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On Julinia; a New Genus of Compound Ascidians from the Antarctic Ocean.

By

W. T. Calman,
University College, Dundee.

With Plates 1—3.

The Ascidian described in the present paper was collected in the Antarctic Ocean by Dr. C. M. Donald, of the whaler "Active," during the Dundee Whaling Expedition of 1892–93, and was placed along with other specimens in Professor D'Arcy W. Thompson's hands. Its large size and remarkable appearance attracted attention, and closer examination suggested its possible identity with the species described by Professor Herdman in his report on the Compound Ascidians of the Challenger Expedition ('Chall. Rep.,' vol. xiv, pp 250) as ——— (?) ignotus. The specimens on which this species was then tentatively founded, two of them in the Challenger collection and the third in the British Museum, were in an unsatisfactory state of preservation, so that Professor Herdman was unable to determine the genus, or even with certainty the family, to which the species belonged. Our specimen was preserved simply in methylated spirit, but on sectioning it was found to be in a better state than might have been anticipated, giving good results with the usual stains and making possible a fairly complete study of its anatomy. In the following description, Maurice's monograph on Fragaroides ('Arch. de Biol.,' viii, pp. 205—495) has been used for comparison

throughout, as being the most complete account we possess of the structure of a Compound Ascidian.

Our species, whose relation to Professor Herdman's —— (?) ignotus I shall again return to, evidently forms the type of a new genus, which at the suggestion of Professor D'Arcy Thompson (under whose guidance this paper has been prepared), I propose to dedicate, under the name of Julinia, to the learned naturalist whose researches on the subneural gland and other anatomical features form classical contributions to our knowledge of the Ascidians.

The specimen was found floating on the surface of the sea in the north of Erebus and Terror Gulf, and Dr. Donald tells me that considerable quantities were seen. As there is nothing in its structure to suggest a pelagic mode of life, the colonies may have been torn from their submarine attachment in shallow water. At the same time the remarkable genus Cœlocormus must be remembered as showing the possibility of a Compound Ascidian leading an unattached life though destitute of any special means of locomotion; moreover in the present case the extreme elongation of the colony renders it improbable that it could have been supported by a narrow base of attachment.

External Appearance and General Features of the Colony.

The colony is irregularly cylindrical in shape (Pl. 1, fig. 1), measuring 78·5 cm. in length and from 1·5 to 2·5 cm. in diameter. One end is torn and ragged, while the other is slightly tapered and smoothly rounded. No attaching fibres such as Herdman describes are preserved. Over large areas, particularly along one side, the surface presents a decayed appearance, the zooids being absent or scattered here and there and partially macerated. Over the rest of the surface the zooids are arranged in fairly regular round or oval systems (Pl. 1, fig. 2), each comprising from about six to twelve zooids. The colour of the whole is yellowish with a tint as though in life it had been orange, the zooids appearing as lighter spots. The buccal orifices are small and six-lobed. In the centre of

each system is a large and irregularly-lobed common cloacal opening.

In a transverse section through the colony (Pl. 1, fig. 9) the zooids are seen arranged round the circumference. From their proximal ends vascular processes pass inwards, branching and interlacing in the interior of the colony. In the further structure of the framework of the colony we have to deal with a number of elements more or less obscure, which may be briefly enumerated but which remain here as in other cases imperfectly understood. Just below the ascidiozooids large numbers of peculiar vesicles occur scattered among the vessels. These vesicles are spherical, from ·3 to ·7 mm. in diameter, and consist of a denser layer of the test-substance surrounding a granular mass of variable size and appearance, in which numerous nuclei are occasionally found. It is possible that these masses may represent degenerating zooids like those described by Maurice in Fragaroides, though their great abundance is rather against this view. Many buds in different stages of development occur scattered among these vesicles. The centre or core of the colony is composed of the spongy test-substance, traversed by the vascular processes, which here run more or less in a longitudinal direction.

In its minute structure the test resembles that described by Herdman in Colella (l. c., p. 78). In section (Pl. 3, fig. 32) it presents a reticulate appearance, the cavities occupied by the large vesicular or vacuolated cells known as "bladder-cells" reducing the matrix to a mere interstitial network. These "bladder-cells" are of common, but not universal occurrence among Compound Ascidians.

In the immediate neighbourhood of the zooids and of the vascular processes the tissue is denser, forming tracts of homogeneous matrix-substance which show, besides a few bladder-cells, small test-cells of the more usual type, with scanty protoplasm and irregular outline. Near the surface of the colony numerous groups of yellowish pigment-cells occur. These are irregularly rounded (probably shrivelled by the spirit), highly refracting, and apparently homogeneous. In

the deeper parts of the colony are found scattered here and there a few small crystalline masses (Pl. 1, fig. 11). These bodies dissolved somewhat slowly and without effervescence in hydrochloric acid, but on account of their small number their chemical nature could not be definitely ascertained, and they cannot be certainly assumed to represent the stellate calcareous spicules, e. g. of the Didemnidæ.

Scattered here and there in the test-substance are groups or nests of rather large cells (Pl. 1, fig. 10) containing rounded deeply staining masses of varying size and appearance. The protoplasm is often very granular, and no nucleus is visible. It is possible that these obscure bodies represent the test-phagocytes of Maurice, to which he ascribes the function of absorbing the dead zooids, but it is to be noted that here they do not occur in relation to the masses described above as possibly representing degenerated zooids.

General Conformation of the Ascidiozooids.

The body (Pl. 1, fig. 3) is divided into two regions, the thorax and abdomen, united by a narrow neck. The thoracic region contains the branchial sac, and varies in shape according to the state of contraction. In the most expanded specimens it is roughly square-shaped when seen from the side, and broadly oval in transverse section (Pl. 2, fig. 14). A number of longitudinal muscle-bands run in the mantle, about four to six on each side. Along the ventral edge of the thorax runs the large undulating endostyle. On the dorsal surface of the animal is the wide atrial orifice, behind which is attached the vesicle described below as probably representing an incubatory pouch. In front of the atrial orifice is the very large atrial languet. The languets belonging to the several zooids of a system meet at their edges to form a composite roof to the common cloacal chamber; seen from the outside this roof is hidden by a continuation of the common test, in the centre of which is the common cloacal opening.

As a result of this arrangement, the individual languets are somewhat irregular in shape. There appears to be no such

continuation of the test-substance on the lower surface of the languets as is seen in Fragaroides. In the abdomen the loop of the gut lies transversely to the dorso-ventral axis, the oval stomach being on the right side. On the dorsal side of the loop is the ovary, containing one or two large orange-coloured ova. In front of this the opaque white vas deferens leads forwards to the cloaca. The heart in its pericardium lies on the ventral side of the abdomen.

Branchial Cavity.—The buccal siphon is short, ending in front in six simple rounded lobes (Pl. 1, fig. 8). It is lined as usual by a reflected portion of the test, and a strong sphincter muscle is developed in its walls (Pl. 2, fig. 21). The oral tentacles (Pl. 1, fig. 8) are twelve in number, situated on a hexagonal ridge surrounding the base of the buccal siphon. Six of the tentacles, situated at the angles of the hexagon, are larger than the others which alternate with them. Separated from the tentacles by the "peribranchial zone" is the "peripharyngeal band," here apparently a simple ridge, not grooved as in Fragaroides. On the ventral side it is continuous with the anterior end of the endostyle, while on the dorsal side it appears to fuse, as in Fragaroides, with the small dorsal tubercle which bears the simple rounded opening of the subneural gland.

The branchial sac is large and comparatively simple. There are four rows of long and narrow stigmata, each row containing about twenty-four slits on each side (Pl. 2, fig. 12). Between the four rows three shelf-like membranes project into the interior of the sac. These are the "lames intersériales" of Maurice, and are continued round the circumference of the sac, being only interrupted by the endostyle. Along the middle of each row of stigmata runs a transverse vessel connected with each of the interstigmatic bars, but not otherwise interrupting the stigmata. A similar arrangement to this occurs in the genus Distaplia. On the dorsal side, but to the left of the middle line, each interserial lamella is produced into a large ciliated languet. Of these the two anterior are placed somewhat nearer the middle line than the third. A similar un-

symmetrical position of the dorsal languets to the left of the middle line occurs according to Herdman in Distaplia rosea, and Maurice describes the same arrangement in Fragaroides.

The branchial sac is connected with the wall of the atrium by small trabeculæ placed at the level of the interserial lamellæ (Pl. 1, fig. 8). These are few in number, apparently only one on each side for each lamella, though I could not ascertain this with certainty. This condition presents the opposite extreme to that seen in Fragaroides, where an almost continuous membrane connects the branchial sac with the body-wall between each row of stigmata. The wall of the branchial sac, in contrast to that of Fragaroides, is here entirely without muscles, except for the longitudinal bands running in the lips of the endostyle and those at the sides of the dorsal sinus.

The minute structure of the interstigmatic bars agrees perfectly with that described by Maurice for Fragaroides. Along each side facing the cleft is the specialised stigmatic epithelium (Pl. 2, figs. 17 and 18), formed by six regular rows of ciliated cells. Each cell forms at its free end a narrow longitudinal ridge along which the cilia stand in a single row. The surfaces of the bar turned towards the branchial and atrial cavities are covered by simple flattened epithelium. In the centre is a blood-sinus bounded by the basement membrane of the epithelium.

The endostyle is large and much undulated. In transverse section (Pl. 2, fig. 20) it shows essentially the same structure as that of Fragaroides. The everted lips are covered by ciliated epithelium. At the bottom of the groove a narrow band of columnar cells bears the enormously long cilia characteristic of the Ascidian endostyle. On each side of the groove is the "glandular" epithelium, consisting of elongated curved cells tapering towards their free ends, and this side wall of "glandular" epithelium is divided into three parts by two small bands of ciliated epithelium. These two bands are here identical in structure, while in Maurice's account of Fraga-

roides the inner or more median of the two appears as a "troisième épithélium vibratile de caractères tout particulières." Just beneath each of the recurved lips of the endostyle a muscle-band runs along its entire length. At the posterior extremity the right lip is continued backwards as a ciliated ridge ("raphé postérieur") which becomes continuous with the œsophageal funnel.

Digestive Tract.—The funnel-shaped opening of the œsophagus into the branchial chamber is large and convoluted (Pl. 2, fig. 13), and the columnar ciliated œsophageal epithelium which covers it is sharply marked off at its edge from the flattened epithelium of the branchial sac. Close to the free surface of the œsophageal epithelium is a layer of deeply staining granules of unknown nature. The œsophagus has thick and convoluted walls (Pl. 2, fig. 19), and its hinder end projects for a little distance into the stomach, where it forms a sort of valvular opening (Pl. 2, fig. 23).

The stomach (Pl. 1, fig. 4) has the form of an oval sac, smooth on the outside but having the lining epithelium thrown into oblique longitudinal folds. This epithelium is very badly preserved in all our specimens, but it can be seen that the cells are columnar and stain deeply.

Embedded in the lining epithelium of the stomach, or lying free in its cavity, are certain large oval or pyriform cells (Pl. 2, fig. 25). These have sharp outlines and very granular protoplasm, stained only faintly by hæmatoxylin and not at all by carmine. The nucleus is large and transparent, and contains a large deeply-staining nucleolus. In one or two cases a small segment, marked off from one end of the cell as shown in fig. 25 *a*, confirms a belief suggested by the nuclear and other characters that these are parasitic Gregarines.

A slight constriction separates the stomach from the intestine (Pl. 2, fig. 23). The latter is of nearly uniform diameter throughout its length, there being no division into duodenum, chylific ventricle, and rectum. Its walls are slightly folded, and it is lined throughout by columnar epithelium. The terminal part projects slightly into the cloacal

chamber, forming what Maurice calls a "pavillon anale," but I have failed to find an anal sphincter, though such in all probability exists.

The intestinal gland is well developed. A series of tubules ramifies over the surface of the intestine (Pl. 2, fig. 24); these are lined by somewhat irregular cells, and unite to form a duct which crosses the intestinal loop to open into the stomach at about its middle (Pl. 2, fig. 23).

Nervous System.—The nerve-ganglion is oval in form, and gives off numerous nerves. Two large nerves in front and a large median nerve (the cloacal), flanked by two smaller lateral ones behind, are easily seen; but it was found impossible to determine the number or arrangement of the smaller lateral nerves.

On the dorsal wall of the dorsal blood-sinus a slight thickening composed of a few small cells (Pl. 2, fig. 22) represents the "cordon viscéral ganglionnaire" of van Beneden and Julin, the remains of the central nervous axis of the larva. In a fortunate series of sections this cord can be traced into connection with the cerebral ganglion, which it enters at the root of the cloacal nerve.

In minute structure the ganglion corresponds exactly with that of Fragaroides (Pl. 2, fig. 21). Outside is a layer of large nerve-cells, finely granular and with conspicuous nuclei, sending processes inwards—the "grey matter" of Julin,—while the interior is composed of the usual fibrillated "punktsubstanz"—the "white matter" of Julin.

Subneural Gland.—The subneural gland (Pl. 2, fig. 21) is very feebly developed, consisting merely of a thin layer of cells lying on the posterior face of the nerve-ganglion, separated from it in the middle line by a space representing the backward continuation of the duct. The tissue of the gland consists of ill-defined rounded cells, many of them containing vacuoles.

The duct, on the other hand, is well developed and normal in structure. As in Fragaroides, its anterior wall is continued back over the posterior face of the ganglion for some

distance, while the ventral wall stops short of this and becomes continuous with the band of glandular tissue. The columnar epithelium lining the duct is reflected at its mouth over the "dorsal tubercle," and in the interior of the duct bears the usual long cilia, one to each cell.

It is interesting to note that the extreme degeneracy of the gland itself (carried to a greater degree, I think, than has been described in any other Ascidian) is not associated with any corresponding reduction in the size of the duct. Indeed, even in other cases where the gland is much better developed (e. g. Fragaroides), the duct, both in respect to size and to the richness of its ciliation, seems out of all proportion to any secretory function we can ascribe to the gland.

Circulatory System.—The heart in its pericardium lies on the ventral side of the intestinal loop (Pl. 1, fig. 4; Pl. 2, fig. 16). It is a thin-walled, somewhat fusiform tube, but I was unable to trace any vessels connected with it. A space lined by endothelium and lying to the left of the pericardium may possibly represent a vestige of the epicardial system. A large blood-sinus runs along the dorsal edge of the branchial sac (Pl. 2, figs. 14 and 22); in its lateral walls run strongly developed longitudinal muscle-bands, while in the middle of its dorsal wall runs the ganglionic nerve-cord above alluded to.

The "vascular process" arises near the hinder end of the abdomen, a little to the left side. In the substance of the test these processes branch and probably anastomose, and finally end in club-shaped dilatations (Pl. 1, fig. 6). In section (Pl. 3, fig. 32) each is seen to consist of a simple tube of ectoderm divided into two by a median partition of thin and apparently structureless membrane. No internal layer of connective tissue, as described by Herdman in Colella, could be distinguished, the processes appearing to be exclusively ectodermal, like the "stolons" of Botryllus (Hjort, 'Mitt. Zool. Stat. Neap.,' x, p. 589), a point which is of interest in connection with the probable origin of buds from them. Numerous blood-corpuscles occur in the cavity of these tubes.

Sexual Organs.—The sexual organs lie on the dorsal side of the intestinal loop, the ovary being external or dorsal to the testis. In all the individuals examined several ova were found in various stages of development, the largest being about ·6 mm. in diameter. They are contained in follicles opening by short canals into the oviduct (Pl. 3, fig. 27). No very young ova were seen, the smallest being about ·1 mm. in diameter, and nowhere did the ovary present in section the bilateral T-shape described by Maurice in Fragaroides and by van Beneden and Julin in Clavellina. Inside the wall of the follicle in all save the youngest there is a loose layer of cells outside the egg-membrane (Pl. 3, fig. 28). This seems to correspond to the inner of the two layers into which the follicular wall splits in Clavellina according to van Beneden and Julin ('Arch. de Biol.,' vi, p. 358), though in the latter case the splitting does not take place until the ovum is nearly ripe. Inside the egg-membrane are the characteristic and problematical "test-cells," with large nuclei each containing several nucleoli, and scanty protoplasm. The vitellus becomes more and more coarsely granular as the egg matures, and in the oldest eggs it is broken up into numerous irregular yolk-masses. The oviduct is a thin-walled tube running up alongside the vas deferens (Pl. 2, figs. 15 and 16).

On the dorsal surface of the animal's thorax, near its junction with the neck and rather to the left of the mid-dorsal line, there is attached by a narrow neck a spherical thin-walled vesicle of about ·5 mm. in diameter (Pl. 1, fig. 3). The wall of this vesicle is composed of two layers, the outer continuous with the ectoderm of the thorax, while the inner seems to be continuous with the wall of the oviduct. It is difficult to make out the exact relations of the parts, but I believe that the lumen of the oviduct opens into the vesicle. This organ occupies the position of the incubatory pouch possessed by so many Compound Ascidians, and its apparent connection with the oviduct naturally suggests that it represents that structure, though I have never found eggs or larvæ in it in a single instance.

In nearly all the specimens examined hardly a trace of

testis could be found, although the vas deferens was usually full of spermatozoa. In one or two cases, however, the testis was developed. It consisted (Pl. 3, fig. 26) of a number of follicles lying in the connective tissue in the intestinal loop, connected by branching tubes with the vas deferens and showing groups of developing spermatozoa in each. In one specimen (badly preserved, unfortunately) the follicles are considerably larger than those figured, and the lower part of the vas deferens is very much distended (to ·3 mm. diameter) with spermatozoa. No definite relation between the states of maturity of ovary and testis such as would suggest the occurrence of protandry or protogyny could be demonstrated.

The vas deferens is usually about ·06 mm. in diameter, and is composed of cubical epithelium, probably ciliated. Its hinder part is much convoluted, though not spirally coiled as in the Didemnidæ; in front it pursues an undulating course to open close beside the anus.

The spermatozoa are linear, slightly curved, about ·001 mm. in length, and bear long flagella.

Buds.—Numerous buds in various stages of development are scattered in the substance of the test just below the layer of ascidiozooids. All of them, even the youngest, lie quite free and unattached, usually alongside a vascular process (Pl. 1, figs. 5 and 6). In no case did I succeed in tracing a connection with the parent animal, and the origin of the buds must therefore be left undecided—more particularly since Hjort ('Mitt. Zool. Stat. Neap.,' x, pp. 588 and 589) has contested Herdman's account of the process of "stolonial" budding in the Botryllidæ. In view of the obscurity still involving many points in the process of bud development in the Tunicata, it did not seem advisible to attempt a detailed examination of our very minute and imperfectly preserved specimens. One point of interest, however, is the presence of several large cells, apparently ova, in all the buds examined (Pl. 3, figs. 29—31). The appearance of these buds with their contained ova closely resembles those figured by Kowalevsky in Distaplia ('Arch. f. mikr. Anat.,' x, 1874). Herdman also

notices the early appearance of ova in the buds in several cases (e. g. Colella), and Hjort (l. c., pp. 604 and 605) has recently called attention to the migration of the egg-cells from the parent animal into developing buds in the case of Botryllus. It is possible that we have here to do with an instance of such migration, but on the other hand, if the buds arise, as seems probable, from the "vascular processes," it is very difficult to see how the ova could have reached their destination.

The youngest buds (Pl. 1, fig. 6, and Pl. 3, fig. 29) consist of a two-layered vesicle, the space between the layers containing loosely scattered cells and one or two ova. Older buds (Pl. 1, fig. 5, and Pl. 3, fig. 31) show the two peribranchial cavities developed at the sides of the branchial sac. In the latter the endostyle can be seen in sections as a pair of ridges near its anterior end. The neural tube lies dorsally to the branchial sac, but its communication with the latter could not be traced. In sections of the abdominal region (Pl. 3, fig. 30) the rudiment of the heart is seen in its pericardium, and on either side of the latter are the two "epicardial tubes," which, as has been already stated, are in this genus absent or rudimentary in the adult, but which in Fragaroides persist as a great space running the whole length of the body. A long "tail" projecting from the hinder end of the older embryos (Pl. 1, fig. 5) is probably the outgrowing vascular process.

Systematic Position.—The unnamed genus referred to by Professor Herdman as —— (?) ignotus, and already mentioned above, is so similar in general appearance as well as in habitat to the form here described that the two must be nearly related, if not identical. The poor state of Professor Herdman's specimens, which accounts for the brevity of his description, may also account for certain discrepancies, as for instance when Professor Herdman describes the tentacles as numerous and equal, and the nerve ganglion as spherical in form. Our species, however, certainly does not belong to the Polyclinidæ, to which Herdman refers his —— (?) ignotus "from the general appearance of the colony and of the ascidiozooids." The genus being certainly nondescript and

requiring a name, I give the animal at the same time a new specific one in the face of what uncertainty remains as to its identity with Professor Herdman's type.

As regards the affinities of the new genus, we are confronted by the fact that the diagnoses of recognised families appear to be somewhat artificial, and certainly do not lend themselves very readily to the reception of new forms. Taking, however, the received families as they at present go, Julinia is obviously excluded from the Polyclinidæ by the absence of a post-abdomen. This negative character, together with the distinct separation of thorax from abdomen, are characters common to the three families of Distomidæ, Didemnidæ, and Diplosomidæ. From the last two families our species is excluded by the absence of retractile muscles in the vascular processes, by the large anal languets (absent in Giard's definition from the Diplosomidæ), by the want of the calcareous spicules so characteristic of the Didemnidæ, and by the characters of the testis and vas deferens, which latter in the Didemnidæ is spirally coiled round the single large testis. Narrowing down our genus accordingly to the Distomidæ, we find that it agrees with that family in the numerous spermatic vesicles of the testis, as well as with individual genera of that family in its incubatory pouch and large atrial languet. The incubatory pouch is common to Colella and Distaplia, while the atrial languet is absent in Colella. The general sum of the characters, then, seems to bring our genus nearest to Distaplia, with which latter genus it further agrees in the characters of the branchial sac with its four rows of long stigmata crossed by intermediate transverse vessels. It differs chiefly from the definition of Distaplia in the form of the colony, as well as in the ascidiozooids being embedded in the test instead of forming prominent knobs or lobes.

The following is the diagnosis of the new genus:

JULINIA, gen. nov.

Colony cylindrical, excessively elongated; ascidiozooids completely embedded in the fleshy or gelatinous test; systems

distinct, with well-developed common cloacal cavities; buccal orifices small, six-lobed; common cloacal orifice large, irregularly lobed; zooids with thorax and abdomen united by a narrow neck; atrial languet very large; vascular process well developed; branchial sac rather large, with four rows of long stigmata crossed by narrow intermediate bars; dorsal languets large, three in number, placed to the left of the middle line; stomach with internal longitudinal folds.

Species.—Julinia australis, n. sp., with the characters of the genus.

Habitat.—Antarctic Ocean.

EXPLANATION OF PLATES 1—3,

Illustrating Mr. W. T. Calman's paper "On Julinia, a New Genus of Compound Ascidians from the Antarctic Ocean."

Reference Letters.

atr. l. Atrial languet. *b.* Bud. *b. l.* Buccal lobes. *b. s.* Buccal siphon. *b. sph.* Buccal sphincter muscle. *bl. c.* Bladder cells of test. *bl. g.* Blood-corpuscles. *br. s.* Branchial sac. *c. cl. o.* Common cloacal orifice. *cl. o.* Cloacal orifice. *cl. n.* Cloacal nerve. *d. l.* Dorsal languet. *d. n.* Dorsal ganglionic nerve-cord. *d. s.* Dorsal branchial sinus. *d. t.* Dorsal tubercle. *ect.* Ectoderm. *end.* Endostyle. *epc.* Rudiment of epicardial system (?) in adult. *epc. t.* Epicardial tubes in bud. *fl.* Outer layer of follicular wall of ovary. *fl''.* Inner layer of follicular wall of ovary. *gl.* Subneural gland. *ht.* Heart. *inc. p.* Incubatory pouch. *int.* Intestine. *int. l.* Interserial lamina of branchial sac. *int. gl.* Intestinal gland. *int. d.* Duct of intestinal gland. *l. m.* Longitudinal muscle-bands. *n. g.* Nerve gland. *œs.* Œsophagus. *œs. f.* Œsophagus funnel. *ov.* Ova. *ovy.* Ovary. *ovd.* Oviduct. *pbr.* Peribranchial cavity. *p. c.* Pigment corpuscles. *per.* Pericardium. *p. ph. b.* Peripharyngeal band. *r. p.* "Raphé postérieur." *st.* Stomach. *t. c.* "Test-cells" of ovum. *t. c'.* Test-cells. *tr.* Trabeculæ from branchial sac to mantle. *tr. b.* Intermediate transverse bars of branchial sac. *ts. t.* Tubules of testis. *v. d.* Vas deferens. *v. p.* Vascular process.

PLATE 1.

Fig. 1.—General view of colony slightly reduced.

Fig. 2.—A single system of ten individuals × 7. In the centre of the system is the irregularly-lobed cloacal orifice; on the outer side of each ascidiozooid the endostyle is seen shining through.

Fig. 3.—A single ascidiozooid seen from the left side × 20. This figure illustrates the form of the branchial sac, the convoluted endostyle, and the relations of the incubatory pouch and atrial languet. In the abdomen the ovary and part of the vas deferens are seen on the dorsal side of the intestinal loop.

Fig. 4.—Ventral view of abdominal region, showing the oblique folds in the stomach wall, and the position of the heart and part of the vas deferens in the loop of the gut. The origin of the vascular process is seen on the lower side of the intestine.

Fig. 5.—Bud lying beside vascular process.

Fig. 6.—Younger bud near club-shaped end of vascular process.

Fig. 7.—View of part of common cloacal chamber seen from within, showing four atrial languets pointing inwards and meeting together. In one individual of the system the oral region is also shown. The roof formed to the common cloacal chamber by these converging atrial languets is not to be seen from the outside, it being covered in its turn by a second roof of test-substance.

