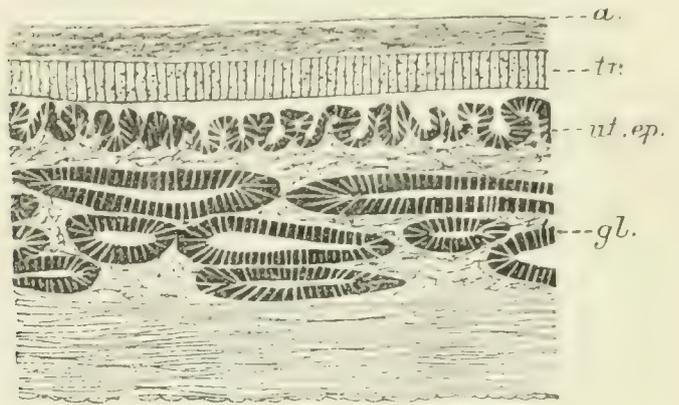


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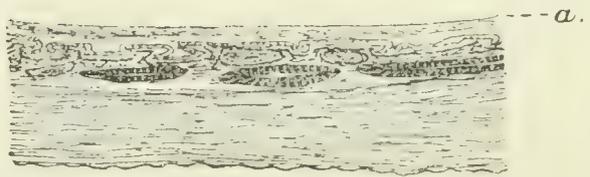
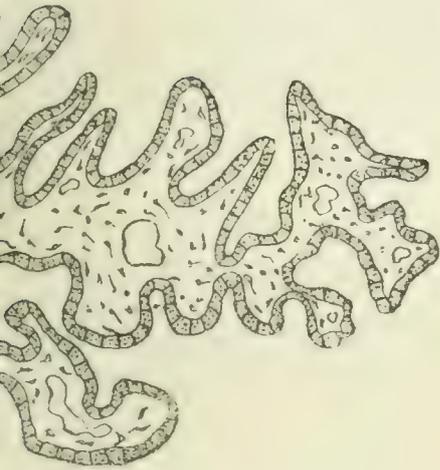


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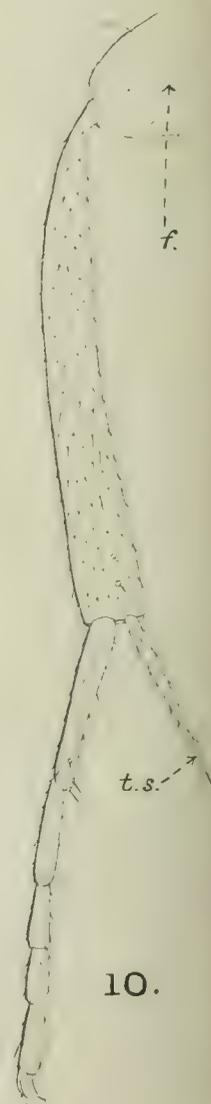
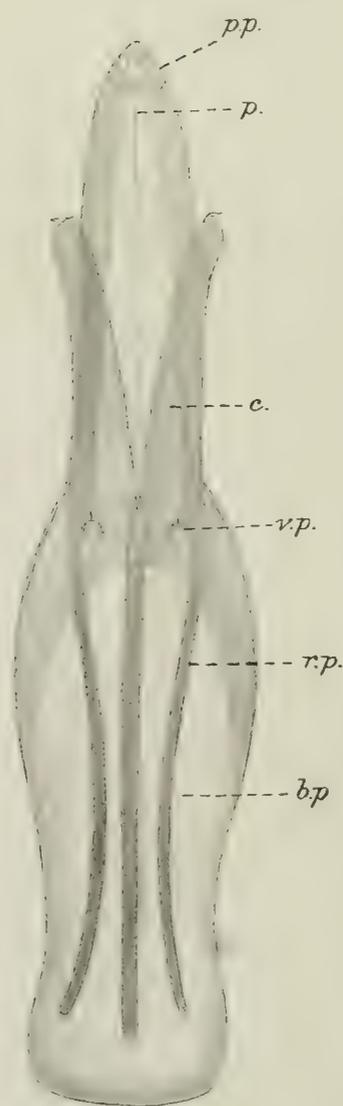
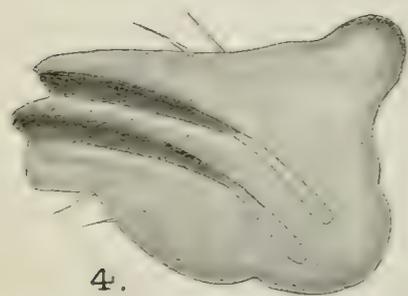
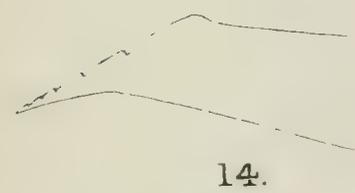
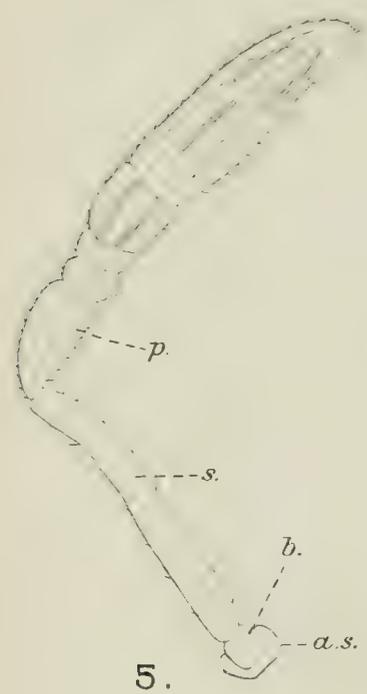
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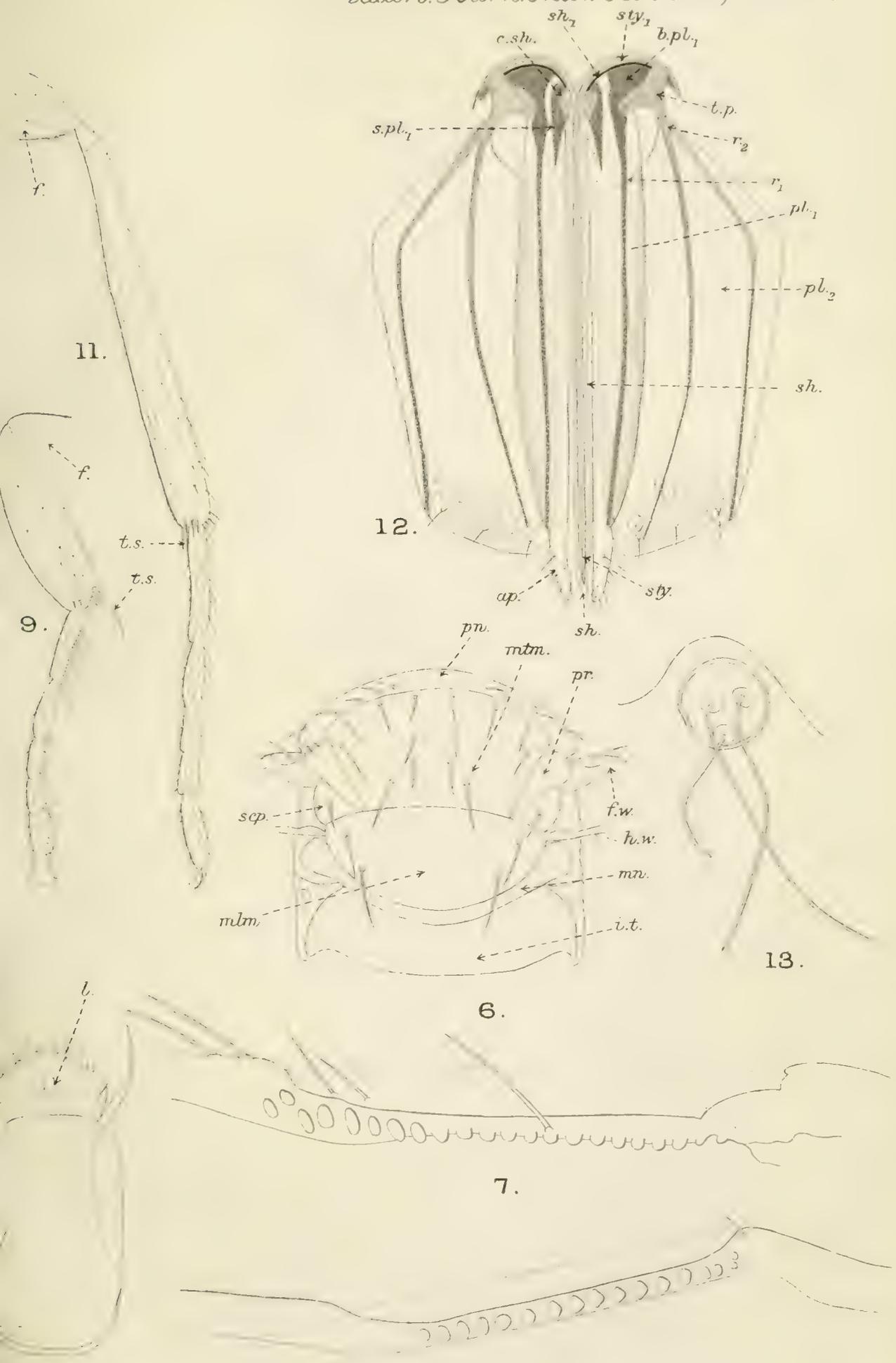




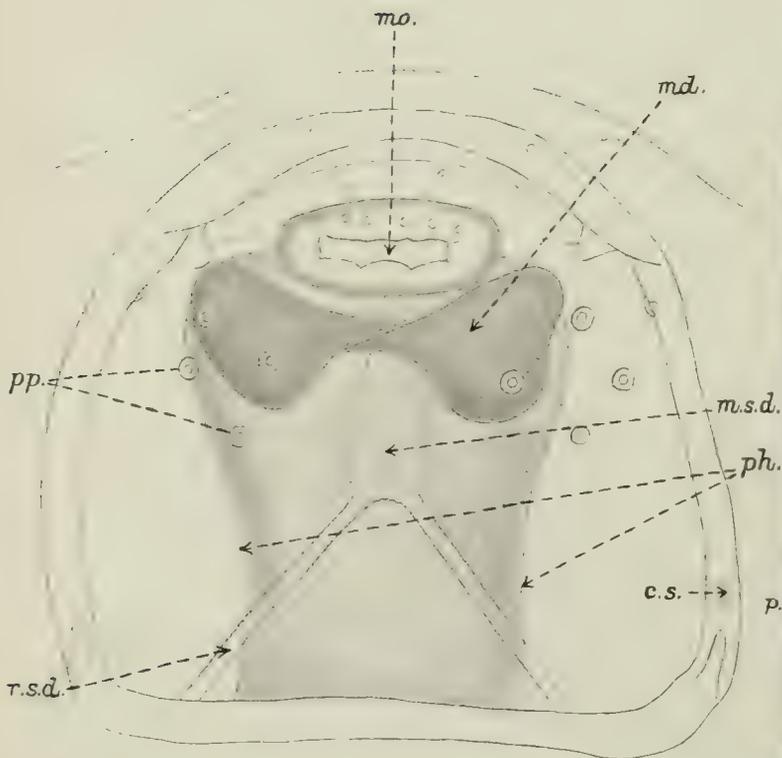
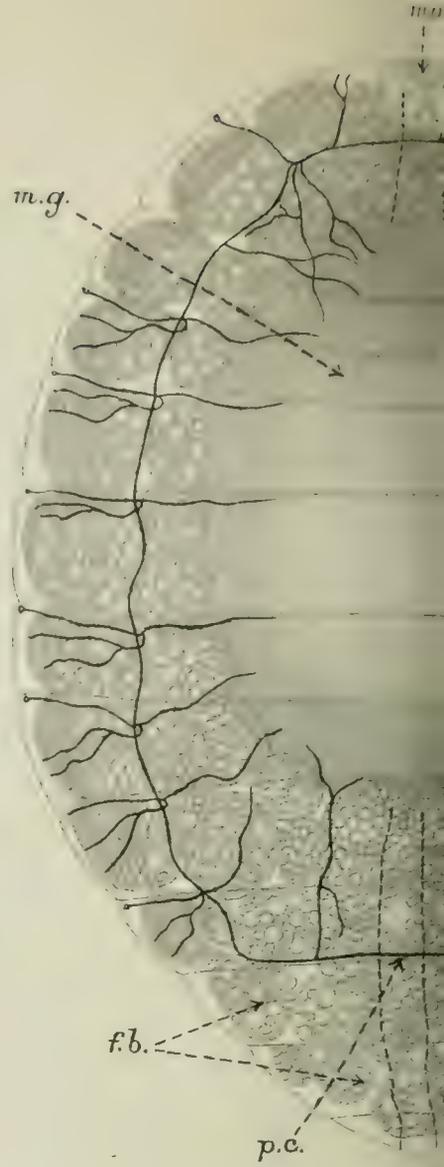
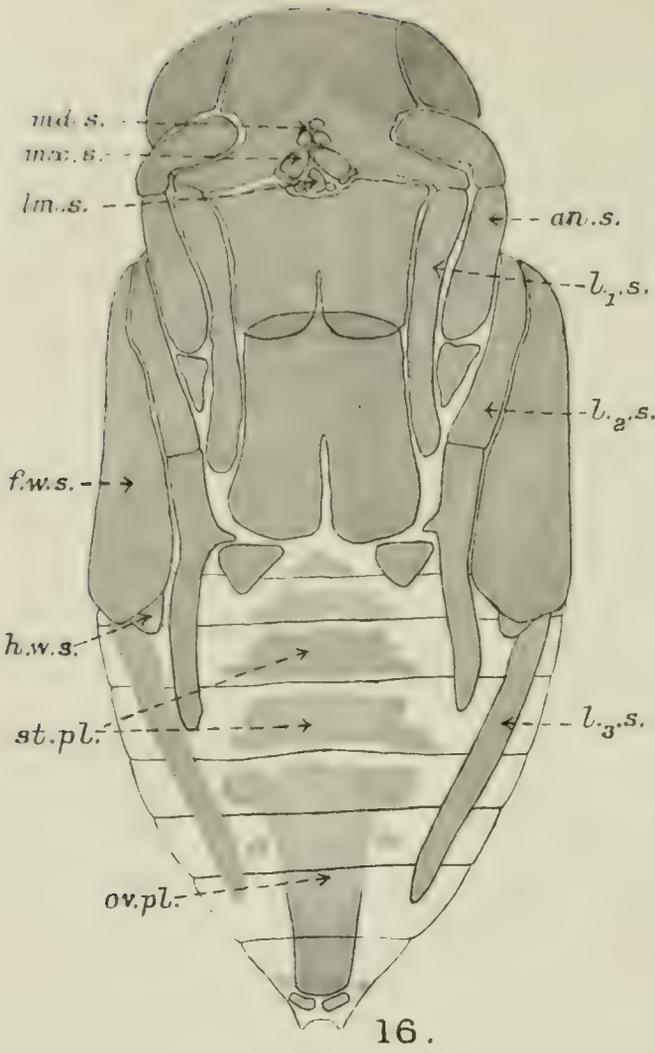
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Huth, London.

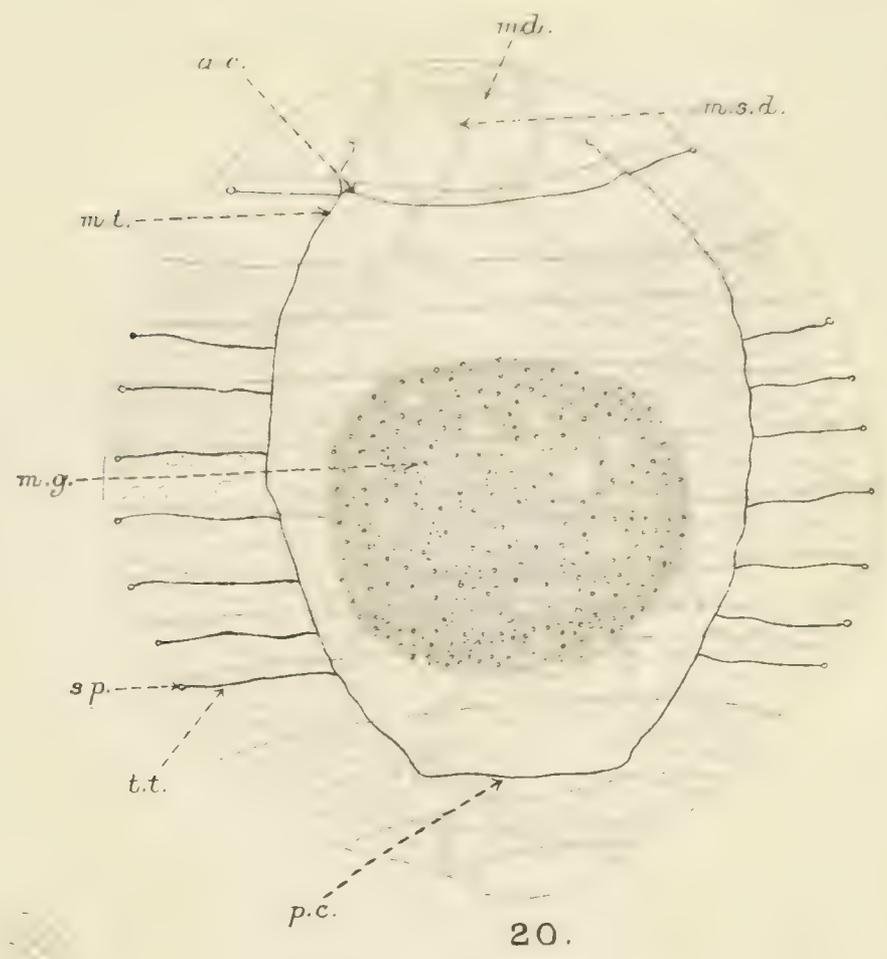
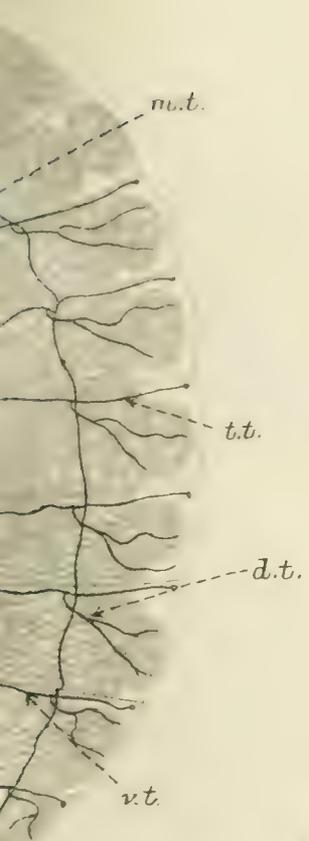


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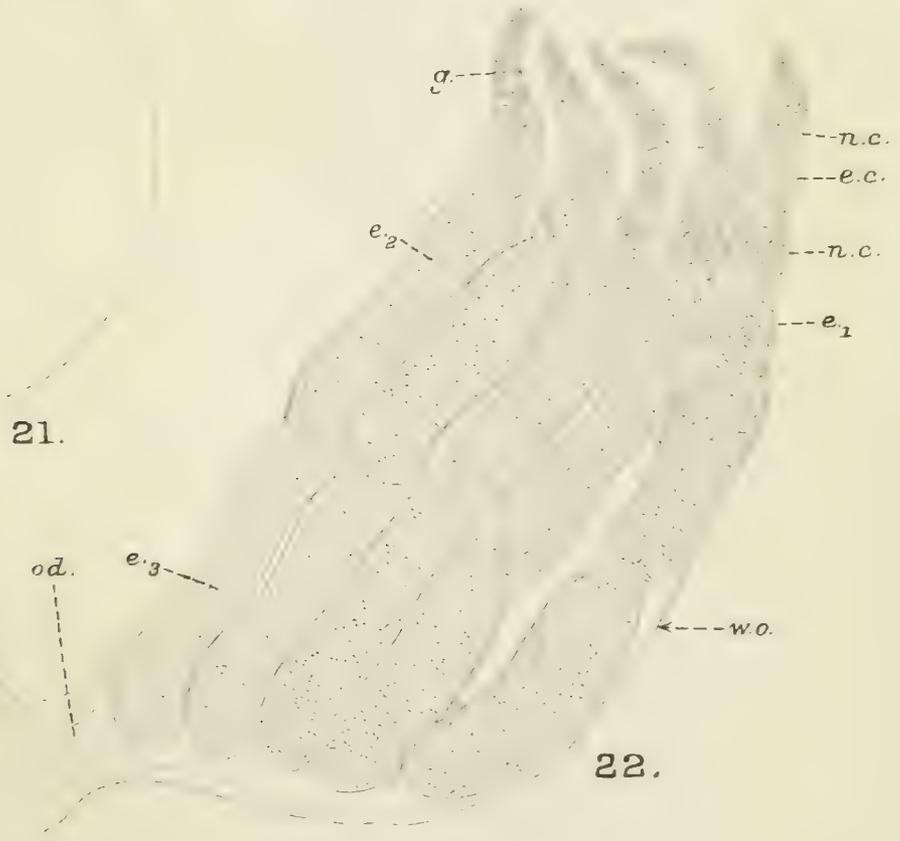
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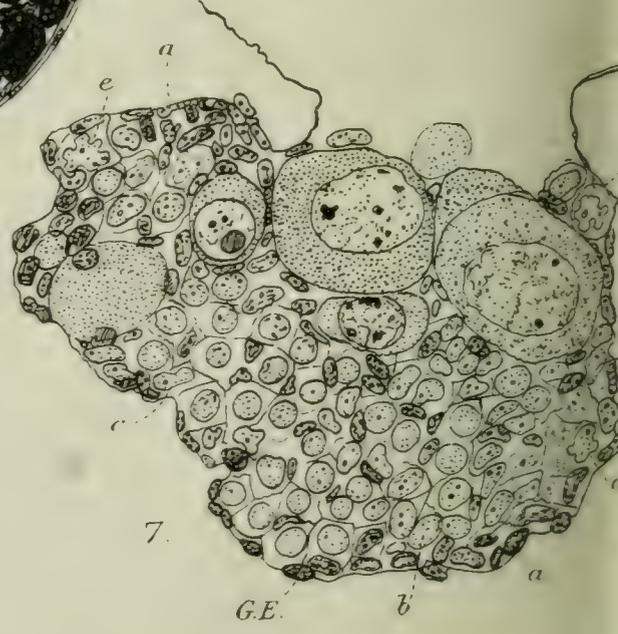
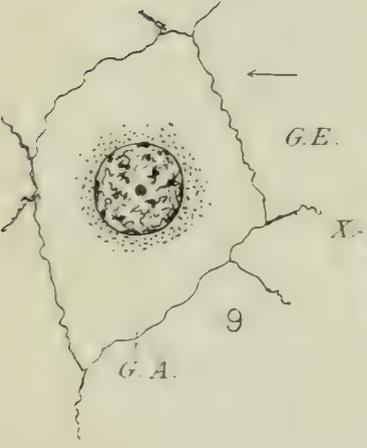
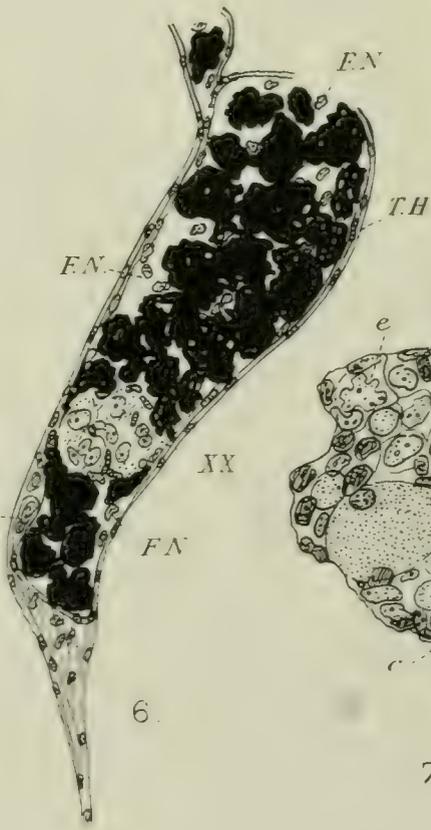
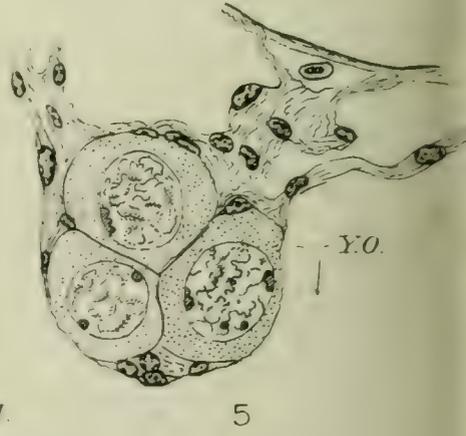
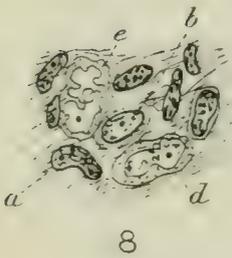
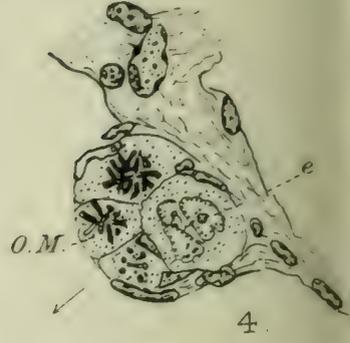
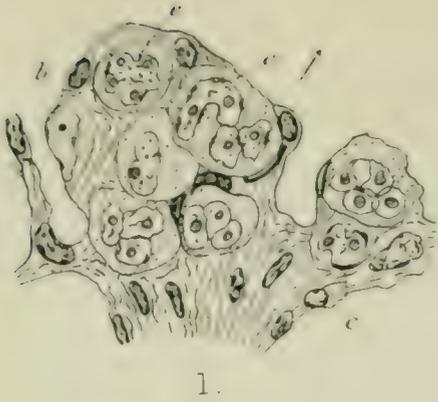


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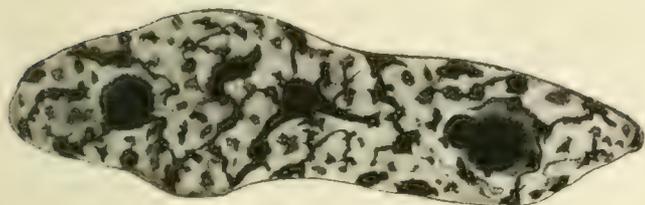