Fig. 8.—Oral region of an ascidiozooid seen from within, showing the six-lobed buccal orifice, the hexagonal ring of twelve tentacles, the peripharyngeal band connected dorsally with the dorsal tubercle and ventrally with the endostyle; the nerve ganglion, the first row of stigmata, and the trabeculæ connecting the stigmatic wall with the atrial wall are also seen.

Fig. 9.—Transverse section through one half of the colony × 4, showing the position of the ascidiozooids, the distribution of the vascular processes through the matrix of the colony, and the zone of problematic vesicles referred to on p. 3 as possibly representing degenerated zooids.

Fig. 10.—Cell elements from the test supposed (p. 4) to be phagocytes. Zeiss, $\frac{1}{12}$th hom. imm., 2.

Fig. 11.—Spicules from test. Zeiss, D. D., 2.

PLATE 2.

Fig. 12.—Internal view of dorsal portion of branchial sac, showing the four rows of stigmata, the transverse vascular bars which cross them, the unsymmetrical position of the three dorsal languets, and the interserial lamellæ with which these latter are connected.

Fig. 13.—The œsophageal funnel and its connection with the endostyle.

Fig. 14.—Semi-diagrammatic transverse section across the thorax at the level of the cloacal opening, showing the proportions of the endostyle and the relations of the dorsal sinus and the degenerate rudiment of the dorsal nerve-cord.

Fig. 15.—Transverse section through the constricted neck between thorax and abdomen, showing the relations of œsophagus and intestine to vas deferens and oviduct.

Fig. 16.—Transverse section through upper portion of abdomen, showing the stomach and intestine in relation to vas deferens and oviduct, and to the heart, pericardium, and (?) epicardial tubes.

Fig. 17.—Transverse section of an interstigmatic bar, showing the contained blood-sinus, the characters of the ciliated epithelium on its lateral edges, and of the flattened epithelium on its inner and outer sides. Zeiss, $\frac{1}{12}$th hom. imm., 2.

Fig. 18.—Surface view of an interstigmatic bar.

Fig. 19.—Transverse section of œsophagus, showing its folded walls and columnar ciliated epithelium.

Fig. 20.—Transverse section of endostyle, showing the epithelial characters of its lips, side walls, and central groove. Zeiss, E, 2.

Fig. 21.—Ventral section of nerve ganglion and associated organs, showing the buccal sphincter, the ganglion with origin of the cloacal nerve, the gland of Julin, and the course of its duct. The long and well-preserved cilia in the duct are all pointing inwards. Zeiss, E, 2.

Fig. 22.—Transverse section of the dorsal branchial blood-sinus, showing dorsally the rudimentary dorsal nerve-cord, and laterally, near the attachment of the sinus to the thoracic walls, its longitudinal muscular bands. E, 2.

Fig. 23.—Longitudinal section of the abdomen, showing the valvular lip of the œsophagus where it enters the stomach, and the entrance into the latter organ of the duct of the intestinal gland. Some tubules of the testis are again seen within the intestinal loop.

Fig. 24.—Longitudinal section of part of the intestinal gland. The gland is seen lying against the wall of the intestine, while its duct passes off in the opposite direction towards the stomach. Zeiss, $\frac{1}{12}$th hom. imm., 2.

Fig. 25.—Gregarines from stomach. Zeiss, $\frac{1}{12}$th hom. imm., 2.

PLATE 3.

Fig. 26.—A portion of the testis, showing its racemose follicles containing spermatozoa. Zeiss, $\frac{1}{12}$th hom. imm., 2.

Fig. 27.—Part of the ovary, showing one ovarian follicle and a portion of another, both in connection with the oviduct. D D, 2.

Fig. 28.—Peripheral layer of ovum with its test-cells, surrounded by the two-layered follicular wall. Zeiss, $\frac{1}{12}$th hom. imm., 2.

Fig. 29.—Transverse section of a young bud, showing included ova. Zeiss, D D, 2.

Fig. 30.—Transverse section of abdominal region of older bud. In this section we see, besides the œsophagus, the intestine, and a large ovum, the developing heart within its pericardium and the two associated epicardial tubes. D D, 2.

Fig. 31.—Optical longitudinal section of a bud of about the same age as that represented in Fig. 30. The branchial sac and the paired rudiments of the atrium are here shown. D D, 2.

Fig. 32.—Transverse section of a vascular process, with its surrounding space and adjacent portions of the test-substance. Large bladder-cells, small test-cells, and pigment corpuscles are seen within the matrix of the latter.

Hermaphroditism in Mollusca.

By

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With Plates 4—6.

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I. Hermaphroditism in Mollusca.

Hermaphroditism is found to occur in every class of Mollusca except the Cephalopoda and Scaphopoda.

1. Among Amphineura, in all the Neomeniidæ.

2. Among Gastropoda, in four genera of Streptoneura (Valvata, Onchidiopsis, Marsenina, and Entoconcha), and in all the Euthyneura (Opisthobranchia and Pulmonata).

3. Among Lamellibranchia, in various species of Pecten,

Ostrea, and Cardium; in Entovalva, the Cycladidæ, the Poromyidæ, and all the Anatinacea.

1. Amphineura.

(1) Neomeniidæ.—In each of the two contiguous hermaphrodite glands of these animals the ova arise in the axial half of the organ, and the spermatozoa in the lateral half (1).

(2) Cryptochiton.—According to Middendorf (2) this genus is hermaphrodite. I have, however, been able to obtain a specimen in alcohol of this form (C. Stelleri, from the North Pacific), and have found it to present a distinctly unisexual condition, as in Chiton. I have also satisfied myself that the sexes are separate in Chitonellus larvæformis (= fasciatus) and Cryptoconchus porosus (or monticularis). Thus the diœcious condition is universal in the Polyplacophora.

2. Gastropoda.

(1) Valvata.—The hermaphrodite gland of this genus is formed of acini, all producing ova and spermatozoa (3).

(2) Marsenina and Onchidiopsis (of the family Lamellariidæ).—While the hermaphroditism of Valvata has been recognised by a fair number of zoologists, that of the two genera just named has only been affirmed by Bergh (4).

As the number of monœcious Streptoneura is extremely limited, an examination of these two genera was desirable in order that Bergh's discovery might be confirmed and his statement of facts extended. I have only succeeded in obtaining a single specimen of Onchidiopsis greenlandica, and upon it I have made the following observations.

The genital gland occupies the posterior part of the visceral mass. Its duct is provided with an accessory mass formed of closely packed, narrow, tubular cæca; it then bifurcates, and the more lateral branch (the right), after first receiving a large bent pouch (fig. 1, iv), immediately thickens before opening to the exterior, and again receives on its right side a large pouch of flattened form. The aperture of this branch is situated in

the mantle cavity, in front of the anus and more to the right; it is the female orifice (VII).

The other branch immediately presents a glandular mammillated mass with closely packed lobules, and then extends forward under the integument over the neck of the animal as far as a considerable projection of the body-wall on the right side, the penis, which it traverses from end to end (fig. 1, v).

The structure of the reproductive apparatus thus differs from that of Valvata in the presence of glands upon the hermaphrodite portion of the duct, and by the absence of a differentiated "uterus" in the female portion.

The glands of the hermaphrodite duct (fig. 1, x) do not appear to me to be vitellogenous or albuminiparous glands (judging, at least, from the imperfectly preserved specimen which I have examined). I regard them rather as vesiculæ seminales.

As for the two appendages of the female duct, the elongated pouch is a receptaculum seminis (or poche copulatrice), and the flattened pouch (fig. 1, III) is the mucous or jelly gland. Lastly, the glandular mass of the male duct can be termed the prostate, as in other hermaphrodite Gastropods.

If the presence of two genital apertures could not, in itself, demonstrate the hemaphroditism of Onchidiopsis, the structure of the genital gland is sufficient to remove all doubts. This gland is formed of parallel cæca, bifid at their extremities (fig. 3): these terminal divisions are ovogenous, while the proximal portions of the duct are spermatogenous. As a result of this arrangement the gland appears to be composed of a superficial or external female portion, and of a deeper or central male portion. However, these two regions are not demarcated with any regularity, and in the middle portion male and female acini can be seen in sections lying side by side (fig. 2). At all events, the products of the two sexes either do not arise in the same cæcum or they do not arise in the same region of a cæcum.

According to Bergh's observations, Marsenina appears to

be constructed upon the same plan,—that is to say, the genital cæca are female in their terminal portion.

(3) Entoconcha.—This genus, so degraded by parasitism, has unfortunately not yet been studied by the method of serial sections. As is known, there is a female genital gland in the middle region of the body, and a number of spermatogenic capsules further back towards the posterior orifice of the body.

(4) Bulloidea.—The genital gland is here formed of acini, all hermaphrodite (Bulla, Limacina, &c.); but in certain forms (Actæon, Pelta, Lobiger) I have found it composed of distinct male and female acini.

(5) Aplysioidea.—The same acini produce spermatozoa and ova (Aplysia). I have found, however, that the male and female acini are distinct in certain specialised forms; the former (male) occupy the central, and the latter the peripheral region (Pneumonoderma, Clinopsis).

(6) Pleurobranchoidea.—The genital gland is uniformly composed of hermaphrodite acini in Umbrella. But in Tylodina and all the Pleurobranchidæ I find that the acini are either male or female, and that the latter open into the former.

(7) Nudibranchia.—Meckel was the first to notice in certain Nudibranchs that the genital gland is formed of male and of female acini; but he supposed that these acini of different sexes were without communication with one another, and that in the genital ducts there was a male canal embedded in the female one (5).

Nordmann immediately afterwards showed that in Tergipes the ovular acini open into capsules full of spermatozoa; but he took the entire hemaphrodite gland for an ovary merely, and held the sacs full of spermatozoa to be "poches de fécondation" (6).

It was R. Leuckart who first determined and correctly interpreted the constitution of the hermaphrodite gland of Nudibranchs, especially Eolis (7),—i. e. peripheral acini exclusively ovular, opening into central chambers producing spermatozoa. His interpretation has been confirmed since for a great

number of genera by various authors (Hancock, Bergh, Trinchese, and myself); the same is the case with those Nudibranchs in which the male and female acini are easily distinguished under a lens of low power (e. g. Fiona).

It must be noticed, however, that in Eolis (Coryphella) Landsburgii the acini of the hermaphrodite gland produce ova in their distal portion and spermatozoa in their proximal portion (8); this arrangement has been recognised as general in all the Elysioidea (Cyerce, Hermæa, Elysia, Limapontia).

(8) Pulmonata.—In these Gasteropods the genital gland is formed of hermaphrodite acini both in the Stylommatophora (e. g. Helix) and in the Basommatophora (e. g. Auricula).

In Siphonaria (Basommatophora), according to Haller (9), each acinus of the hermaphrodite gland is exclusively of one sex, either male or female. In S. Algesiræ, which I have studied, I have observed that the conformation of the gland is precisely analogous to that found in Onchidiopsis, the Pleurobranchidæ, and the Nudibranchia,—that is to say, the peripheral female acini open into the more centrally situated male acini.

However, this conformation is not so entirely different from that presented by the other Pulmonata. I know cases, in fact, in which the wall of the genital gland already shows a distinct sexual differentiation upon the two sides of the follicles, and in which the female side exhibits projections which are the rudiments of acini of this sex (Amphibola).

It follows, then, from what has been said above, that examples of the various possible modes in which the genital gland is constituted are to be found side by side in all the subgroups of hermaphrodite Gastropods.

3. Lamellibranchia.

(1) Ostrea.—The question of sex in oysters has long been a subject of controversy, and its solution, which presents decided difficulties, is not yet universally recognised.

The first point capable of immediate demonstration is that there are both hermaphrodites and dicœcious oysters.

A. Hermaphrodite Oysters.

a. Ostrea edulis, Linné, the hermaphroditism of which was first demonstrated by Davaine (**10**), and subsequently confirmed by Lacaze-Duthiers, Hock, &c.

b. Ostrea stentina, Payr. (= plicata, Chemnitz); also recognised as hermaphrodite by Lacaze (**11**).

c. Lastly, it has been recently stated that the oyster of the N.W. coast of America is also hermaphrodite; this, I suppose, is Ostrea lurida.

In the genital glands of hermaphrodite oysters the spermatozoa and the ova arise in the same acini, but at different times, so that the products of the two sexes are not often to be seen in the same individual. This perfect alternation is the most striking and distinctive characteristic of the heramaphroditism of oysters; it explains how it has been believed, and how at first sight it would still be possible to believe, that the oyster is unisexual.

The male products are the first to appear. This protandry was discovered by Davaine (**13**), and confirmed by P. J. van Beneden (**14**).

B. Dicœcious Oysters.

a. Ostrea virginica, Lister, from the E. coast of the United States (**15**).

b. Ostrea angulata, Lam. (= lamellosa, Broc.), the "Portuguese" oyster, from the Atlantic (**16**).

c. Lastly, I have made out the separation of the sexes in a third species, O. cochlear, Poli, from the Mediterranean, which belongs to the same subdivision (Gryphæa) as the preceding form.

I have not had the opportunity of studying this species alive, but I have had numerous specimens of it of very different sizes, collected at different times of the year.

Of all these there was not a single individual which pre-

sented both ova and spermatozoa in the genital gland at the same time. Each specimen had the glands either full of the products of one sex exclusively (fig. 8), or else almost empty (fig. 7).

Nevertheless it is impossible to suppose that they represented the successive stages, male and female, of an hermaphrodite condition: in the first place, because the individuals with male glands were no smaller than those with female glands,—there were female specimens smaller than the males, and males and females of every size; in the second place, because the appearance and conformation of the genital glands differs considerably according to the nature of the contents, as in Lamellibranchs of different sexes. The glands with spermatozoa are formed of ramifications having an appreciably constant diameter (fig. 4, II); the glands with ova are more lobulated, and so present a distinct appearance, which shows that O. cochlear is diœcious, like O. virginica and O. angulata.

I will add a word here upon the genital apertures of O. cochlear. They are asymmetrical, that of the left being the more anterior (fig. 6, IX), considerably in front of the adductor muscle. The "fente uro-génitale," discovered by Hoek in O. edulis (17) (where the genital gland opens into the urinary slit), has not in this species the simple appearance which it presents in O. edulis. The genital and renal orifices, although adjacent, are quite distinct; the genital is more anterior than the renal, less distant from the median plane (fig. 7, V), and directed outwards, whilst the renal aperture is directed towards the axis (fig. 7, X).

(2) Cardium oblongum.

Lacaze-Duthiers discovered the hermaphroditism of a species of this genus, C. norvegicum, from the Atlantic. I have further observed this condition in a Mediterranean species, C. oblongum, which is grouped with C. norvegicum in a special division or sub-genus (Lævicardium).

In Cardum oblongum the different acini are each of one sex, either male or female; but the acini of the same sex are

not confined to a particular region (fig. 9) as in Onchidiopsis, for example, although the female acini open into the male acini as in the last-named genus. I have never, however, noticed ova and spermatozoa in the same cul-de-sac, as Lacaze-Duthiers found to be constantly the case in C. norvegicum (18).

(3) Entovalva.

The structural details of the hermaphrodite genital gland are not known. There exists a single gland, not differentiated into sexual regions, on each side (19). Probably, therefore, it is constituted like that of Ostrea and Cardium.

(4) Pecten.

The greater number of species of this genus hitherto examined have been found to be hermaphrodite, viz. P. glaber (20), P. Jacobæus (20a), P. maximus (21), P. opercularis (22), P. irradians and magellanicus (23).

I can myself vouch for the hermaphroditism of P. glaber and maximus. Moreover I have observed that P. flexuosus, Poli, from the Mediterranean, also has the two sexes united in the same individual (fig. 10).

On the other hand, among all the species examined, I have only met with two diœcious forms, viz. P. inflexus, Poli, from the Mediterranean, and P. varius, Linné, from the Atlantic, in which latter form this fact was already known (Humbert, 23*a*).

The hermaphroditism of Pecten is recognised with unusual facility. The anterior part of the visceral mass may be readily seen from the outside to be whiter than the posterior part; the whiter part is the male region. Examination of fresh material, or a section, shows at once that the genital products of different sexes are formed in different regions of the hermaphrodite gland (fig. 10). At the same time, as is already known in the case of certain species, there is only one common genital orifice on each side (opening into the nephridium), and only a single genital duct, which ramifies in the various parts

of the ovotestis (P. glaber, maximus, flexuosus). The male region is thus nearer the genital aperture than the female region.

(5) Cycladidæ.

A. Cyclas.—The hermaphroditism of this genus has been known from the time of v. Siebold (24). But the conformation of the genital organs has not been completely described even by subsequent authors (25); Stepanoff has merely made known that one portion of the gland is male and the other female.

In C. cornea (fig. 12) the genital gland has a superficial position upon each side of the posterior part of the body. It is of scarcely any extent except in length (from liver to nephridium), its height and depth being inconsiderable. It is transversely divided into two portions of different appearance and size, separated by a constriction in which there is merely a ciliated duct uniting the two portions (fig. 11, III). The most anterior division is more elongated and more appreciably lobulated than the other; it extends as far as the liver. This is the male region of the gland; the other is exclusively female (fig. 11, II).

The hermaphrodite gland is thus seen to be here divided into two portions of different sexes; and these are no longer in immediate contact (as in Pecten), but already separated from one another, though connected by the intermediation of a duct.

Lastly, the posterior (female) portion of the gland is continued backwards by an hermaphrodite duct which terminates at the genital orifice. The latter, which has escaped notice hitherto) is situated outside the visceral commissure, near the most ventral point of the nephridium (where the aperture of this organ is placed), and in front of the posterior retractor muscle of the foot (fig. 12, VI).

B. Pisidium (26) and Corbicula (fide v. Jhering in literâ) are also hermaphrodite. The conformation of the genital gland appears to be the same in them as in Cyclas (27).

(6) Anatinacea and Poromyidæ.

Several years ago (28) I made known the hermaphroditism of Thracia, Lyonsia, Lyonsiella (Anatinacea), and of the allied family Poromyidæ (Septibranchia); and at the same time I confirmed its existence in Pandora, in which it had already been once affirmed (29). Having thus demonstrated the monœcious nature of all the genera of Anatinacea which I had examined, I was led to believe in the hermaphroditism of the entire group, a conclusion which accorded well with Lacaze's observations upon Aspergillum (30).

Since that time I have been enabled to study one other genus of this group, Clavagella, although, in spite of numerous efforts, I have not succeeded in obtaining either Anatina or Pholadomya, which appear to be rare. I have observed the same monœcious arrangement in Clavagella as in the other Anatinacea previously studied. There can accordingly be no doubt as to the hermaphroditism of this group, since no genus belonging to it has yet been found to be diœcious.

Like all the Anatinacea, Clavagella possesses two testes, two ovaries, and four distinct genital apertures. The two testes are symmetrically placed in the pedal projection (fig. 15, VIII), i.e. in the ventral part of the body; the two ovaries, which are much more voluminous, compose almost the entire posterior visceral mass as far as the nephridia (figs. 15 and 16).

The genital pores are situated behind; those of the testes on the sides of the base of the foot, ventrally or internally to the visceral commissure (fig. 14, VII); those of the ovaries a little further back, dorsally or externally to the same commissure (fig. 14, IV).

II. Phylogenetic Evolution of the Hermaphrodite Gland in Mollusca.

If any other group of hermaphrodite animals is taken into consideration, one is struck by the general uniformity in the structure of the genital glands. In the Mollusca, on the other

hand, as has been shown above, there is a very great diversity in the structure of the hermaphrodite gland.

Indeed, it was Leuckart who was the first to recognise that among the Euthyneurous (or hermaphrodite) Gastropods the genital gland is not always formed in the same manner, and that different types of structure are recognisable in it. The facts known to us to-day show that such is the case not only for the Gastropoda Euthyneura, but also for all the remaining groups of hermaphrodite molluscs—the Amphineura, Gastropoda Streptoneura, and Lamellibranchia.

Four different principal types can be distinguished in the conformation of the hermaphrodite gland of molluscs, but certain forms are transitional between one type and another.

1. The undifferentiated gland, i. e. with acini completely hermaphrodite.—As development shows (vide infra), the most simple condition of an hermaphrodite genital gland is that in which the gonadial wall is still undifferentiated, and produces ova and spermatozoa side by side. This condition is exhibited in—

(1) Valvata.
(2) A great number of Tectibranchs (e.g. Bulla, Aplysia, Umbrella).
(3) Almost the entire group of Pulmonata.
(4) Ostrea edulis and stentina.

A commencement of specialisation is observable in Neomeniidæ, where each genital gland generally gives rise to male products towards its lateral face, and to ova towards its axial face.

Amphibola among Pulmonates (vide supra) also furnishes a transition to the following condition.

2. Gland with separate male and female acini (but as yet without separation into different male and female regions).—After the undifferentiated condition comes that in which the gonadial surface shows a clearly marked specialisation into distinct male and female acini,—the acini, however, not forming regions of different sex.

This arrangement exists in—

(1) Various Tectibranchs (e. g. Lobiger, Pelta).
(2) Pleurobranchidæ, Tylodina, and Nudibranchia (except Elysioidea).
(3) Siphonaria.
(4) Cardium oblongum.

It must be noticed here that the female acini open into the male acini, and that the acini of the same sex tend to group themselves together, as may already be observed in various Nudibranchs (where the female acini are the most "eccentric," i. e. superficial). This arrangement is clearly marked, and furnishes in Onchidiopsis and Pneumonoderma (vide supra) a transition to the next condition.

3. Gland with separate male and female regions (with a common duct).—The type which now presents itself is that in which the acini of the same sex are all united together in such a way as to constitute in the hermaphrodite gland a male and a female part distinct from one another, these two parts having, nevertheless, a single genital aperture and a common duct.

This conformation is characteristic of—

(1) The hermaphrodite species of the genus Pecten, where the male and female regions are contiguous and form an undivided hermaphrodite gland (fig. 10).

(2) The Cycladidæ, where it is already observable that the two regions are fairly separated, and only connected by their duct (fig. 12), which forms a transition to the next type.

4. Male and female glands in the same individual entirely distinct from one another, and with special ducts.—The acini of each sex can form a region absolutely separate from that constituted by the acini of the other sex, these two regions each having their special duct, and so forming veritable testis and ovary.

At the same time the vas deferens and the oviduct may open into a common orifice (Poromyidæ), or there may be no common orifice for the two glands. This last condition, which

represents the highest specialisation of the hermaphrodite genital gland, is only found in the Anatinacea among Lamellibranchs, and in Entoconcha among Gastropods.

III. Physiological Evolution of Hermaphroditism in the Molluscan Individual.

The hermaphroditism of molluscs is not, so to say, self-sufficient; in other words, the eggs of one individual have to be fertilised by the spermatozoa of another.

Speaking generally, the two kinds of products are not ripe at the same moment in the same individual: an interval of greater or less duration separates the two periods of maturity of the different sexual elements.

Leuckart was the first to make known that in certain Opisthobranchiate Gastropods the period of male maturity precedes that of female maturity (31), i. e. the hermaphroditism of these forms is protandric.

This protandry ought to be regarded as a general phenomenon in Euthyneurous Gastropods. This is notoriously the case in the Pulmonates (32), and it has been recognised in the various Opisthobranchs which have been studied from this point of view, viz. Lobiger (33), the Thecosomatous "Pteropods," e.g. Clio striata (34), Cymbulia (35), Desmopterus (36); Nudibranchs, among which I have observed it in Eolis and Elysia; and lastly I may add Clione limacina (Gymnosomata), in which I have noticed that individuals of a length of 15 millimetres (or less) do not as yet show any ova in their genital gland, but stages in the development of spermatozoa only.

Similarly in Entoconcha testicular capsules are only to be observed in individuals in which the eggs are not yet well developed (37).

For Neomenia, among Amphineura, protandry is equally probable (38).

In Lamellibranchs no general statement can be made with certainty. I have been unable to examine young individuals

of Anatinacea, or hatched specimens of Cyclascornea smaller than 4 millimetres: at this size both spermatozoa and ova are already to be found, and not infrequently even developing eggs among the gills. But according to the old observations of Davaine (39) and P. J. van Beneden (40), Ostrea edulis is protandric.

The alternating activity of the two sexes in the same hermaphrodite Molluscan individual is a perfectly certain fact, and explains various mistakes like that of Saint-Loup, who believed he had found a unisexual organisation in Aplysia associated with external sexual characteristics (41).

It must, however, be remarked that this succession of the two sexual conditions may be more or less rapid according to the particular genera under consideration, and that, consequently, the alternation is better marked in certain organisms than in others. It seems to me to be more appreciable where the hermaphroditism of the genital gland is most complete (i. e. where the ova and spermatozoa are produced at the same spot, as in Ostrea, Aplysia, &c.) than where there are acini or regions of different sex (Nudibranchs, &c.).

As for the general fact that the male precedes the female activity, even in the absence of physiological observations it might have been deduced from the morphological constitution of the genital glands in the adult of a great number of hermaphrodite molluscs. In those forms, in fact, which possess acini or regions of each sex, the male part is always nearest the efferent ducts or the genital aperture: the male products will accordingly be the first to reach them. This arrangement exists in Pecten, Nudibranchs, Pleurobranchidæ, Onchidiopsis, Entoconcha, Pneumonoderma, Clionopsis, &c. Cyclas alone constitutes an exception,—a fact for which I can offer no explanation.

For the rest, our knowledge of the physiological evolution of individual hermaphroditism in other groups of the animal kingdoms shows that protandry is general in all forms which have been examined from this point of view, e. g. Sponges, Plathelminthes (Trematodes and Cestodes), hermaphrodite

Nematodes (Pelodytes, Rhabdonema nigrovenosa), Myzostomidæ, parasitic Isopods, Ascidians and Salps, Myxine, Chrysophris, &c.