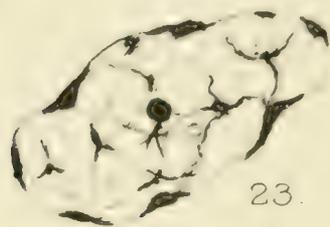
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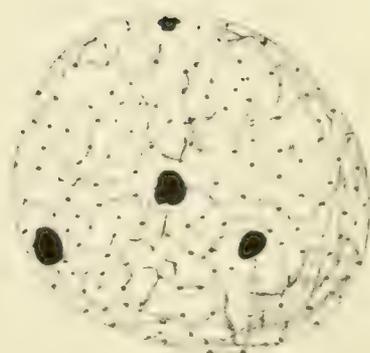
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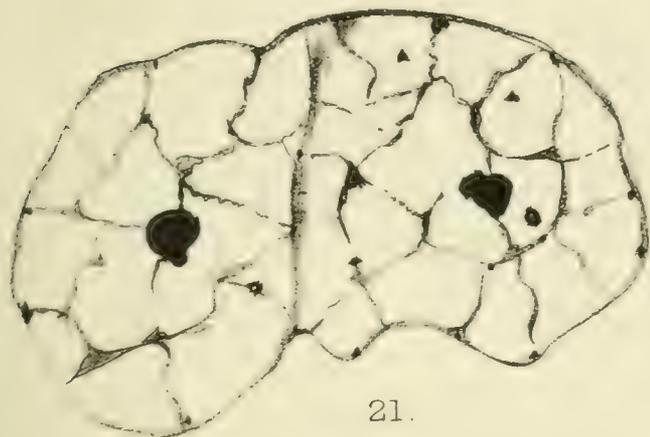
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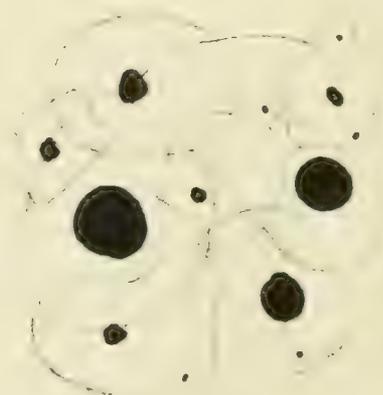
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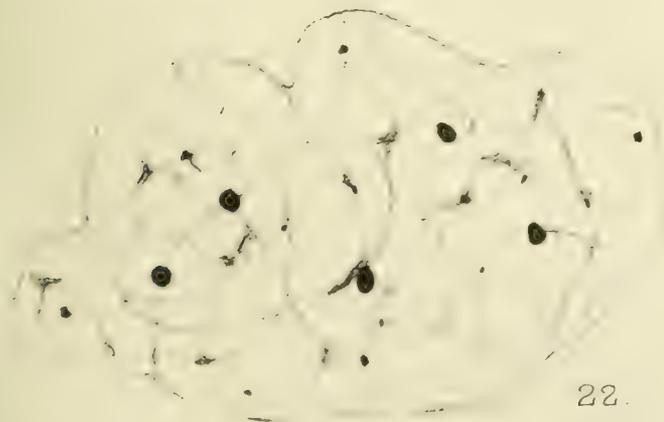
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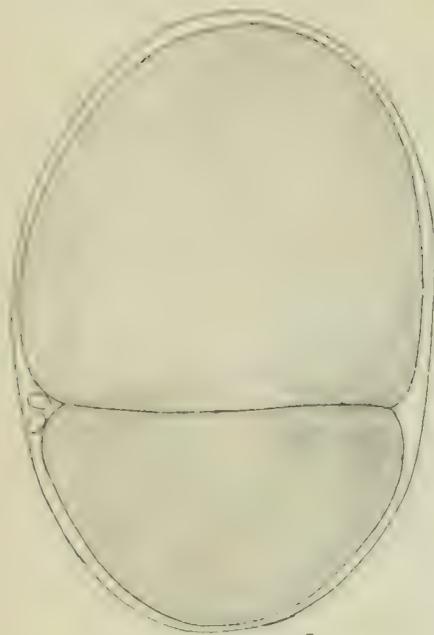
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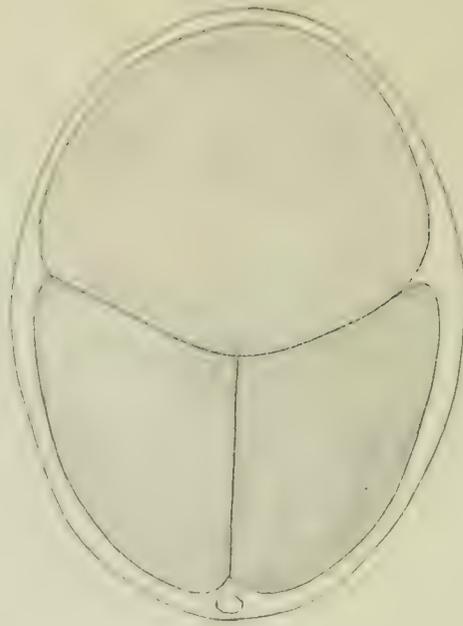
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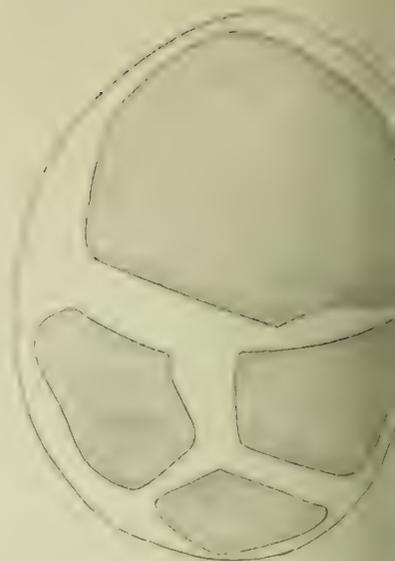
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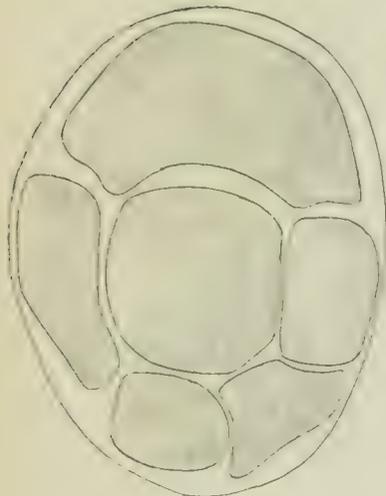
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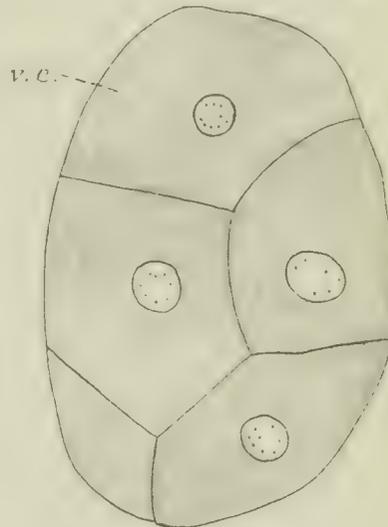
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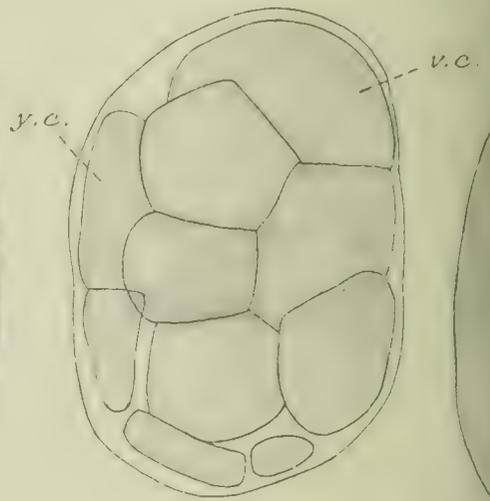
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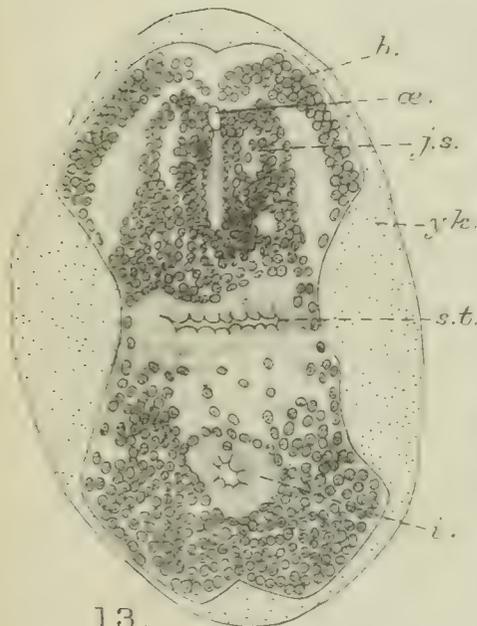
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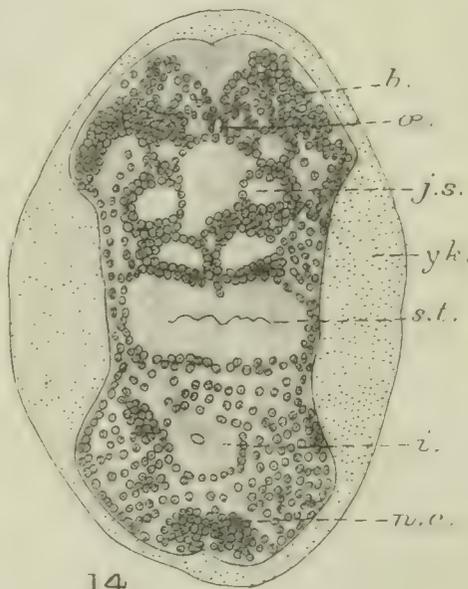
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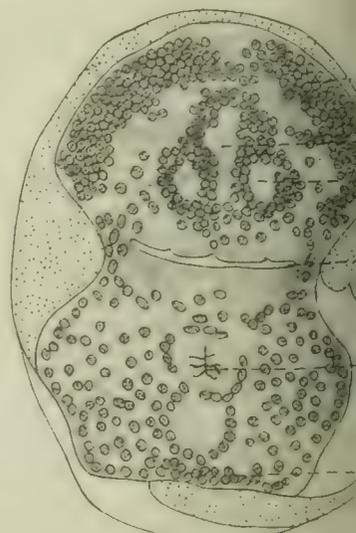
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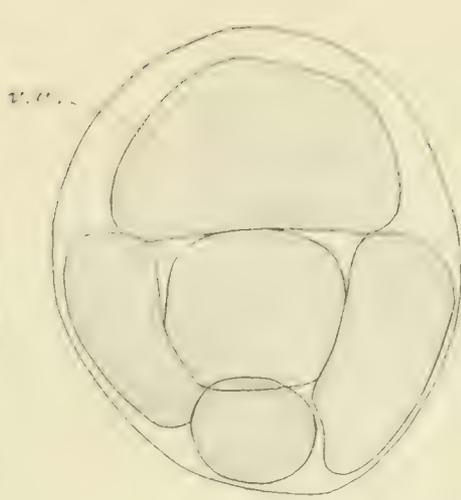
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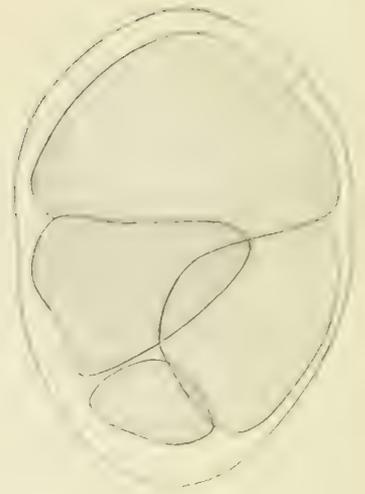
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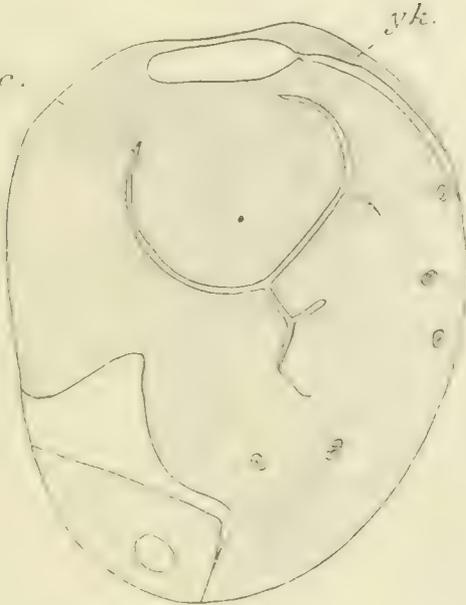
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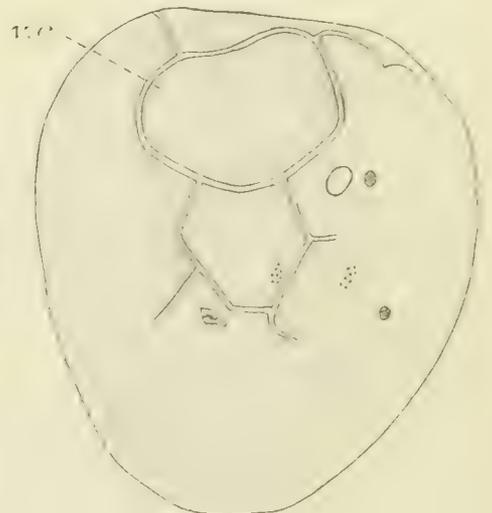
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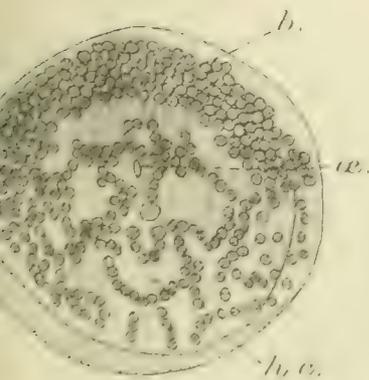
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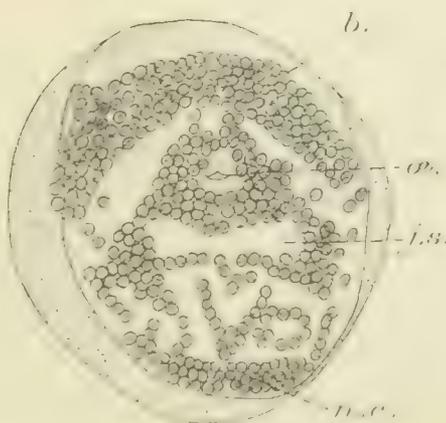
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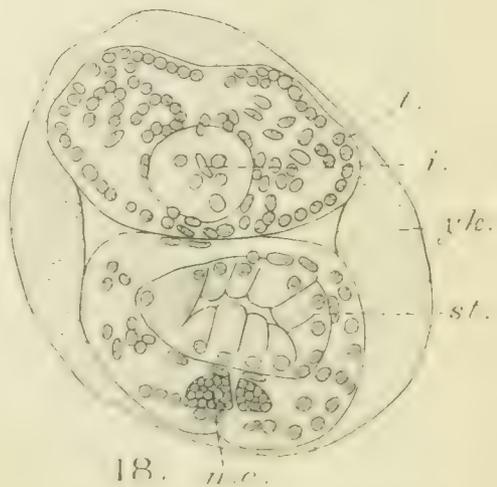
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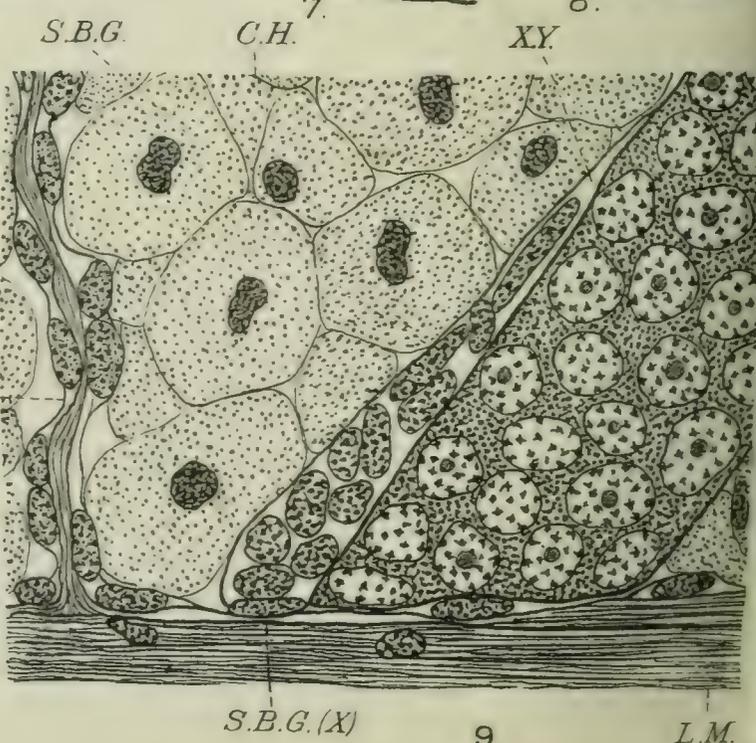
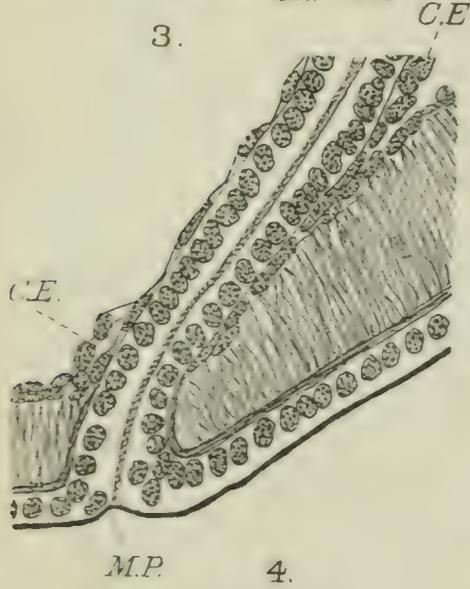
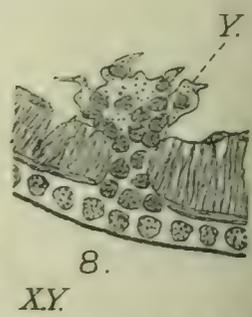
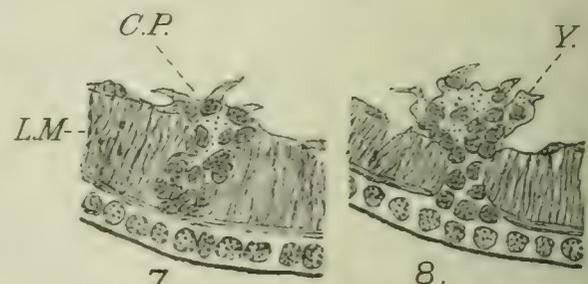
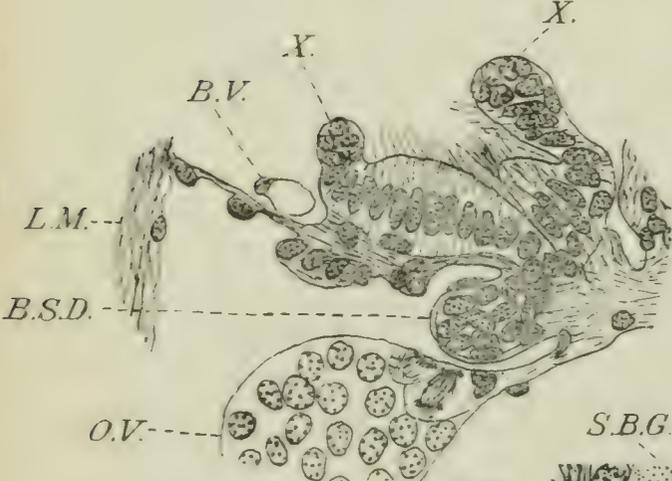
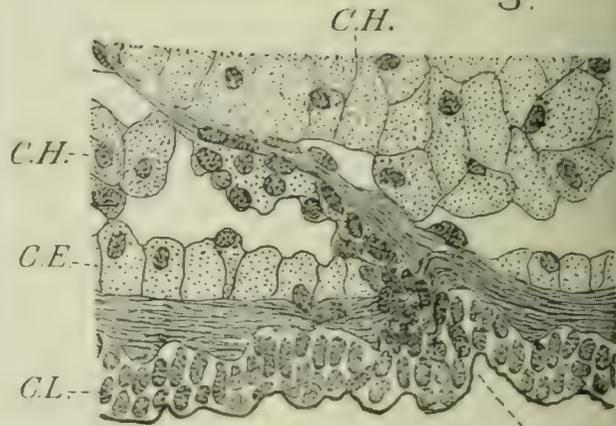
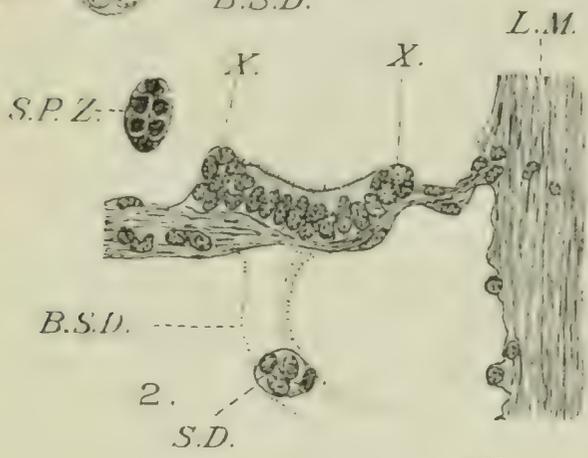
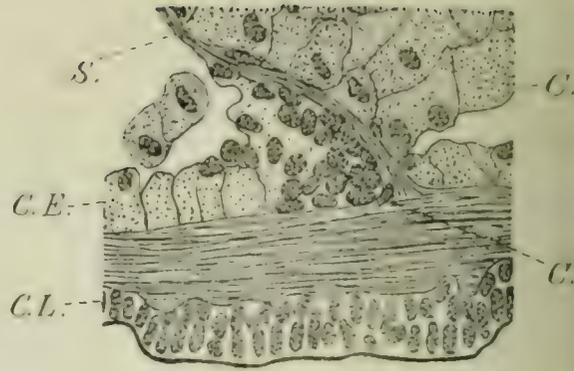
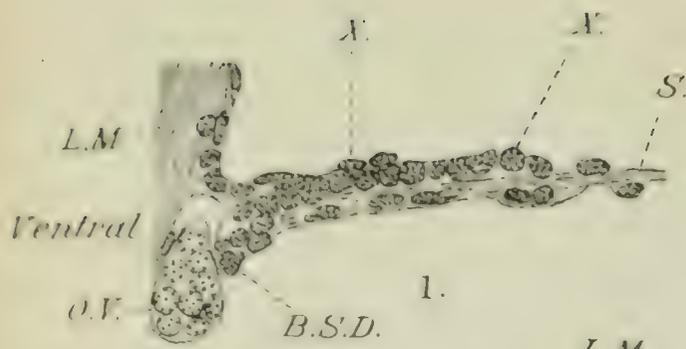


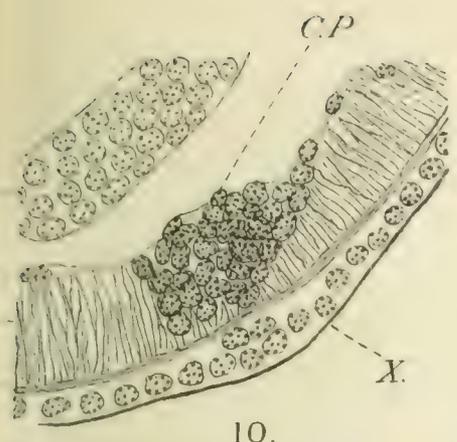
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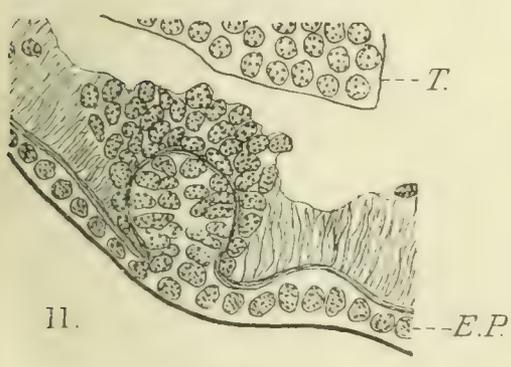
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Huth, Lith^r London

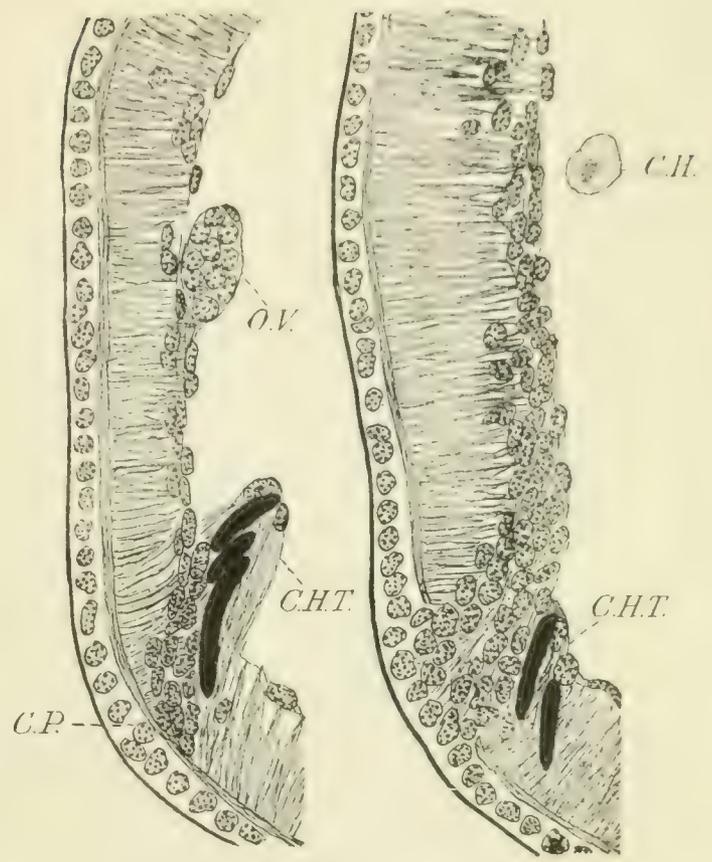




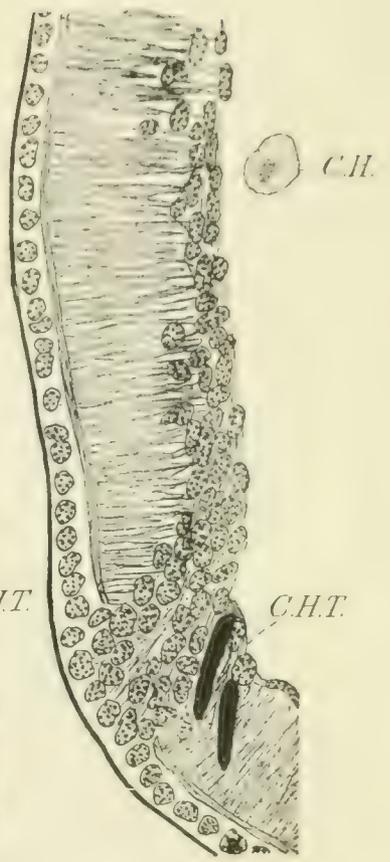
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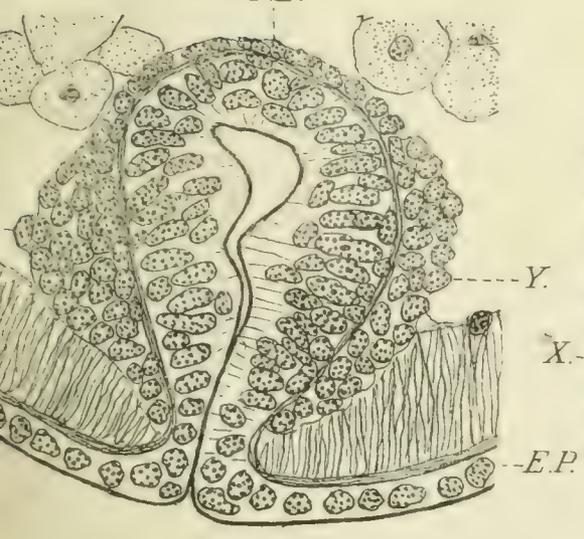
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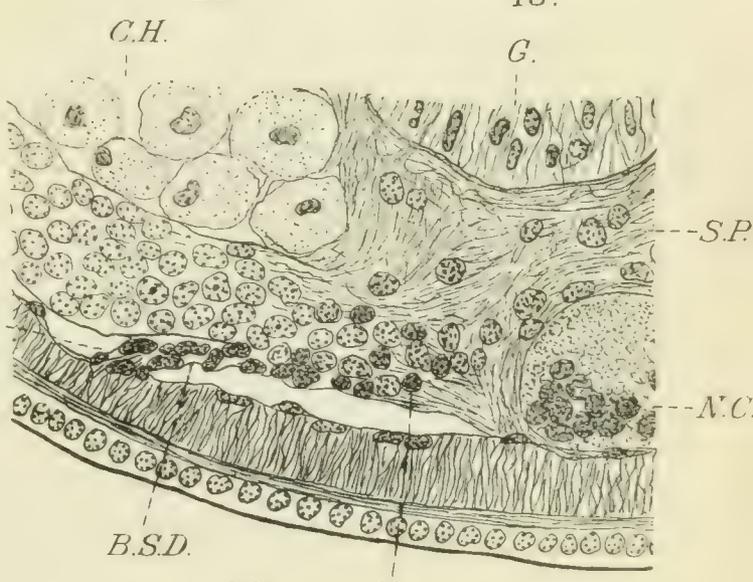
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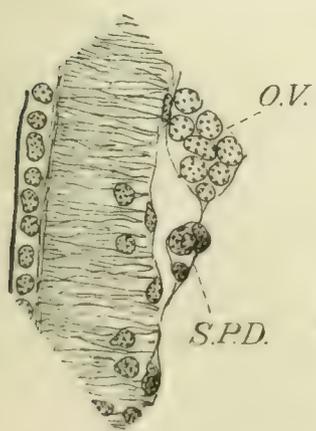
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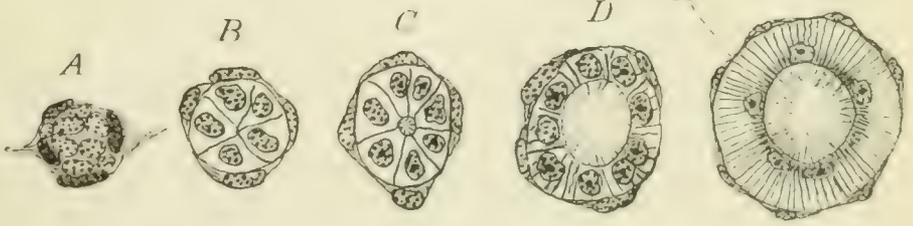
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13.



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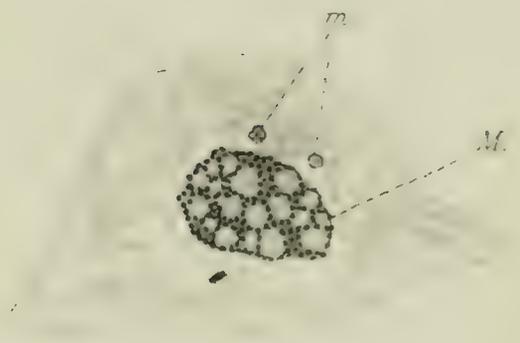
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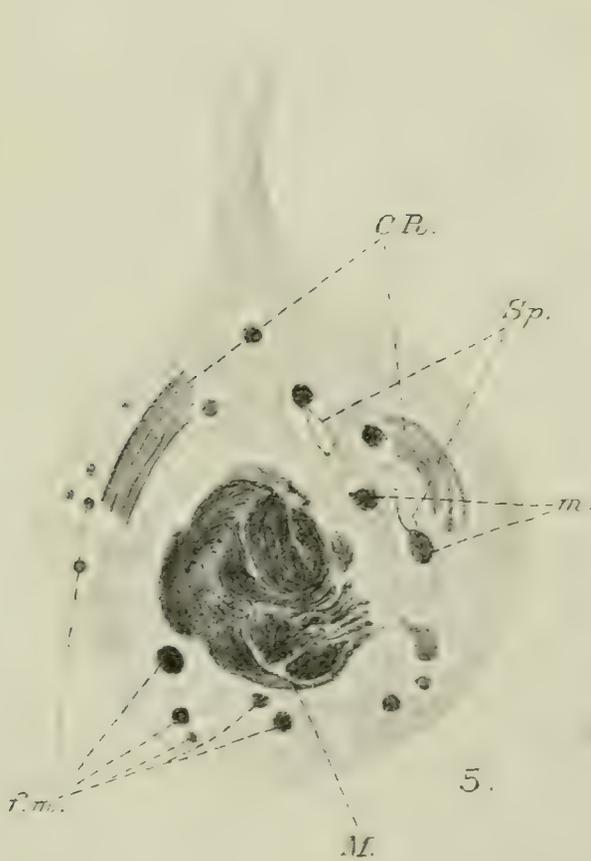
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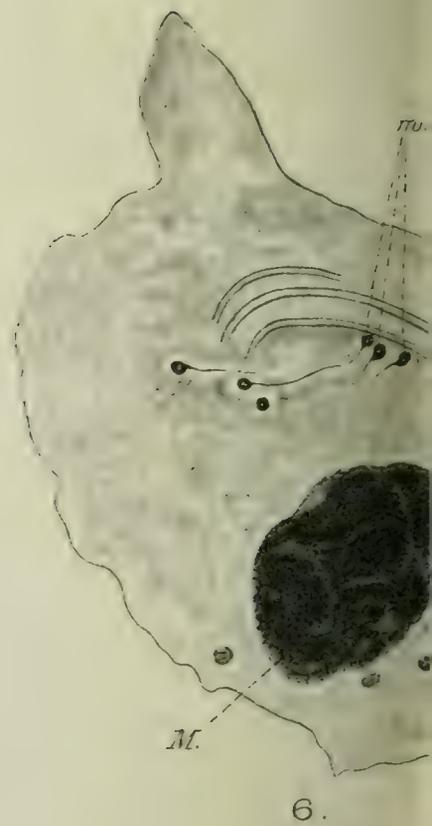
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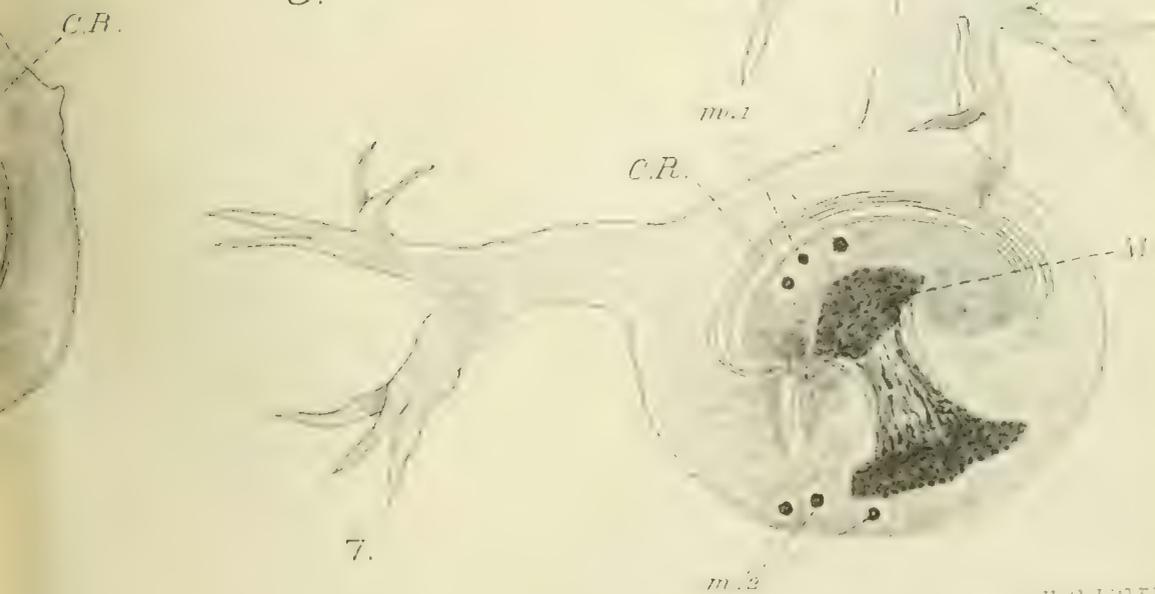
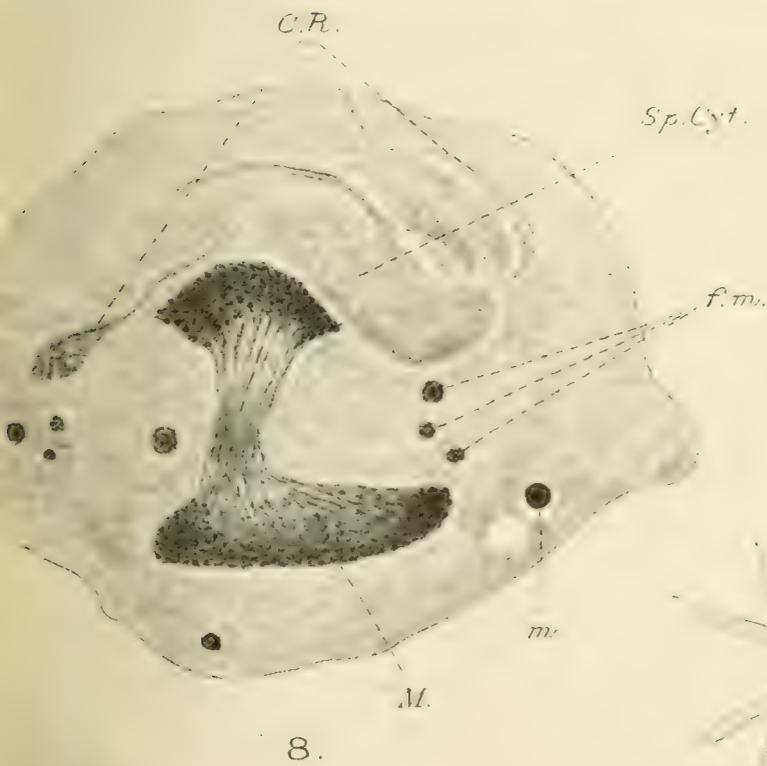
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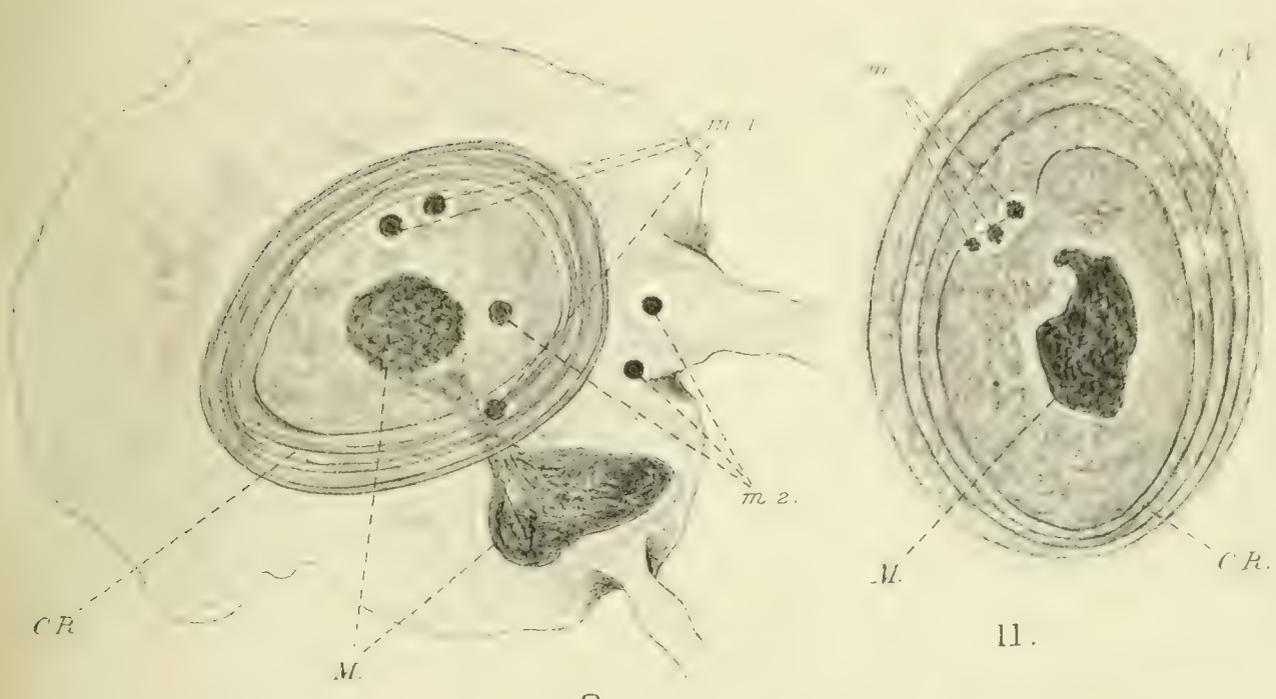
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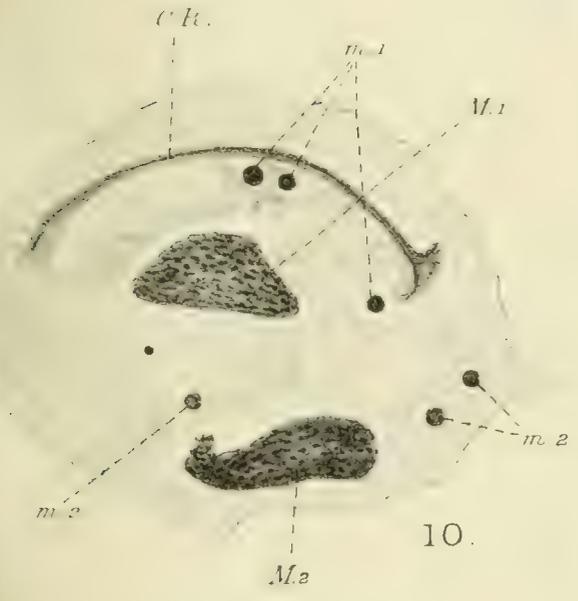


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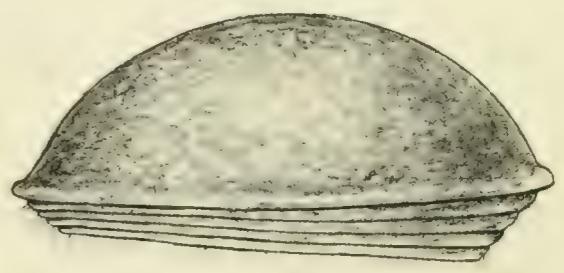


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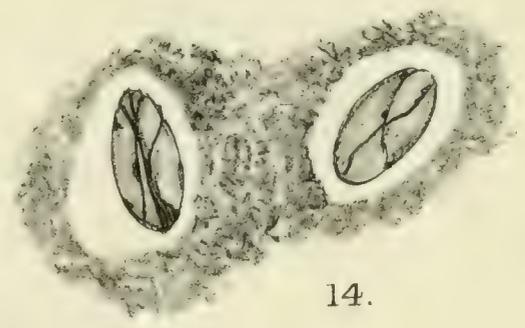
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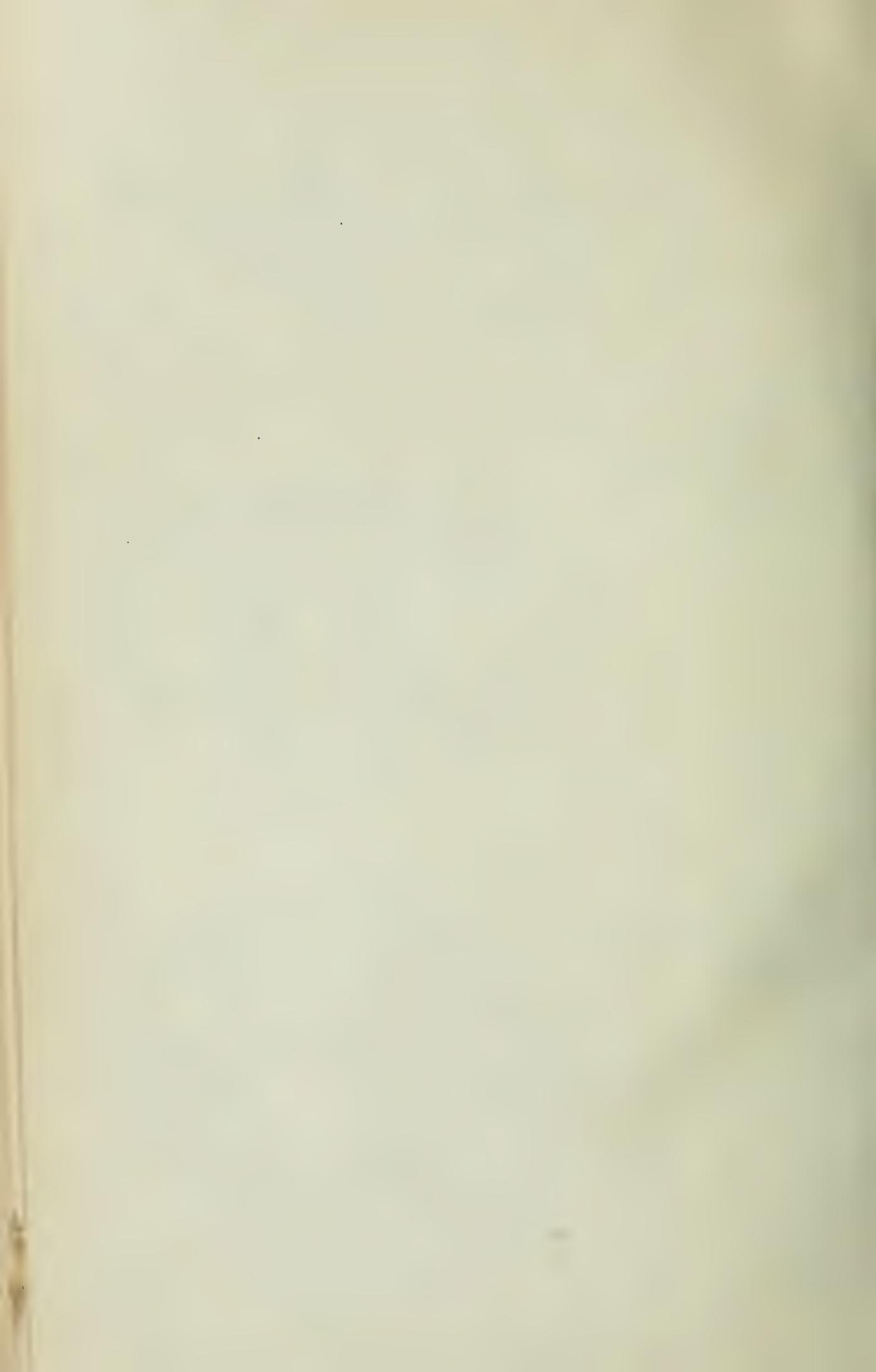
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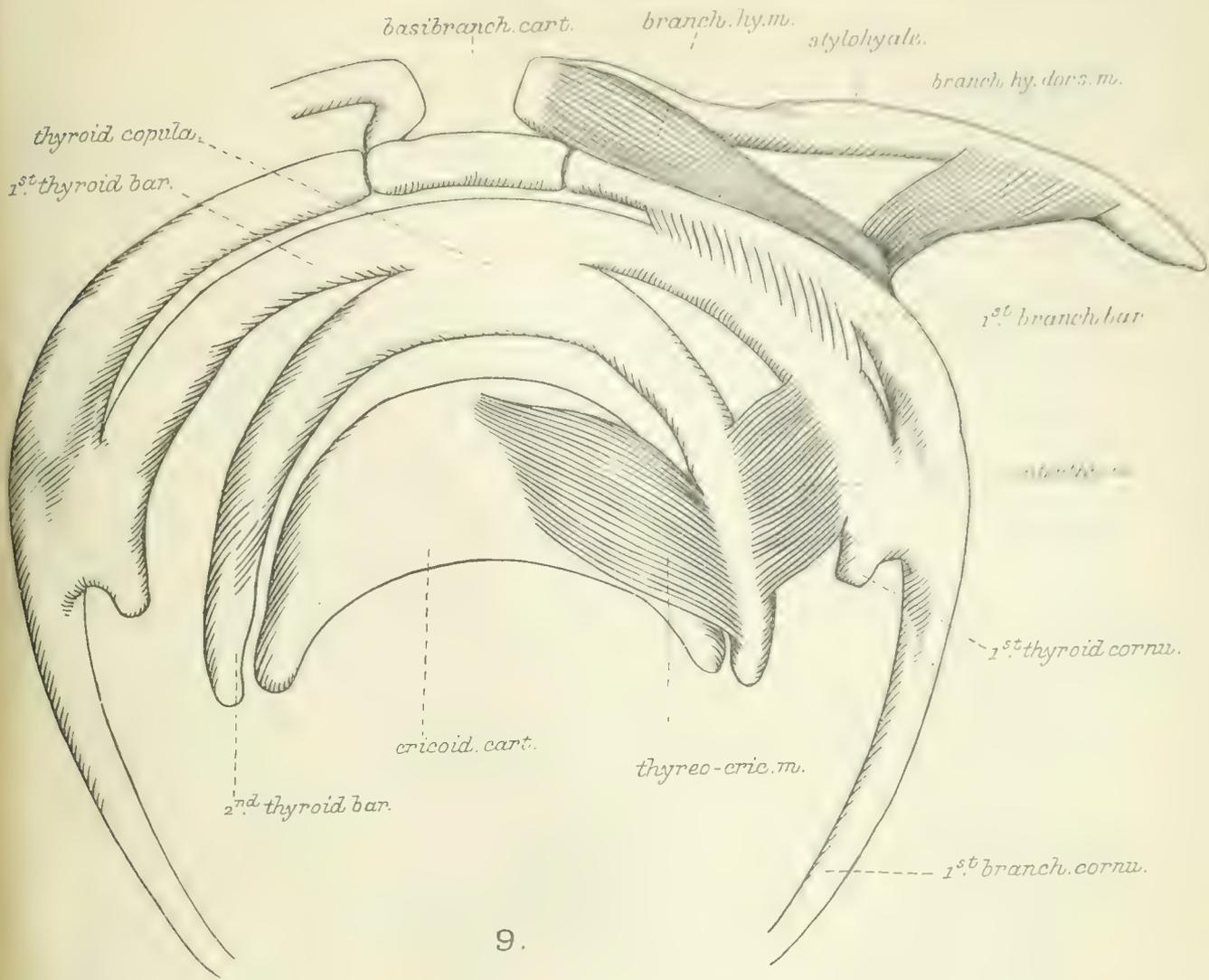


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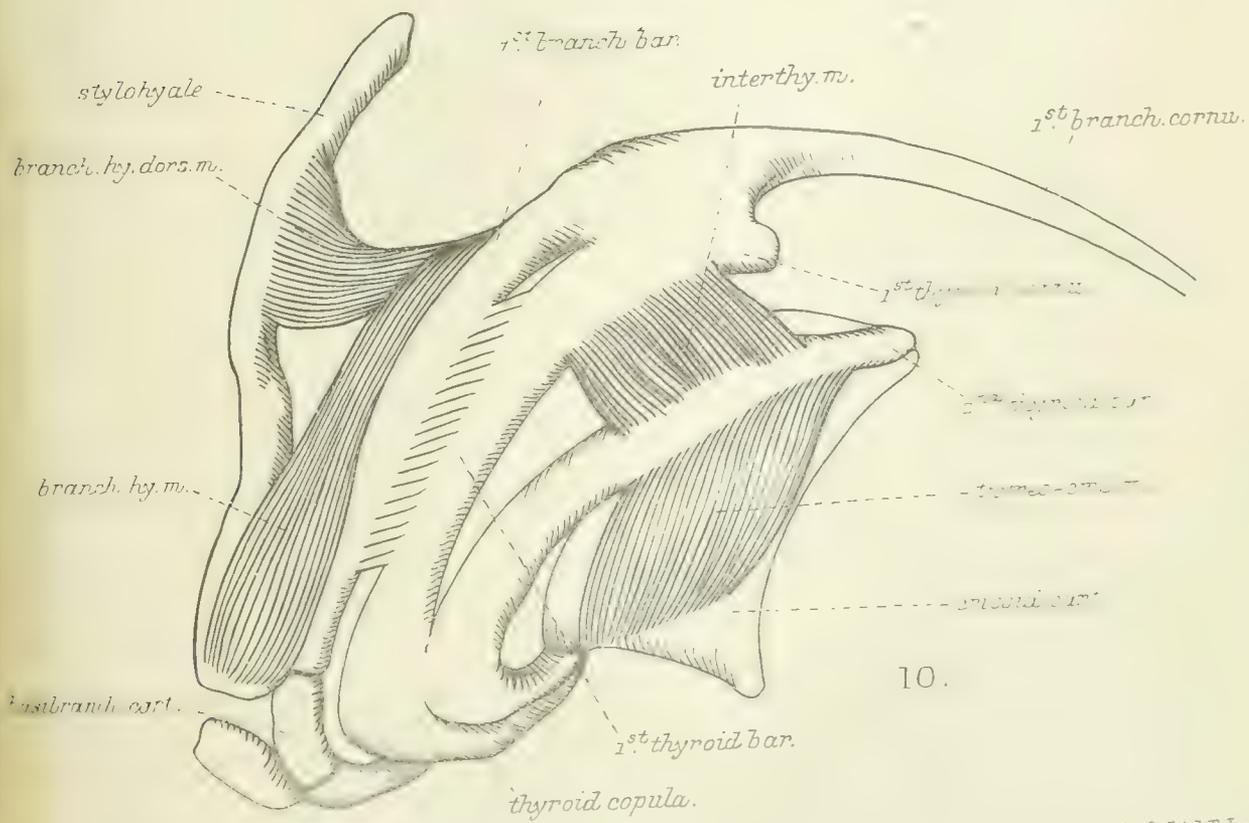
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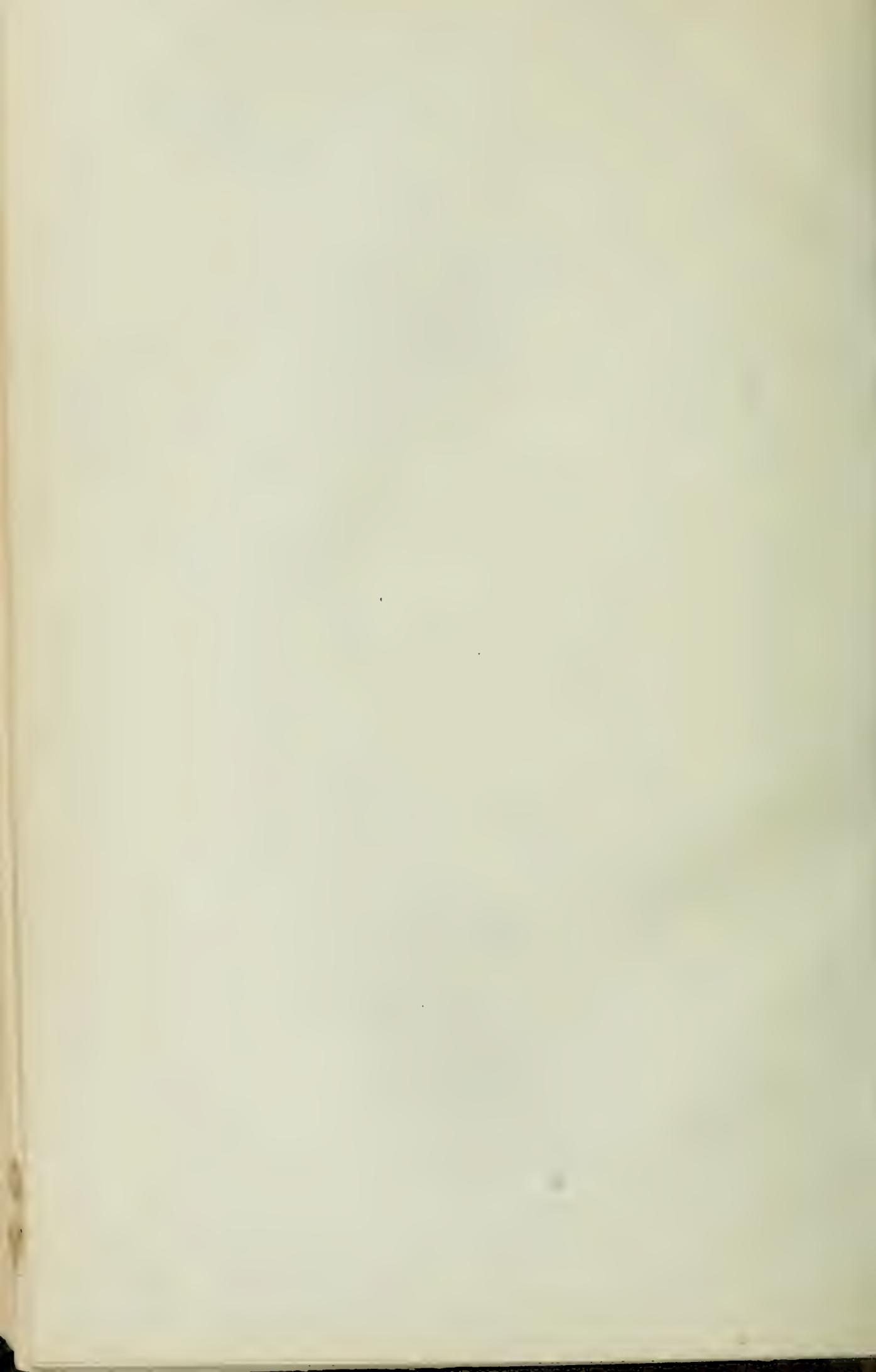