IV. Ontogenetic Evolution of the Hermaphrodite Gland in Mollusca.

According to the theory of the sexuality of the embryonic layers, the female reproductive elements should be of endodermic, and the male elements of ectodermic origin. As regards the Mollusca this view has been maintained by Fol ("Pteropoda" Thecosomata, 42).

But this theory has not been confirmed for any other mollusc, even for any of the nearest allies of the "Pteropods," viz. the Tectibranchs (e. g. Aplysia).

I have not had an opportunity of studying the fresh larvæ of Thecosomatous "Pteropods;" but in sections of various preserved larvæ I have been able to make out that the "corps pyriforme" (which, according to Fol, is the testicular part of the future hermaphrodite gland) neither has the structure of a testicle nor contributes at all to the formation of the genital gland. The latter is unique from its origin, as it is in its final condition. The same is the case in the other molluscs hitherto studied.

However, according to Trinchese (43), in Bosellia (a near ally of Elysia) the ova are not produced in the same part of the body as the spermatozoa, although this author is unable to explain how the male and female parts of the hermaphrodite acini eventually combine with one another. Now in Elysia, specimens of which I have examined at every age from this point of view, I find that the first ova arise in the same acini in which spermatozoa alone were present in the stage immediately preceding. So that it is impossible in this case to hold that the hermaphrodite gland results from the fusion of two male and female parts of independent origin. The same fact may be observed equally well in the "Pteropod" Clione (vide supra).

On the other hand, in a form where the male and female acini are sharply separated in the adult state, viz. Pelta (= Runcina), I find that in the young individual ova and spermatozoa arise side by side on the walls of a common pouch.

I conclude from this that the undifferentiated condition of the hermaphrodite gland is the most primitive, and the morphological observations recorded above (II) lead also to the same conclusion. The Nudibranchs and Pleurobranchidæ (with acini either male or female) are more specialised than Umbrella (with hermaphrodite acini); Onchidiopsis (with acini either male or female) is more specialised than Valvata (with hermaphrodite acini); Pneumonoderma (with acini either male or female) is more specialised than Aplysia (with hermaphrodite acini), &c.; lastly, those Lamellibranchs with male and female glands altogether separated (Anatinacea) are the most specialised (cf. the closure of the mantle, the reduction of the foot, the complexity of the gill, involving the loss of the external layer of the external branchial lamella).

In direct opposition to the theory of the sexuality of the embryonic layers, we find that the hermaphrodite gland has a single origin in the mesoderm (which is itself endodermic), not only in the Opisthobranchs and Pulmonates hitherto studied—where the gland is not yet divided into regions of different sex,—but also in Cyclas (44), where two distinct male and female regions exist (fig. 12, v and xiv). The same mesodermic origin is, moreover, to be observed in the case of both the male and female glands of those forms with separate sexes, such as Chiton, Paludina, and the Cephalopods. These results are also in complete accord with what one finds in other groups of hermaphrodite Invertebrates, e. g. Oligochæta (45), Sagitta, Turbellaria, Plathelminthes, Hirudinea (46).

Lastly, the fact that the wall of the genital gland is in continuity with the cœlomic epithelium (mesoderm) in Nuculidæ (Lamellibranchs), Neomeniidæ (Amphineura), and Cephalopoda, shows that the genital gland, whether male, female, or

hermaphrodite, is an organ possessing a single mesodermic mode of origin, as in all the other Triploblastica.

V. Origin of Hermaphroditism in Mollusca.

It has just been shown that, both from the phylogenetic and ontogenetic points of view, the hermaphrodite condition with separate male and female glands is the most specialised, and the undifferentiated hermaphrodite condition (with gland producing spermatozoa and ova at the same spot) the most archaic. Now this last state is that which most nearly approximates to the unisexual condition, since it only differs in the supplementary production of the elements of the other sex,—a phenomenon which is sometimes to be observed as an abnormality in dicœcious molluscs (e. g. Anodonta and Ampullaria).

The question may, then, be asked in the case of the Mollusca,

1. Whether the hermaphrodite state is not derived from the unisexual; and, in the event of an affirmative reply,
2. Upon which of the sexes the hermaphrodite condition has become established.

In the following pages I shall try to show—

1. That hermaphroditism is not a primitive arrangement in the Molluscan phylum (47), and that it has been derived from the unisexual state.

2. That it has become superimposed upon the female condition.

1. Hermaphroditism has been derived from the unisexual condition.—Let us consider separately the classes in which hermaphroditism is found and those in which the dicœcious state alone exists.

i. In the classes in which hermaphroditism exists it is abundantly clear that the forms with separate sexes are the most archaic, especially in the conformation of their reproductive apparatus (absence of special duct, accessory gland, and penis):

a. Gastropoda Docoglossa and various Rhipidoglossa: the genital gland opens into the kidney.

B. Lamellibranchia Protobranchia (Nucula, &c.): the genital gland opens at the junction of the cœlom (pericardium) and the kidney.

ii. If we consider now the two classes where only the diœcious condition exists, we find that they have preserved many primitive traits, especially in the reproductive apparatus: the genital gland opens into the cœlom (Cephalopoda) or into the kidney (Scaphopoda).

These facts show that in the Mollusca the diœcious condition has preceded the monœcious, and by no means (as Rouzand has tried to make out, 48) that "les Gastropodes unisexués se présentent réellement comme les descendants des Gastropodes hermaphrodites, plus ou moins analogues à ceux qui vivent encore de nos jours."

The molluscs which are certainly the most archaic are diœcious. Their genital organs present a conformation analogous to the primitive disposition observed in the ontogeny of other forms, which can on no account be regarded as the last term of a retrogressive evolution—evolution not being reversible.

But hermaphroditism has been able to establish itself, replacing the diœcious state in Mollusca of different grades of specialisation—

A. In forms where the genital glands open into the cœlom (Neomeniidæ);

B. In forms where these glands open into the kidneys (Pecten);

C. In forms where these glands have special ducts, accessory glands, copulatory organs, &c. (Onchidiopsis, Euthyneura, &c.).

2. Hermaphroditism has been established upon the female organism.—The hermaphrodite condition, then, is secondary in Mollusca. As for the organisation which has given rise to it, I believe it to be that of the female.

Comparative study of the genital ducts and of their development lends support to this opinion, as we shall at once see.

i. In Lamellibranchs, when there is a separate male and

female orifice (Anatinacea), and when the visceral commissure ("connectife cérébro-viscéral") is sufficiently superficial, the female orifice is found lying outside this visceral commissure like the genital orifice—be it male or female—of all the Lamellibranchs (fig. 13). This orifice is accordingly not a new formation.

But the male orifice is within this commissure (fig. 14), and so presents relations to which no counterpart can be found elsewhere; it is consequently a new formation, and if it was necessary for a male orifice to be produced as a new formation it is evident that the starting-point of the hermaphrodite condition must have been supplied by the female organism.

ii. Similarly in hermaphrodite Gastropods the male products are always expelled by a cœnogenetic penial orifice, which is wanting in the archaic diœcious forms (Rhipidoglossa, —as in the Amphineura and Scaphopods), while the female orifice in the same forms lies in the place occupied by the genital orifice (whether male or female) of archaic Gastropods which possess no penis.

And when an approximation of the two apertures, male and female, takes place—the former approximates to the latter in Arion; vice versâ in other cases—the disposition is secondary, and is accompanied by an increased complexity of the ducts and their appendages. In the most primitive cases the two orifices are always widely separated (Bullidæ among Opisthobranchs; Auriculidæ among Pulmonates).

In the development of the Pulmonata the penis and vas deferens are new formations (48a) which arise secondarily, the vas deferens only communicating at a late stage with the genital duct properly so called (physiologically hermaphrodite), which leads to the female orifice; that is to say, the genital organs of Pulmonata develop as female organs, which are eventually modified as as to become hermaphrodite (48b).

The opinion formulated above (that in Mollusca hermaphroditism is grafted upon the female organism) is confirmed by the following fact. In hermaphrodite molluscs, whenever

individuals accidentally present a unisexual genital apparatus, they are always female; it is always the female sex which reappears.

a. Clio (Hyalocylix) striata, without penis, cited above (III, note, according to Schiemenz).

b. Cymbuliopsis calceola, without penis (48c).

c. Agriolimax lævis, without penis, having never been male (adult female; 49).

d. Helix aspersa, without vas deferens, penis, and flagellum (49a).

e. Arion intermedius, without male reproductive organs (49b).

VI. Conclusions.

Granted, then, that in Mollusca hermaphroditism has succeeded a unisexual state, and that it has been superimposed upon the female condition, is this process paralleled in other groups?

1. The origin of hermaphroditism in a unisexual condition.—I have not been able to make any special investigations upon hermaphroditism in the various groups of the animal kingdom. It seems to me, however, that if we examine, by the comparative method, the data which we already possess upon the subject, we must come to the conclusion—opposed to the ordinarily received opinion, which possesses the authority of the names of Huxley, Gegenbaur, Haeckel, Giard, Claus, &c.—that hermaphroditism is secondary, and succeeds a primitively diœcious state.

In fact, like myself, who have reached this result for the Mollusca, so Beard and Delage have already formulated this opinion as concerning two other groups, the Myzostomidæ (49e) and Cirripedes (50). And Fritz Müller, after a more general consideration of the subject, also argues against the primitive nature of the hermaphrodite state, and shows that in the majority of groups the most archaic forms are unisexual (51).

A survey of the various subdivisions of hermaphrodite

animals shows that hermaphroditism is characteristic almost always of specialised forms (52). I will more particularly mention fixation, parasitism, fluviatile or terrestrial life, as specialisations accompanied by hermaphroditism, as the following few examples show:

- Hermaphrodite animals.
  - Fixed (53) or very sedentary — Pecten, Ostrea, Aspergillum, Clavagella; various Serpulidæ (54), Cirripedes, Myzostomidæ, Ascidiæ.
  - Parasites or commensals — Entoconcha, Entovalva, Cestodes, Trematodes, Hirudinea, certain Isopods, Myxine.
  - Fluviatile or terrestrial — Valvata, Pulmonata, Oligochæta.

In support of the opinion that hermaphroditism is a specialisation of the separation of the sexes, it may be remembered that in a great number of unisexual glands individual variations occur in which the elements of both sexes are produced, one of the two kinds of element being an abnormal product. I will cite merely a few instances:

i. Batrachians: female frog (55).
ii. Fishes: female herring (56).
iii. Molluscs: Ampullaria, Anodonta.
iv. Chætopods: ova in the testicle of Lumbricus (57).
v. Crustaceans: female Apus (58), &c.

This phenomenon makes it possible to understand that the hermaphrodite state can easily establish itself in certain circumstances where it is useful for the same individual to give rise to the products of both sexes (59).

2. The establishment of hermaphroditism on the female condition.—I cannot at present affirm that hermaphroditisim has everywhere grafted itself upon the female organisation; nevertheless the phenomenon ought to be exhibited elsewhere than among the molluscs.

As a matter of fact, there are several other groups in which the male sex is preserved in the form of individuals, either degraded or not, when there are no longer any females,

but only normal hermaphrodite individuals. This is the case with—

i. Various Myzostomidæ.

ii. Certain parasitic Isopods, e. g. the Cryptoniscidæ.

iii. Various Cirripedes, e. g. Scalpellum, &c.

And the study of one of these Cirripedes has led Delage to the same conclusion as that which I have deduced from the study of hermaphrodite molluscs: "Au début, les Sacculines étaient des animaux à sexes distincts, dont les femelles sont devenues hermaphrodites beaucoup plus tard" (60).

Lastly, the same seems to be true among osseous fishes in Serranus, according to Günther (61), viz. that the hermaphrodite individuals are transformed females. This has been confirmed by Brock, who has specially studied the genital organs of fishes. He concludes that "die hermaphroditischen Knochenfische sind weibliche Individuen, in deren Ovarium sich an Stelle einiger Ovariallamelle ein Hoden sich entwickelt hat (62). And, on the other hand, the vas deferens of Serranus is not homologous with that of other osseous fishes (63); it is, accordingly, like that of the Mollusca Anatinacea and Pulmonata (vide supra, v, 2, ii), a new formation grafted on the female condition.

In these different cases the establishment of the hermaphrodite condition has been probably characterised by the following successive stages:—Production of spermatozoa in a part of the ovaries; reduction of the size of the males and of their number (hyperpolygyny); then complete replacement of the female by the hermaphrodite form; and, lastly, the total disappearance of the degraded males.

Summing up.

A. The study of Mollusca, Myzostomidæ, Crustacea, and Pisces shows that in these groups the separation of the sexes has preceded hermaphroditism; various cases in other groups tend to show that this is true universally; and the same conclusion applies to plants.

B. In Mollusca, Crustacea, and Pisces, at least, hermaphroditism is grafted upon the female sex.

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32. Since Eisig ("Beiträge zur Anatomie und Entwickelungsgeschichte der Geschlechtsorgane von Lymnæus," 'Zeitschr. f. wiss. Zool.,' Bd. xix, p. 310), &c. According to Babor ("Ueber den Cyclus der Geschlechtsentwickelung der Stylommatophoren," 'Verhandl. Deutsch. Zool. Gesellsch.,' 1894, p. 57), Agriolimax lævis and Limax maximus furnish exceptions and are protogynous.

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47. This opinion, which I expressed several years ago for the Mollusca in general ('Comptes rendus,' t. cx, p. 1083), is also shared by Brock for the Pulmonata ('Zeitschr. f. wiss. Zool.,' Bd. xliv, p. 374), and has been since adopted by Lang ('Lehrbuch der vergleichenden Anatomie,' p. 816).

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48b. Brock.—Loc. cit., pp. 372, 374.

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ment chez tous les Crustacés hermaphrodites, est l'état primitif dans le développement ontogénétique et phylogénétique."

51. F. Müller. "Die Zwitterbildung im Tierreich," 'Kosmos,' Bd. xvii, pp. 321—334.

52. In addition to the various examples given further on, it may be remarked that the only hermaphrodite forms among Echinoderms are the Synaptids, which are the most highly specialised by the reduction of the calcareous plates, and the disappearance of the madreporite and the ambulacral tubes. Similarly in Pisces the hermaphrodite forms (Serranidæ) belong to the most specialised types of Teleosteans; see Cope, 'The Origin of the Fittest,' 1887, p. 331.

53. Though descended from free ancestors, as their larvæ show.

54. Some Spirorbis, Protula, Pileolaria, Laonome.

55. Bourne.—"On Certain Abnormalities in the Common Frog (Rana temporaria)," 'Quart. Journ. Micr. Sci.,' vol. xxiv, p. 83, pl. iv.

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59. Although it is yet a very rare practice to compare the phenomena presented by the two organic "kingdoms," it may be added here that hermaphroditism, properly so called, is also a characteristic of specialised plants, and that it is wanting, speaking generally, in aquatic plants and the most ancient of terrestrial plants; see especially Errera et Gevaert, "Sur la structure et les modes de fécondation des fleurs," 'Bull. Soc. Botan. Belg.,' vol. xvii, p. 164.

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EXPLANATION OF PLATES 4, 5, & 6,

Illustrating Dr. Pelseneer's paper on "Hermaphroditism in Mollusca."

Fig. 1.—Hermaphrodite reproductive apparatus of Onchidiopsis greenlandica, dorsal view. I, ovotestis; II, oviduct; III, mucous gland; IV, receptaculum seminis; V, penis; VI, spermiduct; VII, female aperture; VIII, prostate; IX, sperm-oviduct; X, vesicula seminalis.

Fig. 2.—Transverse section of the ovotestis of Onchidiopsis.

Fig. 3.—Part of the ovotestis of Onchidiopsis, slightly magnified, showing male (white) and female (grey) portions.

Fig. 4.—Ostrea cochlear, male, left view. I, rectum; II, testis; III, internal plate of left gill; IV, internal plate of right gill; V, pallial suture; VI, adductor muscle; VII, auricle; VIII, ventricle.

Fig. 5.—Ostrea cochlear, female, left view. I, liver, seen by transparency through the ovary; II, auricle; III, pallial suture; IV, abductor muscle; V, intestine; VI, ventricle.

Fig. 6.—Ostrea cochlear, ventral aspect, mantle and gills removed. I, right genital aperture; II, right renal aperture; III, visceral ganglia; IV, outline of the mantle; V, adductor muscle; VI, visceral commissure; VII, branchial nerve; VIII, left renal aperture; IX, left genital aperture; X, palps (the lines of adhesion of the gills to the visceral mass are indicated by dotted lines).

Fig. 7.—Transverse section of a female Ostrea cochlear. I, intestine; II, right lobe of the mantle; III, nephridium; IV, branchial axis; V, genital aperture; VI, visceral commissure; VII, external plate of the right gill; VIII, internal (adaxial) plate of right gill; IX, visceral commissure (left half); X, renal aperture; XI, reno-pericardial duct; XII, ovary; XIII, pericardium; XIV, ventricle; XV, left lobe of the mantle; XVI, rectum; XVII, auricles joined together.

Fig. 8.—Transverse section of a male Ostrea cochlear. I, right lobe of the mantle; II, branchial axis; III, testis; IV, external plate of right gill; V, visceral commissure; VI, internal plate of right gill; VII, visceral commissure; VIII, genital aperture; IX, nephridium; X, testis; XI, pericardium; XII, ventricle (on the pericardial wall of which are modified epithelial cells = pericardial glands); XIII, left lobe of the mantle; XIV, rectum; XV, auricle; XVI, intestine.

Fig. 9.—Transverse section of the ovotestis of Cardium oblongum.

FIG. 10.—Transverse section of the abdomen of Pecten flexuosus, showing male and female halves of the ovotestis. I, intestine.

FIG. 11.—Longitudinal section of the right genital gland of Cyclas cornea (the anterior part is below). I, epithelium of the visceral mass; II, testis; III, part of the genital duct between testis and ovary; IV, ovary.

FIG. 12.—Cyclas cornea, left view, without left gill and pallial lobe. I, anterior retractor of the foot; II, anterior adductor; III, palp; IV, foot; V, ovary; VI, genital aperture; VII, visceral ganglion; VIII, posterior adductor; IX, posterior retractor of the foot; X, kidney; XI, rectum; XII, auriculo-ventricular slit; XIII, ventricle; XIV, testis; XV, liver mass.

FIG. 13.—Plan of nervous system, and genital and renal apertures in an ordinary Lamellibranch I, cerebral ganglion; II, visceral commissure; III, pedal ganglia; IV, genital aperture; V, renal aperture; VI, visceral ganglia.

FIG. 14.—Plan of nervous system, and genital and renal apertures in Anatinacea. I—III, and V, VI, as in Fig. 13; IV, female aperture; VII, male aperture.

FIG. 15.—Transverse section of Clavagella passing through the male apertures. I, ovary; II, aorta; III, mantle; IV, branchial axis; V, visceral commissure; VI, spermiduct; VII, foot; VIII, testis; IX, pad of adhesion of the reflected internal lamella of the gill; X, right male aperture; XI, branchial axis; XII, ovary; XIII, auricle; XIV, intestine; XV, pericardium.

FIG. 16.—Transverse section of Clavagella (posterior to Fig. 15), passing through the female apertures. I, ovary; II, aorta; III, intestine; IV, ovary; V, visceral commissure; VI, foot; VII, pad of ciliated adhesion of the internal reflected lamella of the gill; VIII, female aperture; IX, branchial axis; X, nephridium; XI, auricle; XII, mantle; XIII, pericardium.

A Description of the Cerebral Convolutions of the Chimpanzee known as "Sally;" with Notes on the Convolutions of other Chimpanzees and of Two Orangs.

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With Plates 7—11.

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Preface.

The brain of the interesting and well-known chimpanzee "Sally," which lived for eight years in captivity at the Zoological Gardens in London, has been recently figured and described by Mr. Beddard (11) in his important memoir on the anatomy of this ape, which he considers to be T. calvus; there are, however, several features about this brain which deserve further notice.

After the brain had been described it was purchased by Professor Lankester for the Oxford University Museum; and my attention was particularly directed to it when comparing the convolutions of the anthropoid apes with those of man, for the purpose of exhibiting in the museum a series of anatomical preparations illustrating the relations of man to the apes.

One of the most striking features about "Sally's" brain is the absence of a well-defined "occipital operculum," the presence of which was supposed by Gratiolet to mark off the chimpanzee brain very strongly from that of the orang, in which it is generally absent. With this disappearance of the operculum is connected certain other modifications in this region of the brain, the most evident of which is the diminution in the extent of the so-called "Affenspalte" or Simian fissure, and the consequent resemblance of this region of the brain to that of the orang and of man. Gratiolet placed the chimpanzee further from man than the orang, but "Sally's" brain is considerably more human than that of any orang hitherto figured, and it becomes a matter of great interest to compare the gyri and sulci in the brain of this specimen with those of the more ordinary chimpanzee on the one hand and with those of man on the other. In making these comparisons I have the advantage over Beddard in that I am able to make use of the valuable and extensive researches of Dr. D. J. Cunningham, published in 1892, just after Beddard's communication to the Zoological Society; and in the present paper I shall adopt Dr. Cunningham's nomenclature, which is in agreement generally with that employed on the Continent.

My great indebtedness to Dr. Cunningham's work is sufficiently evidenced by my constant reference to it.

I. Historical.

Descriptions, more or less detailed, of the brain of the chimpanzee are fairly numerous. I append a bibliography of the more important papers; but amongst those described I have only been able to light upon two brains bearing any close resemblance to that of "Sally." One of these is described and

figured by P. Broca (6).[1] In the figure here reproduced (fig. 19) it will be seen that the "operculum" is absent on both sides. The "Affenspalte," or Simian fissure (*b. b.*), or "external perpendicular fissure" as he called it, is not interrupted on the left side, as the second "pli de passage" does not rise to the surface. But on the right side the annectant gyrus (2) becomes superficial, and separates the Simian fissure (*a. a.*) into two parts. The first "pli de passage" (*I.*) is present on both sides. It will be seen below that another explanation of these arrangements is possible. It may be well to note that the brain belonged to a young male.

The second brain to which reference may be made was described in considerable detail by Dr. Joh. Müller (9) in 1888, one of whose figures is here reproduced (fig. 25). In it the "operculum" is absent on both sides, the occipital lobe and the region of the Simian fissure being more complex than in the ordinary chimpanzee, but not so folded as in Broca's chimpanzee, or as in "Sally," for the second annectant gyrus does not come to the surface on either side. This brain also was taken from a young male, which still retained its milk teeth.

Neither of these authors gives any information as to other anatomical characters of the animal to which the brain belonged to enable us to state whether it belonged to the so-called species T. calvus, or T. niger.

As long ago as 1866 Sir Wm. Turner (4) described a couple of chimpanzee brains presenting characters which at the time were of peculiar interest, on account of certain views held by Gratiolet above referred to. One of the brains, figured on p. 680 of the paper, had but a feebly developed operculum on the right side, and therewith presented certain other peculiarities, but there is no special resemblance to that of "Sally." The object of the paper was to show that Gratiolet's opinions as to the annectant gyri in the chimpanzee were founded on insufficient material.

[1] I have to thank Sir Wm. Turner for most kindly calling my attention to this paper.

2. Introduction.

In looking over the stock of chimpanzees' brains in the museum of the Royal College of Surgeons,—for which privilege I wish to express my thanks to Prof. Stewart, who most kindly gave me every assistance in the matter,—I came across a specimen in the museum stores which bears considerable resemblance to that of "Sally," as well as to that figured by Turner, in that it has lost its occipital operculum, but only on the right side, as it is well developed on the left. This brain belonged to the late Prof. John Marshall, but has not been described by him so far as I have been able to ascertain. It came into the possession of the Royal College of Surgeons in 1891, but I have been unable to trace its origin. I have thought it worth while to give a figure of the right hemisphere here (fig. 20).

On the supposition that the peculiarities of "Sally's" brain are specific, and characteristic of T. calvus, I wrote to Prof. Herdman, of Liverpool, for I heard that he had purchased one of Garner's chimpanzees which died on its arrival in England; it was possible that this was T. calvus, and Prof. Herdman most generously acceded to my request to be allowed to examine the brain, and forwarded it to me. I was, however, disappointed, for it does not present characters marking it off for that of ordinary chimpanzees (see fig. 36), and it is as yet uncertain whether the specimen from which it was taken is T. niger, T. calvus, or a third species. In his letter to me Prof. Herdman writes: "Garner declared that this animal was different from calvus, and also from niger. . . . I saw the dead head (after being skinned), and thought it might be calvus."

Thus we have no positive evidence to show whether the peculiarities of "Sally's" brain are characteristic of the species T. calvus; that they are not due to the age or sex of the animal seems certain from the specimens of Broca and Müller, and it can scarcely be maintained that they are connected with "Sally's" greater intelligence, for both Broca's

and Müller's specimens were quite young, and no particular intelligence has been attributed to them. The only suggestion remaining is that in the species T. calvus the brain does present peculiarities, and that Broca's and Müller's specimens belonged also to this species.

On the other hand, we are still uncertain that there is more than one "species" of chimpanzee. Mr. Beddard, from a careful study of the muscular anatomy of "Sally's" limbs, from a comparison of the skull, brain, and other organs, considers that the animal belonged to the species T. calvus, and shows reasons for distinguishing it from the animal described, anatomically, by Gratiolet and Alix, and named T. aubryi. Nevertheless, the differences in muscular anatomy, &c., recorded in the ordinary chimpanzee and in these two other forms, can scarcely be held to establish with absolute certainty the specific distinctness of these three forms: the variations in muscular anatomy in man appear to be every bit as great as in these, and till sufficient specimens of the black-faced bald chimpanzee, known as T. calvus, on the one hand, and of the flesh-coloured, black-red haired form (T. niger), have been as fully and as carefully described as Beddard has done for "Sally," we shall not be entitled—at least we ought to hesitate—to be positive about the "specific" differences of these three forms.