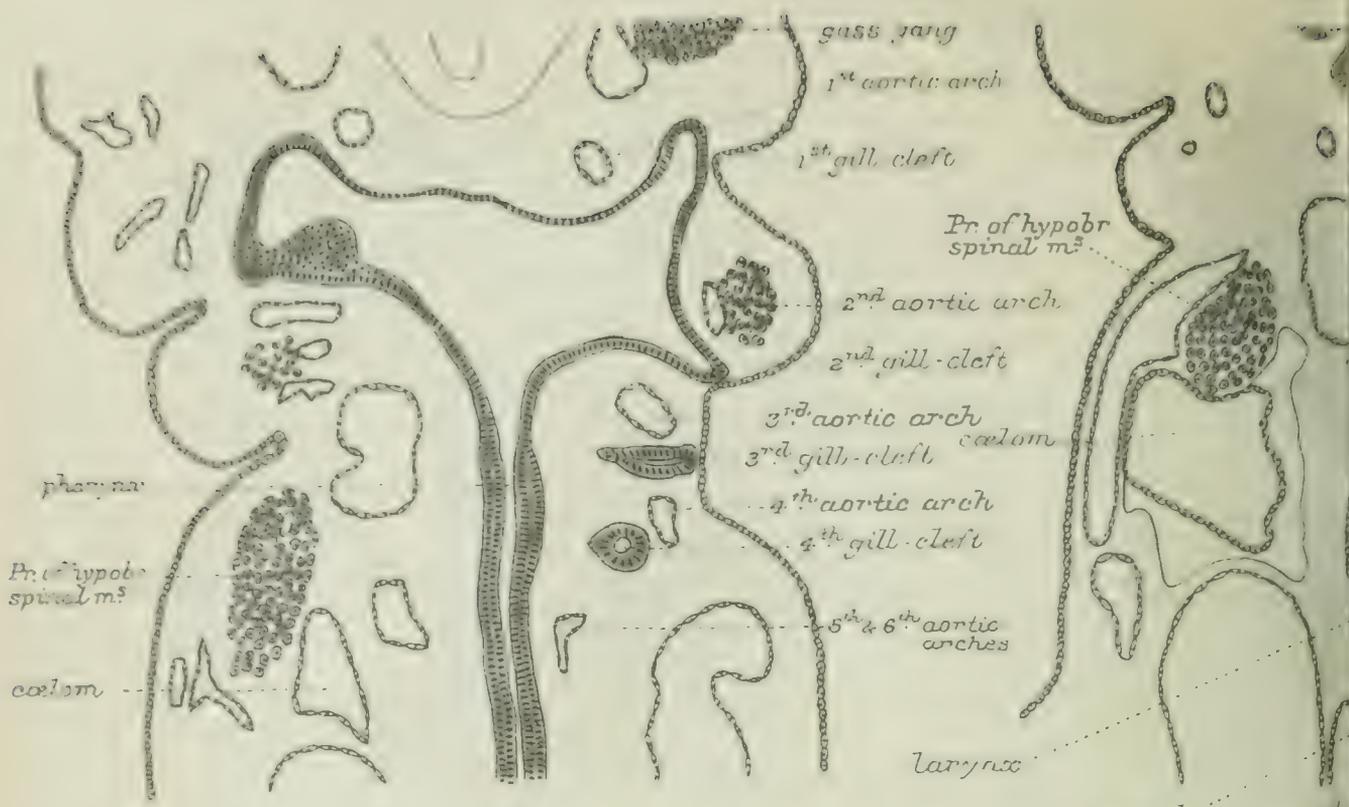
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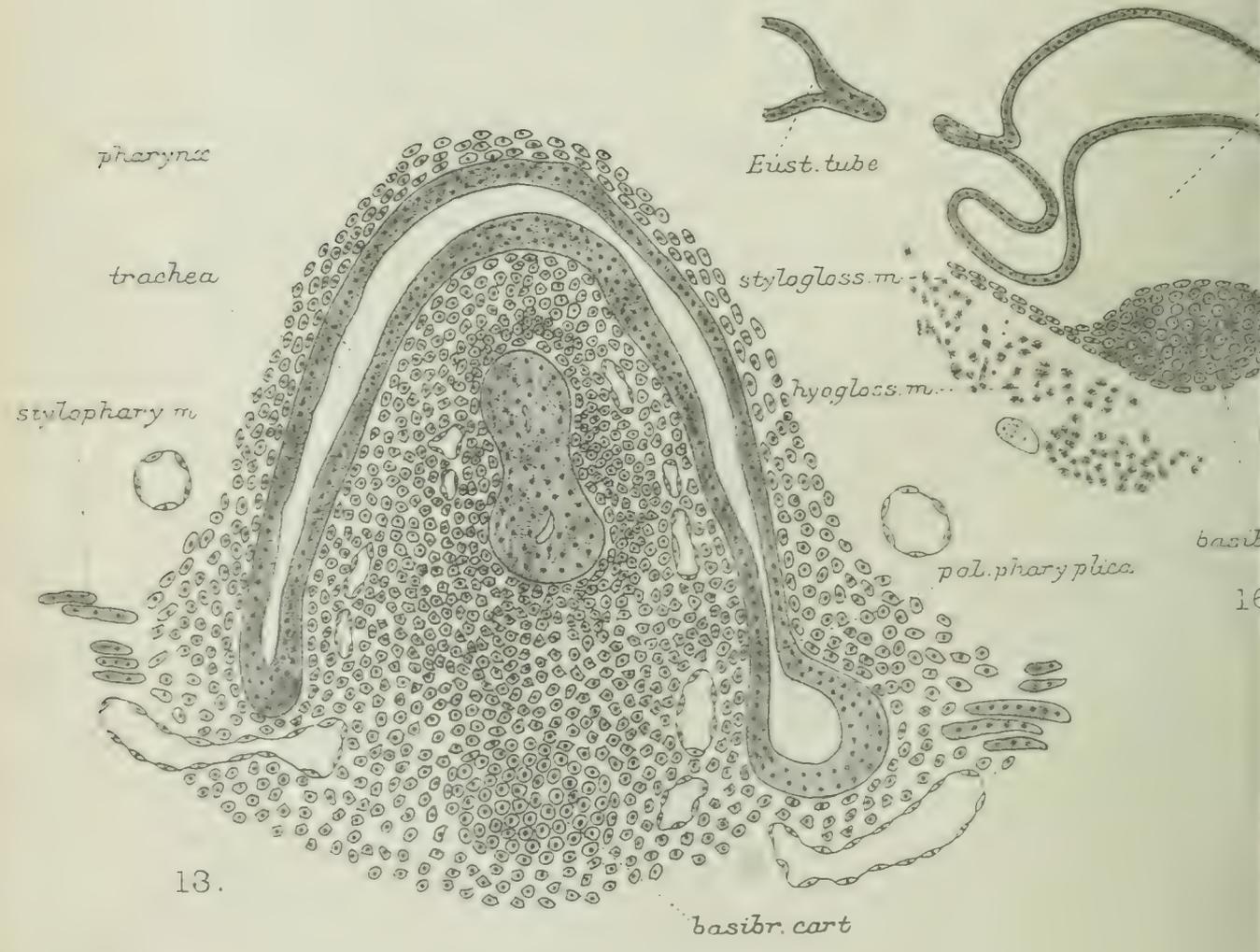
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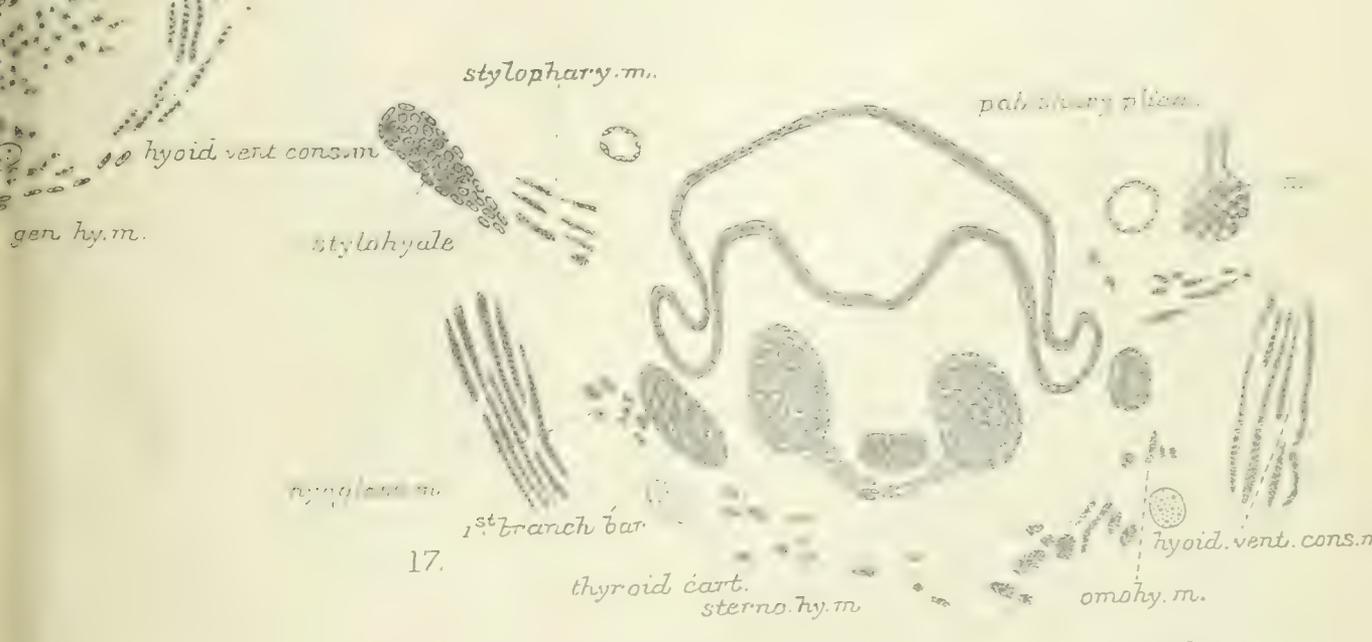
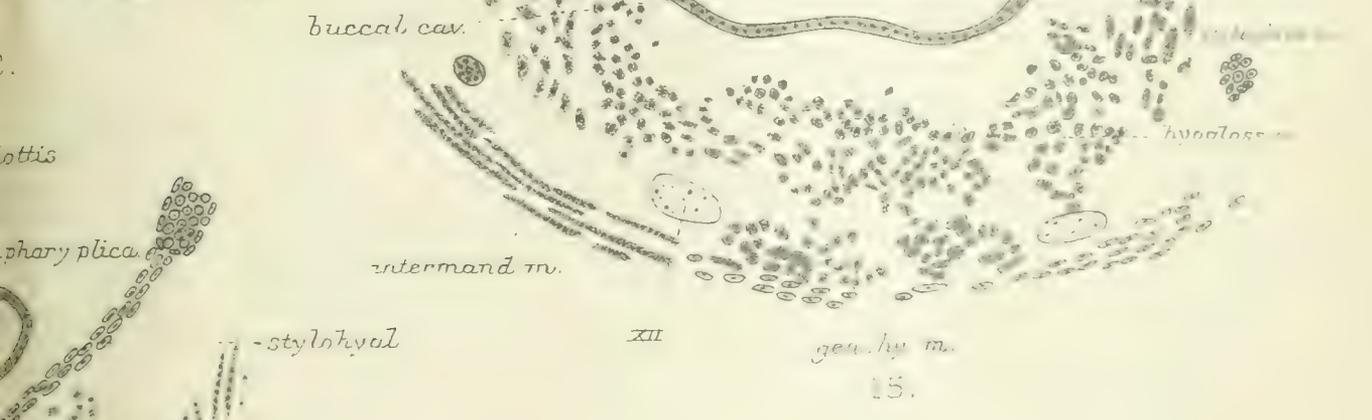
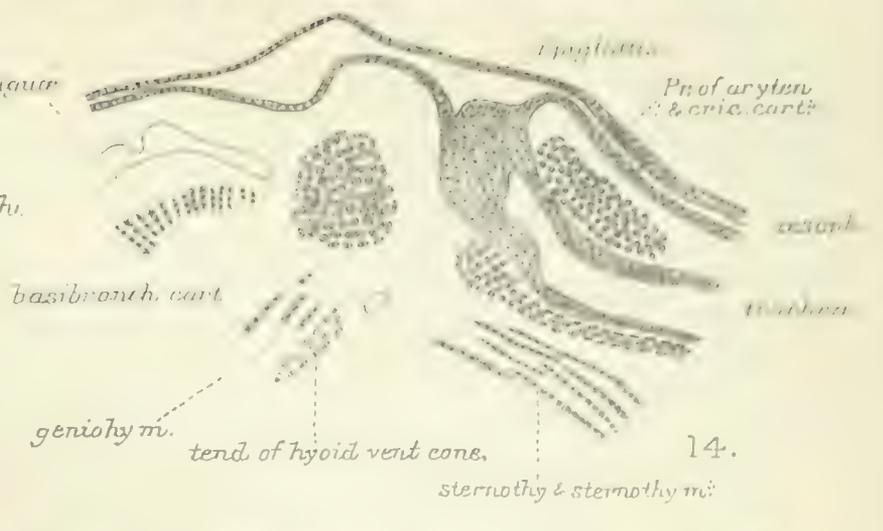
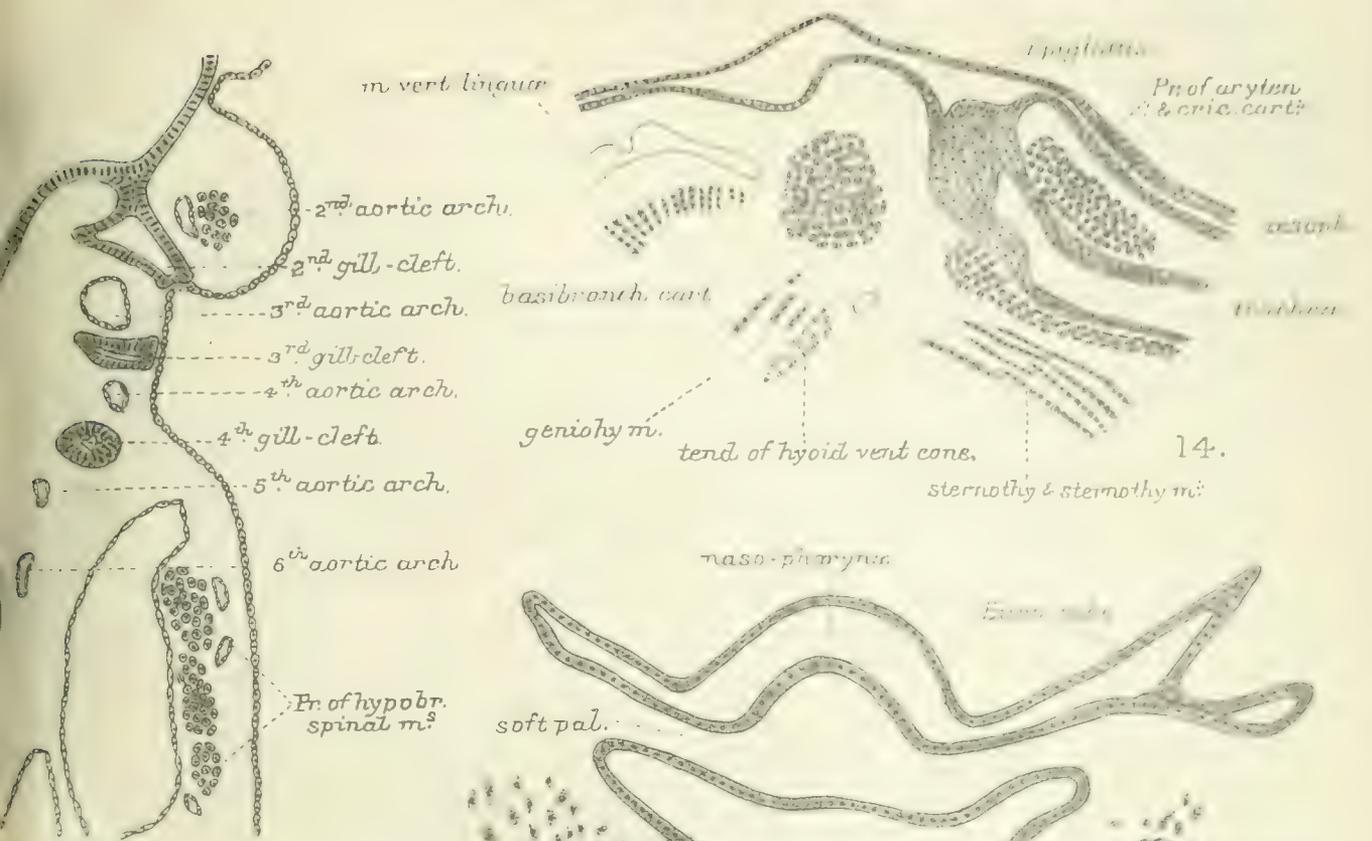


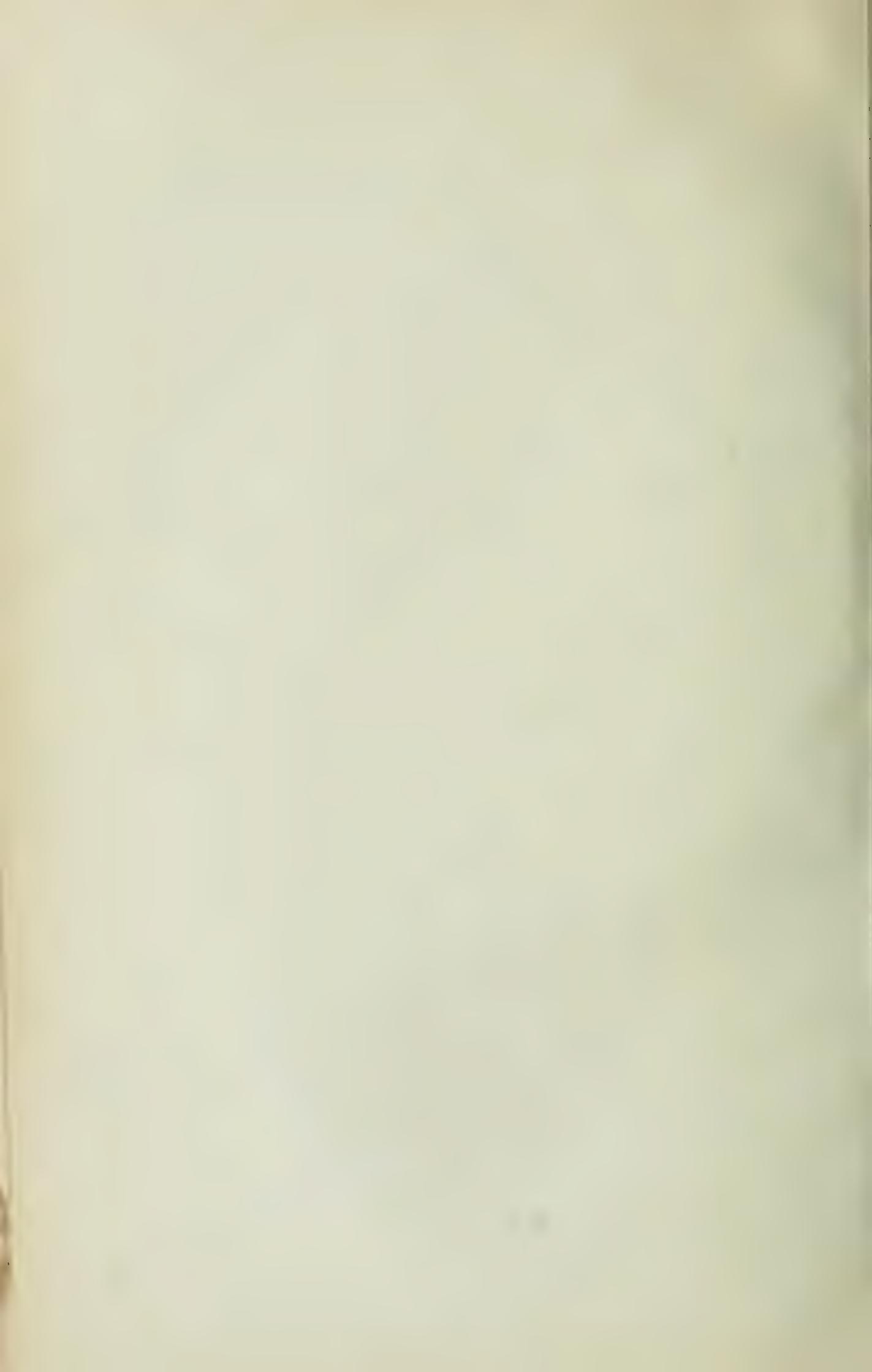


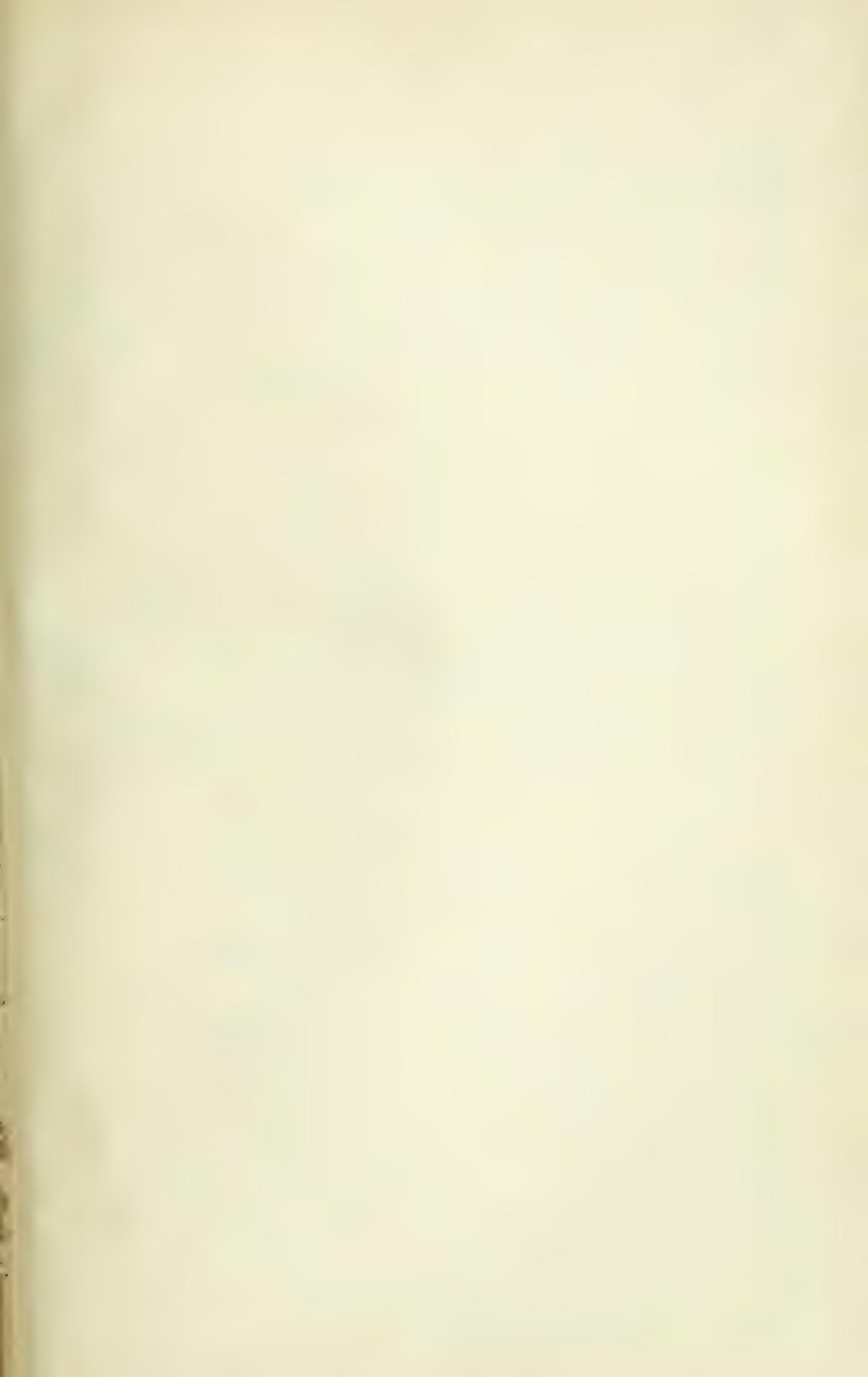
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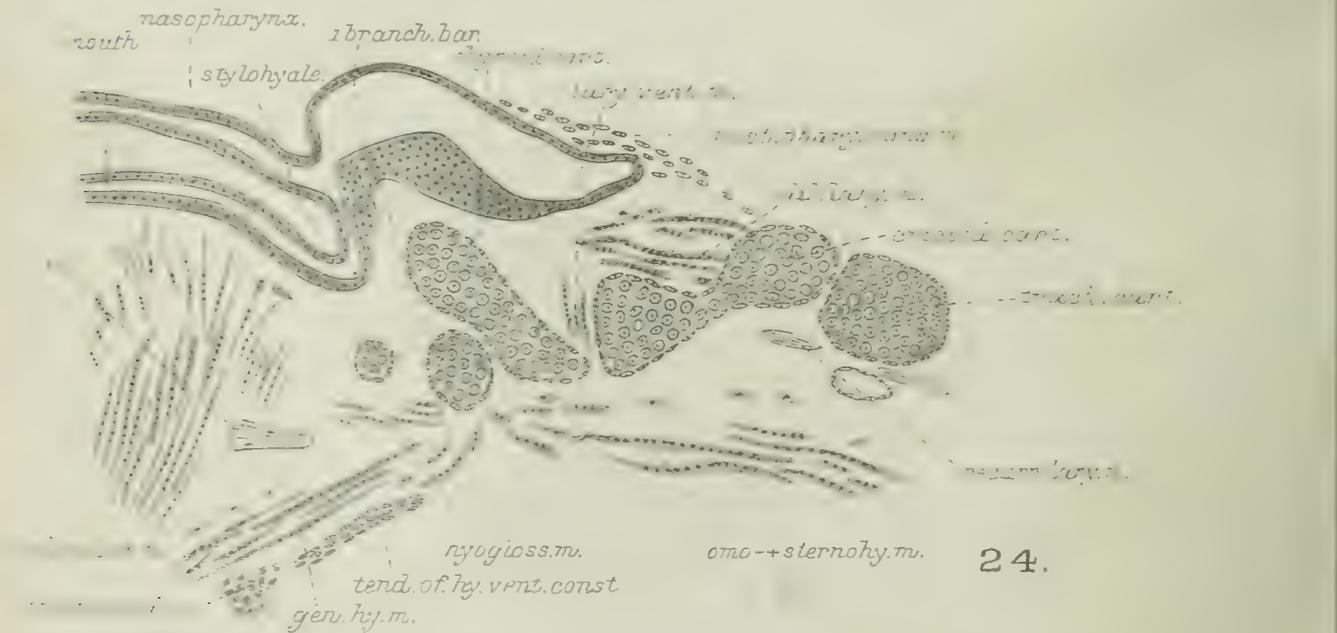




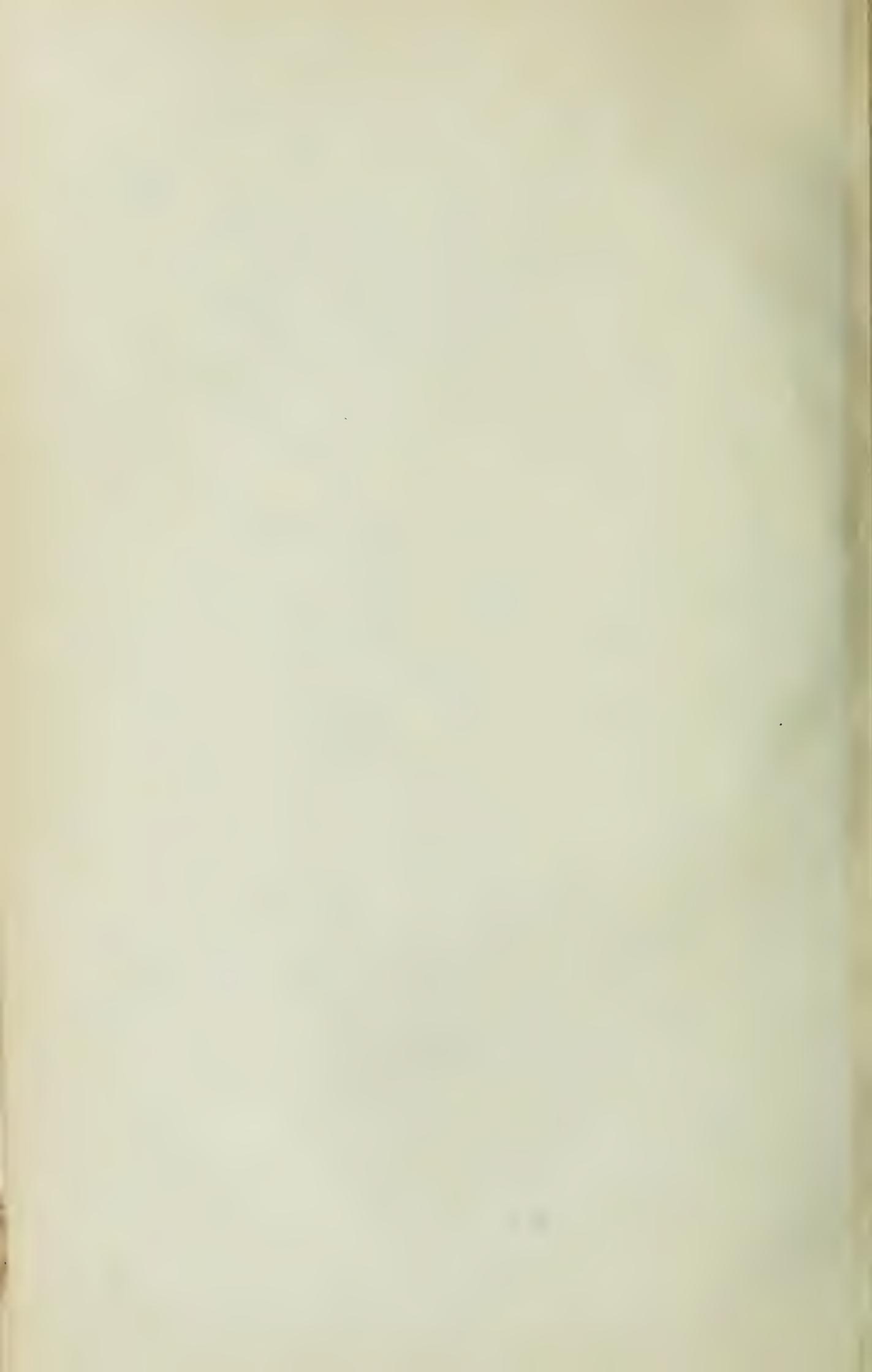
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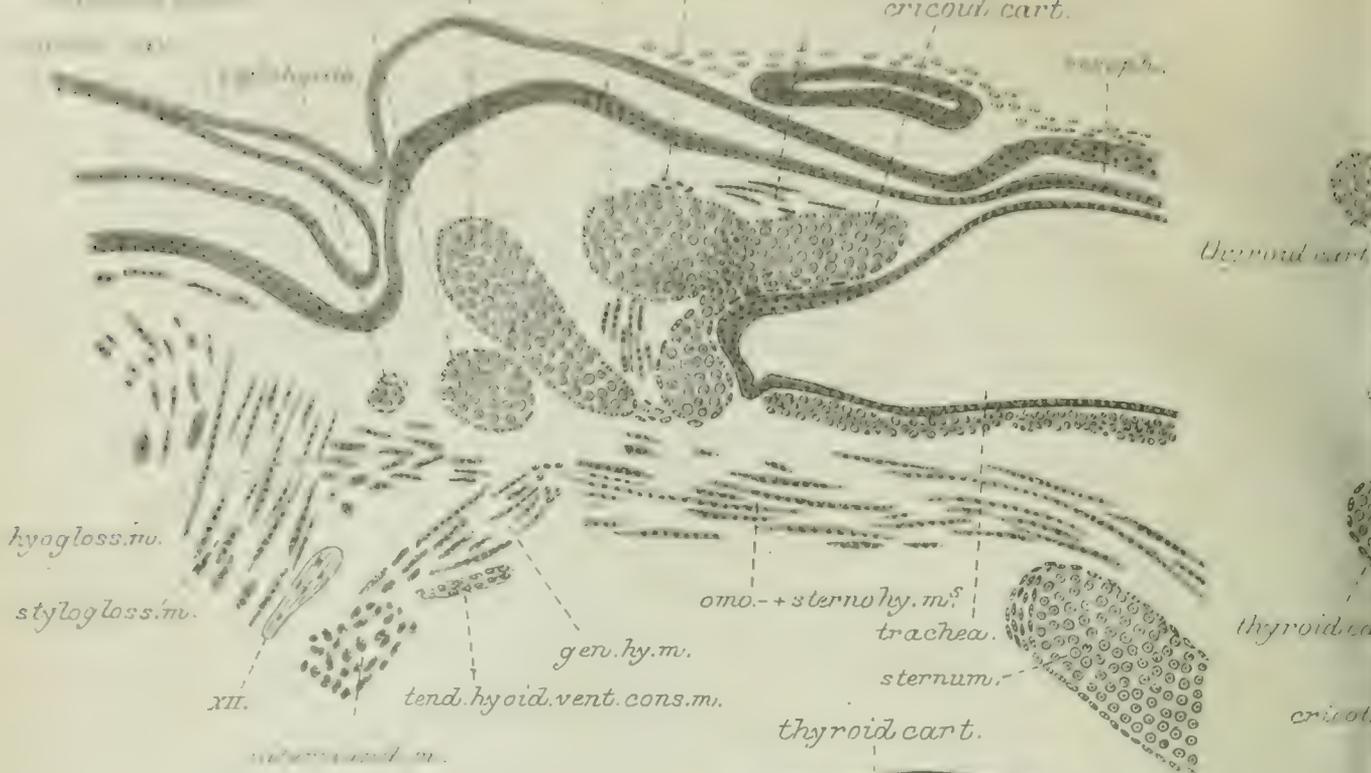


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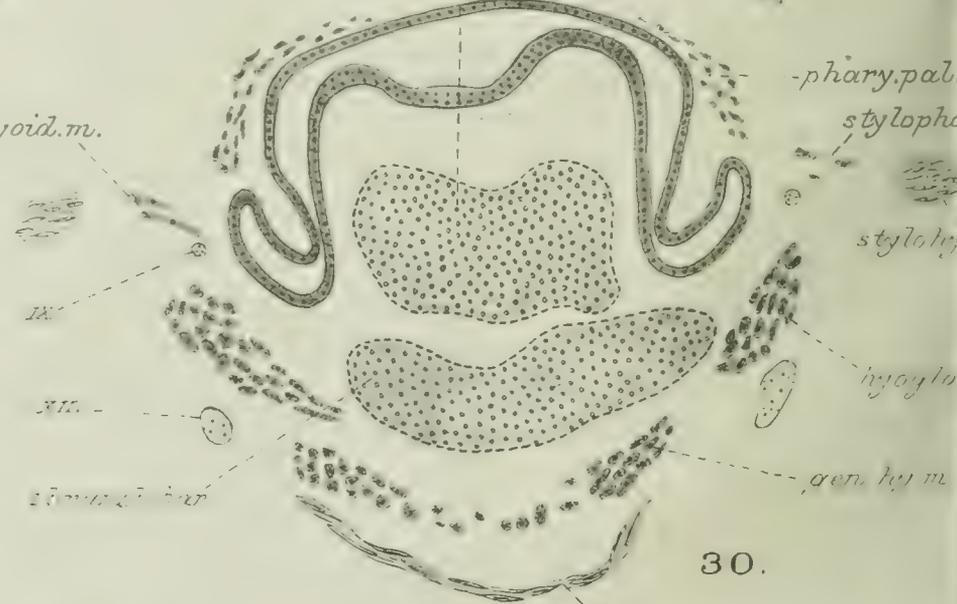
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 thyroid.cart. interary.m.

cricoid.cart.



28.

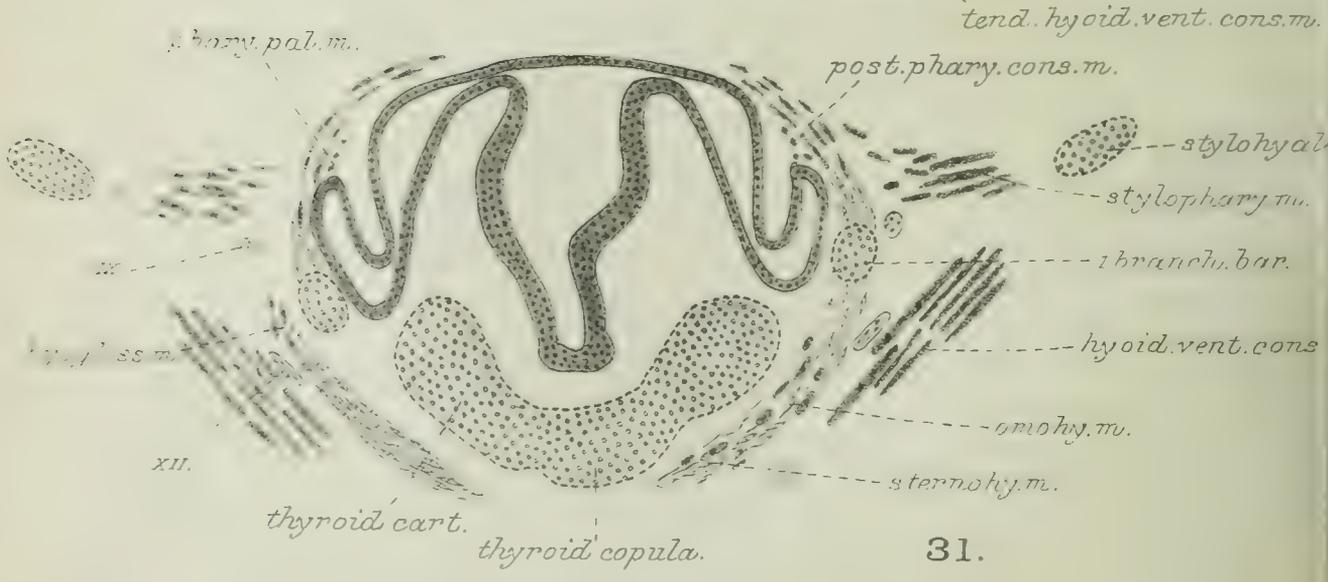
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30.

phary.pal.m.

post.phary.cons.m.

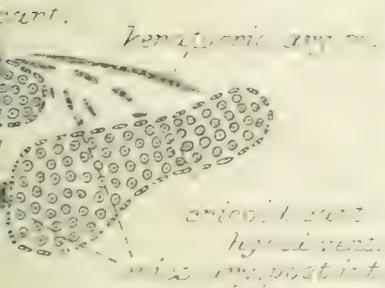


31.

lacrimal amp. m.



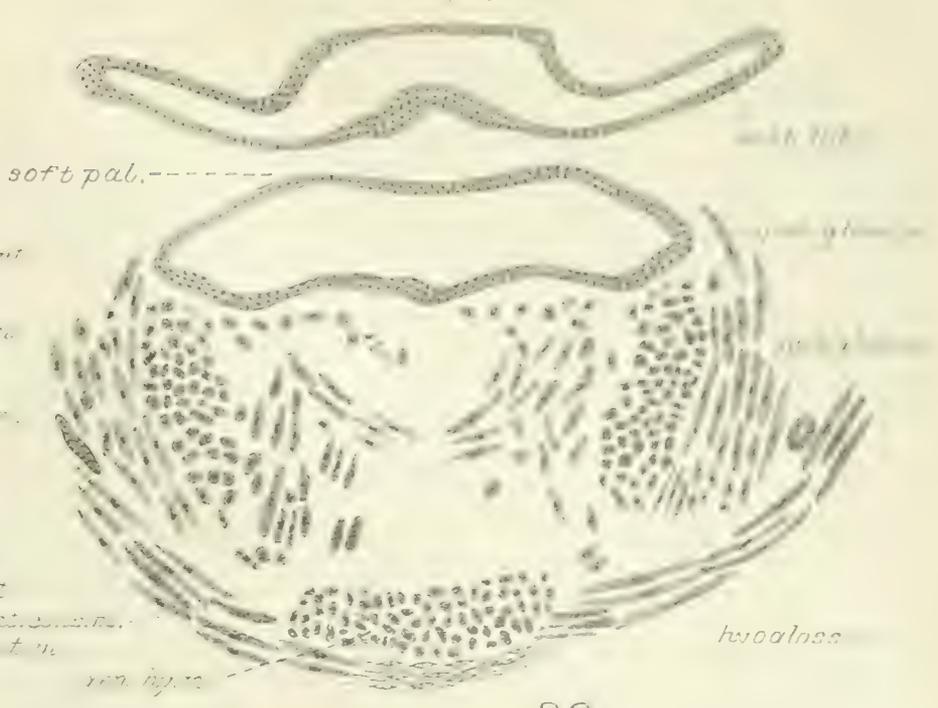
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kerato-crioid amp. m.
crioid cart.
hyal. cart.
mix. m. post. int. m.

27.

nasopharynx



soft pal.---

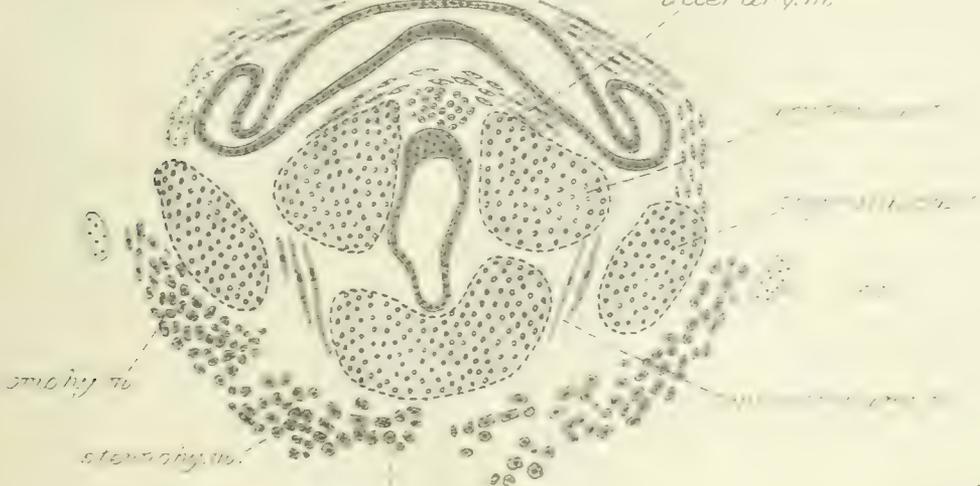
twoaloss

29.

interary cart.

post. phary. cons. m.

interary. m.



sternohy. m.

sternohy. m.

32.

sternohy. m.

pal. phary. plica.

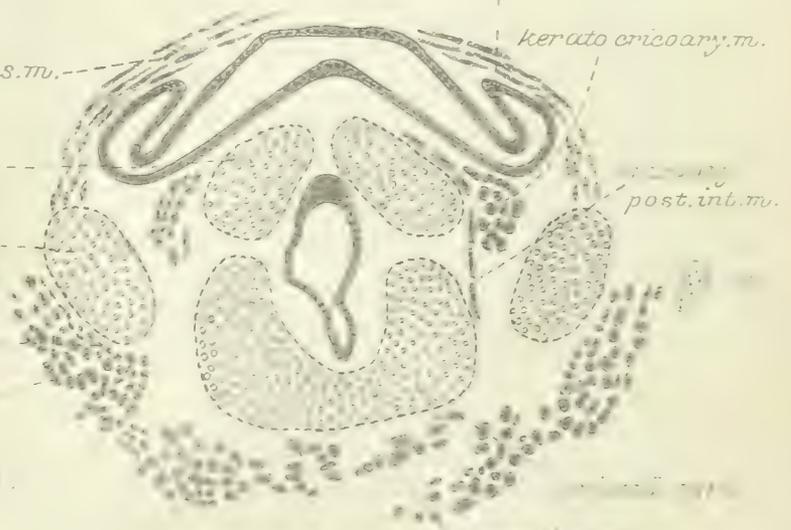
kerato-crioid m.

post. phary. cons. m.---

aryten. cart.---

thyroid cart.---

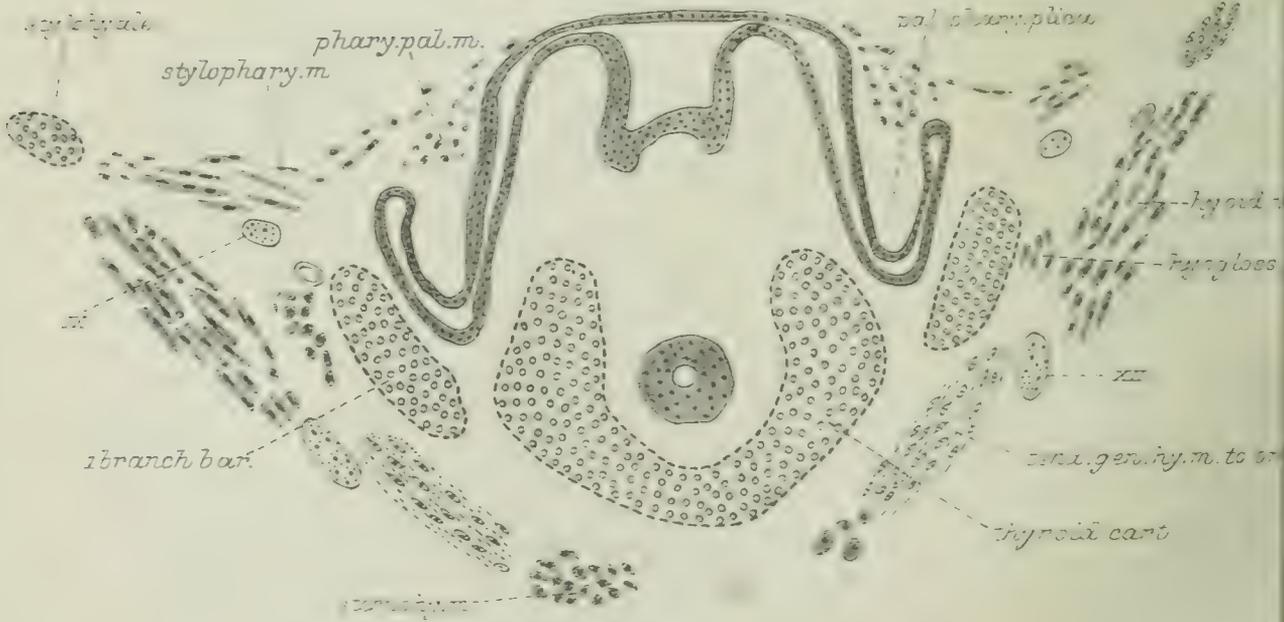
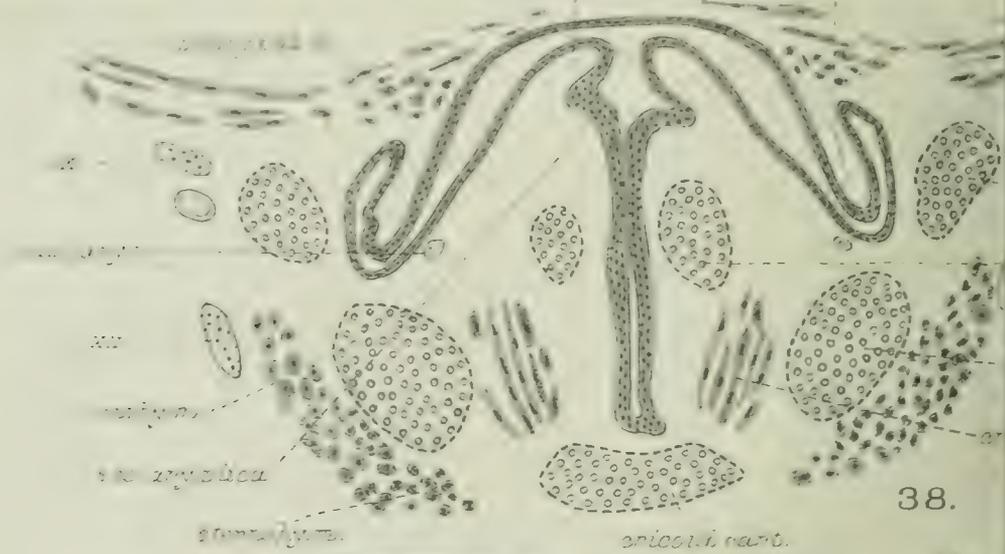
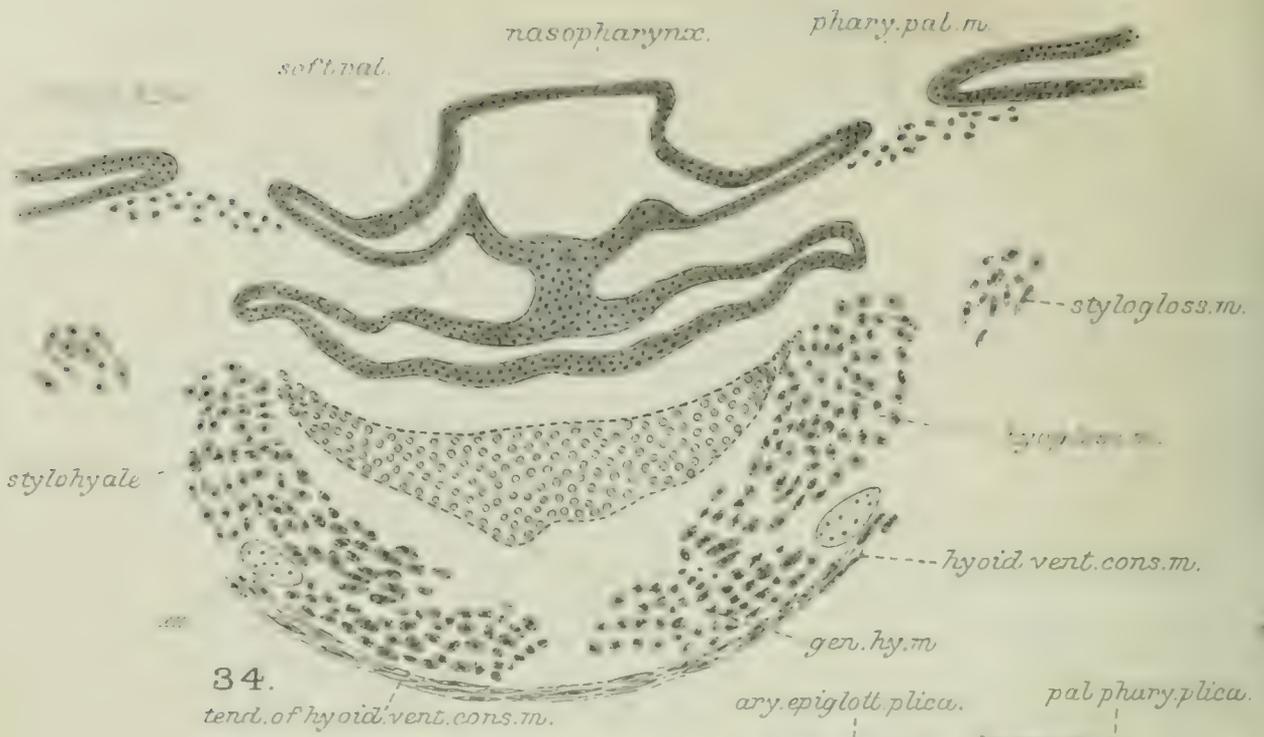
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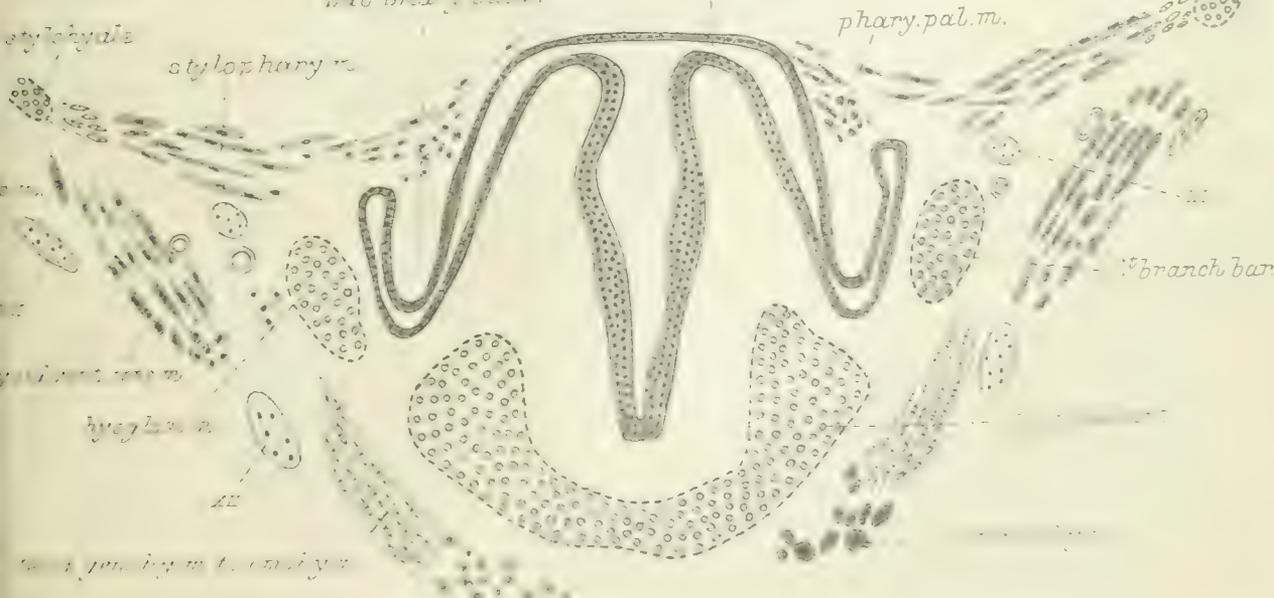
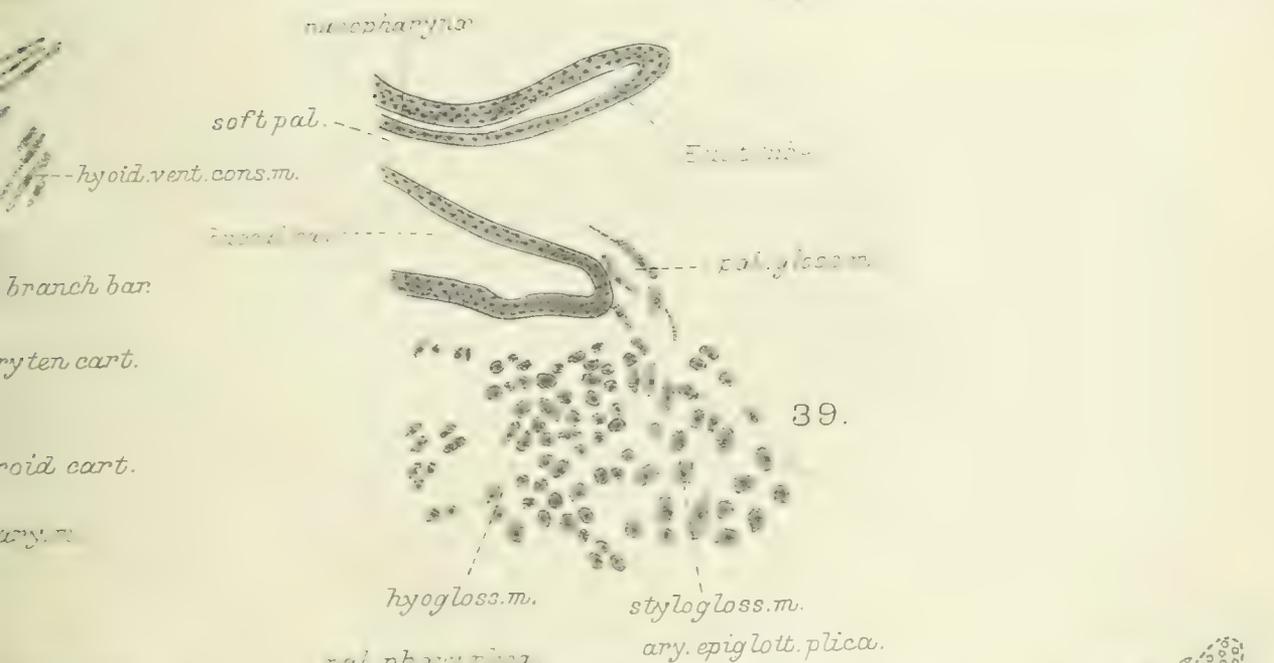
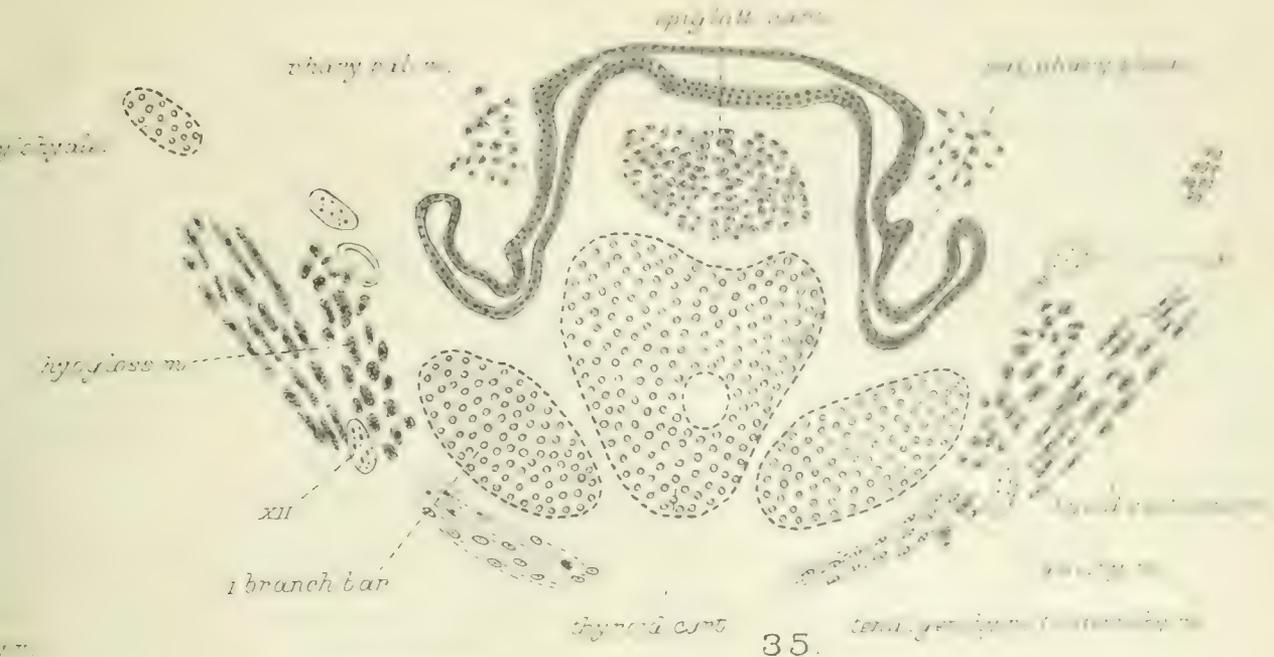


post. int. m.