As we shall see, the convolutions of the chimpanzee's brain are subject to variations as wide as occur in man, and some of them are of importance. Such features, too, as the shape of the ear are extremely variable, every stage in the folding of the margin being exhibited, from the unfolded condition to a state closely resembling man's ear.

For the present, at least in this paper, the chimpanzee "Sally" is regarded as being merely a "variety" of the single species T. niger.

3. Description of "Sally's" Brain.

The general features, dimensions, and weight of "Sally's" brain have been described by Beddard; I have but little to

add. He found that the brain "weighed, after removal of the pia mater and after an immersion of four months in spirit, $8\frac{3}{5}$ oz.; it had been allowed to dry for an hour and a half before weighing, it was then damp, but not wet."

He gives the weights of brain of two other chimpanzees (T. niger) as $6\frac{1}{2}$ oz. and $6\frac{3}{5}$ oz.

The brain described by Müller weighed $6\frac{4}{5}$ oz. (213 grammes), whilst that described by Symington (10), though not bearing any particular resemblance to "Sally's" brain, weighed as much as $8\frac{1}{2}$ oz. after preservation in spirit; both these authors tabulate the weights of the brains previously recorded. Marshall's specimen (1861) (2) weighed under similar circumstances as much as 9 oz. But the weight of the brain fresh is considerably more; thus Symington's was 13 oz., Marshall's 15 oz., and Chapman's (7) 10 oz. 10 grains. In all these cases the animal was young, and Symington suggests from analogy with man, and from comparison of cranial cavities of young and adult chimpanzees, that the weight of the brain in the latter is 15 oz.

I have not deemed it necessary to weigh the various brains to which I shall have occasion to refer.

Beddard gives the length of "Sally's" brain as 103 mm., and that of the hemisphere alone as 100 mm. Müller's measured 92 mm. and Symington's 95 mm., so that "Sally's" appears to be the largest hitherto carefully measured, as one would expect, since the animal is the oldest chimpanzee whose brain has been described.

The general appearance of the brain is more like that of man on a small scale than is that of an ordinary chimpanzee (see figs. 1, 2, 3). The frontal lobes are wider and more rounded anteriorly. But we must not lay too much stress on this point, for there is considerable variation in the shape of the frontal lobes when many chimpanzee brains are examined. There is one point which Beddard remarks upon, and which differentiates "Sally's" brain from any other chimpanzee's brain described and figured, viz. the very slight "keel" or ridge on the inferior or orbital surface of the frontal lobes where they

touch in the median line. In none of the brains which I have examined is this keel absent; it is present in the " Sally "-like brain in the Museum of the Royal College of Surgeons referred to above; it was present in the brain described by Müller.

A front view of " Sally's " brain thus presents a great contrast with that of any other chimpanzee hitherto examined, and recalls that of an orang or man (see figs. 5—7).

Since it is the posterior part of the brain which is so characteristically developed, I will commence with this region.

4. The Parieto-occipital Fissure.

This fissure in " Sally's " brain presents certain features of interest, chiefly in that it is divided into two portions, one of which, the mesial or " internal " parieto-occipital, is confined to the mesial surface; the other extends on to the upper surface of the hemisphere, and may be termed the " lateral " parieto-occipital fissure, as the term " external " has been used in rather a different sense from that which I wish to indicate here. Viewed from the upper surface (figs. 2, 10), the parieto-occipital on this left side appears as a deep, well-marked fissure about 15 mm. long, extending rather obliquely outwards after coming on to the surface, which it cuts at a distance of 75 mm. from the anterior end. This superior or lateral portion of the parieto-occipital is bounded by an " arcus parieto-occipitalis " (Gratiolet's " seconde pli de passage "), which is, as usual, marked out laterally by the ramus occipitalis of the intra-parietal (p^4.) and anteriorly by a small mediad of this branch (*n.*), the præarcal branch. But there is another portion of the parieto-occipital fissure which does not reach the upper surface, and which Beddard overlooked. It is seen in fig. 2 lithographed from a photograph, and also in fig. 10, just in front of the lateral portion. When the mesial surface of the hemisphere is examined (fig. 14), it is seen that the first-named portion of the furrow (*lat. p. o.*) is nearly entirely cut off from the remaining vertically disposed part of the furrow (*mes. p. o.*), for the anterior limiting gyrus passes very deeply down at *a.*, and disappears from view, so that the superficial and the vertical

portions of the parieto-occipital are connected by a short but deep sulcus.

The vertical portion of the fissure, after thus joining the superior portion, forks dorsally as shown (at *b.* and *c.*); below the fork it passes straight downwards on the mesial surface, and is continued on to the tentorial surface of the hemisphere, where it joins the calcarine fissure—the gyrus cunei being quite deep. We thus have the ≺-shaped arrangement of this system of fissures which Cunningham regards as typical for man, but which he denies to the apes.

On the right hemisphere (figs. 2 and 11) the lateral part of the parieto-occipital is entirely separated from the mesial vertical portion, and is completely surrounded by gyri (see fig. 15); its mediad end dips for only a very little distance downwards in the mesial face, and the vertical portion—the main part of the fissure (*mes. p. o.*)—comes up to the upper surface of the hemisphere in front of the fissure in question. So entirely cut off is the upper part of the parieto-occipital that I at first took it for a part of the Affenspalte, but its depth and other relations, especially when compared with the arrangement on the left side, show it to be a portion of the parieto-occipital, as Beddard describes it. He, however, overlooked the gyrus which cuts it off from the vertical part of the fissure, which also he did not recognise.

The separation of the parieto-occipital fissure into two distinct portions appears to be a rare occurrence. I do not find it mentioned in descriptions of chimpanzees' brains, and only rarely is it recorded for man. Cunningham does not say very much about it. He figures the "normal" arrangement on p. 43, where the fissure, at a point some distance below its upper end, gives off two branches, one anterior and one posterior, to the main track, which is separated from the calcarine fissure by a superficial gyrus cunei; but he represents no gyrus intercuneatus.

What has happened in "Sally"? The hinder of the two branches of the parieto-occipital appears to have been cut off—partially on the left, entirely on the right—from the rest of the fissure. Cunningham represents several varieties in the arrange-

ment of the parieto-occipital in man, but in none of them do we find quite these relations. In three instances in which the fissure is duplicated (see his figures on p. 40), the portion which cuts into the upper surface of the hemisphere lies in front of the vertical portion, and not behind it as in "Sally." In one case (his fig. 18) this "doubling" is brought about by the gyrus cunei rising to the surface and the "stem" of his ≺-shaped fissure being prolonged into the cuneus. This was only met with in 4 out of 127 hemispheres examined. In the other case (his fig. 19), which he found only once in the course of his research, the gyrus intercuneatus is oblique and becomes superficial, the upper part of the parieto-occipital being prolonged downwards in front of the main part of the fissure.

Now, in "Sally" it appears to me that the cause of the "doubling" is the same, viz. the rising up of the gyrus intercuneatus (*a.* in my figures), but the upper part of the original fissure cuts downwards into the cuneus, behind the main part of the fissure, which extends into the precuneus and only just reaches the surface.

This part of the parieto-occipital fissure lying on the upper surface and separated from that on the mesial surface may be termed the "superior or lateral parieto-occipital."

I wish to avoid the term "external," which would perhaps be more appropriate, since the term "external parieto-occipital" has been used in a variety of senses. The only true sense in which the expression should be used, as Cunningham and others have pointed out, is to refer to that part of the vertical incision which appears as the upper surface of the hemisphere, and varies in extent, naturally, with the depth of the incision. But the term has had two other significances attached to it, especially by English writers, namely, to imply (1) true Affenspalte (Bischoff's "fissura perpendicularis externa," in the fœtal human brain)—in this sense it is used by Beddard; and (2) the groove between the anterior free edge of the operculum and the parietal lobe.

We will now examine the conditions of the parieto-occipital fissure in the normal chimpanzee brain.

In a specimen (947 *f*) in our Museum (see fig. 22) the operculum is normally developed. When this is turned back the Affenspalte is exposed, and in front of it the deep parieto-occipital fissure is seen, extending about three quarters of an inch across the brain (fig. 23). On the mesial face this descends for nearly half an inch (fig. 24), but is cut off from the rest of the parieto-occipital by a conspicuous gyrus (*a.*). The main portion of the fissure lies in front of it, and only just cuts the edge of the hemisphere (*mes. p. o.*). The anterior portion of the fissure (*mes. p. o.*) does not branch. Here, when the operculum is removed, we have the same arrangement as in "Sally."

In another specimen (fig. 26), in which the edge of the operculum is directed obliquely backwards at its mesiad end, we have a further development of the gyrus intercuneatus, so that the very deep "lateral" parieto-occipital (*lat. p. o.*) scarcely bends round on to the mesial face at all, and lies almost entirely below the operculum, cutting off the intra-parietal from reaching the Affenspalte. In fact, it looks at first sight as if it were the "Affenspalte," and as if the gyrus marked *a.* were the "first annectant gyrus." Such, indeed, it appears to be, for in many cases the "external perpendicular fissure" of authors is not the same fissure as is here called "Affenspalte," although it has been homologised with it. The "internal" or mesial parieto-occipital (*mes. p. o.*) enters the calcarine fissure, the gyrus cunei being, as far as I can see, absent (fig. 27).

In a brain referred to by Rolleston,[1] in which on the right side the operculum is less developed than usual (see fig. 30), the parieto-occipital fissure is very deep, and extends for nearly an inch across the surface of the hemisphere (fig. 32). Here it is limited by a well-defined "annectant" (arcus). Seen from the mesial surface (fig. 21) there is no trace of a division of the fissure into two portions. On both right and left sides there is but a single, nearly vertical cleft—cut off by the gyrus cunei

[1] Rolleston, "On the Affinities of the Brain of the Orang-outang," 'Nat. Hist. Rev.,' 1861.

from the calcarine fissure—but presenting no lateral branches, nor gyri coming to the surface.

This seems to be the simplest condition, and closely resembles the arrangement in the lower monkeys; some other of our specimens of chimpanzees exhibit this condition; in others we have seen the gyrus intercuneatus interfering with the continuity, and giving rise to two more or less independent fissures.

In a brain at the College of Surgeons (No. 1338, I a*), the left hemisphere exhibits this simple arrangement, whilst the right (1338, I *a*) possesses the more complicated condition.

In the human brain, the separation of the parieto-occipital fissure into two appears from Dr. Cunningham's researches to be rare, but in the brain (950) exhibited as a typical human brain in the Oxford Museum, and represented here by fig. 16, we have on the left side an arrangement repeating, I believe, that in " Sally," namely, the uprising of the gyrus intercuneatus (*a.*) so as to separate a lateral parieto-occipital (*lat. p. o.*) from the remainder (*m. p. o.*). On the right hemisphere the parieto-occipital cuts into the upper surface for about an inch, forming the true " external parieto-occipital," and this part is continuous with the vertical portion. But in the left hemisphere there lies in front of the " transverse occipital fissure" or Affenspalte, between it and the vertical part of the parieto-occipital, a transversely placed fissure about an inch in length (*lat. p. o.*), bounded anteriorly by a gyrus which is partially overlapped by the " arcus parieto-occipitalis."

5. The Parietal Convolutions.

The fissure of Rolando (" fissura centralis ") has the usual wavy character; it does not enter the Sylvian fissure on either side, nor does it pass on to the mesial face of the hemisphere. The distance of the upper extremity of the fissure from the anterior end of the hemisphere (between vertical plates) is 45 mm. There appears to be some confusion in Beddard's paper with regard to the position of this fissure of Rolando. In the plate xxiii, fig. 3, the index line from F. R. is carried to the upper

end of the calloso-marginal fissure. This appears to be a slip, yet his measurement of the fronto-Rolandic length given on p. 200 is 54 mm., which is really the distance of the calloso-marginal fissure from the anterior end of the cerebrum. The fissure is thus in front of the middle of the cerebrum and not behind it, as Beddard states.

In the normal chimpanzee here figured (fig. 1) the length of the cerebral hemisphere is 95 mm., the fronto-Rolandic length is 55 mm., so that the Rolandic fissure is behind the middle of the hemisphere.

The intra-parietal fissure of Turner may be divided, according to Cunningham, into three constituents: (1) An anterior post-Rolandic vertical fissure, or "sulcus postcentralis." (2) A more or less horizontal furrow, or "ramus horizontalis" ("interparietal" of Ecker), passing backwards and (3) as a prolongation which bounds the arcus parieto-occipitalis and passes on to the occipital lobe—the "ramus occipitalis."

These three constituents may or may not be continuous in man, and to them is added, usually as a separate furrow, a "superior postcentralis," lying more or less parallel to the Rolandic furrow, and dorsal of the inferior postcentralis.

In "Sally," as will be seen (figs. 10, 11), this is also the arrangement; the inferior postcentralis (p^1.) is continued a short distance upward beyond its junction with the ramus horizontalis (p^3.), and above this fissure is a small but well-marked furrow, representing the superior postcentralis (p^2.); this is continuous on the left side with an oblique furrow (*p. s.*) lying parallel to and above the ramus horizontalis, whilst on the right side the corresponding furrow is parallel with the long axis of the brain.

This secondary sulcus (*p. s.*) in the superior parietal lobe is represented in Cunningham's drawing (by the letter *c.*), and is a very constant furrow in the chimpanzee, though variable in form.

The superior postcentralis (p^2.) is in most of the published figures of chimpanzee brains, as well as in several of those examined by me, a much more definite feature (see figs. 30, 36,

20), having a course more nearly parallel with the Rolando than is the case in " Sally's " brain. In both of Cunningham's figures (pp. 203, 204) this furrow continues the direction of the inferior postcentralis (p^1.).

Cunningham states (p. 230) that the union of superior postcentralis (p^2.) with inferior is the usual condition in chimpanzee; and finds them separated in only two of the brains out of eight examined, and that only on one side. In "Sally," in Müller's chimpanzee, and two others from the Royal College of Surgeons, as well as in Herdman's, I find the fissure marked by Cunningham as upper part of superior postcentralis, separate from the rest of the system, and in all these instances it loses its parallelism to Rolando, becomes more or less oblique, and enters into connection with the furrow *ps.* (secondary sulcus of sup. par. lobule).

6. The Region of the Affenspalte.

The most interesting feature in " Sally's " brain, however, is the condition of the hinder end of the intra-parietal fissure.

In the normal chimpanzee brain, and in the lower apes, the hinder part of the parietal lobe is overlapped by a comparatively thin fold of the occipital lobe, known as the " occipital operculum." This lies flat against the parietal lobe, and thus conceals a greater or less amount of the latter. The deep cleft, extending nearly directly backwards and slightly downwards, existing between the operculum and the parietal lobe, is known as the " Affenspalte," or Simian fissure (figs. 27, 29, show its direction well). But some confusion has resulted from the application of this term to the superficial groove between the edge of the operculum and the parietal lobe. This superficial groove has also wrongly been termed the "external parieto-occipital" fissure, since it is apparently continuous with the "internal" fissure of that name. The true "Affenspalte" lies behind this latter fissure, and the two are independent.

The parieto-occipital fissure is a vertically placed fissure in the median surface of the hemisphere, into which it extends

for a considerable distance, and as such is visible also on the upper surface for a greater or less extent, according as the incision is deeper or shallower; that part of the parieto-occipital (= Huxley's occipito-parietal) which is seen in the upper surface is the only fissure to which the term "external parieto-occipital" is properly applied.

I have shown above that this part of the parieto-occipital may become separated from the so-called "internal" parieto-occipital; but it can always be distinguished from the true Affenspalte, or Simian fissure, or subopercular furrow.

In "Sally's" brain (figs. 10, 11) the ramus occipitalis (p^4.) sends off a small mediad branch (*n.*) in front of the parieto-occipital fissure, as is very generally the case in chimpanzees, the existence of which was first mentioned by Rolleston and later described by Sir Wm. Turner (4). This small fissure, therefore, is of historical interest; further, it helps to mark out definitely the so-called "pli de passage supérieur externe" which Gratiolet claimed for chimpanzee's brain.

The ramus occipitalis in "Sally" now curves outwards, forming the outer limit of the "pli de passage" or annectant gyrus or "arcus parieto-occipitalis," and enters the "Affenspalte."

The arrangement on the two sides of the brain is not identical. The above description refers to the right side.

On the left side (fig. 10) the ramus occipitalis appears to bifurcate, a condition represented in some of Cunningham's figures. The shorter, outer or laterad fork enters the "Affenspalte;" the more conspicuous mediad fork (*x.*) passes backwards on to the occipital lobe in nearly a straight line.

On the right side the ramus occipitalis also appears to bifurcate, but this is not the case. Owing to the fact that the parieto-occipital fissure has become divided into two portions—the "lateral" portion lying on the upper surface (*lat. p. o.*), whilst the "internal" portion is almost confined to the mesial surface and only just reaches the upper surface (*mes. p. o.*)—the fissure (*n.*), which at first sight might be taken for the mediad fork of the ramus occipitalis, is, as we have seen, in

reality the præparieto-occipital branch of the intra-parietal fissure, which forms the anterior limit of the "arcus parieto-occipitalis." This branch (*n.*), which Beddard's figure represents as joining the parieto-occipital, is separated by a gyrus from this, but as it is overlapped by the superior parietal lobule, its true relations are not seen in a view from above (as, for example, in a photograph).

After giving off this small branch the intra-parietal fissure (p^1.) curves slightly outwards and enters the Affenspalte, the well-marked obliquely-transverse groove so evidently the remains of the more extensive fissure of the normal chimpanzee. The hinder boundary of the Affenspalte on each side is a low ridge only slightly above the level of the opposite side of the furrow ; this is the remains of the operculum.

In describing the terminations of the ramus occipitalis, Cunningham states that the two forks into which it divides are frequently almost at right angles to the main stem and nearly in line with one another, giving rise to a transversely directed fissure, which he identifies with the "occipitalis transversus" of Ecker.

The condition of affairs on "Sally's" left hemisphere (fig. 10) is, indeed, not very unlike the left side of the figure of the human brain given by Ecker, in which the fissure marked there *o.*, into which the intra-parietal falls, consists of two parts, one laterad of the intra-parietal, which soon forks; the other, mediad, lies behind the parieto-occipital. The question arises, do these two parts correspond to the two fissures in "Sally's" brain, the laterad (Affenspalte) and the mediad (*x.*), which I have described as a continuation of the ramus occipitalis? In other words, may the "occipitalis transversus" be composed of two parts originally independent? This is a view held by Eberstaller.

There is no doubt but that in the apes the bifurcation of the intra-parietal is an independent thing which may or may not become connected with the Affenspalte. In some cases the outer, in other cases the inner fork enters the Affenspalte, or both forks. Thus in Cunningham's figure, p. 203, right side, the laterad fork is independent, the mediad joins the Simian fissure

but again leaves it, whilst, as we see in "Sally" on the left side, it is the laterad fork which enters the Affenspalte. Cunningham shows that this is the case in the ape, and one of our chimpanzees (fig. 31) somewhat resembles the left side of the brain of Cebus albifrons figured by him in illustration of the point (on p. 223 of his memoir).

Amongst our specimens of chimpanzees it is very frequently the case that the intra-parietal does not bifurcate, or if it does the resulting figures are coextensive with Affenspalte. The ramus occipitalis in our specimens usually passes under the operculum and enters the Affenspalte. In one case, however (see figs. 22, 23), the parieto-occipital fissure makes so deep an incision in the hemisphere that it cuts off the intra-parietal from the Affenspalte. We have here a condition similar, as I believe, to that described by Dr. Cunningham for Cebus capucinus, which he uses to support his view as to the homology of the "occipitalis transversus" with the bifurcation of the ramus occipitalis. According to him the fissure regarded as ramus occipitalis lies nearly at right angles to the rest of the intra-parietal fissure, and the parieto-occipital fissure falls into the system at this angle (loc. cit., fig. 45, p. 222).

But it appears to me, from observations on C. robustus, that another explanation may be given of the fissure he regards as "ramus occipitalis," for in our specimen of this monkey, if the occipital lobe be pressed backwards it is seen that the parieto-occipital fissure is so deep that it extends more than halfway across the hemisphere, and receives, nearly in its middle, the intra-parietal. So that what in C. capucinus is regarded by Cunningham as "ramus occipitalis" is in C. robustus the outer part of the parieto-occipital. The Affenspalte, as Cunningham shows, is quite independent of this transversely placed fissure, as it is too, in our chimpanzee (947 *f*) just referred to.

Now in the human brain do we find any close similarity between the arrangement of the convolutions in this region and those of "Sally"?

I have already referred to Ecker's diagram of a generalised

condition of the human fissures, which is closely similar to "Sally," the left hemisphere in each case being compared. Again, the left side of the brain figured by Bischoff (and reproduced in fig. 17) is like the right hemisphere of "Sally" (fig. 11) so far as the "Affenspalte" is concerned. The same oblique direction is to be noted. On the right side of the same brain, too (fig. 18), we have a very similar arrangement, but the intraparietal fissure (p^4.), before entering the "Affenspalte" (transverse occipital) sends inwards a short branch (z.) behind the "arcus parieto-occipitalis."

In a human brain in the Oxford Museum, to which I have already referred, we have on the left side (fig. 16) a condition of the transverse occipital which seems to support Cunningham's view. The intra-parietal appears to curve round the "arcus," and nearly reaches the mesial fissure, where a rather irregular furrow passes backwards from it; this small portion (x.) resembles a part of the "mediad fork" of the ramus occipitalis of "Sally's" left side (on fig. 10). The "transversus occipitalis" in this human brain would then be, according to Cunningham, the upper (mediad) fork of the intra-parietal. But the general direction of the "occipitalis transversus" is so very similar to that on the right side of "Sally's" brain that it is possible to explain this irregular furrow (x.) by supposing it to be an independent adventitious furrow on the occipital lobe, such as occurs in "Sally's" left hemisphere, which there has gained a connection with one of the forks of the ramus occipitalis.

It is still, in fact, a debateable point as to whether there is in the adult human brain a representative or homologue of the "Affenspalte" of the ape. There are, indeed, three chief views on the subject: (1) Ecker regards his "sulcus occipitalis transversus" as such; (2) Eberstaller (to quote from Cunningham) draws a distinction between the upper and lower limbs of the sulcus occipitalis transversus of Ecker. In it we can distinguish a medial and a lateral segment by the point of union with the sagittal portion of the intraparietal furrow. The former (x. in my figure) bounds the arcus parieto-occipitalis

behind, without, in the majority of cases, reaching the mesial border; the latter is the sharply descending end of the arch-like "fissura interparietalis." Ecker's view, originally shared by Cunningham ('Journ. Anat. Physiol.,' 24), is now combated by him, chiefly, it appears, on embryological grounds, partly on those of comparative anatomy. (3) He would regard the transverse occipital as merely a portion of the "intra-parietal system" which has nothing to do with the Affenspalte. It is due to the bifurcation, in fact, of the ramus occipitalis; the Affenspalte, according to him, is only very rarely represented in man. The "fissura perpendicularis externa" of Bischoff makes its appearance in the human fœtus about the fifth month (according to Ecker and Cunningham); it is a "complete" fissure impressing itself upon the ventricle, so that the outer wall is bulged inwards by it. Some time later, apparently during the sixth month, it disappears. Now all these authorities are agreed that this external perpendicular fissure of the human fœtus is the homologue of the "Affenspalte" of the ape. But it is only a transient furrow, though "complete," and its place is occupied later on (seventh or eighth month) by the terminal bifurcation of the intra-parietal fissure, which is not a "complete" fissure, that is, it does not leave its impress upon the wall of the ventricle; this second or replacing fissure becomes the "transverse occipital."

For these and other reasons Cunningham denies the possibility of the suggestion that the latter fissure can be the homologue of the Affenspalte.

Nevertheless it must be borne in mind that with regard to the "completeness" of the Affenspalte, Cunningham (p. 69) "cannot tell whether or not it (the elevation of the wall of the ventricle) exists in the anthropoid brain. Specimens of these are so valuable that I am unwilling to destroy those I have got, even in a determination of a point of this importance." He has only observed the bulging inwards of the outer wall of the ventricle at the bottom of the Affenspalte in the Sooty Mangaby and in Cebus.

As the Oxford Museum possesses several chimpanzees' brains,

Professor Lankester very generously gave me permission to dissect one of them to ascertain what was the fact with regard to this point. I sliced the hemisphere in planes parallel to the median plane till I cut through the posterior cornu of the lateral ventricle. I find that the "Affenspalte" does not cause a bulging in the roof or side of the ventricle. In the figure (fig. 9) there appears to be such a bulging, but this is a portion of the 'calcar avis' passing on the roof of the ventricle, and the slight ridge (*x*) just in front of it lies distinctly in front of the Affenspalte. Müller, who represents dissections of the brain of a chimpanzee, does not figure or describe any such bulging. But even if there were such an elevation of the outer wall of the ventricle in the lower apes, it does not seem to me a consequence that the higher apes would present one. Even if in the ordinary chimpanzee with a well-marked operculum and deep Affenspalte the impress of the latter is exhibited by the outer wall of the ventricle, it does not necessarily follow that in the condition in which the Affenspalte is present in "Sally," the same impress would be left.

As the operculum disappears, and the Affenspalte becomes proportionately shallower, it seems quite possible that the bulging of the ventricular wall should get less and less marked.

In the human brain the "occipitalis transversus," as we have seen, replaces the "fissura perpendicularis externa" after the disappearance of all trace of the latter. It does not however follow, because there is no actual continuity of the two grooves, that there is no genetic relation between them. The obliteration of the earlier furrow is probably due to the growth of the nerve tissue bringing about a thickening in the wall of the ventricle, and "filling up" (if we may use the expression) the furrow; at the same time the bulging on the inner face becomes smoothed out. But the furrow again makes an effort —inheritance is too strong for the vegetative growth,—and succeeds in obtaining its permanent position as the "occipitalis transversus," which is relatively shallower and does not cause any bulging inwards of the wall of the ventricle.