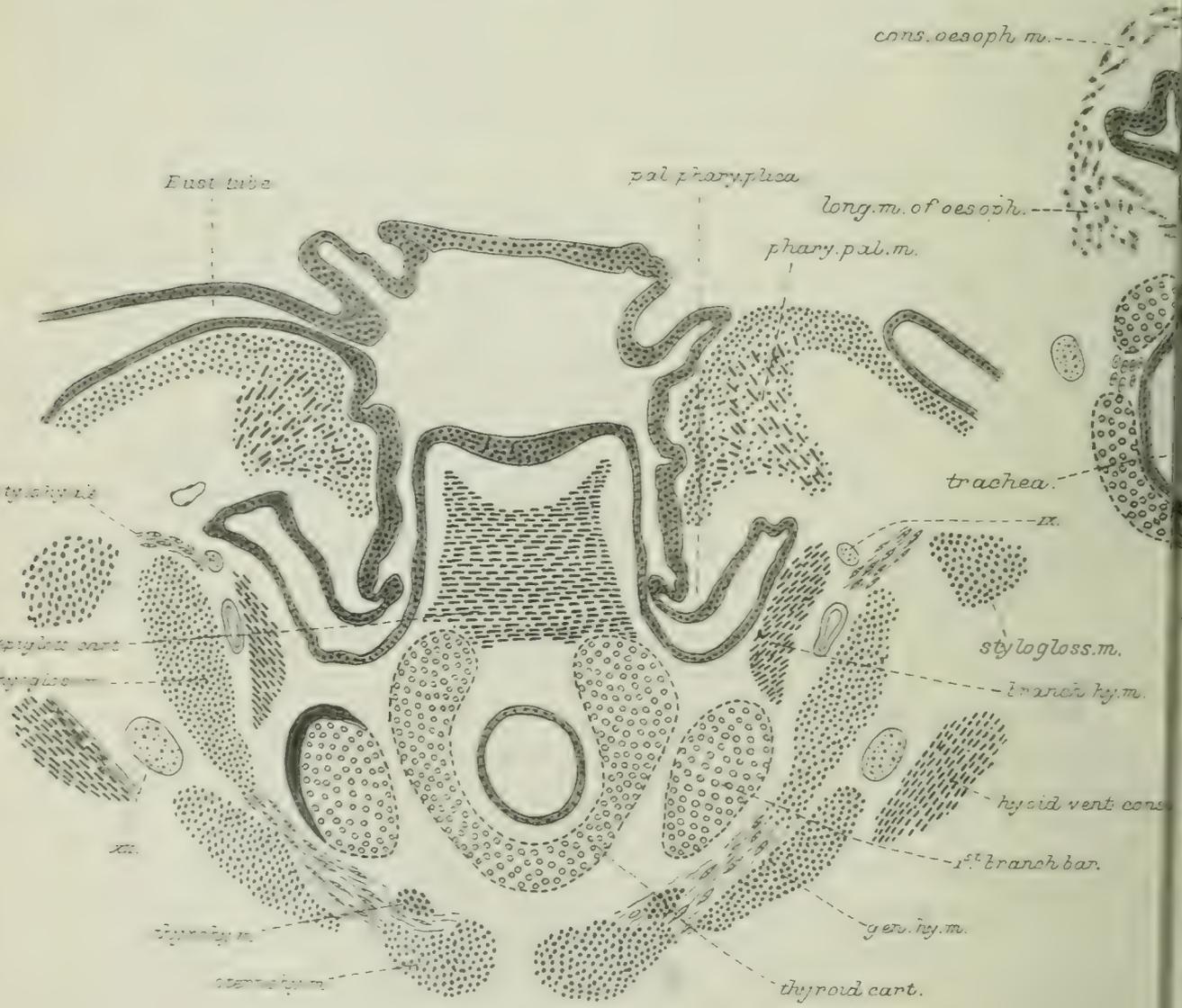
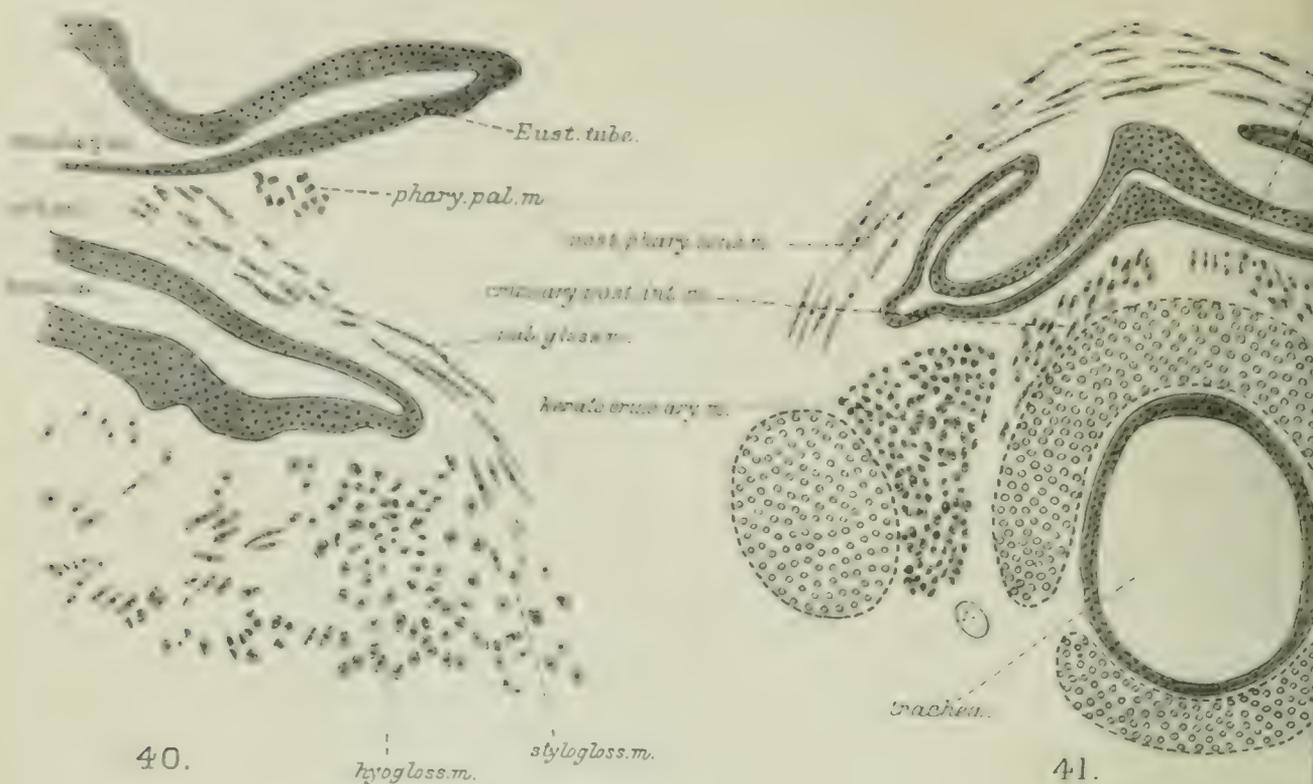
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sternohy. m.

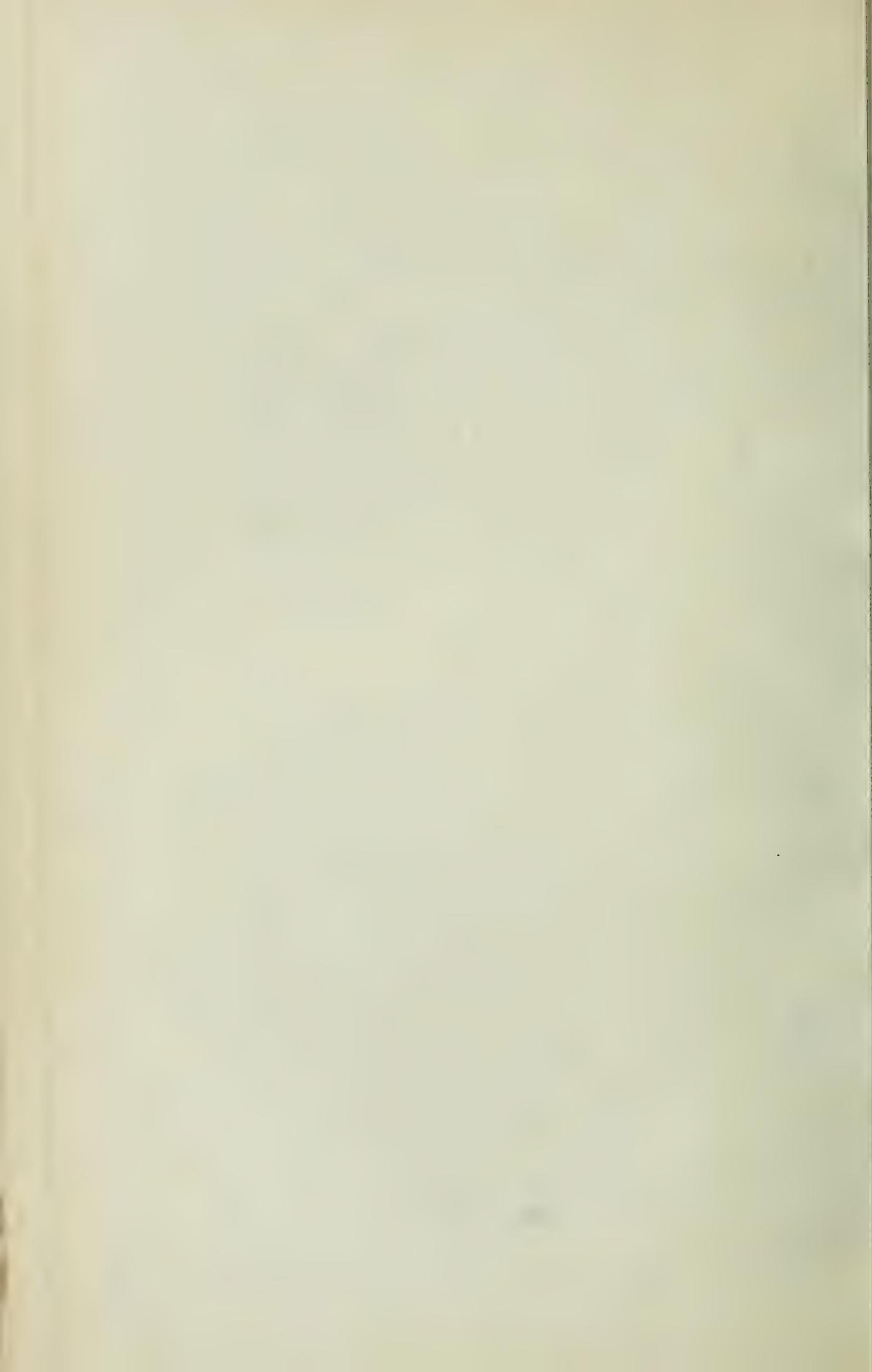








43.



pharynx

pharynx

ventr. aort.
sub larynx

pharynx
pharynx
pharynx
pharynx
pharynx

46

pharynx

sub larynx
1st thyroid bar

der. aorta

1st aortic bar

2nd thyroid bar

6th aortic arch

der. aorta

vent. aort.

1st aortic arch

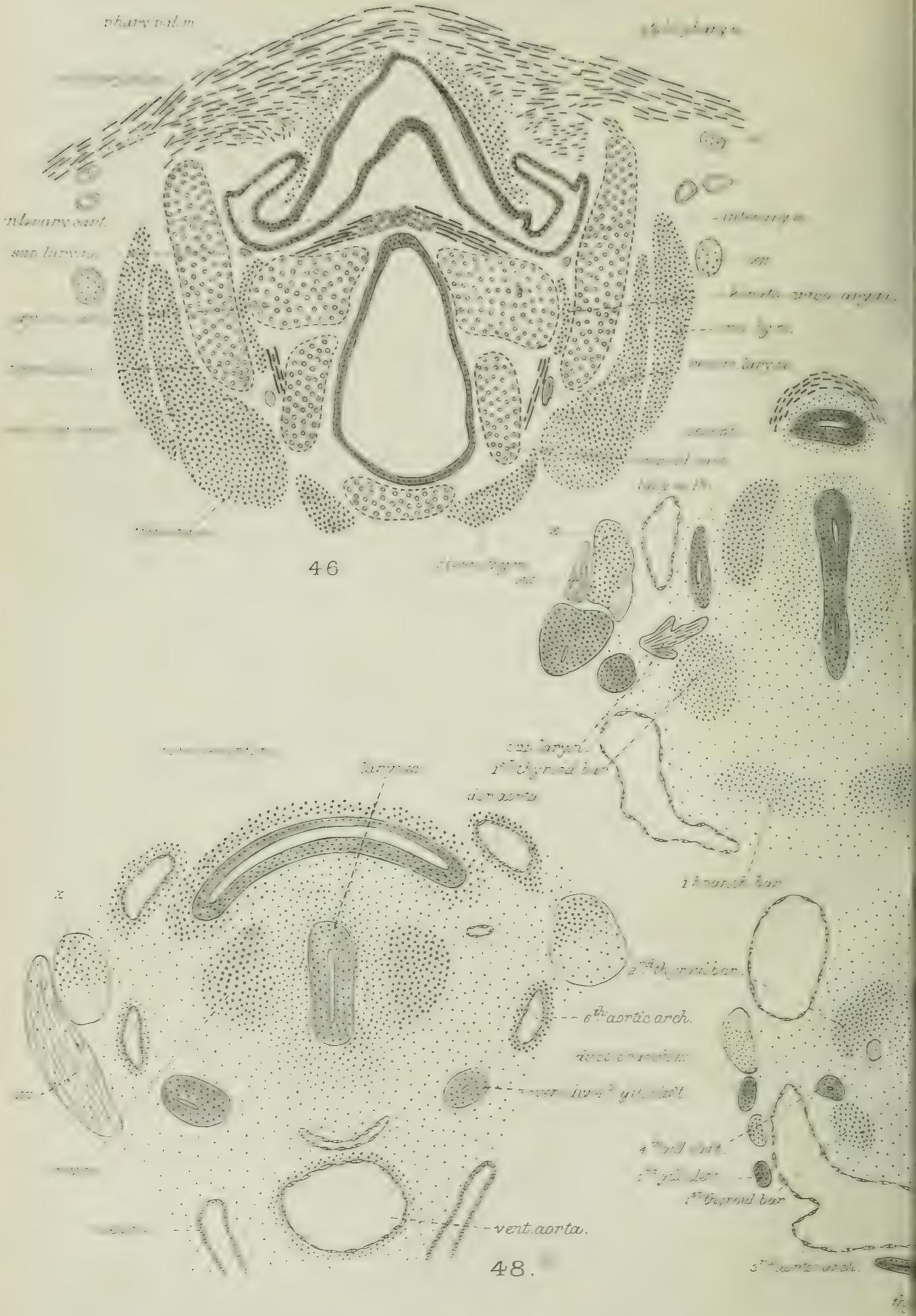
2nd thyroid bar

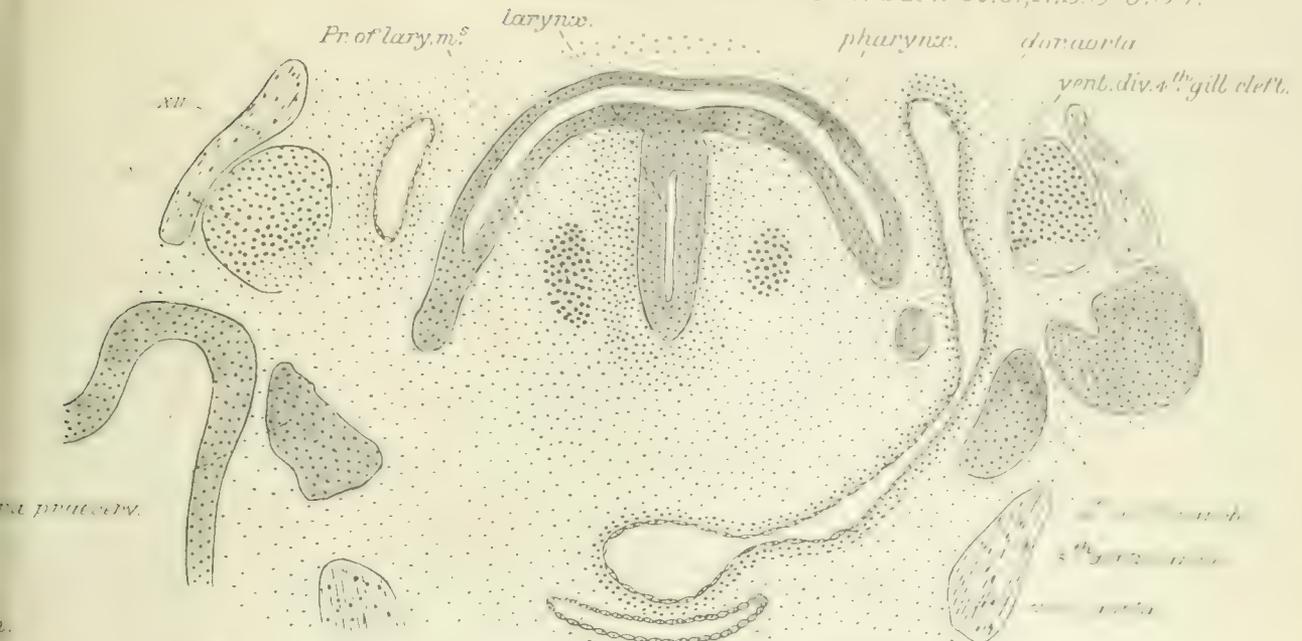
1st thyroid bar

vent. aorta.

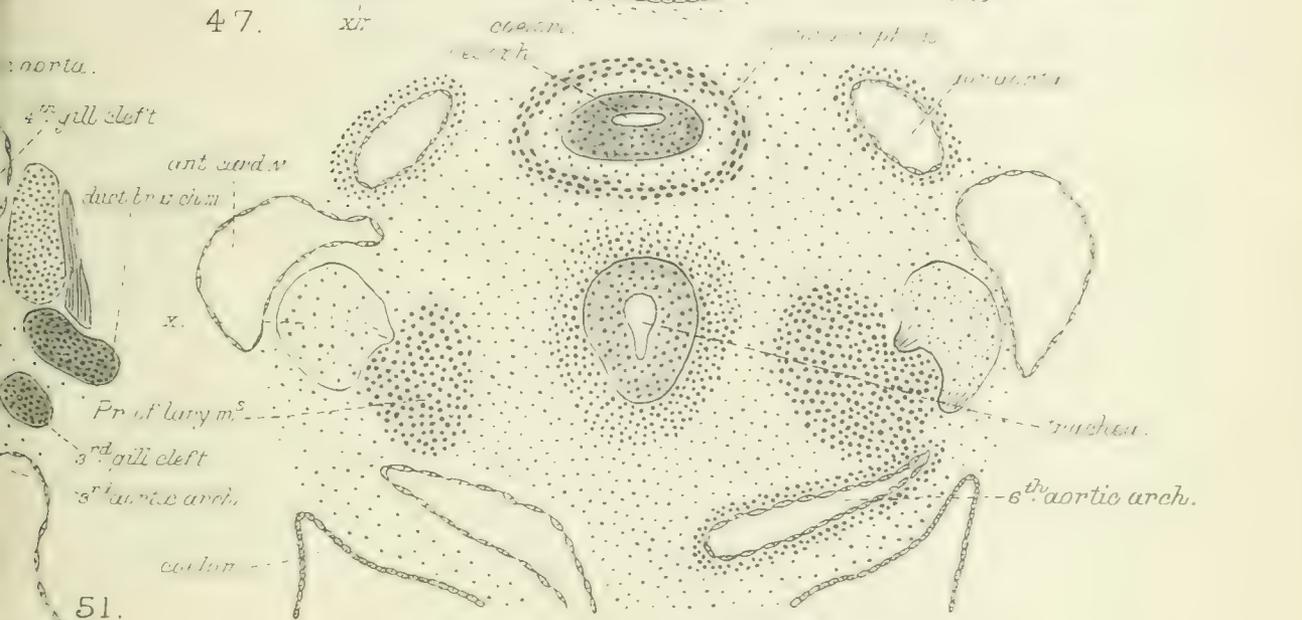
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3rd aortic arch

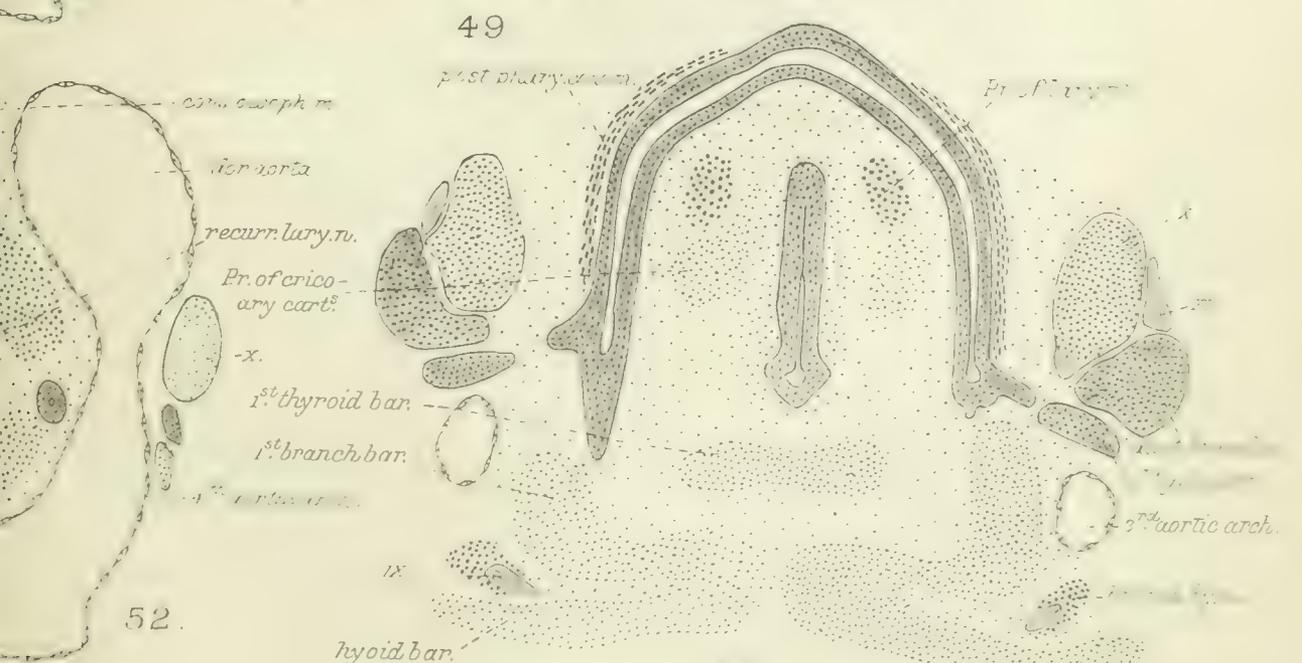




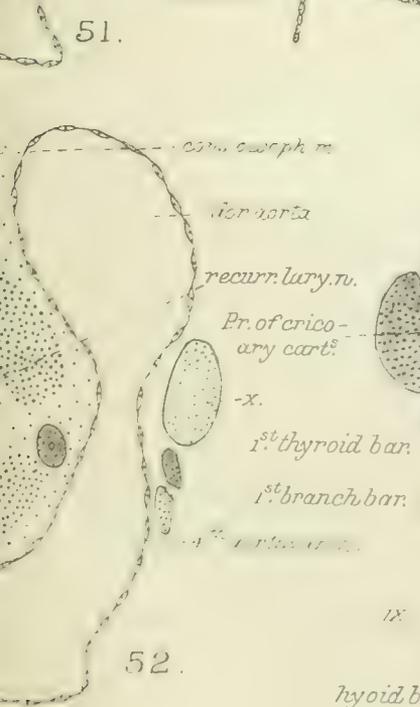
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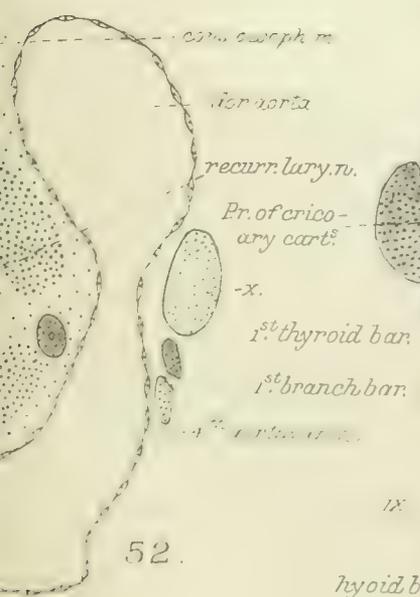
49



50.

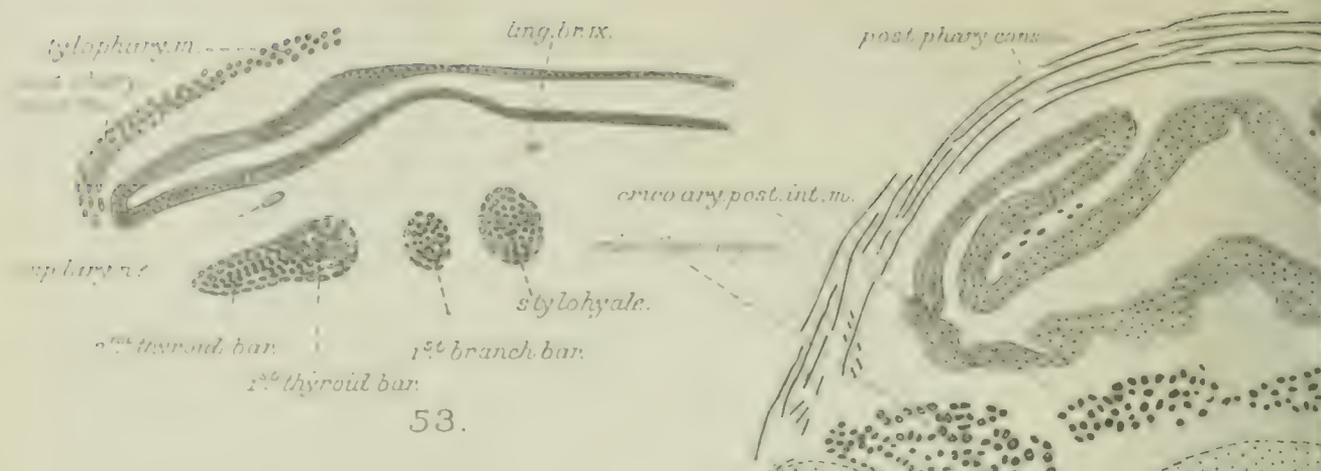


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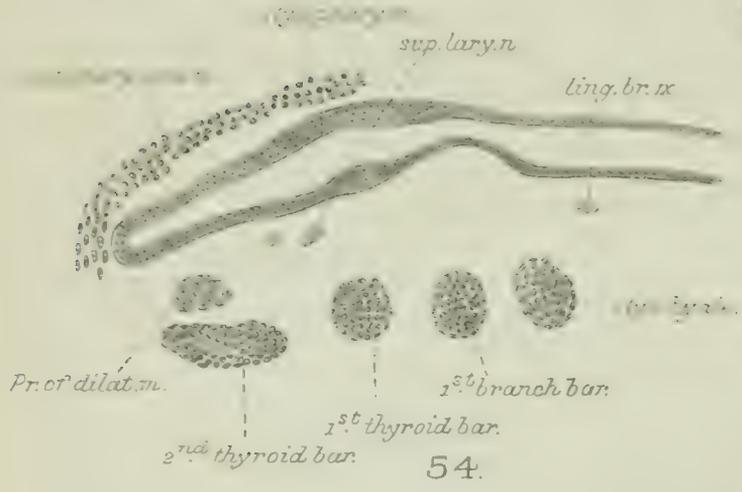
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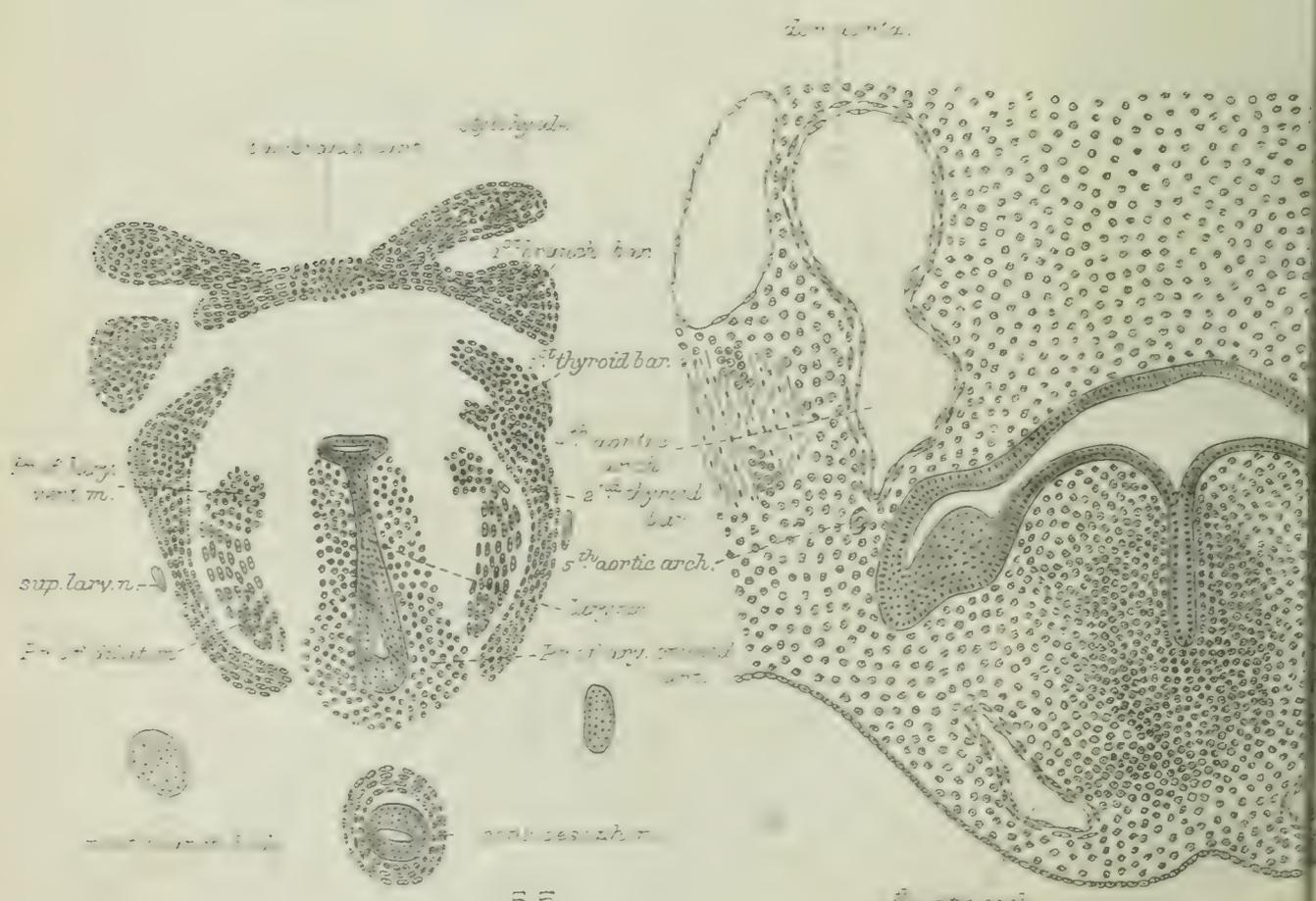


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56.

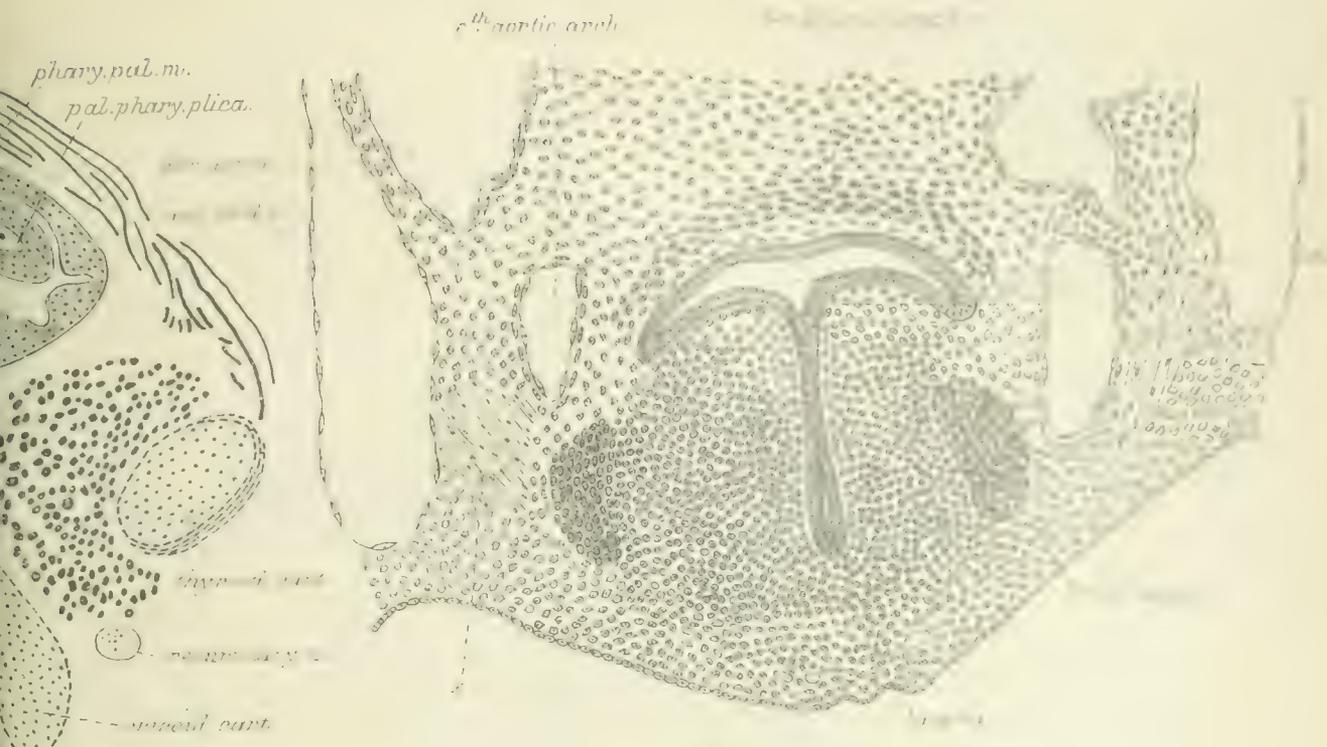


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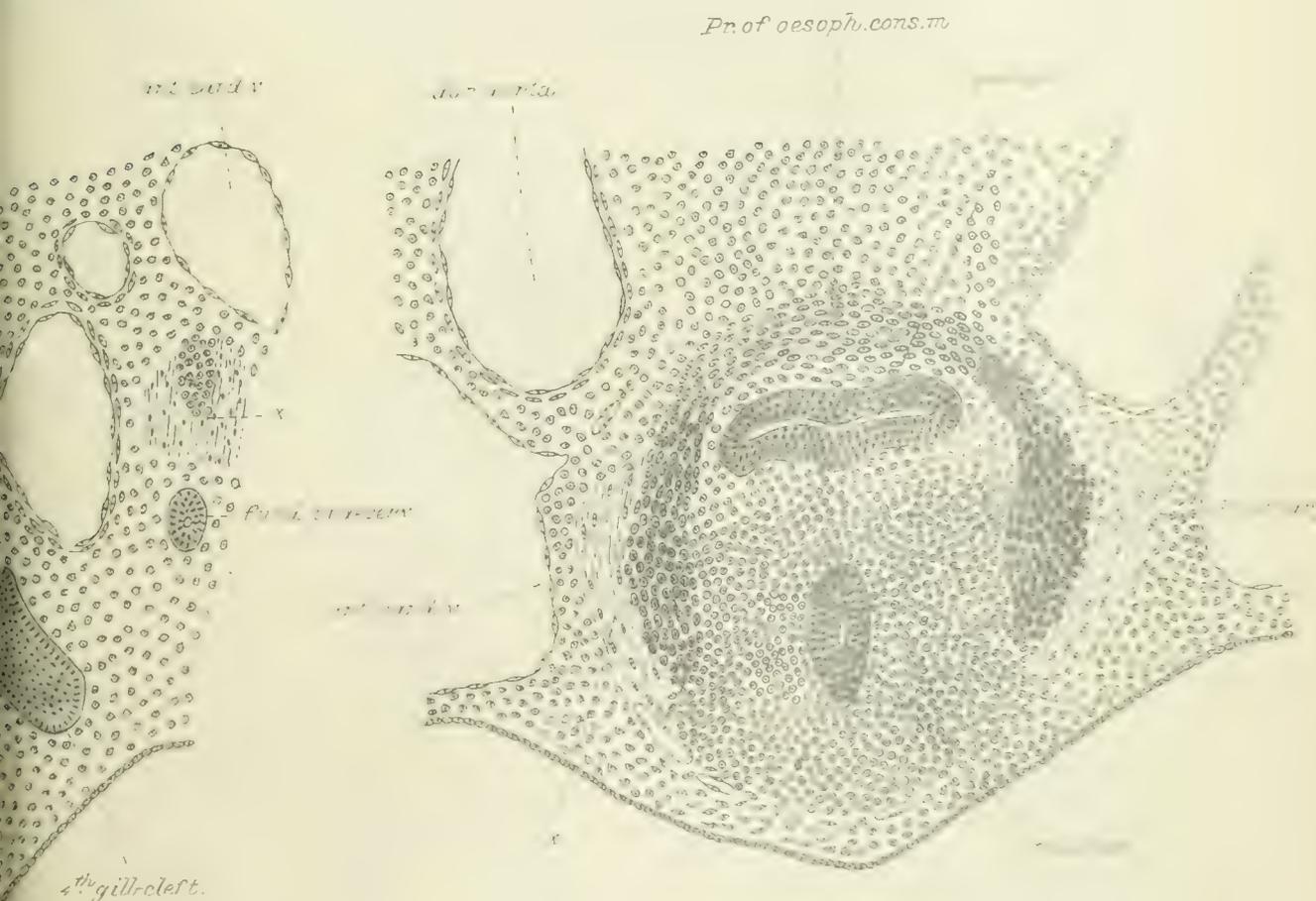


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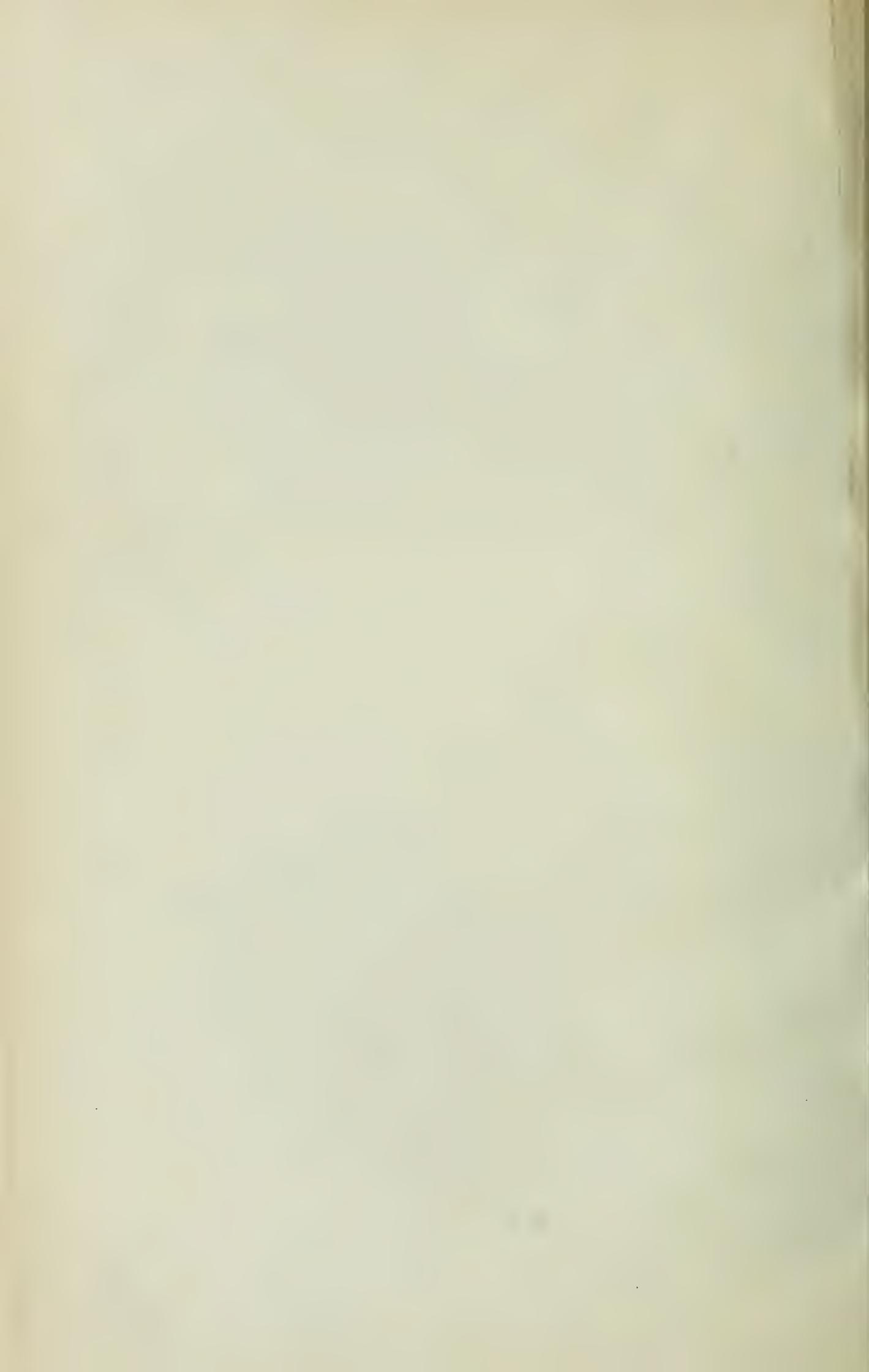
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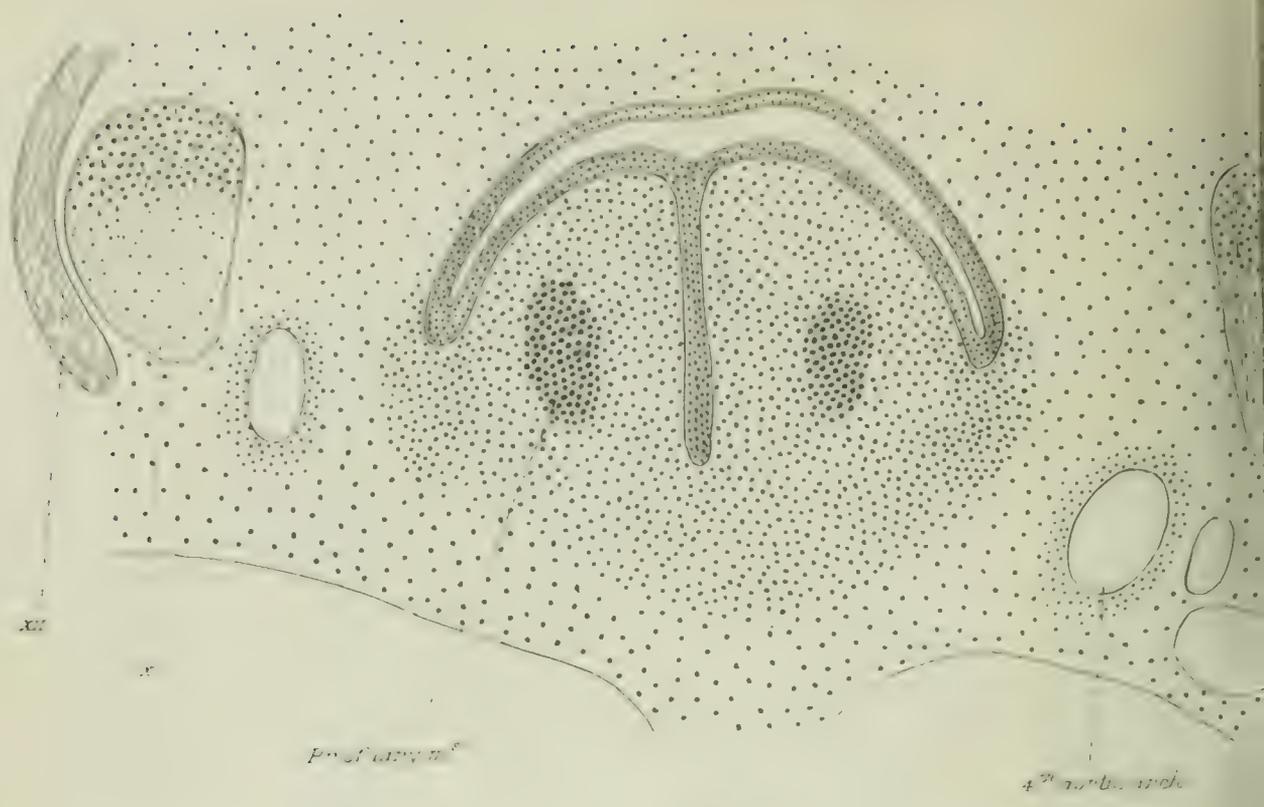


58

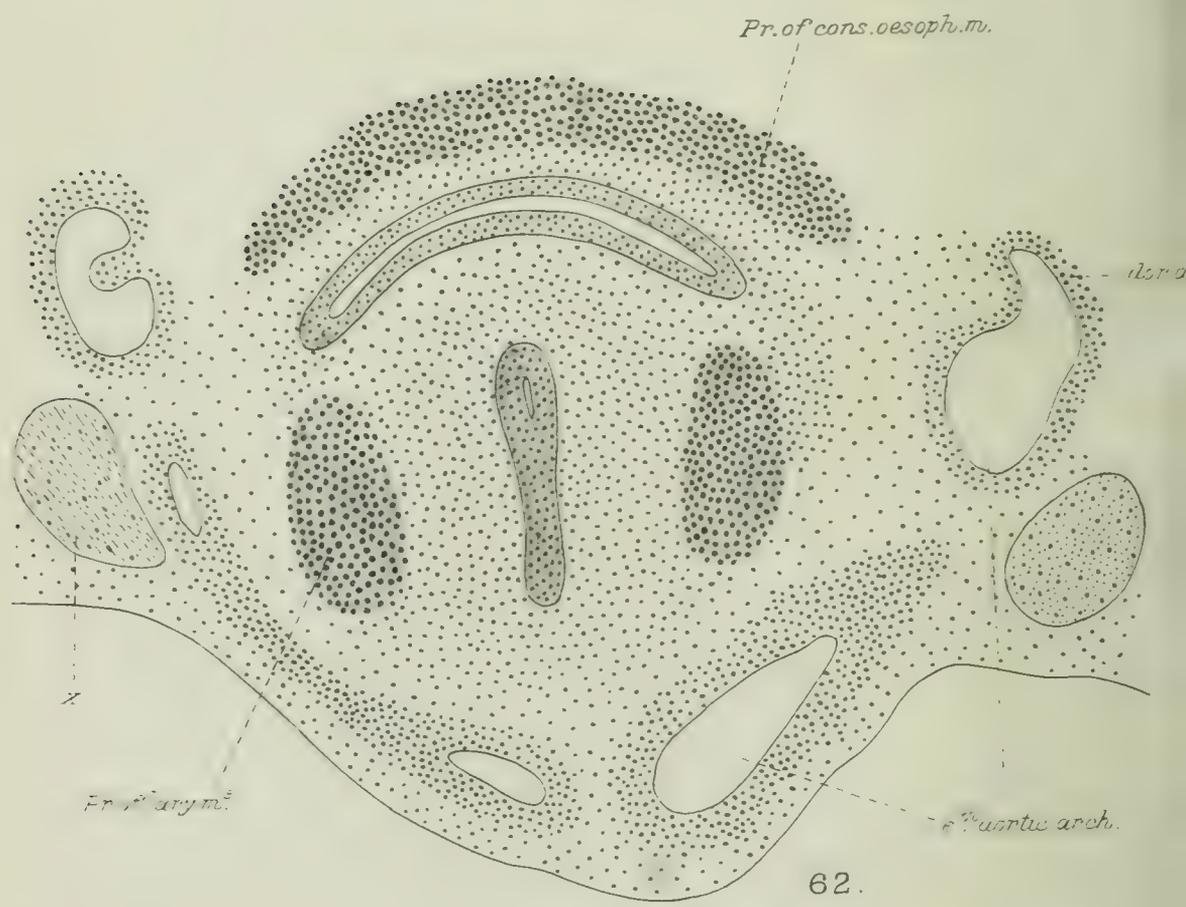


59





60.

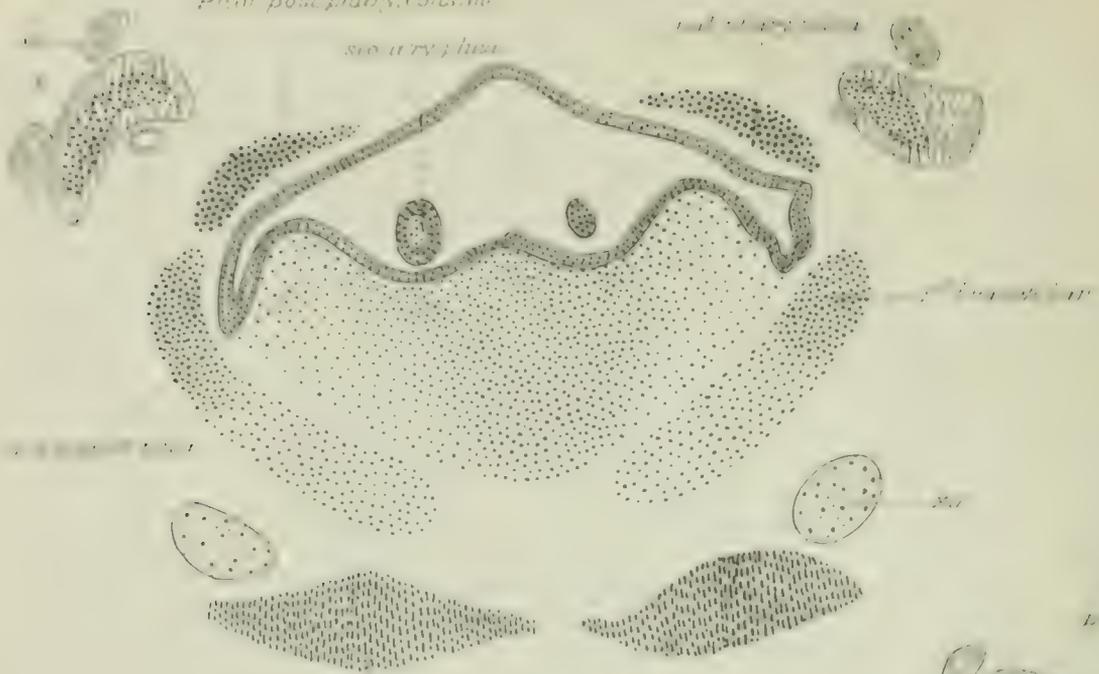


62.

Pr. of post phary. cons. m.

so. a r y p h a

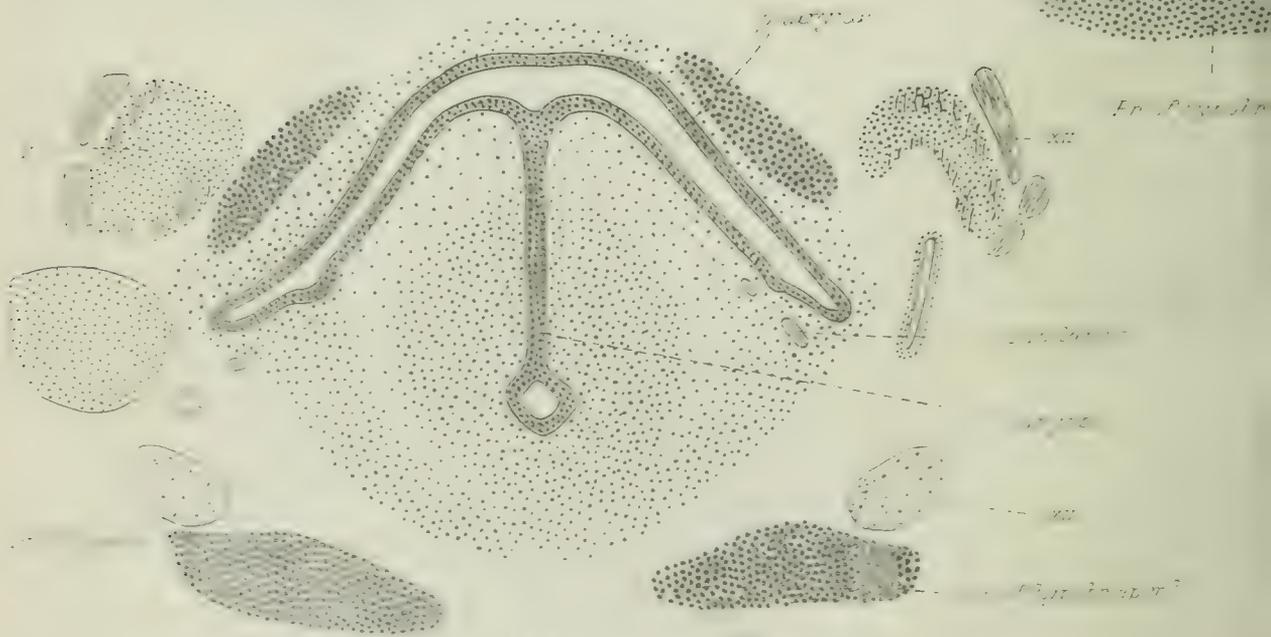
int. of pharynx



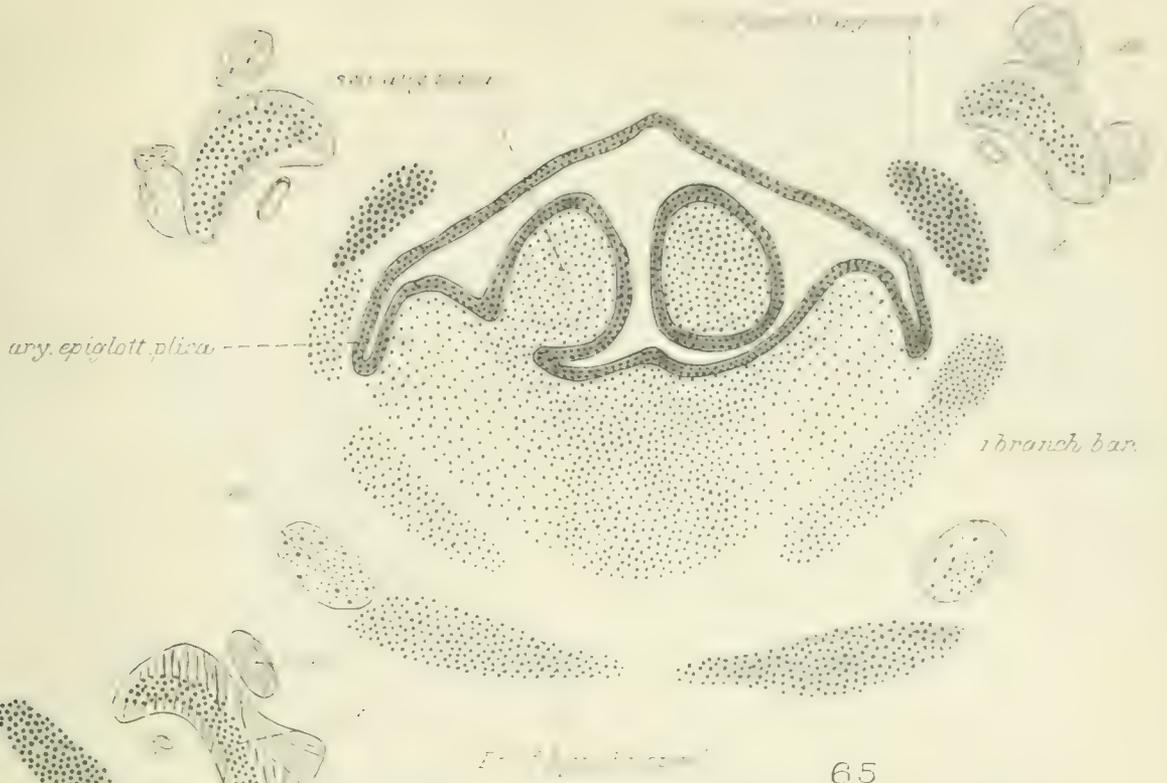
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Pr. of hypobr. sp. m. 5

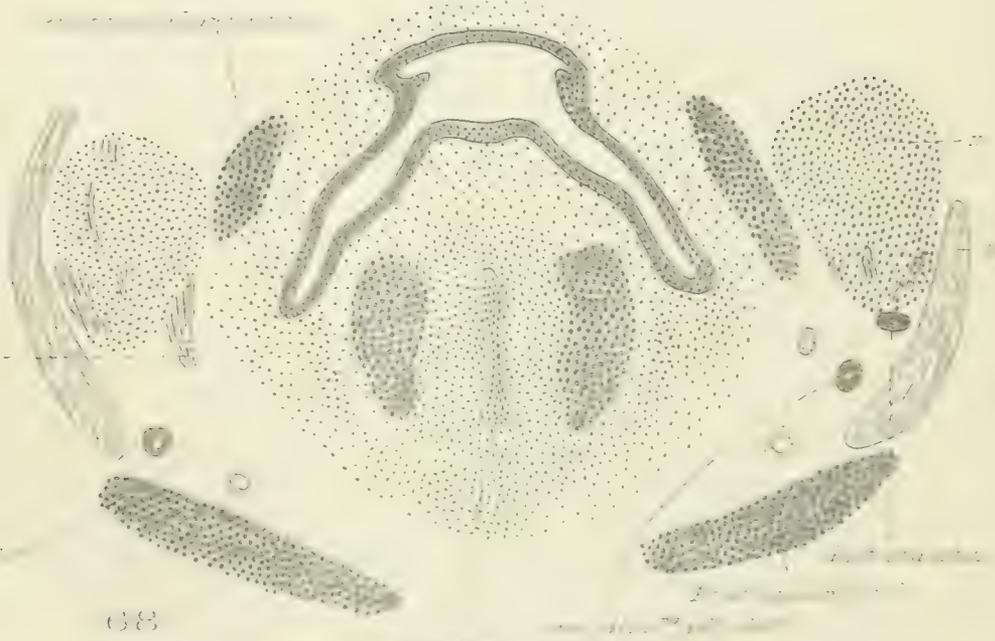
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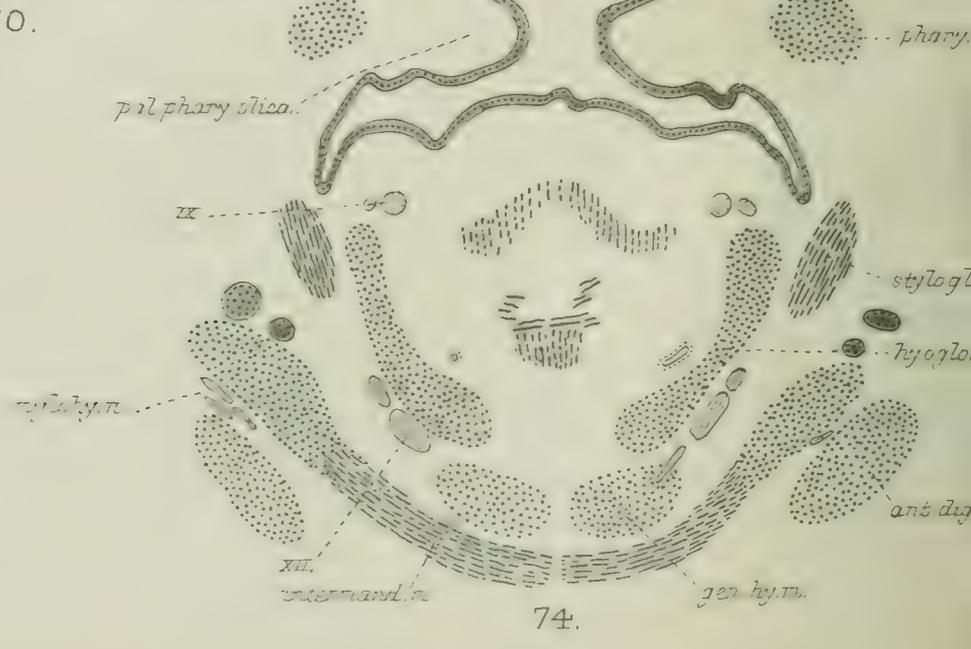
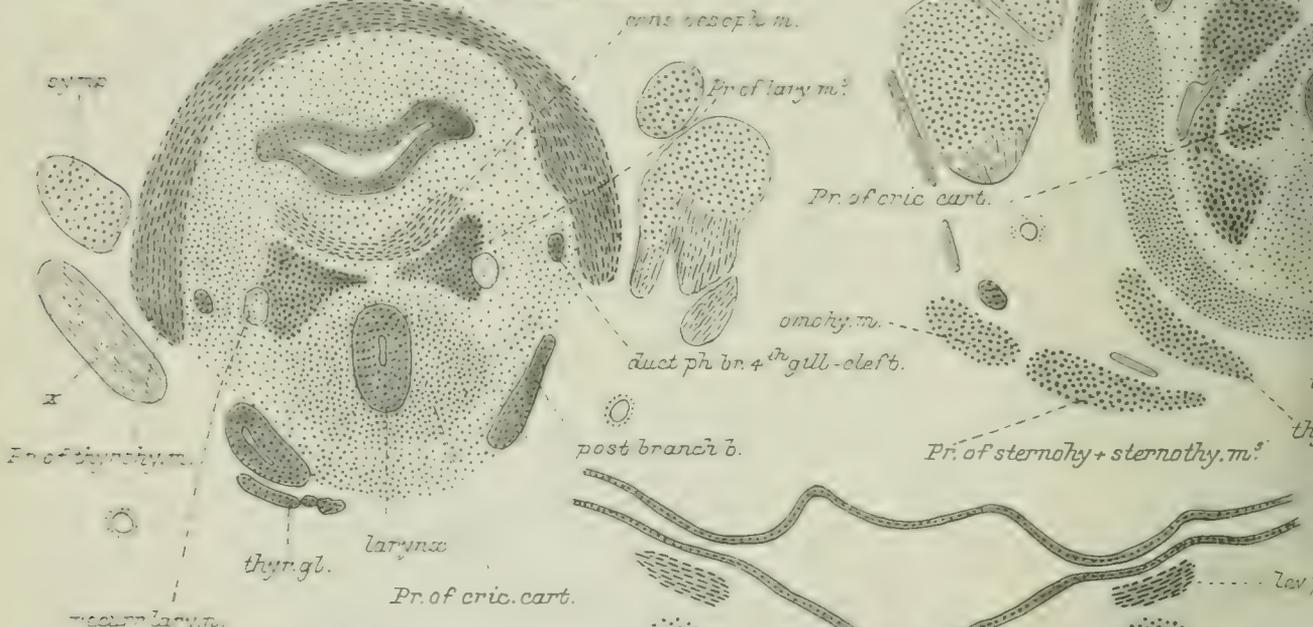
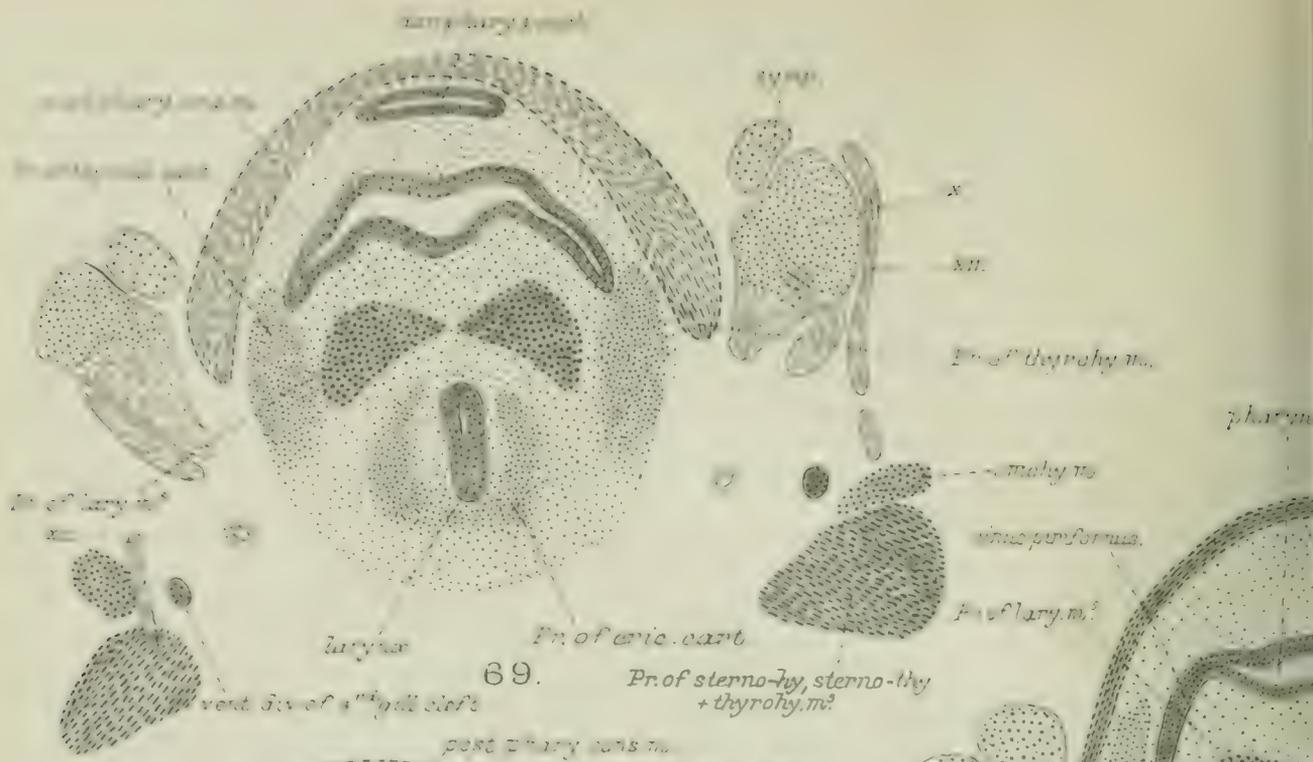