We know that ontogeny does not by any means follow

slavishly the exact steps of phylogeny: the mode of formation of the heart in mammals, for example, differs from that of birds, nevertheless the two hearts in their entirety are surely homologous and homogenetic; yet the ontogenetic condition cannot represent a functional phylogenetic stage.

In the ontogeny of some Vertebrates the œsophagus becomes closed at a certain period of development, and it again opens out to form the permanent œsophagus. We do not deny the homology (homogeny) of these two structures.

It appears to me that the facts which have been gathered together with regard to the Affenspalte in the chimpanzee, and the "occipitalis transversus" in man,—the fact also that there is considerable variation in position and extent of the fissure in both animals, and that parallel variations occur in both,—are so strongly in support of Ecker's view, that the mere fact of a slight difference in ontogeny is not sufficient to overturn the homology.

Further, what is the course of development of the "Affenspalte" in the ape and monkey? We are, I believe, absolutely ignorant on the matter.

In the human brain (fig. 17) it seems at first sight pretty evident that the sulcus occipitalis transversus is formed by the bifurcation of the intra-parietal (p^4.); the angle formed by the two branches of the fissure lends its weight to this view. But compare with it fig. 18. Here p^4. gives off a branch (z.) behind the arcus parieto-occipitalis, which, there is little doubt, is the mesiad fork of the intraparietal, whilst the laterad fork enters the transverse occipitalis. This fissure itself closely resembles the "Affenspalte" in "Sally" (fig. 11). Thus Cunningham's interpretation would apply to fig. 17, and Ecker's to fig. 18.

Now, in some chimpanzees, such as that represented by figs. 30 and 32, we have an "Affenspalte" below the operculum (fig. 32) into which there falls the ramus occipitalis (p^4.), and at this point an angle occurs in the "Affenspalte." There is, in fact, a very close resemblance to the condition in the human brain (fig. 17), which Cunningham would explain as

bifurcation of the intraparietal. How are we to distinguish between the two? We can scarcely regard the subopercular furrow of the chimpanzees (fig. 32) as anything but the Affenspalte. Therefore, why should we not apply the same name to an apparently similar furrow in fig. 17?

In fig. 31, again, we have an arrangement somewhat like that in the human brain (fig. 18), viz. the ramus occipitalis (p^4.) gives off a post-arcal branch, which is distinct from the (greater part of the) Affenspalte. The figs. 17 and 18 represent two sides of a human brain, and figs. 31 and 32 the two sides of a chimpanzee's.

I do not pretend to decide the matter. It would be great presumption on my part, with my slight experience of human cerebral topography, to express any decided opinion on a matter on which such authorities as Eberstaller, Ecker, and Cunningham are at variance. I merely wish to bring forward other facts and arrangements of these fissures which appear to me to have some bearing on the question.

7. The Occipital Lobe.

The occipital lobe itself is more furrowed in "Sally's" brain than in the ordinary chimpanzee, and bears some resemblance to that of Müller's brain.

The old terms given by Gratiolet to the gyri in the neighbourhood of the parieto-occipital fissure, and to which so much importance was at first attached—the annectant gyri, or "plis de passage"—are now gradually being discarded. Since Rolleston and Turner showed their presence in chimpanzee, where they are usually concealed by the operculum, the differential importance of them has gone; and the names, though still sometimes employed, appear to be giving way to more descriptive and systematic terms.

The pli de passage supérieur externe and pli occipital supérieur are now called by one name, "gyrus occipitalis primus" (Ecker), or "arcus parieto-occipitalis" (Eberstaller).

This arcus parieto-occipitalis is well seen on the right hemisphere, where it curves firstly round the lateral extremity of

the "lateral parieto-occipital," and is continuous with the gyrus occipitalis primus, which bends round the mediad extremity of the Affenspalte.

The gyrus occipitalis secundus (*O* 2) (deuxième pli de passage externe) lies behind the Affenspalte (in fact, is the operculum) and curves round its laterad extremity, becoming continuous with the "angularis."

The third occipital gyrus lies below the oblique fissure (the sulcus occipitalis inferior), and also passes into the third temporal gyrus.

The calcarine fissure just cuts the edge of the occipital lobe, but is more extensive on the left than on the right hemisphere.

8. The Sylvian Fissure.

The Sylvian fissure in "Sally's" brain is less vertical than in the brain figured by Müller, though, as Beddard has remarked, it is more vertical than in common chimpanzees. The "Sylvian angle" in Dr. Cunningham's sense is 55° in "Sally;" the brain received from Professor Herdman has rather a smaller angle, viz. 50°, whilst normally it is, according to Cunningham, 54·5°.

With regard to the "anterior limb" of the Sylvian fissure, Beddard has quite rightly figured it as a short, nearly horizontal fissure (Pl. 23, fig. 2, *F. s. a.*). A comparison of a series of chimpanzee's brains led Cunningham to believe that it is the anterior boundary of the fronto-parietal operculum, and therefore is the homologue of the "ramus ascendens" in man. He gives reasons for his conclusion that in Troglodytes the only opercula covering the island of Reil are the fronto-parietal and the temporal. The two anterior ones, frontal, and orbital of man, are absent; and he suggests that part of the orbital lobule in the ape may represent the island of Reil in man.

In "Sally" the insula is distinctly limited anteriorly by a portion of the frontal lobe, which is at a much higher plane than the insula, and separated from it by a deep fissure; but it does not sensibly overlap the insula. However, in other

chimpanzee brains this portion of the frontal lobe below the "anterior limb" is much more developed, and forms practically an operculum—overlapping, though only to a slight extent, the insula.

Cunningham states that the submerged portion of the insula presents very little trace of an anterior boundary, and that it ascends gradually along an inclined plane until it finally reaches the free surface of the frontal lobe. I do not find this to be the case at all generally. In two brains in the Oxford Museum this overhanging anterior lobe exhibits more or less distinct traces of a subdivision into two lobules.

The most distinct case was photographed, and is represented in fig. 8. Here the "anterior limb" of the Sylvian fissure is forked; one branch is nearly vertical (*Sy'.*), and forms as usual the boundary of the fronto-parietal operculum; it is the "ramus ascendens" of the anterior limb. Just below it there is another slight but distinct fissure (*Sy''.*) separating a small triangular lobe from the rest of the anterior boundary of the insula (*fr. op.*). This little lobe, I suggest, is the "frontal operculum" or pars triangularis, and the second fissure is the homologue of the "anterior horizontal limb" of the Sylvian fissure. Below it the remainder of the lobe will then correspond with the orbital operculum, which here distinctly overlaps the insula.

A second brain (Oxford Museum, 947 *f.*) shows somewhat similar conditions, but in a less degree. These may be compared with Cunningham's fig. 1, pl. iv, of the brain of a human fœtus of the eighth month.

The brain represented in fig. 8 resembles still more closely the figures given by Schäfer in 'Quain's Anatomy' (tenth edition, vol. iii, part 1). He shows that on the right side of this human brain the two branches of the anterior limb of the Sylvian fissure practically join at their commencement, i. e. the "pars triangularis" or frontal operculum is here less developed than on the left side, where it entirely separates the two rami. The greater development on the left side of the pars triangularis may be in relation with the "centre of speech."

It appears to me particularly interesting to find in the chimpanzee a condition so closely resembling that on man's right side.

Cunningham regards the inferior frontal convolution of the ape as representing non-covered insula, and the "sulcus fronto-orbitalis" as the homologue of the anterior limiting sulcus of the insula, and compares it with fœtal human brains at a certain stage. But the position and relations of the fronto-orbital fissure (*e. o.*) with regard to the "anterior limb" of Sylvius (limit of fronto-parietal operculum) seem to me to be very different. In most of the chimpanzees it passes upwards from the orbital surface of the frontal lobe some distance in front of the anterior end of the Sylvian fissure, and curves upwards and forwards, extending considerably above the so-called "anterior limbs." It is easy to understand how the folding backwards—formation of orbital operculum—in the fig. 9, pl. iv of Cunningham's memoirs would give rise to the state of things represented in his fig. 1 of the same plate representing human fœtal brain.

Here the fissure supposed to correspond with the fronto-orbitalis joins the "anterior ascending limb" of the Sylvian fissure, and then curves backwards. From the facts referred to above, and represented in the photograph (fig. 8), it seems to me that the sulcus fronto-orbitalis may have some other meaning. May it not be part of the system of orbital furrows which has extended upwards?

9. Frontal Lobe.

A system of furrows, more or less parallel to, and in front of, the fissure of Rolando, constitutes the "præcentralis."

This præcentral fissure arises, according to Cunningham, in three pieces—vertically, a "præcentralis inferior," and a "præcentralis superior," with a horizontal ramus, which is usually connected with the upper part of the former.

On the right hemisphere of "Sally's" brain (figs. 11, 12) the sulcus præcentralis inferior (*p. c. i.*) is divided into an upper and a lower portion by a submerged gyrus situated just above

the junction of the fissure with the s. frontalis secundus (f^2.); the upper portion of this vertical fissure appears to represent the "ramus horizontalis" (*h.*). The præcentralis superior (*p. c. s.*) is a very well marked, transversely-placed furrow, seen best in the view of upper surface. The lower end lies behind the upper end of the ramus horizontalis. It is in free communication with the distinct "sulcus frontalis primus" (or superior) (f^1.), which runs straight forwards and bifurcates anteriorly.

Lying in front of this is an oblique furrow, with its posterior end directed towards the mesial fissure, which it almost reaches: the anterior end bifurcates, and lies in front of, but mediad of, the end of the sulcus frontalis primus. What is this fissure?

It is represented in the left hemisphere by a smaller and unbranched furrow.

Is it a portion of the frontalis primus? or is it a representative of the "sulc. frontalis mesialis"?

According to Cunningham, the disjointed portions of the sulcus frontalis primus lie in exactly the opposite direction, i. e. the posterior ends of each are laterad (outside) of the anterior end of the preceding. From the fact that these disjointed pieces are slightly variable, it will perhaps be safer to leave the matter open, for hitherto the "s. frontalis mesialis" is characteristic of the human brain, and has not been met with in the ape.

In front of this, again, is a curved furrow which is probably another portion of the s. front. primus.

The s. frontalis secundus (or inferior) has been carefully identified by Cunningham, and he has conclusively shown that the sulcus rectus of the monkey's brain is the homologue of the s. frontalis inferior, and not of the s. frontalis medius as Eberstaller believed. Cunningham therefore agrees with Gratiolet's views, which are at variance with some more modern views, that the inferior frontal lobe is present in the apes.

In "Sally" the sulc. frontalis inferior (secundus) (fig. 12, f^2.)

is well developed. Posteriorly it is continuous with the præcentralis inferior, whence it runs nearly directly forwards for some distance and then bends downwards in front of the orbito-frontalis (*e. o.*), when it divides into a fore-and-aft nearly horizontal fissure (*w.*), and can be traced forwards to the frontal pole. The anterior branch appears to be the fronto-marginalis of Wernicke.

In his description of this fissure in man, Cunningham states that the hinder limb of the fork lies between the two anterior limbs of the Sylvian fissure. Granting that the s. frontalis inferior is identical in man and apes,—and in "Sally" it closely resembles the arrangement figured by Cunningham on p. 248 for man,—the hinder branch of the fork ought to have the same relation to the fronto-orbital furrow (*e. o.*) as it has in man to the anterior horizontal limb of the Sylvian fissure, if Cunningham's identification of the fronto-orbitalis is the true one. But here in "Sally" it has not this position; a fact which is adverse to Cunningham's interpretation of these other furrows. But in other chimpanzees this fissure marked "*w.*" is not related to the "s. front. inferior," but to the "medius," as in fig. 35, where the s. frontalis inferior is short and its anterior end is surrounded by a curved fissure—part of the "medius" (*f. m.*)—which passes downwards and divides near the orbital surface of the brain into a fore-and-aft horizontal furrow (*w.*), which can be traced round to the frontal pole. This appears to be the s. fronto-marginalis of Wernicke, and this brain resembles in this respect the one figured by Cunningham (fig. 68, p. 290). In other cases the "medius" runs in the same direction, but does not divide (fig. 38).

The s. frontalis medius in "Sally" is unconnected with the s. præcentralis inferior, and is quite a distinct fissure on both hemispheres.

Turning now to the left side of "Sally's" brain (figs. 10, 13), we find the sulcus præcentralis inferior (*p. c. i.*) is a well-marked continuous furrow extending from nearly the Sylvian fissure upwards for about half the surface of the hemisphere. There appears to be no ramus horizontalis, unless it is represented by

a small oblique furrow (*h.*), recalling the "præcentralis medius" of some authors.

Cunningham in some cases finds in man the ramus horizontalis nearly vertical, or composed of a forked furrow. This he considers is merely an extreme condition to which the term "præcentralis medius" has sometimes been applied. The other frontal furrows are normally developed.

It may be worth while to refer to some of the variations in the arrangement of these furrows in our other chimpanzee brains. In most of them the ramus horizontalis is continuous with the præcentralis inferior; it is most characteristically developed in the brain represented in figs. 33, 34. In fig. 38, again, this latter furrow is very well developed, but has no anteriorly directed horizontal branch. Probably the upper part of it, together with the short posterior horizontal (*h.*) branch, represents the ramus horizontalis. In fig. 28 the ramus horizontalis is continuous with what appears to be a portion of the frontalis primus. It is in both these cases more or less vertical.

The frontalis secundus (f^3.) is continuous with the præcentralis inferior, except in fig. 28 and on the brain represented at fig. 30. In the former the frontalis secundus is connected with a downwardly directed vertical furrow, which is very much more distinct in fig. 33. This condition may be compared with that of an eighth month human fœtus figured by Cunningham on p. 250.

In the brain represented in figs. 30, 34, on the right side the præcentralis inferior (*p. c. i.*) is provided with a large ramus horizontalis (*h.*) composed of an anterior sagittal and a posterior constituent directed obliquely upwards. The s. frontalis medius (*f. m.*) and the "inferior" (f^2.) are separate from the præcentralis and are nearly simple furrows; but the "inferior" bifurcates at its extremity. This bifurcation probably represents the s. fronto-marginalis. S. frontalis primus (f^1.) is in three separate pieces, the hindermost being continuous, as usual, with the s. præcentralis superior (*p. c. s.*).

On the left side of the same brain (fig. 33) a very peculiar

association of furrows occurs, somewhat like that in fig. 35. The ramus horizontalis is connected, as usual, with the s. præcentralis inferior. The s. frontalis medius is a curved fissure, concave downwards, lying some little distance in front of the præcentralis inferior; from this is given off a branch which runs forward—a modification of the ordinary forking of this fissure. But where is the "inferior"?

Just below the medius and lying parallel to the ramus horizontalis is a very short fissure (separated from the præcentralis inferior by a gyrus, which just reaches the surface), crossing the top of a vertical fissure of considerable extent. This latter passes downwards nearly to the anterior limb of Sylvius. This fissure appears to represent the sulcus diagonalis, and the horizontal one at its upper end the s. frontalis inferior. We have in this chimpanzee brain a condition resembling the fœtal human brain of eight months figured on p. 250 by Cunningham—the "inferior," however, being shorter in the chimpanzee.

The brain represented by fig. 38 is of interest in that the s. frontalis secundus is divided into two pieces by a broad gyrus, one portion remaining continuous with the præcentralis inferior—a condition resembling that of a seventh month human fœtus figured by Cunningham on p. 276, fig. 63; and it is probable that the part of the curved longitudinal fissure in fig. 33, labelled? f^2., is a similarly disjointed portion of the "frontalis secundus."

It is unnecessary to enter into further detail, as an examination of the figures will show the interpretations which I put upon the various fissures.

10. Results of the Comparison of a Series of Chimpanzee Brains.

1. The parieto-occipital fissure, originally a simple and single incision on the mesiad side of hemisphere, frequently in the chimpanzee and more rarely in man becomes divided into two fissures by the gyrus intercuneatus becoming superficial. Of these two portions, the superior, or lateral parieto-occipital,

may come to be more or less entirely on the surface of the hemisphere (figs. 11, 16, and 31), whilst the lower or mesial portion lies on the mesial surface. This "lateral parieto-occipital" is independent of the Affenspalte, and is not synonymous with what is frequently termed in text-books the "external parieto-occipital."

2. The occipital operculum in the chimpanzee presents very great variations in its size; the two hemispheres of a given brain frequently present differences in respect of this feature (cf. figs. 10, 11, 19, 20, 25, 26, 30). In "Sally" this operculum is practically absent, and the subopercular groove or "Affenspalte" is fully exposed.

3. This "Affenspalte," or Simian fissure of the ape, seems to be homologous with the "sulcus transversus occipitalis" of Ecker, and to be independent of the bifurcation of the intraparietal fissure.

4. In some chimpanzees there exists a "ramus ascendens" and a "ramus horizontalis" of the anterior limb of the Sylvian fissure; these enclose a "pars triangularis" or frontal operculum overlapping the insula (see fig. 8). Further, I find that the anterior boundary of the insula is more or less distinctly marked, and that there is a distinct and sudden elevation of the frontal lobe at the anterior margin of the insula. In other words, an "orbital operculum" is present in some chimpanzees, though it may be feebly marked in some specimens.

5. There is a fissure in "Sally's" brain which may possibly be the homologue of the sulcus frontalis mesialis (figs. 10 and 11, "$?f^1$."), though I have provisionally taken it to be a disjointed portion of the sulcus frontalis primus, since the former sulcus has not hitherto been recognised in any ape brain.

6. Various arrangements of the frontal fissures in chimpanzees resemble those recorded in man, either adult or fœtal.

11. List of the more Important Memoirs dealing with the Brain of the Chimpanzee.

1. Gratiolet.—'Mémoire sur les Plis cerebraux de l'homme et des Primates.'

2. Marshall, J.—"On the Brain of a Young Chimpanzee," 'Nat. Hist. Review' (new ser.), i, 1861, p. 276.

3. Bischoff, T. W.—"Die Grosshirnwindungen des Menschen," 'Abh. d. k. Bayer. Akad. d. Wiss. Math. Phys. Classe,' x, 1866.

4. Turner, W.—"Notes more especially on the Bridging Convolutions in the Brain of the Chimpanzee," 'Proc. Roy. Soc. Edin.,' v, 1866.

5. Bischoff.—"Ueber das Gehirn eines Chimpanse," 'Stzber. d. Wiss. Math. Phys. Classe d. k. Akad. Bayerish.,' 1871, München.

6. Broca.—"L'Étude sur le cerveau du gorille," 'Rev. d'Anthrop.,' 1878.

7. Chapman.—"On the Structure of the Chimpanzee," 'Proc. Acad. Nat. Sci. Philadelphia,' 1879, p. 52. (Gives literature.)

8. Parker.—"On the Brain of a Chimpanzee," 'Medical Record,' 1880.

9. Müller.—"Zur Anatomie des Chimpanse Gehirns," 'Arch. f. Anthropologie,' xvii, 1888, p. 173.

10. Symington.—"On the Viscera of a Female Chimpanzee," 'Proc. Roy. Phys. Soc. Edin.,' x, 1890, p. 297.

11. Beddard.—"Contributions to the Anatomy of the Anthropoid Apes," 'Trans. Zool. Soc.,' xiii, 1893.

12. Cunningham.—"Contribution to the Surface Anatomy of the Cerebral Hemispheres," 'Cunningham Memoirs,' vii, Roy. Irish Acad., 1892.

12. The Brain of the Orang-outang.

The arrangement of the frontal convolutions in this ape is so varied, and the fissures have in many specimens such peculiar relations, that Dr. Cunningham, in the few remarks he makes on the subject (loc. cit., p. 295), confesses his uncertainty as to the accuracy of the identification which he places upon these fissures.

As the Oxford Museum possesses two brains of the orang, and as these differ from one another, as well as from that described by Cunningham, it has seemed to me worth while to place on record the plan of these convolutions, and to endeavour in the light of these new variations to arrive at a more accurate determination of these fissures. Even if I am

not successful in this—and as so great an authority as Dr. Cunningham has confessed his inability to thoroughly establish his identifications I feel some hesitation in believing that I should be more successful—I shall at any rate have added new facts to the comparatively small amount of knowledge that we possess concerning the orang's brain.

Of the two orangs' brains one is historical, being that described and figured by Rolleston in 1861 (**2**). It is very well preserved.

The other brain, however, is rather soft and slightly injured on the right side. It is, further, rather distorted, the hemispheres being pressed over to the left side, so that nearly all the temporo-sphenoidal lobe is thrust under the lower surface, against which it is flattened. It appears to have belonged to a young individual, as it is not nearly so large as those hitherto figured. The cerebral hemispheres measure 84 mm. in length, but it is useless to give other measurements owing to the distortion of the brain.

This brain will be referred to as Brain No. 1, that described by Rolleston as No. 2.

Orang's Brain No. 1 (figs. 42—45).

The Frontal Lobes.—On the left side (figs. 42 and 44) the s. præcentralis inferior (*p. c. i.*) is continuous above with an obliquely placed fissure, directed upwards and forwards (*h.*), which I would identify as the ramus horizontalis, since the posterior end of the s. frontalis medius lies below the anterior end of it. About midway along its length, the s. præc. inferior gives off an anteriorly directed furrow, which I would identify as a part of the s. frontalis secundus (f^2.). Such an arrangement I have described above in a chimpanzee (fig. 38); and which, moreover, is very similar to that figured by Cunningham for an adult man on p. 262 of his memoir.

The s. præcentralis superior (*p. c. s.*) seems to be in two portions, the lower of which, lying parallel to the ramus horizontalis, is continuous with the s. frontalis primus (f^1.), which in this hemisphere is represented by only this one fissure.

The s. frontalis medius (*f. m.*) is a simple fissure passing forwards nearly horizontally, its hinder end having precisely the typical position below the ramus horizontalis, from which it is separated by a deep-lying gyrus.

The s. frontalis secundus (f^2.) is in two parts, the chief of which lies just below the s. frontalis medius and passes forwards to the frontal lobe, giving rise to the "s. fronto-marginalis" of Wernicke. The other part of the fissure to which I have referred above is in continuity with the s. præcentralis inferior.

On the right side of this brain (figs. 43 and 45) the furrows on the frontal region have rather a different arrangement. The sulcus præcentralis superior (*p. c. s.*) is single and of considerable length, occupying the position of the two furrows of this name on the left side. It lies parallel to the fissure of Rolando, and at about half its length is joined by a longitudinal fissure, which appears to be the main part of the sulcus frontalis primus (f^1.).

The sulcus præcentralis inferior (*p. c. i.*) is also fairly well developed; upwards it lies in front of the sulcus præcentralis superior, and below it nearly reaches the Sylvian fissure, from which it is separated by a deep gyrus. Passing from it anteriorly are two horizontally directed furrows, the upper of which appears to be the ramus horizontalis (*h.*), the lower and shorter is probably a part of the sulcus frontalis secundus (f^2.).

Where, now, is the sulcus frontalis medius? It ought to lie below the ramus horizontalis. There is a furrow in this position which curves upwards round the anterior end of the ramus horizontalis, part of which possibly represents this sulcus frontalis medius. Unfortunately the brain is so ill preserved in this region that the exact arrangement of fissures on the lower part of the frontal lobe cannot accurately be made out.

Orang No. 2 (figs. 39—41).

In Rolleston's specimen the upper part of the right hemisphere has been removed, but is preserved. The following is the arrangement:—On the left side (figs. 39 and 41), lying in

front of and nearly parallel to the upper part of the fissure of Rolando is a conspicuous fissure which passes for some distance forwards, nearly parallel with the median fissure, for about half the length of the lobe. This appears to represent a portion of the sulcus frontalis primus (f^1.), together with the uppermost part of the sulcus præcentralis superior (*p. c. s.*).

At a point a short distance behind its anterior termination a fissure passes downwards and slightly backwards, nearly parallel with the fissure of Rolando, and before it ceases gives rise to another fissure directed nearly horizontally forwards (*h.*). A triradiate fissure results; the lower, posteriorly directed limb ends freely, the upper limb joins the sulcus frontalis primus. This triradiate fissure appears to represent the "ramus horizontalis" which is connected with the vertical limb of the sulcus præcentralis inferior (*p. c. i.*).

The sulcus præcentralis inferior (*p. c. i.*) passes downwards and backwards in the usual way, and near its lower end is apparently connected with a branch of the sulcus frontoorbitalis (*e. o.*), from which, however, it is separated by a deeplying concealed gyrus.

Behind the præcentralis inferior lies a particularly long sulcus diagonalis (*d.*) which reaches very nearly up to the ramus horizontalis, and occupies a similar position and has about the same extent as the same fissure in the orang's brain figured by Cunningham, and reproduced here on fig. 46.

The arrangement of these fissures is very similar to that figured for a human fœtus of the seventh month on p. 268, fig. 60, of Dr. Cunningham' memoir.

This system of fissure is no doubt very peculiar—unusual, though not unknown; connections take place in such a way that we have a practically continuous series of furrows representing sulcus præcentralis superior, sulcus frontalis primus, ramus horizontalis, and sulcus diagonalis.

Between the upper end of this composite furrow and the Rolandic fissure is a short, obliquely placed furrow (*a.*), which appears to be the "sulcus præcentralis marginalis." The

direction of this furrow does not seem to agree with the view that it is an isolated portion of the s. frontalis primus.

Beyond the anterior end of the "sulcus frontalis primus" is a rather short furrow, lying nearer to the median fissure, to which it is parallel. It does not appear to be a separate portion of the frontalis primus, for such portions are usually oblique, and the upper lateral end is placed below, laterad of, the end of the upper part of the frontalis primus. It appears to be possible that it represents the "sulcus frontalis mesialis," which has hitherto been regarded as peculiar to man, and has not been recorded for any anthropoid brain, and I do not wish to insist upon this interpretation. The fissures in the orang are so variable that it is quite possible that this one is merely a portion of the s. frontalis primus.

The s. frontalis medius and s. frontalis secundus are comparatively short but distinct furrows having a longitudinal direction. Both are independent of the vertical fissures.

The "s. fronto-orbitalis," which in Cunningham's figure is so conspicuous, is in both our orangs almost limited to the orbital surface, only a small part coming on to the outer surface. This portion presents the peculiarity that it appears to pass into the sulcus præcentralis inferior, from which, however, it is in reality separated by a deep gyrus.

Parietal and Occipital Lobes.—With regard to the "Affenspalte" our two orangs illustrate two different conditions. In the small brain (No. 1) there is on each side a well-developed operculum, and the intra-parietal passes into the Affenspalte below it. But in Rolleston's specimen, as he described, the operculum is less developed; the "arcus occipitalis," or annectant gyrus, comes to the surface, so that the parieto-occipital fissure is cut off from the Simian fissure.

The arrangement of the "intra-parietal system" is exhibited in the diagrams; the right hemisphere of the one brain resembles the left hemisphere of the other, and vice versâ. There is nothing special to be noticed about them. Rolleston fully described the specimen thirty-three years ago.

List of the more Important Works dealing with Orang's Brain.

1. Gratiolet.—Loc. cit. supra.
2. Rolleston.—"On the Affinities of the Brain of the Orang-outang," 'Natural Hist. Review,' 1861.
3. Bischoff.—"Ueber das Gehirns eines Orang-outang," 'Stzb. Math. Phys. Class., 17 June, 1876, d. k. Bayer. Akad.,' i.
4. Cunningham.—Loc. cit. supra.

EXPLANATION OF PLATES 7—11,

Illustrating Dr. W. Blaxland Benham's paper, "A Description of the Cerebral Convolutions of the Chimpanzee known as 'Sally;' with Notes on the Convolutions of other Chimpanzees and of Two Orangs."

The following abbreviations have the same significance throughout the figures.

a. Gyrus intercuneatus (except fig. 19). *aff.* "Affenspalte" or Simian fissure. *arcus.* Arcus parieto-occipitalis, or "seconde pli de passage" of Gratiolet, or first annectant gyrus. *calc.* Calcarine fissure. *c. c.* Corpus callosum. *c. m.* Calloso-marginal fissure. *d.* Sulcus diagonalis. *e. o.* Sulcus fronto-orbitalis. f^1. Sulcus frontalis primus. f^2. Sulcus frontalis secundus. *f. m.* Sulcus frontalis medius. *fr. op.* Frontal operculum, or pars basilaris. *h.* Ramus horizontalis of the præcentral system. *I. P.* Intra-parietal fissure. *I. R.* Insula of Reil. *i. t.* Inferior transverse fissure of Rolando. *k.* Keel of the orbital lobule. *lat. p. o.* Lateral portion of the parieto-occipital fissure. *m. p. o.* or *mes. p. o.* Mesial portion of the parieto-occipital fissure. *n.* Præarcal branch of the intra-parietal fissure. *O.* I. Superior occipital convolution. *O.* II. Second occipital convolution. *O.* III. Third occipital convolution. *op., operc.* Occipital operculum. *orb. op.* Orbital operculum, or pars orbitalis. p^1. Sulcus postcentralis inferior. p^2. Sulcus postcentralis superior. p^3. Ramus horizontalis of the post-central system. p^4. Ramus occipitalis of the post-central system. *p. c. i.* Sulcus præcentralis inferior. *p. c. s.* Sulcus præcentralis superior. *P. O.* Parieto-occipital fissure. *p. s.* Secondary fissure in the superior parietal lobule. *R.* or *r.* Fissure of Rolando, or sulcus centralis.

S. or *Sy.* Sylvian fissure. *S'*. Its anterior limb. *sy'*. Ramus ascendens of the anterior limb of the Sylvian fissure. *sy''*. Ramus horizontalis of the anterior limb of the Sylvian fissure. *temp.* Temporo-sphenoidal lobe. *t'*. Parallel fissure. *w.* Sulcus fronto-marginalis of Wernicke.

Fig. 1.—Dorsal view of a brain of an ordinary chimpanzee. (From a photograph, reduced.) The operculum is well developed, and conceals the "Affenspalte."

Fig. 2.—Dorsal view of the brain of "Sally," for comparison with Figs. 1 and 3. The operculum is very feebly developed, and the "Affenspalte" is fully exposed. The parieto-occipital fissure is divided into two, more distinctly on the right than on the left side. The position of the fissure of Rolando is to be noted, compared with Fig. 1. The frontal lobes are very much fuller than in the ordinary chimpanzee, so that the shape of the hemispheres more nearly resemble those of man. (From a photograph, reduced.)

Fig. 3.—Dorsal view of the "Hottentot Venus;" from a photograph of Gratiolet's figure, reduced to the same size as those of the chimpanzee.

Fig. 4.—Side view of "Sally's" brain, from a photograph.

Fig. 5.—Front view of an ordinary chimpanzee's brain, rather larger than natural size. The orbital region of the frontal lobe on each side is greatly compressed, forming a "keel" (*k.*).

Fig. 6.—Front view of "Sally's" brain, in order to exhibit the almost entire absence of the "keel." Much less of the temporo-sphenoidal lobe is seen.

Fig. 7.—Front view of a European brain, for comparison with Fig. 6. Reduced. The keel is very similar to that in "Sally."

Fig. 8.—View of the "island of Reil" of the right side in a chimpanzee (Oxford Museum, No. 947 *l.*). The temporo-sphenoidal lobe has been pulled downwards. *Sy'*. The "ramus ascendens," and *Sy''*. Ramus horizontalis of the anterior limb of the Sylvian fissure. *fr. op.* Frontal operculum. *orb. op.* Orbital operculum. (From a photograph.)

Fig. 9.—View of a longitudinal section of a chimpanzee's brain (Oxford Museum, No. 947 *f.*). (From a photograph.) The section passes along the posterior cornu (*p. cornu*) of the lateral ventricle, and is intended to show the relation of the Affenspalte to the wall of the ventricle. *cal. av.* Portion of the calcar avis.

Figs. 10—15 illustrate the surface anatomy of "Sally's" brain. They are drawn life size, and the position and shape of the sulci are represented with the greatest care.

Fig. 10. View of the left hemisphere, as seen when looked at from above and slightly laterally. The arrangement of the sulci on the frontal, parietal, and occipital lobes are shown.

Fig. 11. View of the right hemisphere, as seen when looked at from above and slightly laterally.

Fig. 12. View of the right hemisphere, seen laterally but also with the upper surface shown, so that the result is a projection of the surface. The shaded portion indicates the orbital surface of the frontal lobe.

Fig. 13. The left frontal lobe, viewed laterally but with the dorsal surface shown. Rather less of the orbital region is represented than in Fig. 12.

Fig. 14. Inner (mesial) surface of the hinder part of the left hemisphere, in order to exhibit the condition of the parieto-occipital fissure (*P. O.*), which is nearly divided into two parts (*lat. p. o.*) passing on to the upper surface, and (*mes. p. o.*) at *a.*; the gyrus intercuneatus, however, does not come quite up to the surface. *b.* and *c.* Branches of the *mes. p. o.* in the præcuneus. In this and the following figure it will be noted that the calcarine and parieto-occipital fissures meet.

Fig. 15. Inner (mesial) surface of the hinder part of the right hemisphere. The gyrus intercuneatus (*a.*) is here superficial, and divides completely the parieto-occipital fissure into the mesial (*mes. p. o.*) and lateral (*lat. p. o.*) portions.

Fig. 16.—Dorsal view of the left hemisphere of a human brain (Oxford Museum, No. 950), reduced from a photograph, in order to illustrate the condition of the parieto-occipital fissure. It is to be compared with the right side of "Sally's" brain. *m. p. o.* and *l. p. o.* are the two parts of the parieto-occipital fissure. *a.* is the gyrus intercuneatus. The "transverse occipital" is labelled "Affenspalte" for comparison.

Fig. 17.—The hinder part of the left hemisphere of the human brain figured by Bischoff (tracing), for comparison with that of "Sally," more especially with regard to the condition of the "Affenspalte" or transverse occipital.

Fig. 18.—The hinder part of the right hemisphere of the human brain figured by Bischoff (tracing), for comparison with that of "Sally."

Fig. 19.—A tracing of the chimpanzee brain figured by Broca. *a. a. a.*, *b. b. b.* The Affenspalte on right and left side respectively, divided into two. 2. 2. The "arcus." *I. I. I.* The occipitalis primus.

Fig. 20.—The right hemisphere of the chimpanzee's brain presented by Professor John Marshall to the Royal College of Surgeons in 1891. On the left side an operculum is well and normally developed, and hides the Affenspalte; but on the right side this fissure is exposed by the diminution of the operculum, and in this resembles "Sally."

Fig. 21.—The inner (mesial) surface of the hinder part of the right hemisphere of the brain, No. 947 *a.*, represented at fig. 30. The parieto-occipital fissure is quite simple.

Fig. 22.—The left hemisphere of a chimpanzee's brain (Oxford Museum, No. 947 *f.*), to illustrate the condition of the parieto-occipital fissure and neighbouring parts. The operculum conceals the Affenspalte and some portion of the lateral parieto-occipital.

Fig. 23.—The hinder portion of the same brain (947 *f.*), with the operculum turned back and the "Affenspalte" exposed. The intraparietal fissure (*I. P.*) enters the parieto-occipital (*lat. p. o.*), which is separated from the Affenspalte by a large gyrus—a part of the arcus. The shaded parts indicate those which are concealed by the operculum in Fig. 24.

Fig. 24.—The mesial surface of the hinder part of the same hemisphere (947 *f.*), to further illustrate the relations of the parieto-occipital fissure. In this instance this fissure does not meet the calcarine.

Fig. 25.—A tracing of the chimpanzee's brain figured and described by Müller.

Fig. 26.—The hinder part of the right hemisphere of a chimpanzee (Oxford Museum, 947 *i.*). The operculum is intermediate, as regards the size and development, between the usual condition and that of "Sally;" the "Affenspalte" is, however, concealed. The "lateral parieto-occipital" (*lat. p. o.*) is separated from the mesial parieto-occipital (*mes. p. o.*) by the gyrus intercuneatus (*a.*).

Fig. 27.—The mesial surface of the same brain (947 *i.*), to further illustrate the condition of the parieto-occipital fissure. The shaded part is the under surface of the occipital lobe. *b. c. y.* are three branches of the mesial portion of the parieto-occipital. *a.* The gyrus intercuneatus. This figure also shows the distinction of the affenspälte from the parieto-occipital fissure. Note that the calcarine and parieto-occipital fissures meet.

Fig. 28.—Projection view of the dorsal and lateral surfaces of the left frontal lobe of the chimpanzee exhibited in the Court (Oxford Museum, No. 910).

Fig. 29.—The mesial surface of the hinder part of the right hemisphere of a chimpanzee's brain (exhibited in the Oxford Museum, No. 910), to illustrate the arrangement of the parieto-occipital fissure, which does not join the calcarine. The shaded portion indicates the lower surface of the occipital lobe.

Figs. 30—34 illustrate the brain of a chimpanzee (Oxford Museum, No. 947 *a.*). Natural size.

Fig. 30. Dorsal view. It illustrates two stages in the diminution of the operculum. On the right side a condition very similar to that of "Sally" is seen on turning back the small operculum, as in Fig. 32. On the left the operculum is larger, and the condition of the fissures somewhat peculiar.

Fig. 31. The hinder part of the left hemisphere, with operculum turned back. The shaded parts indicate the portions ordinarily concealed by that structure. The parieto-occipital, which is undivided, passes for a considerable distance over the surface of the hemisphere. The Affenspalte is divided into two pieces, the outer of which receives the intra-

parietal fissure (p^4.). It is possible that another interpretation might be placed on these fissures.

Fig. 32. The hinder part of the right hemisphere, with the operculum turned back. The shaded portions are those now exposed. Cf. Sally's brain.

Fig. 33. Projection view of lateral and dorsal surfaces of the frontal lobe of the left side. The "frontalis secundus" is apparently separated into two parts, the hinder (f^2.) being joined by the "diagonalis" (*d.*).

Fig. 34. Projection view of the lateral and dorsal surfaces of the right hemisphere.

Fig. 35.—Projection view of the dorsal and lateral surfaces of the right frontal lobe of a chimpanzee (Oxford Museum, 947 *i.*). The dotted ring indicates a slightly injured area. The præcentralis inferior (*p. c. i.*), the ramus horizontalis (*h.*), and the frontalis secundus (f^2.), are continuous.

Fig. 36.—Dorsal view of the right hemisphere of Professor Herdman's chimpanzee. Natural size. It is quite normal. As the membranes were not completely removed it was not possible to completely trace out all the fissures in detail.

Fig. 37.—Right frontal lobe of the same brain (Professor Herdman's).

Fig. 38.—Projection view of the dorsal and lateral surfaces of the left frontal lobe of a chimpanzee (Oxford Museum, No. 947 *f.*).

Figs. 39—46 illustrate the surface anatomy of the orang's brain.

Figs. 39—41 refer to Rolleston's orang, referred to in the text as II.

Figs. 39 and 40 are dorsal views of the right and left hemispheres.

Fig. 41. Projection view of the left frontal lobe. The right side of the brain had been dissected, but the upper surface had been sliced off and is preserved. The character of the Affenspalte and neighbourhood may be contrasted with that in Figs. 42 and 43. The peculiar condition of the "præcentral fissure" is noteworthy. *a.* Sulcus præcentralis marginalis.

Figs. 42—45 refer to the small orang in the Oxford Museum, referred to in the text as I.

Fig. 42. Dorsal view of left hemisphere. Natural size.

Fig. 43. Dorsal view of right hemisphere. Natural size. The shape of the frontal lobe is noteworthy.

Fig. 44. Projection view of the left frontal lobe.

Fig. 45. Projection view of the right frontal lobe. The dotted portion is injured.

Fig. 46. A copy of Cunningham's fig. 10, p. 294, for comparison of the frontal convolutions with those in other orangs. I have retained Cunningham's lettering.

On the Inadequacy of the Cellular Theory of Development, and on the Early Development of Nerves, particularly of the Third Nerve and of the Sympathetic in Elasmobranchii.

By

Adam Sedgwick, F.R.S.

It is now more than ten years ago since I first pointed out the inadequacy of the cellular theory of development. That I did so in a very guarded manner need hardly be said; but now, after ten years of mature work, I feel justified in giving a stronger expression to the views which I then formed, and which all my subsequent work has amply confirmed. My words then (in 1883) were as follows:—"In short, if these facts are generally applicable, embryonic development can no longer be looked upon as being essentially the formation by fission of a number of units from a single primitive unit, and the co-ordination and modification of these units into a harmonious whole. But it must rather be regarded as a multiplication of nuclei and a specialisation of tracts and vacuoles in a continuous mass of vacuolated protoplasm." Again, in 1888, in the preface to my "Monograph on the Development of the Cape Species of Peripatus,"[1] I wrote: "It would appear, indeed, that in Peripatus the cells of the adult, in so far as they are distinct and sharply marked off structures, are not, as appears to be generally the case, present in the earliest embryonic stages, but are gradually evolved as development proceeds. In other words, the cell-theory, if it

[1] 'Studies from the Morphological Laboratory of the University of Cambridge,' vol. iv, part 1.

implies that the adult cells are derived from embryonic cells which have been directly produced by the division of the ovicell, does not apply to the embryos of Peripatus."

In the days when these words were written it was a general belief among leading histologists and physiologists that the connections which were known to exist in some cases between adult cells had arisen secondarily, and that the primary condition brought about by the cleavage of the ovum was a complete separation from one another of these units, of which the body was supposed to be composed. There has been, no doubt, a change of opinion since those days, and although many biologists would still maintain that cleavage is complete and results in the formation of separate units which later become connected, there is a constantly increasing number who would consider themselves misrepresented if one imputed to them this belief not long ago universal, and the belief which was supposed to follow from it, that the first stage in the evolution of the Metazoa was a colonial Protozoon. But, as I have said, opinions have changed since those days, and I quote my words, written then, to show that I have long held the view which I am now expressing, and that I was among the first to attack a theory which had even then passed its stage of usefulness, and is now holding men's minds in an iron bondage. For although opinions have changed on this important subject, and although there are some who think that they have escaped from the domination of this fetish of their predecessors, yet as a matter of fact the cellular theory of development is still rampant, still blinds men's eyes to the most patent facts, and still obstructs the way of real progress in the knowledge of structure.

In order that I may not be met with the statement that such a state of things exists only in my own imagination, that I am putting up a dummy merely to knock it down again, it is necessary that I should give some proof that this hypothesis has still the power which I ascribe to it. What is the cellular theory of development? I am not concerned with what its authors held; what we want to know is, what is the present

form and extent of it? What is the point of view which it compels its votaries to take?

It is not easy to answer this question; it is, in fact, as difficult to answer as that other question so often asked of the teacher by his pupil—what is a cell? The source of the difficulty is that we are dealing with a kind of phantom which takes different forms in different men's eyes. There is a want of precision about the cell-phantom, as there is also about the layer-phantom, which makes it very difficult to lay either of them. Neither of these theories can be stated in so many words in a manner satisfactory to every one. The result is that it is not easy to bring either of them to book.

To answer the question—what is the cellular theory of development?—the best plan will be to consider for a moment the ideas which are taught to the student of biology, and which influence him in his future work. We tell him that the cell is the unit of structure, that an organism may consist of a single cell, or of several cells in association with one another: we draw the most fundamental distinction between the two kinds of organism, and we divide the animal kingdom into two great groups to receive them. As a proof of the importance which we attach to this feature of organisation we assert that a man is nearer, morphologically, to a tapeworm, than a tapeworm is to a paramœcium. We tell him that the various structures present in a protozoon are all parts of one cell, whereas in a metazoon the various parts are composed of groups of cells which differ from one another in structure. Finally, when we ask him in the examination to tell us the principal differences between hydra and vorticella, we consider that he is very inadequately prepared if he does not sum them up by saying that hydra has tissues composed of definite cells and is multicellular, while vorticella is without definite cellular tissues and is unicellular. Carrying on the idea thus implanted in his mind as to the fundamental importance of the cell, we tell him about the neuro-epithelial cell and the myo-epithelial cell, and we point out their primitive distinctness,—an idea which is still further impressed upon him when he studies the

connection between nerve and striated muscular fibre. Finally, when he comes to study embryology, the importance and distinctness of the cell meets him at every step, from the complete cleavage which he is led to believe is primitive, to the development of nerves according to the views of His.

So much for the student in the schools: now for the investigator in the laboratory. He studies the ovum and maintains its absolute isolation in the organism; or he examines epithelial cells and draws them as isolated structures separated by sharp boundary lines; or he labours to prove the continuity between the nerve and muscle, or between the nerve and secreting cell: so much is he dominated by the idea of separate cells that he considers that the burden of proof rests rather with the man who asserts such continuity than with him who denies it. Or, if he be an embryologist, he will talk of, and figure, the proliferation of cells at the primitive streak; he will describe the nascent ganglion cell sending a process from the developing spinal cord into the anterior root, and he will figure it; he will talk of mesenchyme cells, and figure them for the most part separate from one another.

I take it that this is a not unfair account of the training a zoologist receives at the present day, so far as the cell is concerned, and of the ideas which dominate him in his later work. He believes that the cell is the unit of structure, and that it forms the basis of organisation in the Metazoa; it is the functions of the cell and the relations which it enters into with other cells which forms an important subject of current biological investigation. Who, then, can deny that the cellular theory of development is still a living power in the school of biology? That it blinds men's eyes to the most patent facts, and obstructs the way of real progress in the knowledge of structure, it will now be my endeavour to show. For this purpose I shall deal on this occasion with the origin and structure of two tissues of the Vertebrate embryo—the so-called mesenchyme and the system of peripheral nerve-trunks. My results are the product of many years' work, and will, I hope, be published in greater detail and with figures on a future occasion.

The So-called Mesenchyme Tissue of Elasmobranch Embryos.

This tissue is always described as consisting of branched cells lying between the ectoderm and the endoderm. The cells are spoken of as being separate from one another, and from the adjacent ectoderm and endoderm, excepting at points where they are supposed to arise from one of the primary layers. And not only are they described as being separate cells, but they are actually drawn in the author's figures as separate from each other. This is, perhaps, the best instance that can be given of the bondage in which the cellular theory holds its votaries. For what are the facts? The separate cells have no existence at all! In their place we find, on looking into the matter, a reticulum of a pale non-staining substance holding nuclei at its nodes. It is these nodes, with their nuclei, which are drawn by authors as the separate branched cells of the mesenchyme, and they are constrained by this theory, with which their minds are saturated, not only to see things which do not exist, but actually to figure them. Another erroneous view due to the same cause is the view that this mesenchyme tissue is not continuous with the ectoderm or with the endoderm; whereas, as a matter of fact, the opposite is the case, for the primary layers are simply parts of this reticulum in which the meshes are closer and the nuclei more numerous and arranged in layers. These are facts of which anyone with an unbiassed mind can convince himself by the simple inspection of a Selachian or an Avian embryo, and they would have been recognised long ago had it not been for the dominating influence of the cellular theory of development.

The current views as to the origin of this tissue show just as conspicuously the influence of the same theory. It is said to arise by the budding-off and migration of cells from the walls of the embryonic cœlom, from the primitive streak, and from the neural crest; and the space between the ectoderm and endoderm into which these cells migrate is described as being empty of structural elements. What are the facts? The

space between the layers is never empty; it is always traversed by strands of a pale tissue connecting the various layers, and the growth which does take place at the places mentioned is not a formation of cells, but of nuclei which move away from their place of origin and take up their position in this pale and at first sparse reticulum which exists between the layer. As this reticulum, which has always existed, becomes infested with nuclei it increases in bulk, and forms the conspicuous reticulate tissue which is by some authors called mesenchyme. The primitive streak, the walls of the cœlom, and the neural crest, and, as Goronowitsch[1] has shown, parts of the ectoderm, are growing points where nuclei, not cells, are produced. These facts I described long ago in the development of Peripatus, and it is the recognition of the same processes taking place in the Vertebrata in an even more conspicuous manner that has induced me to again call attention to their importance.[2]

The Origin of Nerve-trunks and the Fate of the Neural Crest.

If there is one point more than another on which the cellular theory of development has led anatomists completely astray, it is upon this one. We may take it that the new views upon the origin of the peripheral nerves began with Balfour's discovery of the structure which is generally called the nerve crest. Before that discovery nerves were supposed to develop in situ in the mesoderm; after it, there were two principal views as to the origin and growth of nerves: one of these was that cells of the central organ grew outwards as strings to the periphery; while, according to the other, nerve-fibres are the elongated

[1] 'Morpholog. Jahrb.,' Bd. xx, 1893.

[2] At the same time Peripatus shows certain features more clearly than the Vertebrate; I would refer especially to figs. 24 *d* and 26 *d* on pl. v of my Monograph, in which, while the so-called ectoderm and endoderm are obviously parts of the same layer, or tissue: they are separated by a region in which the vacuoles are larger, the protoplasmic strands less numerous, and nuclei are conspicuous by their scarcity.

processes of cells either of the central organ or of the ganglia. Both these views are erroneous; and if both were not inspired by the cellular theory of development, they were both promulgated at a time when that theory was at its zenith. The earlier view, that nerves were developed in situ from the mesoderm, was much nearer the truth.

The nerve crest does not, as was first stated by Balfour and afterwards by all authors on the development of nerves, give rise exclusively, or even principally, to nerves and ganglia. It gives rise to nuclei which spread out in, and add to the mesoblastic reticulum, which at all times, i.e. from the very beginning, exists between the layers, and to nuclei which become the nuclei of the rudiments of nerve ganglia. The nerves are developments of the reticulum; they are elongated strands of the pale substance composing the reticulum, with some of its nuclei; and their free ends branch out into the fibres of the reticulum, and are added to by the latter falling into the line of the growing nerve. Neither they nor the ganglia appear until the nerve crest is breaking up. The reticulum further gives rise certainly to smooth muscular fibres, connective tissues, and blood-vessels, and probably also to striated muscle. It is also continuous with all the so-called epithelial tissues of the embryo; indeed this latter substance is to be regarded as consisting only of one or more layers of nuclei embedded in the outer part of the reticulum, which is rather denser than elsewhere in correspondence with the greater density of the nuclei. Nerves are a gathering up, so to speak, of the strands of the reticulum into bundles, and are formed in that way; or, to put the matter in another way, nerves are a special development of the reticulum along certain lines. These special developments are generally marked by an increase in the number of nuclei, such increase being particularly great in the neighbourhood of the ganglia.