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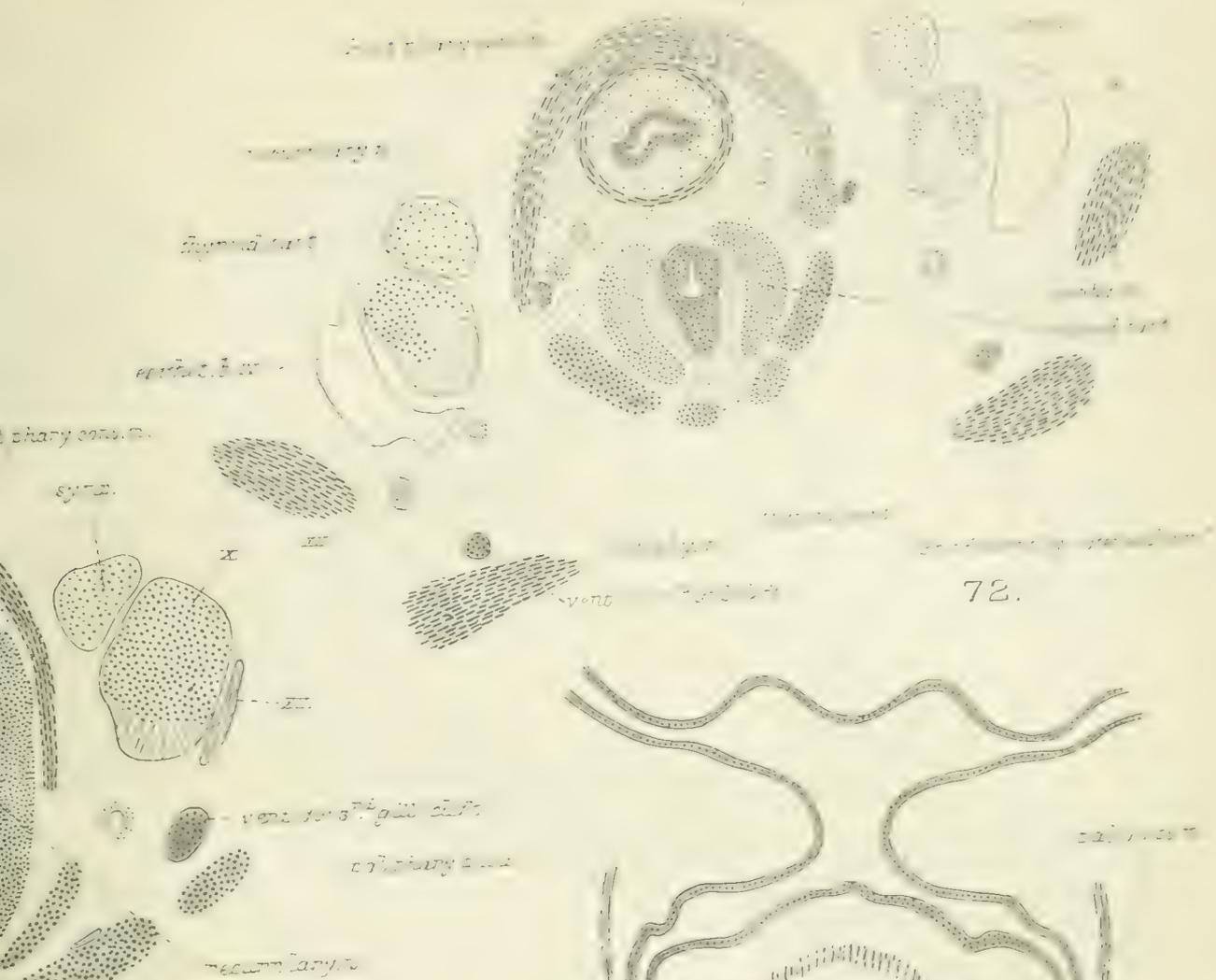


dor. phary. pouch.

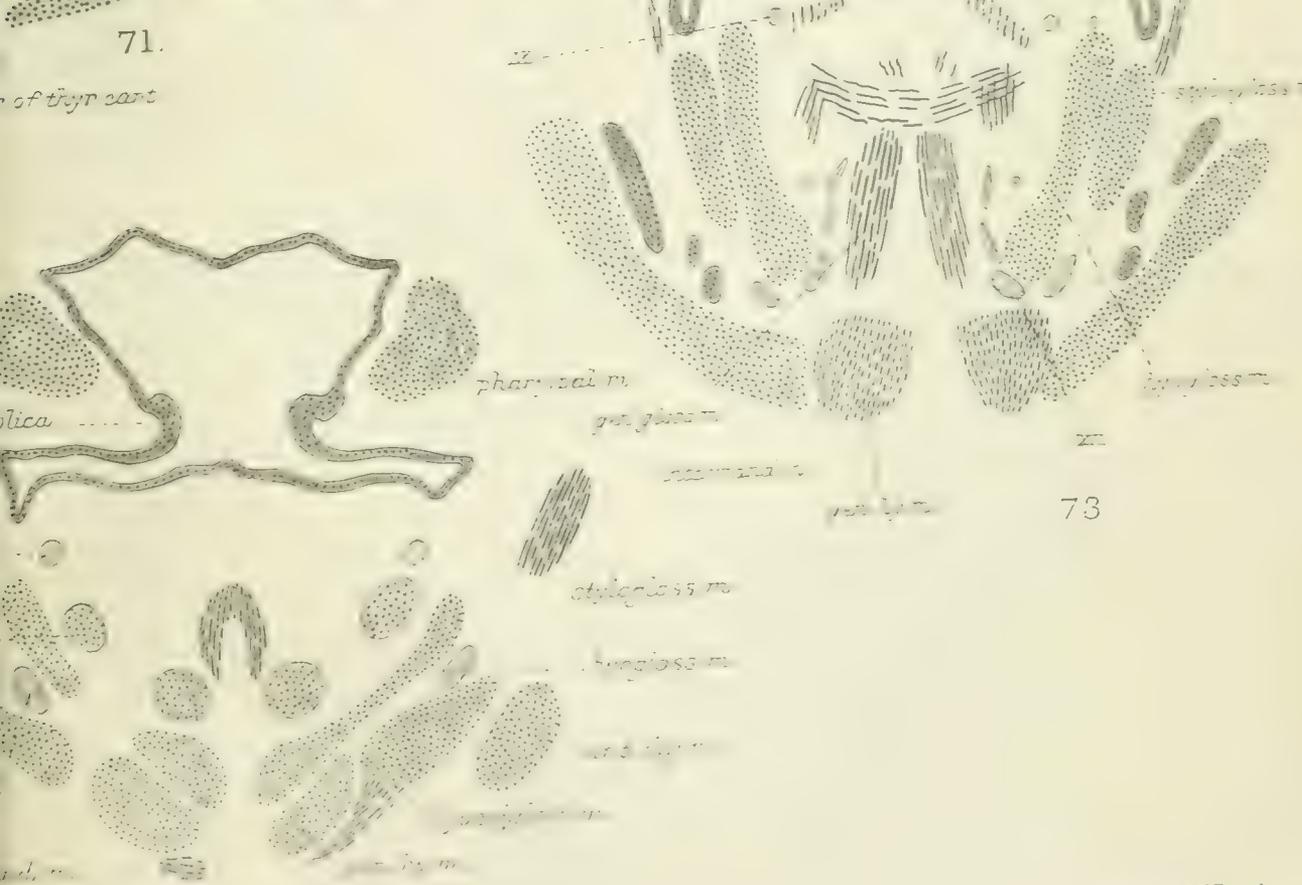




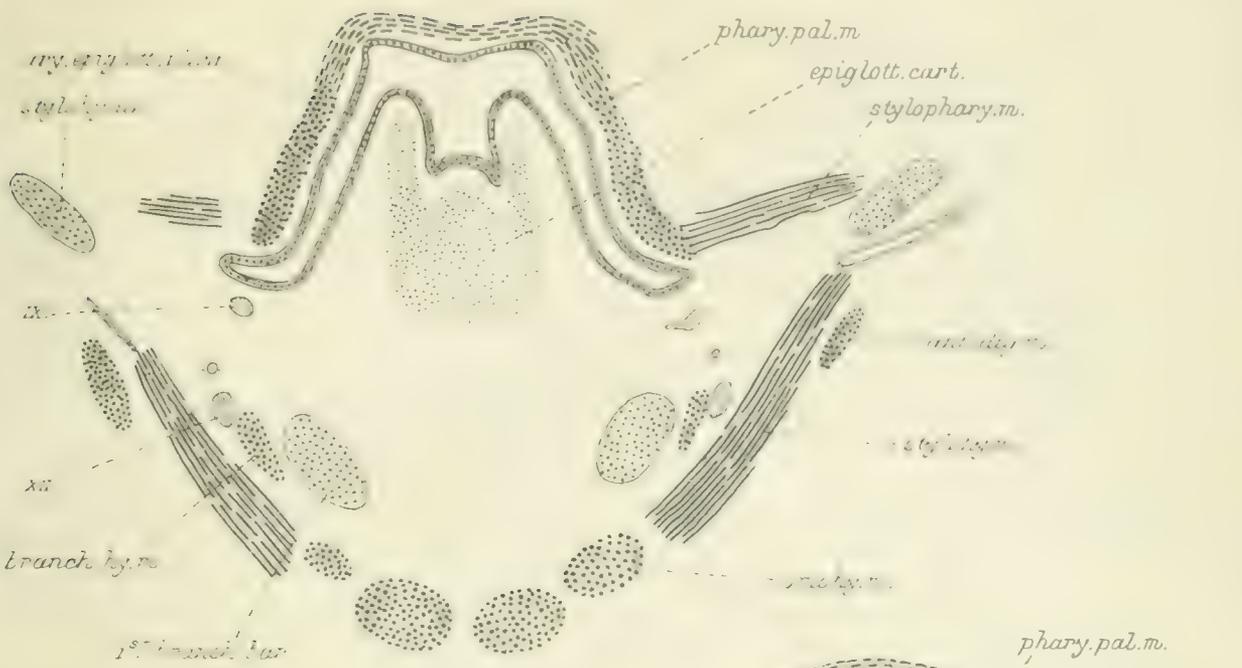
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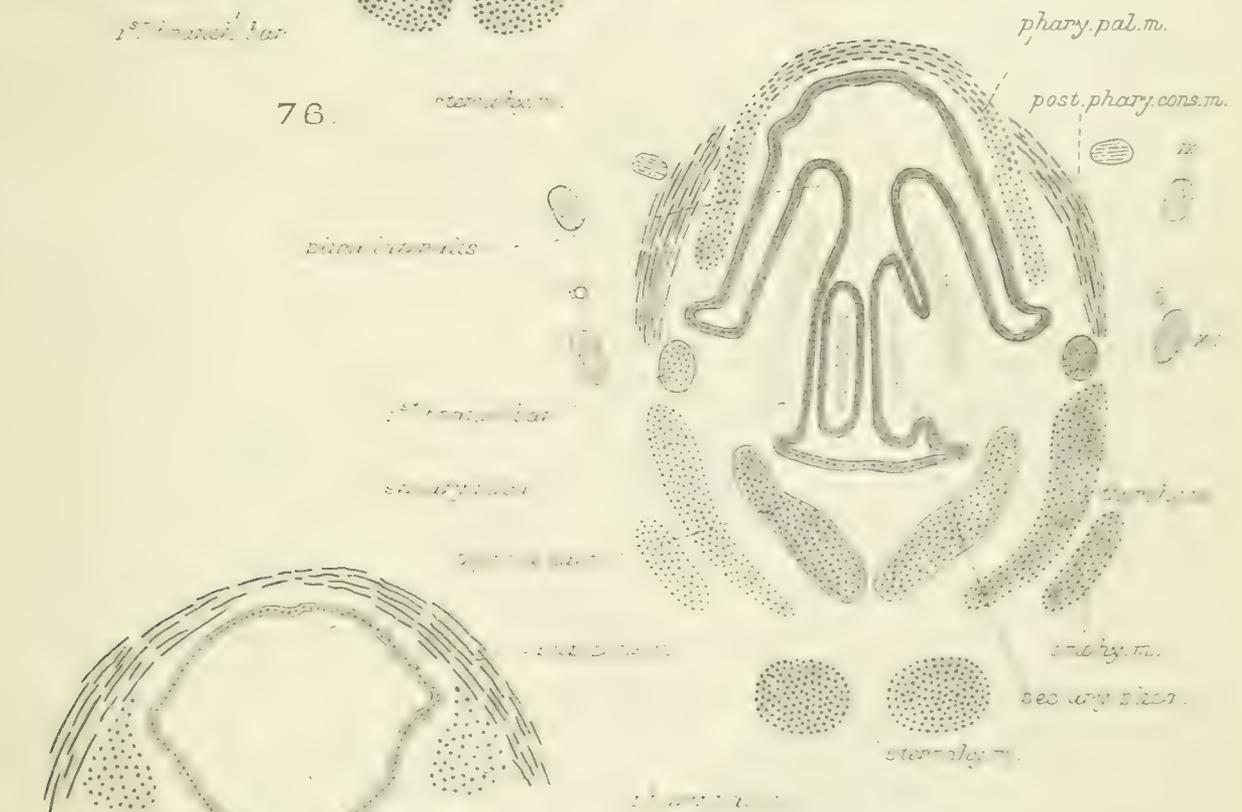
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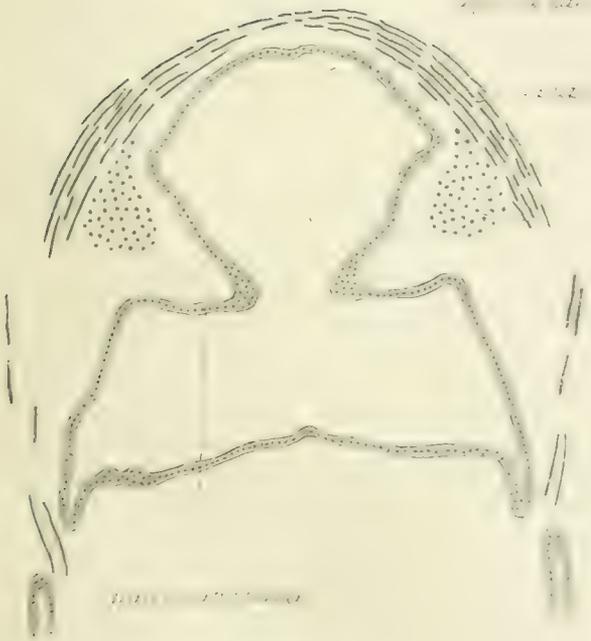
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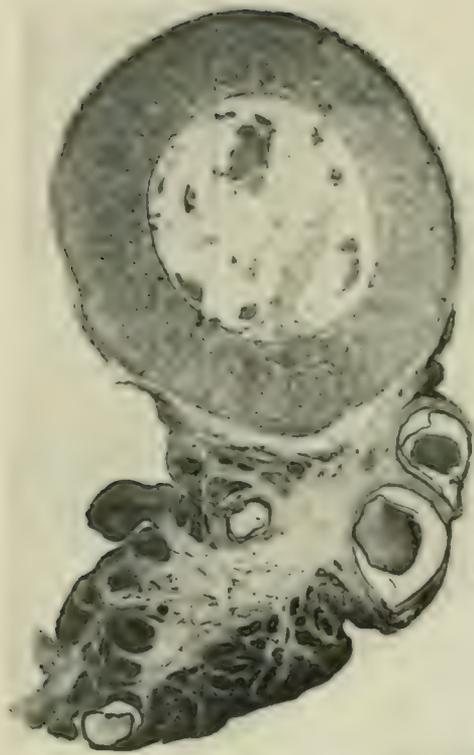


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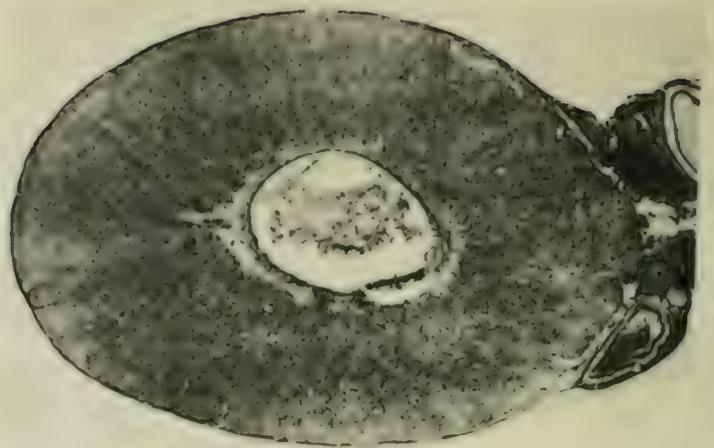


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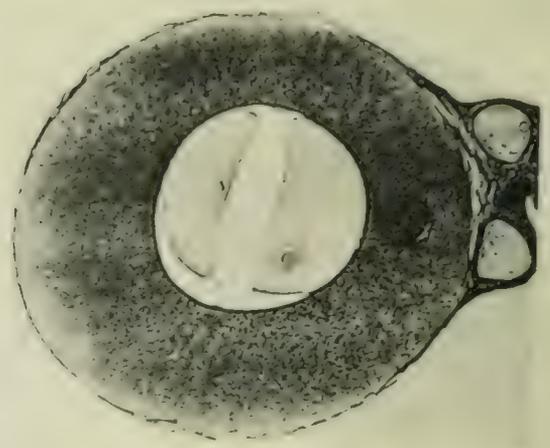




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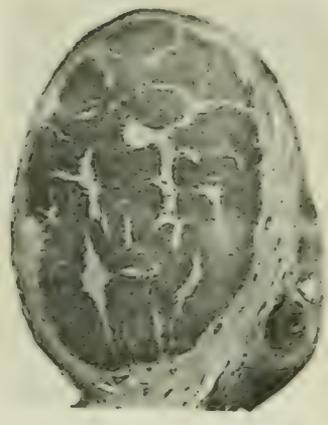
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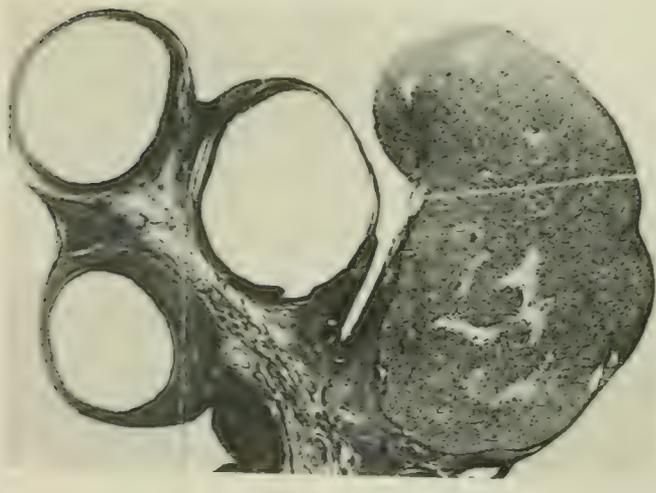
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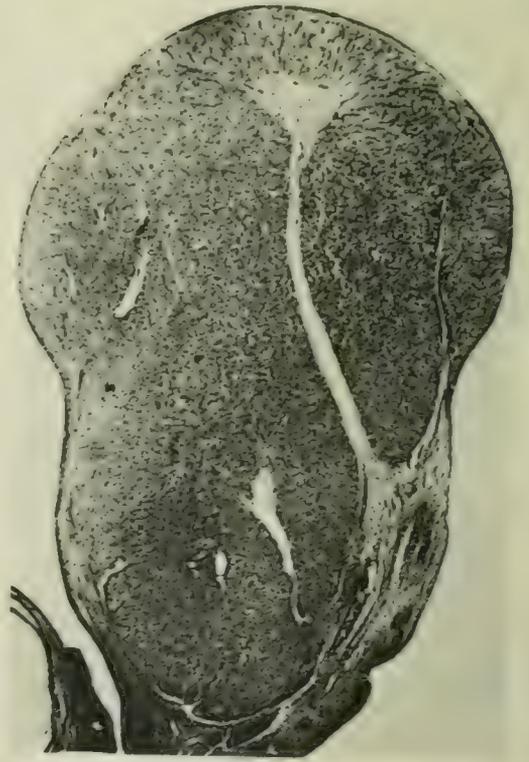
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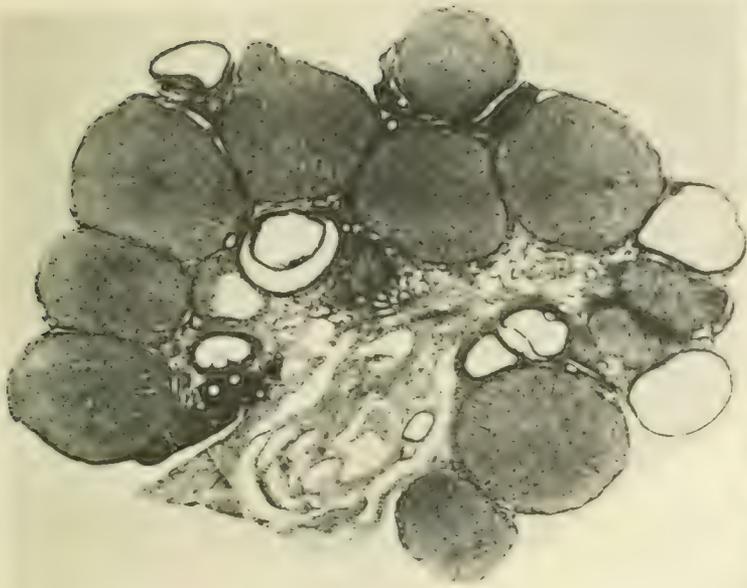
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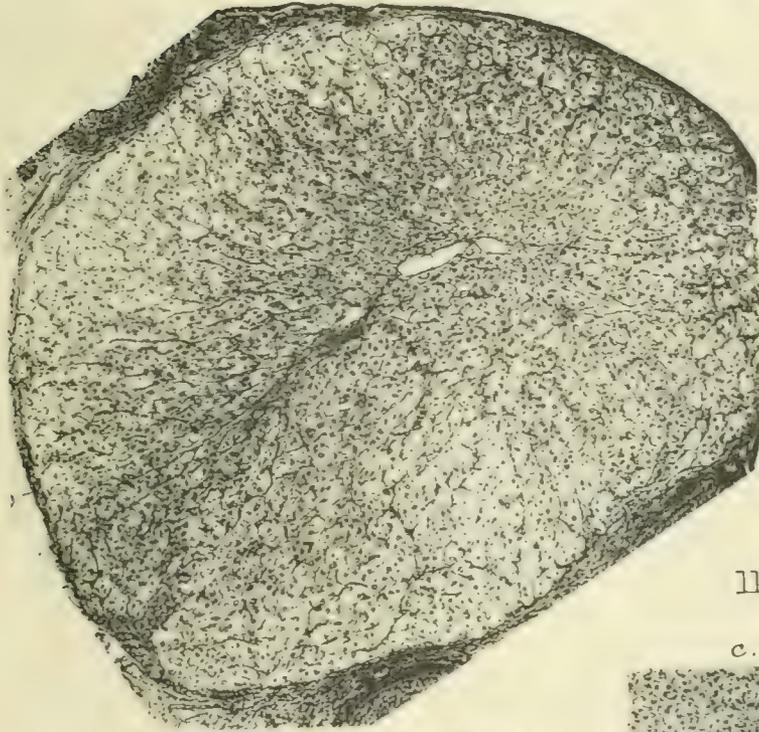
C.H.O.D. photo.

8.



10.

12.



11.

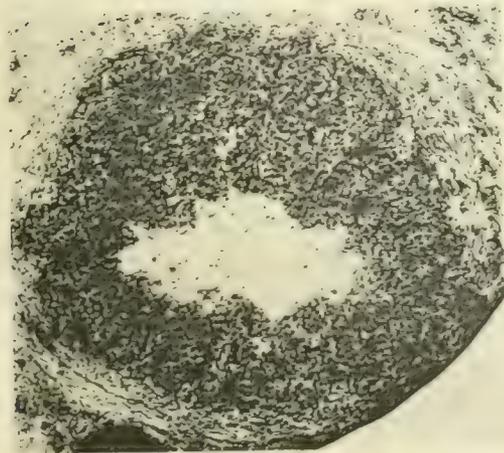


c.

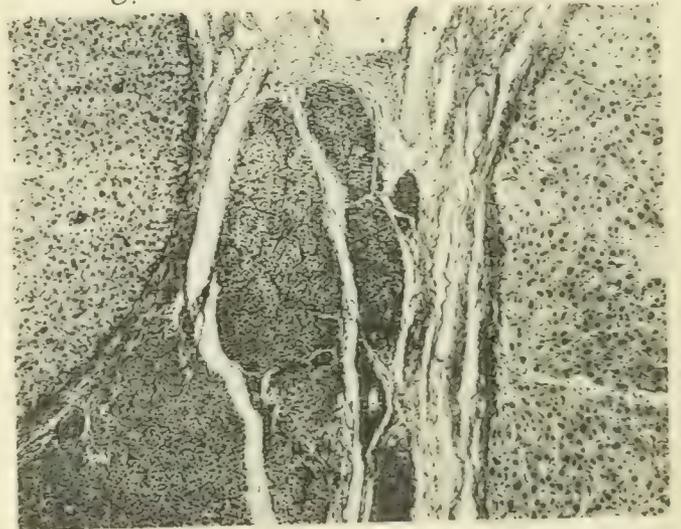
b.

a.

9.



13.



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