To sum up the matter, the nervous and muscular tissues are, as they were in Peripatus (see my Monograph, p. 131), special developments of the same primitive reticulum, a com-

munity of origin which renders their adult relations perfectly intelligible. Further, I have no hesitation in saying that His' descriptions of the development of nerve-fibres as processes of central or ganglionic nerve-cells, does not apply to Selachians; inasmuch as nerves are laid down long before any trace of nerve-cells can be made out. The neuroblasts of His and of other authors are nuclei lying in a substance which, after death caused by the ordinary reagents, has usually a fibrous structure. This substance is continuous with, and therefore a part of, the reticulum outside. The cell-processes which have been described as growing out from the neuroblasts are merely parts of this reticular substance, the fibres of which become arranged more or less in the direction of the long axis of the nuclei, and the meshes correspondingly drawn out and narrowed. Many of His' drawings even show that this is so, and an inspection of the specimens leaves no doubt at all about the matter. In short, the development of nerves is not an outgrowth of cell-processes from certain central cells, but is a differentiation of a substance which was already in position; and this differentiation seems to take place from the medullary walls outwards to the periphery, both in the anterior and posterior roots, and to precede, or to proceed pari passu with, the development of other tissues. The nerve crest is, then, to be regarded as a centre for the growth of nuclei, which spread into the body of the embryo and become concerned in the formation of many tissues, nervous tissues amongst the rest. There are many other such centres for the production of nuclei; for instance, I may mention the walls of the cœlom, the caudal swellings, and in the Amniota the primitive streak. All these centres of growth are in so-called epithelial tissues. This is, of course, necessitated by the fact that Selachian embryos are at one stage composed entirely—or almost entirely—of these so-called epithelial tissues; as are many embryos, e. g. those of Peripatus and of Amphioxus.[1] These facts will be dis-

[1] The significance of this epithelial structure of the young embryo—this

puted by many morphologists, but they are easy of proof by the simple inspection of good preparations to minds not warped by the cellular theory as ordinarily taught. In fact, had it not been for the undue persistence of this hypothesis beyond the time of its fruitful life, they would have been recognised long ago, and much needless waste of labour in trying to make the facts of nerve-development conform to the theory would have been saved.

The nerve-crest in Selachians (Scyllium, Acanthias, Raia, and Pristiurus) is, as I pointed out some time ago ("Notes on Elasmobranch Development," 'Quart. Journ. of Micr. Sci.,' vol. xxxiii), from its first appearance, in three pieces.[1] The first of these pieces reaches from the region of the fore-brain to the hind brain. The posterior limit of it is marked in older embryos by the root of the trigeminal nerve. It gives rise to the reticulum of the front part of the head, and contributes to that of the mandibular arch. The following nerves are formed within its limits:—The trigeminal and its branches, which include the so-called ramus ophthalmicus profundus with the ciliary ganglion and the third nerve (see below). Very possibly other nerves, viz. the fourth, the sixth, and the olfactory, may be also developed from this part of the reticulum, but I have no observations on this point.

The manner in which these nerves are laid down may be described as follows:—When the nerve-crest, which in this region of the head very early spreads ventralwards on each side of the brain, is breaking up into the reticulum, certain tracts of it remain unaltered and characterised by a greater density of nuclei. These tracts mark the course of the future nerves and the sites of the future ganglia. They them-

collection of the nuclei at the surfaces, as it may be described—I hope to consider in another place. Now, I may merely hint that it is probably due to the impress of some well-marked larval phase in earlier stages of evolution (see my article on "von Baer's Law, &c.," in 'Quart. Journ. Micr. Sci.,' vol. xxxvi).

[1] Goronowitsch ('Morph. Jahrb.,' Bd. xx) has recently found the same fact for the bird, but he makes no reference to my results on this point.

selves continue to break up, but a kind of core remains which constitutes the foundation of the future nerve and ganglion. The Gasserian ganglion, the ophthalmicus profundus, the mandibular branch of the fifth, and the ciliary ganglion thus gradually emerge from the remains of the nerve-crest—are, so to speak, crystallised out of it. At first they have the form of dense cords of nuclei; but they soon acquire some of the non-staining fibrous substance, which makes its appearance as a rule in their central portions, so that for a time sections of these nerves exactly resemble in appearance sections of the nerves of Invertebrata, e. g. Peripatus, Chiton, &c. This description holds for an embryo of 35 mm., beyond which stage I have no observations. The nuclei which have peeled off, leaving the nerve-trunk below, give rise to the muscular and connective tissues of the parts concerned, the reticulum of which is freely continuous with that of the nascent nerve, especially at the free end of the latter. It thus becomes apparent that these tissues—nervous, muscular, connective, and vascular—are all developed in continuity.[1]

While the Gasserian ganglion, the mandibular branch of the fifth, the ophthalmicus profundus, and the ciliary ganglion all crystallise out of the nerve crest; the third nerve does not do so. It arises as a differentiation of the reticulum formed by the breaking up of the nerve crest, and it first makes its appearance as a forward projection of nuclei from the ciliary ganglion. This, by a gradual differentiation of the reticulum, extends itself until it reaches the base of the mid-brain, with which it becomes continuous by means of an increase in the pale fibrous strands which pass between the medullary wall and the reticulum. The third nerve is at first a cord of nuclei and rather dense pale substance. The third nerve,[2] there-

[1] The continuity of the embryonic tissue which will give rise to the nervous and muscular tissues is well seen in the embryo of Peripatus capensis, and I have already hinted at this fact in my Monograph on the development of that species at pp. 131 and 133, and figured the tissue as nerve musc., pl. x, fig. 5.

[2] It will be evident, if my observations are correct, that I have found an earlier stage of the third nerve than Dohrn describes in his sixteenth study. In

fore, presents this interesting and remarkable peculiarity in Scyllium and Acanthias; it grows or is differentiated from the ciliary ganglion to the floor of the mid-brain, and not in the opposite direction, as has hitherto been supposed. The proof of this is to be found in the fact that in a Scyllium and Acanthias embryo of 10 to 11 mm. the third nerve can be seen projecting forwards from the ciliary ganglion, and ending in front in the reticulum, short of the floor of the mid-brain. The ciliary or profundus ganglion is at one time—when it is first laid down—in contact with the ectoderm. Later it is shifted inwards, but remains connected for a time with the ectoderm by a cord of cells, which eventually disappears. This point has been seen by van Wijhe.

The embryonic medullary wall[1] is connected with the reticulum by pale fibres similar to those which compose the reticulum, and the nerve-roots, both anterior, posterior, and cranial, are special enlargements of such connecting strands. They are formed at a time when no structures which could be called cells by any but a fanatical devotee of the cellular theory are present, either in the medullary wall or in the ganglionic rudiments, and in a manner which, if closely followed, renders it quite impossible to speak of growths one way or the other, excepting that one can make one assertion—the pale fibrous substance which marks the nerve appears both in the anterior and posterior roots and in the cranial nerve-roots next the central organ, at a time when the white matter (which is composed of this pale fibrous substance) first appears as a thin layer, and in continuity with such white matter. The differentiation outwards proceeds from this point, and the free end of the nerve-rudiment always ends by branching out into the fibres

my fuller paper dealing with this subject I hope to examine Dohrn's results in detail.

[1] Inasmuch as the nerve-crest is derived from the medullary wall and gives rise to mesodermal structures, the medullary wall itself gives rise, in part, to mesoderm.

of the reticulum. The only exception to this rule is the third nerve of Scyllium and Acanthias (and probably others), which is undoubtedly differentiated from the ciliary ganglion to the floor of the mid-brain; but this is, perhaps, more an apparent exception than a real one, because the ciliary ganglion belongs to the fifth nerve and the order of fibrous differentiation is normal, viz. from the root of the fifth nerve, through the ciliary ganglion, to the floor of the mid-brain. I commend this observation on the development of the third nerve to the physiologist, with a view to a renewed investigation of its functions. It is rendered the more interesting by the fact that in Lepidosiren it is commonly stated that the area of the third nerve is supplied by the ophthalmic branch of the fifth, the third nerve being absent.[1]

I have already, in my 'Notes on Elasmobranch Development,' stated my reasons for believing that the views put forward by Hensen as to the origin of nerves were nearer the truth than those of any other zoologist. I have, in this paper, shown not only that the network required does exist, but also how it arises, and how it gives rise to the rudiments of the peripheral nerve-fibres. Minot, in his 'Human Embryology,' p. 624, says that Hensen's theory of the origin of nerves "cannot be adopted because the outgrowths of the nerve-fibres have been observed; moreover, Altmann has pointed out that the fibres seen in the embryonic mesoderm are really processes of the mesoderm cells, and, as shown in the excellent fig. 2 of his plate, are quite distinct both from the ectoderm and endoderm." (The italics are mine.) This passage is, according to my work, full of errors; for I maintain, as the result of long and careful observation, extending over many years, that the outgrowth of nerve-fibres from cells in the ganglia and medullary wall not only has not, but cannot

[1] This statement rests on Hyrtl's work. It must, however, be remembered that his specimen was confessedly rotten in its nervous tissues, and by the fact that v. Wijhe ('Nied. Arch. f. Zoologie,' Bd. v, 1882) has found the third nerve in Ceratodus. Parker does not deal with the brain and nerves in his memoir on Protopterus.

be observed; that the fibres in the embryonic mesoderm are not processes of mesoderm cells (as they are always figured), which have no existence, but are parts of the reticulum which has always existed from before cleavage onwards, connecting together the various parts of the developing ovum, and that this reticulum is not separate from ectoderm and endoderm, but freely continuous with both, they being but parts of it. The almost universal practice of drawing this reticulum as composed of separate branched cells is a most remarkable instance of the manner in which a theory can blind men's eyes to the most obvious facts.

Before concluding this general account of my work, I may mention one or two other points of general interest which I have noticed. Firstly, I may mention that in Scyllium there are a number of anterior roots next the head, varying in number from three to five, according to the age of the embryo, without posterior roots. They no doubt give rise, as has been suggested by others, to the so-called anterior roots of the vagus. Secondly, Balfour was quite correct in the account he gave of the origin of the sympathetic ganglia in Elasmobranchs.[1] The ganglia arise as swellings on the posterior roots of the spinal nerves, and soon become removed from the latter, so as to form isolated masses connected with the spinal nerves by a cord. These masses eventually become united longitudinally into a chain. I may add to Balfour's account this fact, viz. that no sympathetic ganglia are found within the area of extension of the vagus ganglion. Or, if I am not correct in applying the term "vagus ganglion" to the posterior part of the vagus—the part which lies dorsal to the gill-slits and gives off the branchial nerves, it would be better to say that sympathetic ganglia are not found in the region of the branchial slits, but begin immediately behind these structures. Thus, in an embryo of 22 mm. the vagus ganglion and branchial

[1] I have not examined mammals on this point, but I think Paterson's memoir ('Phil. Trans.,' 181) does not carry conviction. On the contrary, there is, I think, in it internal evidence which inclines me to the view that he has not got to the bottom of the matter.

region of the fore-gut ends at the level of the fifth anterior root, and the first posterior root and the first sympathetic ganglion occur at the level of the sixth anterior root. In older embryos, in which the branchial region extends much further back and overlaps a number of fully-formed spinal nerves, the original sympathetic ganglia which were formed in connection with the ganglia of these spinal nerves thus overlapped are found to have disappeared. The first sympathetic ganglion appears always to be just behind the branchial region, as in the adult, and sympathetic ganglia are formed in Scyllium in connection with nerves which are without a posterior root.

Gaskell reproaches v. Wijhe with not knowing the true meaning of a sympathetic ganglion, and one is tempted to ask, does Gaskell himself know much more about it, or throw any light upon the question? He says ('Journal of Physiology,' vol. x, p. 162) that a sympathetic ganglion is the ganglion of the anterior root of a spinal nerve which has travelled to a variable distance from the central nervous system. As Dohrn (seventeenth study, 'Naples Mit.,' Bd. x) very properly insists, this view is at variance with the known developmental history of the ganglion—which I am able to confirm so far as its nuclei are concerned, and with the reservations necessitated by the views set forth in this paper—and I am now able to state that it is at variance with the fact that sympathetic ganglia are entirely absent from those spinal nerves in which the posterior root fails to reach its full development. In fact, one may say of these ganglia that they are always absent when the posterior roots are not developed.

With regard to the fate of the neural crest described in this paper, I should mention that I strongly hinted that it gave rise to nuclei which entered the reticulum in my 'Notes on Elasmobranch Development,' p. 581, published in 1892; and that Goronowitsch arrived independently at the conclusion that it broke up into mesenchyme in the bird, and published his results at some length in 1893 ('Morph. Jahrbuch,' Bd. xx); but Goronowitsch failed to recognise the reticulum, and he was

unable to appreciate the full significance of the facts he described in their bearing on the question of the origin of nerves. Platt approximated to the truth with regard to the third nerve in her account of it as growing from the ciliary ganglion to the brain, but retained the error of her predecessors in regarding it as a cellular object, and not as a differentiation of the reticulum.

Minot has a characteristic comment on Platt's statement. He says ('Human Embryology,' p. 639), "This view rests probably on erroneous interpretation of observation, for it cannot be admitted that a motor nerve is formed by ganglionic fibres"! (The italics are mine, as is also the note of admiration.)

On Benhamia cœcifera, n. sp., from the Gold Coast.

By

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With Plate 12.

Some time this year I received a large specimen of Benhamia from Professor Jeffrey Bell for examination and identification. My best thanks are due to the authorities of the British Museum for their permission to make the examination.

The worm, which turns out to be a new species, to which I give the name Benhamia cœcifera, was collected by Captain Torry at Axim, in the Fantee Country, on the West Coast of Africa.

We already know a number of species from this side of Africa, as well as from the East Coast and inland, and we know pretty certainly that this continent is the home of the genus.

This new species is of considerable size, measuring 510 mm. (20 inches), with a diameter of 17 mm. at the clitellum, and gradually diminishing posteriorly. Its average diameter is about 12 mm., and it tapers only very slightly to within a few segments of the hinder end, where it rounds off. There are 310 segments.

The colour of the worm is dirty brown (in spirit), and the hinder end is not very sensibly lighter than the rest of the body.

In each segment there is a circle of small dark brown pig-

ment spots at the level of the chætæ, giving the impression at a casual glance that the chætæ are in a circle round the body. A similar style of pigmentation has been noted by Michaelsen in the case of B. affinis and others.

The dorsal pores are large and conspicuous, commencing after the 4th segment; as usual these are absent (or obliterated) in the clitellar region. When the animal was handled a considerable quantity of a brown fluid issued from the pores.

The chætæ, which appear as black dots, have the usual arrangement, and present no pecularity of shape or ornamentation. They are invisible, if indeed they are present, in the 2nd segment. As I had only one specimen, which had to be returned to the British Museum, I could not, of course, examine the body-wall microscopically. The four couples are equidistant from one another, being about 2 mm. apart. Some of the anterior segments, except the first three, are biannulated, the first annulus being nearly twice as large as the second, and bearing the chætæ.

The prostomium is not dovetailed into the peristomium; the latter is not more than half the size of the 2nd segment.

The clitellum covers eleven segments, viz. XIII to XXIII inclusive; the intersegmental groove is still distinct detween the 13th and 14th segments, but elsewhere, at any rate dorsally and laterally, the limits of the segments composing the clitellum are not recognisable, except by bands of darker pigment. As in all species of this genus, there is a ventral area in the middle of the clitellar region which in this instance presents specific characters. In Segments XIII, XIV, and XV the clitellum is "complete," but in the remaining segments it ceases ventro-laterally, i. e. just below the outer series of chætæ, and forms a distinct margin (fig. 1, *m.*) limiting a more or less rectangular depression, the "ventral field." This is wider anteriorly than posteriorly, being about 12 mm. across on Segment XVII and about 8 mm. on Segment XXII. Anteriorly the ventral field is bounded by the hinder margin of Segment XV.

Within this "ventral field" lie the four prostate pores, a pair on each of the Segments XVII and XIX. The two pores of

each side are connected by a longitudinal groove, as in other species of the genus. But what marks this species as distinct from all others hitherto examined is the presence and arrangement of numerous small pits, no doubt having some function in relation to copulation. The figures give a better idea of the arrangement of the pits than any detailed description. It will be seen that for the most part they form transverse rows on Segments XV, XVI, XVII, XIX, XX, XXI, XXII. There is a single median pit on Segment XXIII, and on the 18th segment three short longitudinal rows occur, one median and a pair of lateral rows.

"Copulatory organs" ("pubertäts Tuberkeln") have been noted by Michaelsen in various species, especially B. affinis and B. inermis. In the latter they are paired, and occur on segments in front of the clitellum, but they appear from his figures and descriptions rather as tubercles than as pits, and have altogether a different arrangement.

These pits in the present worm mostly have a well-defined margin, not raised above the general level of the surface. In others there appears to be a papilla on one side of the pit, projecting to a greater or less extent into the cavity of the pit, as shown in fig. 2, *B. C.* But in no case do the papillæ project to the exterior, nor are they visible except on careful inspection.

Two pairs of similar pits occur in relation to the spermathecal pores, viz. on Segments VII and VIII (fig. 1, p^1. p^2.). The anterior pair lies on the hinder margin of the segment, the posterior pair on the anterior margin of the second annulus—this one is more laterally placed than the former. In the opened worm there were no sacs or other structures projecting into the cavity of the worm's body; the pits appear to be limited to the body wall.

The spermathecal pores have the usual position on the boundaries of Segments VII-VIII and VIII-IX, in a line with the inner series of chætæ.

The oviducal pore is small, but it is visible just in front of the ventral (inner) chætæ of Segment XIV. On Segment XIII a pair of circular whitish areas, each with a central depression,

is situated just behind and slightly laterad of the inner chætæ. These probably have some copulatory significance. They are, however, different from the "pits" above mentioned. Internally in this segment is a low but rather extensive muscular prominence occupying the whole length of the segment, which, I presume, is the wall of some structure opening by the above-mentioned pore.

Turning now to the internal anatomy, there are the following points to be noted. As in other species of the genus, certain of the anterior septa are very much thickened, viz. the septa 9/10, 10/11, 11/12, 12/13, and 13/14; the next two septa are also thick, but less than the foregoing five. In front of the first of these thickened septa, the viscera are wrapped together by a delicate membrane, due no doubt to the thin septa of these segments being pushed backwards by the large gizzards; owing to the tenuity of the septa here, and to the fact that they are ruptured in merely turning the organs aside, it is difficult in a dissection to trace them out. The first septum in the body is distinct, and separates Segments IV and V; consequently septa 5/6, 6/7, 7/8, and 8/9 are represented by the enveloping membrane just referred to.

The Alimentary Tract.—Amongst the internal organs the most noticeable feature of novelty is presented by the intestine. The two gizzards, characteristic of the genus, are of large size, and appear to belong to the Segments V and VI. The œsophagus is narrow, and provided with the usual three pairs of "calciferous glands" lying in Segments XV, XVI, and XVII. Whether carbonate of lime is present, as has been determined for other species, I am not in a position to affirm, as I did not think it worth while to explore the glands. As is frequently the case, the first gland on either side is the smallest. In the genus Benhamia the wall of these glands presents a variety of patterns, owing to its folding; these have not been figured carefully, but have been described by Michaelsen and by Horst as horizontal folds in some cases, vertical folds in others. But in the present species the foldings are very elaborate, so that the surface of the gland, as well as

its shape, suggest a much convoluted cerebral hemisphere (see fig. 4).

Each gland is, on the whole, reniform, and connected to the narrow œsophagus by a short, narrow, but distinct duct. The glands are supplied by a large vessel from the dorsal trunk, and are further closely connected to the septa.

In the 20th segment the gut enlarges suddenly and forms a wide, thin-walled, dark brown coloured "sacculated intestine," constricted of course by the septa. In Segment XXIX, however, the intestine takes on the peculiar character (unknown in its details in any other earthworm) which is referred to in the specific name "cæcifera." In each segment, commencing at the 29th, the intestine gives rise to a finger-shaped diverticulum on each side, which is directed upwards and arches over the dorsal blood trunk (fig. 3, *c*.).

These cæca extend as far as the 52nd segment, the last half-dozen gradually diminishing in size, till the intestine resumes the ordinary sacculated condition. Each such cæcum arises by a comparatively broad base from the upper surface of the side of the main gut, and gradually narrows as it curves upwards, to end in a blunt rounded apex. The apices of the pair of cæca of any segment overlap each other above the dorsal vessel. These cæca are thin-walled, and quite unlike the well-known cæca which lie in the 26th segment of the ordinary Perichæta.

The generative organs conform to the usual Benhamia type.

There are two pairs of sperm-sacs, in segments XI and XII, each being much divided and having a lobulated appearance.

The two pairs of prostates are very long, narrow, yellowish tubes of the usual acanthodriloid pattern; the muscular duct is very delicate at its origin from the gland, enlarging in its slightly winding course, to pierce the body wall: they lie in segments XVII and XIX (fig. 5).

I could detect no penial chætæ; these special chætæ have been noted as lacking in other species of Benhamia. It is a point on which I laid some stress in my note on "The Genera

Trigaster and Benhamia,"[1] where I pointed out the various differences between the two genera. Some of these differences have been bridged over by various species of Benhamia, though in my opinion the genus Trigaster still stands as a valid genus.

Though I could find no penial chætæ on careful examination of the partially dissected individual, I should not like to affirm that they do not exist. There are several strong bands of muscle in the Segments xvii to xxi (fig. 5), arising close to the nerve-cord and passing outwards to be attached to the body-wall near the dorsal midline; they have a similar arrangement to those figured by me for Moniligaster (indicus) robusta, A. G. B.,[2] and it is possible that special chætæ may lie below these muscles—but I did not wish to do too much injury to the specimen.

The spermathecæ, which lie in Segments vii and viii, differ in size, as has already been noted in several species. The anterior pair are smaller than the posterior pair, and whereas the latter is provided with a small ovoid diverticulum, those of the 7th segment are without one (fig. 6). Each spermatheca consists of a thin-walled, somewhat ovoid sac, with a longish and comparatively stout, muscular, glistening duct, which widens as it approaches the body-wall.

I found no structures in these segments corresponding with the pits noticed in the account of the external anatomy.

The vascular system calls for a few remarks, but I have not attempted to make a complete study of this system; nevertheless, from what I have observed and from what previous observers have noted, I believe the vascular system of Benhamia and Acanthodrilus would repay a careful study on the lines of and in relation to Dr. Bourne's thorough account of the vascular system in Megascolex[3] and Moniligaster.[4]

[1] 'Ann. Mag. Nat. Hist.,' 1890, p. 414.

[2] 'Quart. Journ. Micr. Sci.,' vol. xxxiv, pl. 32, fig. 3.

[3] "On Megascolex cœruleus, &c.," 'Quart. Journ. Micr. Sci.,' vol. xxxii, p. 49.

[4] "Moniligaster grandis," 'Quart. Journ. Micr. Sci.,' vol. xxxvi.

In Benhamia cœcifera the dorsal vessel gives origin to four pairs of very large hearts, lying in Segments IX, X, XI, XII. These may be, and I suspect are, connected also with a supra-intestinal trunk, as Horst has described in certain species of this genus; but I could not, without injury to the worm, determine the point.

In Segments VII and VIII two other pairs of hearts arise; these are smaller, and differ in their appearance from the posterior pair, being more irregular in their swellings. The large posterior hearts remain greatly distended till close to the ventral trunk, to which they are joined by a short narrow vessel (fig. 8). The heart in Segment VIII has a less diameter and a longer, narrow vessel connecting it with the ventral trunk. The heart in Segment VII is much shorter. About midway between the dorsal and ventral trunks it suddenly narrows; this narrow part opens into a small spherical dilatation, whence arise two delicate vessels; one goes forwards to the side of the second gizzard, the other continues downwards to enter the ventral vessel. This anterior heart recalls most curiously the condition of the heart in Segment IX of Megascolex figured by A. G. Bourne (loc. cit. pl. 8, fig. 4).

The dorsal vessel terminates here in Segment VII after giving rise to the hearts (figs. 7, 8), but its place appears to be taken by a pair of "latero-longitudinal" vessels (I use this term topographically, and with no implication as to their homology with those so named by Bourne in Moniligaster). These (*l. l. v.*) arise, as far as I could determine, as branches of the dorsal trunk just in front of the third pair of hearts, i. e. in Segment IX, and each runs forwards to the pharynx, giving off vessels to the gizzard and other structures.

The blood-vessels on the body-wall present a feature which I have not seen noticed in any previous account. In each segment there is a small vessel having a circular direction along the inner face of the body-wall. I do not know its relation to the main trunks, but, as it is readily recognisable with the naked eye: on each side of its course, it gives rise to a fairly regular series of small globular dilatations; these were more

readily noticeable along the dorsal wall, but could be traced all round (fig. 9). Viewed under the microscope it is seen that each dilatation is connected by a short narrow vessel to the circular vessel; and from the dilatation a vessel passes away, which soon dips into the muscular coat of the body wall and then subdivides. I have not traced them further; but the regular arrangement and large size of these dilatations, which recall those previously described and figured in the case of Lumbricus, Trigaster, &c., is deserving of mention.

The nephridial system consists of a series of "micronephridia,"[1] arranged in a row around the inner surface of the body-wall, near the hinder septum of most (probably all) of the segments. This nephridial system is specially abundant in Segments XIII, XIV, and XV, and most markedly towards the ventral surface of these segments.

Summary.

Benhamia cœcifera is, then, characterised (1) by the number and arrangement of the peculiar copulatory pits on the ventral field; (2) by the possession of a number of peculiar finger-shaped cæca arising from the intestine; (3) by the form of the foldings of the wall of the calciferous glands, and possibly (4) by the termination of the dorsal vessel in Segment VIII; (5) by the extent of the clitellum; (6) by the position of the first dorsal pore between Segments IV and V.

Affinities.

In its size (length 510 mm.) and number (310) of segments it might be grouped with certain other large species of the genus found on the West Coast of Africa, viz. B. Schlegelii, B. Buttikoferi, B. rosea, and B. inermis. But it differs from each of these in the above characters. Firstly, it is marked off by the forward position of the first dorsal pore; this structure has been found of specific value in our European

[1] This term, employed by Vejdovsky, is preferable to my term of "plectonephridia," for we have no evidence that in every case there is a true network any more than we have a real network in all "capillary networks."

earthworms, Allolobophora and Lumbricus, and is probably of similar value here.

In extent of the clitellum (Segments XIII—XXIII inclusive) it recalls B. rosea, Mich., in which it ceases on the 22nd segment. But in this species the paired spermathecal and oviducal pores are connected by transverse grooves; further, penial chætæ are present, and the shape of the "ventral field" is quite different. Again, no mention is made of copulatory pits or tubercles.

To B. inermis, Mich., our present species presents a greater likeness, in that there are no penial chætæ but there are copulatory tubercles ("pubertäts-tuberkeln"); these are, however, arranged on quite a different plan. There appear to be about twelve or more pairs of them; the first pair on Segment VIII, then follow seven pairs situated at the intersegmental grooves of as many segments, all in front of the clitellum; there are four more pairs within the clitellar region. Each pit leads into a sac projecting into the body cavity. This association of the presence of copulatory papillæ and pits with the absence of penial chætæ is worthy of note.

Another large species, B. itoliensis, is known only from its anterior end. This species was found at Itoli, on the Victoria Nyanza, and though in general it appears to resemble B. cœcifera, yet the possession of penial chætæ and absence of copulatory pits (as well as other features) serve to distinguish the two from one another.

EXPLANATION OF PLATE 12,

Illustrating W. Blaxland Benham's paper, "On Benhamia cœcifera, n. sp., from the Gold Coast.

Fig. 1.—Ventral view of genital segments of Benhamia cœcifera. (Nat. size.) The segments are numbered. All the genital pores are shown. *spth.* Spermathecal pores. p^1. p^2. Copulatory pits in their neighbourhood. *cop.* Copulatory (?) organs of a different nature on XIV. *m.* Latero-ventral margin of clitellum bounding the "ventral field." The arrangement of the copulatory pits, represented as small dots, is shown.

Fig. 2.—Three copulatory pits from the "ventral field." In *B.* and *C.* a papilla (*p.*) is more or less exposed in the pit; in *A.* no papilla is to be seen.

Fig. 3.—A portion of the intestine exhibiting the characteristic finger-shaped cæca (*c.*), arching upwards over the dorsal blood-vessel (*D. v.*); in two cases the blood-vessels (*v.*) to this cæca are shown.

Fig. 4.—The three pairs of calciferous glands (*gl.*), with their vessels from the dorsal trunk (*d. v.*).

Fig. 5.—The prostate duct (*d.*), with copulatory muscles (*m.*), which are seen passing into the next segment. *pr.* A portion of prostate. *n. c.* Ventral nerve-cord. *spt.* Septum.

Fig. 6.—The two spermathecæ of the right side. *d.* Muscular duct. *div.* Diverticulum.

Fig. 7.—The three most anterior pairs of hearts, lying in Segments VII, VIII, and IX. The dorsal vessel (*D. v.*) is seen to terminate in Segment VII. *spt.* Septum 9/10.

Fig. 8.—The same three hearts seen partially from the side, showing the relations ventrally. *l. l. v.* Latero-longitudinal vessel arising in Segment IX. *g.* Its branch to gizzard. *g'.* Branch from first heart to the gizzard. *s.* Swelling from which this arises. *V. v.* Ventral vessel.

Fig. 9.—A small portion of the body-wall of a segment, to show the dilatations on the circular vessel (*c.*), as well as the position of the micronephridia (*n.*). S^1., S^2. The anterior and posterior septa.

A Re-investigation into the Early Stages of the Development of the Rabbit.

By

Richard Assheton, M.A.

With Plates 13—17.

INTRODUCTION.

It is now nearly fifteen years since Ed. van Beneden[1] published his account of the development of the early stages of the rabbit; and although I do not see that one can as yet describe every detail of even the earliest embryology of the rabbit strictly epigenetically, still it seems probable that van Beneden took far too little heed of the extrinsic causes which may direct the course of development.

The effect upon the development of the presence or absence of such structures as the albuminous layer or zona radiata has been almost entirely ignored, apparently because they are matter outside the ovum. So, again, the size and structure of the uterus have hardly received their proper share of attention.

The present paper and my other "On the Causes which lead to the Attachment of the Mammalian Embryo to the Walls of the Uterus," tend, I hope, to show how many of the details of the earliest stages may be ascribed to the direct maternal influences. That is to say, the inherited force is an energy

[1] 'La maturation de l'œuf,' &c., Bruxelles, 1875; "La formation des Feuillets chez le lapin," 'Arch. de Biologie,' vol. i, 1880.

which would of itself produce not a specific embryo, but an amorphous monster unless directed by the influence of (in the rabbit) inanimate coats and the walls of the uterus.

I have come to the following conclusions upon certain disputed points.

(i) Van Beneden's description of the segmentation I consider to be inaccurate.

(ii) I find no trace of van Beneden's blastopore.

(iii) I find no trace of a "gastrulation."

(iv) There is no evidence in support of Robinson's[1] speculations concerning the existence of a hypoblastic wall to the blastocyst surrounded subsequently by the epiblast.

(v) Rauber's layer fuses with the inner layer of epiblast as described by Balfour[2] and Heape.[3]

(vi) This fusion has but slight morphological significance, since its existence and disappearance are caused mechanically by ontogenetic conditions.

(vii) The growth round of the hypoblast is apparent only, being due to the presence of a zone of specially active epiblast surrounding the embryonic disc, which zone is to be considered to be the equivalent of the träger in other rodents.

I have endeavoured to show how important is the albuminous layer, and how I believe that it is possible to account for many details of change up to the end of the sixth day by strictly epigenetic processes; and since these processes during this time are almost all directed by environment as between the embryo and the maternal influences rather than between cell and cell of the embryo itself, it follows that the palingenetic features of the development must be reduced to a minimum.

This paper is based upon the examination of upwards of 300 embryos between the 24th and 168th hours. The embryos have been examined fresh, and after treatment with various reagents —Perenyi, osmic acid, picric acid, Flemming, Hermann, chromic

[1] 'Quart. Journ. Micr. Sci.,' vol. xxxiii.

[2] 'Comparative Embryology,' vol. ii.

[3] 'Quart. Journ. Micr. Sci.,' vols. xxiii and xxvi.

acid, &c., and sections have been examined of all stages from the 30th hour onwards.

A large portion of the work for the present paper, and the three or four other papers I hope to publish shortly, was carried on while I held the post of Demonstrator of Zoology in the Owens College.

To the kind consideration of the late Professor Arthur Milnes Marshall I am indebted for many opportunities for work which I should otherwise have found difficult to obtain.

The remainder of the work has been done in the Morphological Laboratories of Cambridge through the permission of Mr. Sedgwick, to whom I wish to express my gratitude for the said permission and for his kindness in reading my papers and offering many valuable criticisms.

CHAPTER I.

Segmentation of the Ovum.

Since the accuracy of van Beneden's account of the early stages of the development of the rabbit depends to a great extent upon the way in which the earliest cleavage planes succeed one another, I think it advisable to give in some detail the results of my own researches on this point. In fact the very first segmentation division, according to van Beneden, gives rise to an important question, which is as follows: is there any essential difference between the two first segments, and do the descendants of one segment give rise to the epiblast cells, and the descendants of the other to the hypoblast cells?

To this van Beneden replied that there is always a difference in size between the two first segments, and that the smaller of the two is the more granular, and that from that one are derived all the cells of the inner mass, and that the larger clearer segment gives rise to cells which gradually surround the descendants of the small segment and form the wall of the future "blastodermic vesicle."

From the descendants of the small segment van Beneden

derived the hypoblast and subsequently the mesoblast; from the descendants of the larger, the epiblast.

As far as the conversion of the whole of the inner mass into hypoblast and mesoblast is concerned, van Beneden has changed his opinion, admitting that Kölliker's (and others—Balfour, Heape) careful research in this matter is a truer account than his own.

Again, Heape, on the mole, has shown that a different interpretation may be placed upon the fact as seen in the rabbit, although he finds that there is a difference in the size of the two first segments.

The results of my researches show that there is, if not always at any rate very frequently, a difference in size between the first two segments; sometimes the difference is very marked, but more usually it is to be found only by careful measurement. In one rabbit I examined the following results were obtained.

Rabbit 48, was killed $24\frac{1}{2}$ hours after coitus. In the thin-walled part of the Fallopian tube of the right side I found four embryos at about 30 mm., 40 mm., 42 mm., 50 mm., from the funnel mouth of the Fallopian tube respectively.

No. 1, the highest up, had not as yet divided. The ovum was spherical with a distinct nucleus, the ovum not occupying the whole space within the zona radiata.

No. 2, the second on the right side, was divided into two segments, but this I did not measure.

No. 3, the third and lowest on the right side, was also in two segments. I could detect no differences at all in colour or texture, but on measuring the longest and shortest diameters of each the following results were obtained.

One of the segments measured as follows: longest diameter ·1 mm., shortest diameter ·076 mm. The other segment: longest diameter ·096 mm., and shortest ·068 mm.

The specimens were examined and measured in the fresh condition in a drop of aqueous humour of the rabbit. The measurements were taken with a micrometer eye-piece, No. 3, and Zeiss D objective, which gave a magnification of $\frac{1}{250}$ mm. for each division in the eye-piece.

In giving the measurements I have reduced these to actual measurements, giving them in decimal fractions of one millimètre.

No. 4 was also in two segments, but was not measured; each segment was composed of an inner denser and outer clearer part. In the Fallopian tube of the left side I found six ova, which were scattered along an area between two points 35 mm. and 65 mm. from the upper end of the Fallopian tube. The whole length of the Fallopian tube was about 100 mm.

Three of the ova were in the wide thin-walled upper part, and the other three were just within the lower thicker-walled portion of the Fallopian tube. No. 1, that is to say the one nearest to the funnel end, was unsegmented. It appeared, however, to be on the point of dividing (fig. 1). It did not occupy the whole of the cavity within the zona radiata. It was spherical, and two polar bodies lay between the ovum and the zona, the one larger than the other. The nucleus, however, though not very distinct, undoubtedly seemed to be double or dumb-bell shaped.

The diameter of the ovum was ·11 mm.

No. 2 (v. fig. 3) was in two segments. The difference in size between these two was very marked. It was quite obvious without measurement. This specimen showed the greatest difference I have hitherto met with. There was no perceptible difference in density or in colour, either while fresh or after treatment with nitrate of silver followed by picro-carmine and aniline blue-black. The measurements of the two segments of this specimen were as follows:

Of the larger the longest diameter was ·101 mm., the shortest diameter ·088 mm. Of the smaller the longest diameter was ·08 mm., and shortest was ·064 mm. The nuclei were clear and round.

No. 3 was in two segments, but far more equal in size. Nuclei round and clear and distinct. Measurements were—

The larger segment	.	.	·1 mm. × ·078 mm.
The smaller segment	.	.	·098 „ × ·074 „

I could detect no difference except as regards size.

No. 4. I could make nothing of this one; it may have been an unfertilised ovum or pathological.

No. 5 was in two segments. Here again the only difference I could detect was in size, as follows:

Larger segment .	. . .	·086 mm. × ·072 mm.
Smaller segment	. . .	·084 „ × ·066 „

No. 6, the lowest down, that is to say the nearest the uterus on the left side, was still unsegmented, being spherical. Both polar bodies were visible between the ovum and the zona pellucida.

The ova from another rabbit, No. 51, were examined fresh in aqueous humour of the rabbit.

In the left Fallopian tube I found only one ovum, situated almost midway between the two ends. This specimen was not yet segmented, but the ovum had retracted from the zona pellucida, which seems to be a sign that it was a fertilised ovum.

In the right oviduct I found four ova.

No. 1, the one nearest the funnel opening of the Fallopian tube, was in two segments. I could determine no difference in character, but on measurement a slight difference in size was made evident.

Larger segment	. . .	·0924 mm. × ·070 mm.
Smaller segment	. . .	·0920 „ × ·064 „

After being carefully examined and measured while fresh, this specimen was transferred to a 2 per cent. solution of osmic acid for thirty seconds, and subsequently stained with a mixture of aniline blue-black and picro-carmine, and mounted in glycerine jelly—a very pretty preparation. At no period could I detect any difference except in size between the two segments.

In this specimen the two polar bodies were placed far apart from each other, a peculiarity I have not noticed in any other (v. fig. 4).

No. 2 was also in two segments, and was examined, measured,

and drawn in aqueous humour. The result of measurement was—

Larger segment	·099 mm. × ·068 mm.
Smaller segment	·090 „ × ·060 „

No. 3 was also in two segments, and was examined, measured, and drawn in aqueous humour. Result of measurement:

Larger segment	·100 mm. × ·079 mm.
Smaller segment	·096 „ × ·071 „

No. 4, the nearest specimen to the uterus, was as yet unsegmented, possibly an immature or unfertilised one, as the ovum completely filled the space within the zona pellucida. I could not find any trace of polar bodies.

All three specimens from this rabbit, which were in two segments, I examined most carefully, to find, if possible, evidence of the marked difference in appearance described by van Beneden. I treated all with 2 per cent. osmic acid and used weak staining solutions of picro-carmine and aniline blue-black, of picro-carmine, of Beale's carmine and aniline blue-black respectively, but to no purpose. They were then mounted in glycerine jelly, and now, after the lapse of a year, I still fail to find any difference between the two segments.

From another rabbit I obtained two specimens in the two-segment stage, but was unfortunately only able to make a very cursory examination. One of these I thought I distinguished as having a larger darker segment and a smaller clearer one, but I could only examine it while lying on the folds of the Fallopian tube, the unevenness of which might easily have caused one segment to appear darker. So I cannot advance even this instance in support of there being a marked difference in appearance between the two segments.

Another rabbit, with ova aged 25½ hours, gave me four, of which two were in the two-segment stage, and two showed four segments. Here again there was no perceptible difference. I did not measure these while fresh. One I have since measured,

and find that the longitudinal and transverse axes of the one measure exactly the same as those of the other.

A tabular view of the measurements of these two first cleavage segments may be of interest, showing how the size of one specimen may vary with that of another, and the variation in size of the two segments of one and the same specimen.

	Larger Segment.		Smaller Segment.	
	Longest Diameter.	Shortest Diameter.	Longest Diameter.	Shortest Diameter.
Rabbit 48—				
Specimen No. 1 .	·100 mm.	× ·076 mm.	·096 mm.	× ·068 mm.
„ „ 2 .	·101 „	× ·088 „	·080 „	× ·064 „
„ „ 3 .	·100 „	× ·078 „	·098 „	× ·074 „
„ „ 4 .	·086 „	× ·072 „	·084 „	× ·066 „
Rabbit 51—				
Specimen No. 1 .	·0924 „	× ·070 „	·0920 „	× ·064 „
„ „ 2 .	·099 „	× ·068 „	·090 „	× ·060 „
„ „ 3 .	·100 „	× ·079 „	·096 „	× ·071 „

Each segment of each specimen when examined immediately after the death of the animal showed a denser, more granular inner portion, and a clearer, almost hyaline outer layer, the nucleus being situated in the denser inner portion (v. fig. 2). This difference is more evident in the early stages of segmentation, up to the time that there are twelve to sixteen segments, than later.

Stage with Four Segments.

The second plane of cleavage seems to be at right angles to that of the first. It appears that the two segments divide about, if not exactly, at the same time. This occurs about twenty-five or twenty-six hours after coition. The time between the segmentation of one into two and between two into four appears to be nearly two hours. Since there may be some difference in size between the two primary segments, it follows that there is also very frequently a difference in size between

the cells of the four-segment stage, and, as one would expect, it may appear that two segments are small and two larger, as in fig. 6.

Development between the 26th and 72nd Hours. Stages of Segmentation between Four-Segment Stage and Commencement of Cavity of the Blastodermic Vesicle.

The further cleavage of the four segments does not occur in each segment at the same moment. A stage of five segments or even seven may often be found.

In a rabbit killed 27½ hours after coitus (Rabbit 36) I found in the right Fallopian tube two ova in the usual locality for this age, which is about 40 mm. above the uterus; one of them was in five segments, the other in seven. The five-segment one is shown in fig. 7.

In this specimen it will be seen that two (L^2.) spheres are almost exactly the same size, and that these are considerably larger than two (S^3.) of the remaining three, and slightly larger than the third (S^2.). This specimen I surmise may have been one in which the two primary segments were unequal, and that each of these divided into the approximately equal spheres, and that at the moment of examination one of the daughter cells of the primary smaller (?) one had divided again into two very nearly equal spheres (S^3.).

It should be noted that in van Beneden's account of the process the larger primary segment gives rise to the more rapidly dividing daughter cells forming his so-called epiblast; while in this case it seems to be the descendant spheres of the smaller primary segment which appear to be the more ready to undergo division.

Fig. 14 is a camera lucida drawing of the other embryo of the same Fallopian tube, seen as a transparent object. In this specimen one segment (L^2.) is larger than the others. The four marked S^3. were approximately equal, and slightly larger than the two (L^3.). May this be interpreted as follows? The ovum which gave rise to this embryo divided into two

slightly unequal spheres. The smaller of these two divided into two, each of which has divided into two, the result being four approximately equal spheres (S^3., S^3., S^3., S^3.). The larger primary spheres divided into two, one of which is still undivided (L^2.), the other having divided into two (marked L^3.). Here again there seems to be a tendency for the descendant spheres of the smaller primary to undergo division first.

In the Fallopian tube of the left side of the same rabbit I found six embryos, of which some were in seven segments, others in eight. I had not time, unfortunately, to measure all these or draw them carefully. The embryos of this rabbit seem to be unusually far advanced for their age.

The stage with eight segments is a very common one to find between the 29th and 44th hours. This may be accounted for by there being a rather long resting stage after the production of the eight segments.

One rabbit (Rabbit No. 19) presented a very curious condition of its Fallopian tube, a condition, I believe, that has been noticed before, but I cannot remember by whom. The rabbit was a very large, healthy English doe. Both Fallopian tubes were almost filled with ova. On examining the Fallopian tubes with a lens before opening I imagined I had come across a marvellous find of embryos—in all about fourteen "ova" were to be seen shining through the wall of the left Fallopian tube. Of these only three turned out to be embryos, and were mingled with at least twelve apparently disintegrating unfertilised ova. The right Fallopian tube likewise contained a number of disintegrating ova, as well as four perfectly normal embryos.

Some of these unfertile ova were more thickly coated with albumen than is usual for normal eggs, possibly due to their having been a long time in the Fallopian tube. It is a matter of curiosity why these unfertilised or pathological ova had not passed down the Fallopian tube, but had allowed the fertile ova to pass them, as one at least had succeeded in passing the whole twelve bad ova. One can hardly believe that all these ova had left the ovary at the same time and together with

the ova which became fertilised and developed into normal embryos.

The corpora lutea are often very difficult to make out at this early stage, but I am pretty certain that there were certainly not more than five corpora lutea in one ovary and six in the other.

Fig. 9 is drawn from one of the fertile ova of this rabbit. I have reproduced it here to show the appearance of the denser inner and clearer outer layer of protoplasm of each sphere, described above as occurring in the earlier stages.

Possibly this appearance is constant in later stages, but as the cells get smaller the difference becomes less easy to distinguish.

I have avoided the use of the terms macrosphere and microsphere, because a morphological significance is sometimes given to these terms which I think is not advisable, at any rate in the case of the rabbit.

At the same time it is necessary, in discussing van Beneden's description and deduction, to pay especial attention to the question of the fate of the segments derived from the two first spheres.

It is hardly necessary to point out the very great difficulty in determining the fate of the descendant segments of the two primary segments when size is the only character on which we can rely, and when even in size there may be no difference. Again, if the ovum may sometimes divide into two segments of different size, may not the two primary segments also segment unevenly?

I can see no way of determining the question except by watching the division of one specimen, and this is, as far as I have tried, impossible. Even if it were accomplished, it must be under conditions which can hardly be called normal, and therefore not a very safe ground on which to base either facts or theory.

Certainly, in cases where the primary division is so unequal as in fig. 3, the subsequently formed segments could, on the whole, probably be classed as larger ones derived from the

large primary segment (fig. 3, *L.*), and smaller ones derived from the small primary segment (v. fig. 3, *S.*). If, then, we rely upon the only character at present possible, that is to say, upon the size of the segment, we are bound to conclude that not only does the process of segmentation proceed with great irregularity, but that also there is no evidence of the descendants of the larger primary segment ever forming a cap and subsequently enveloping the descendants of the smaller primary segment.

I believe, rather, that descendant segments of the larger primary segment become intermingled quite irregularly with the descendants of the smaller primary segment, for this undoubtedly affords a better explanation of the appearance of embryos like that shown in fig. 12 or fig. 15.

Fig. 11 is an outline drawing of a specimen taken from a rabbit which was killed at the completion of the 39th hour. In this animal seven of the eight embryos found were in the eight-segment stage. It is extremely difficult to measure the segments with sufficient accuracy to be of any service, and although I measured them I shall not give the results. In this case, as shown by the fig. 11, there was very little difference in size, and none, so far as I could judge, in texture. There was one curious feature which is worth mentioning, but I do not attach any importance to it. The larger polar body was visible between the embryo and the zona radiata. The smaller of the two was inside, or rather mingled with the segments of the embryo. It is quite possible that this frequently occurs, for the polar bodies seem very often to disappear entirely. It would be of interest to determine whether, when this is the case, the polar bodies, ever under the altered conditions, acquire a renewed activity and give rise to segments whose descendants become part of the embryo.

I have as yet no evidence as to which sphere commences the next series of cell division.

At about the 47th hour the embryo has the typical morula form, and is made up of a number of segments (sixteen to twenty), which very frequently present great diversity in size.

Nor is there any regularity, as far as I have observed, as to the location of the large and small spheres. Figs. 12, 15, and 17 show this very well.

Fig. 12 was drawn with camera while the specimen was still fresh in a drop of aqueous humour of the rabbit.

After the drawing had been made the specimen was placed in Perenyi's fluid and subsequently freed from the zona pellucida with fine needles. The segments were then separated one from another. In all there were seventeen segments, and of very different sizes.

Fig. 13 shows three of the segments thus separated. Except in size I could detect no difference.

Another specimen from the same Fallopian tube was placed in $\frac{1}{2}$ per cent. solution of silver nitrate for two minutes, and after having been washed in water and exposed to the sun for a few hours was embedded in paraffin and cut.

Fig. 20 is from a section through about the centre of this specimen. The nuclei of the individual segments are not at all distinct, excepting where they have been cut almost through their centres, as no other stain except silver nitrate has been used.

(Of all methods of fixing the early segmenting stages, I believe none answer so well, as far as concerns the preservation of the correct shape of the spheres, as a weak solution of silver nitrate allowed to act for not more than two minutes. Next to silver nitrate I believe osmic acid, 2 per cent., is best.)

Fig. 15, which is a specimen from a rabbit killed sixty-six hours and a half after coition, exhibits in a very remarkable way the great difference in size which may sometimes occur between the several segments.

From the forty-fifth to the seventieth hour the segmentation proceeds slowly, and, I am inclined to think, sometimes very irregularly, as shown by the last-mentioned specimen (fig. 15).

In sections of these stages I do not notice anything particularly remarkable, except that I have completely failed to find any constant character whereby the inner cells can be distinguished from the outer. Fig. 21 is of a section through the

centre of a rabbit embryo aged seventy-six hours and a half. This was preserved in Perenyi's fluid, stained with borax carmine, cut and mounted in series. The one I have drawn is the fourth of eight which pass through the actual embryo itself.

The embryo at this stage is composed of a number of cells or segments, as far as I can see all similar in character, though varying a good deal in size.

The cells in the centre are no doubt pressed closely together in the living state, the several clefts, with the possible exception of one, being artificial. This just-mentioned exception, the more regular and continuous slit marked *C. BL.*, is in all probability the first commencement of the slit which ultimately enlarges into the cavity of the blastodermic vesicle.

In only one specimen have I found certain cells to take the stain better than others. I have drawn two figures (18 and 19) from the series of sections in which this occurs, in order to show how such an appearance as that described and figured by van Beneden (pl. iv of his paper) may arise, and at the same time to show how the interpretation put on it by him cannot be held to be sound.

In this specimen the embryo has contracted very much, and is lying quite free from the zona pellucida and albuminous layer.

In fig. 18 the majority of the cells at the surface are seen to be slightly darker than the mass inside, with the exception of two at the point *x*. This is undoubtedly like van Beneden's figure of an optical section (pl. iv, fig. 1). But if we look at another section, fig. 19, we find here that again the cells of the surface layer have mostly stained darker, with the exception of one at *x'*.

Hence we are obliged to believe that in this specimen there existed at least two of van Beneden's blastopores, for the light-coloured cells which show at the surface in fig. 18 are at almost the opposite pole to that at which the lighter-coloured cells of fig. 19 show at the surface.

In sections of other specimens of about this age (seventy-two hours) fixed with osmic acid 2 per cent., I have failed also

to find differences of any value between the inner and outer cells. The same may be said of sections of specimens treated with silver nitrate.

Fig. 16 is a drawing through the centre of a specimen from a rabbit seventy-two hours after coition, fixed with nitrate of silver $\frac{1}{4}$ per cent., exposed to the light and stained with picro-carmine.

Summary up to the Seventy-second Hour.

The original description of the segmentation stages by Bischoff I believe to be in the main correct. I cannot find any evidence to support van Beneden's view of the origin of the inner mass of cells from a smaller, more darkly staining primary segment only; or for the origin of the outer layer of cells from a larger, more lightly staining primary segment only; or, again, for the growth of the descendants of one of the two primary segments round the descendants of the other primary segment.

It must be borne in mind that the very fact of van Beneden's description of the later stages of development (the origin of the mesoblast and hypoblast from the inner mass, and that of the epiblast entirely from the outer layer) having been shown to be wrong by several authors (Kölliker,[1] Heape, Balfour, &c.), made it almost certain that van Beneden's description of the segmentation stages was incorrect, or, at any rate, that the interpretation he put upon the supposed facts could no longer be held to be sound.

My account is briefly as follows:

1. The ovum about the twenty-fourth hour after coition divides into two segments, one of which is usually larger than the other, there being much variation in this respect.

2. Each of these segments again divides about the twenty-sixth hour after coition, each dividing very nearly at the same time. These four segments now resulting may vary in size.

3. The third series of divisions takes place about the twenty-

[1] A. Kölliker, 'Festschrift zur Feier des 300 Jährigen bestehens der Julius-Maximilians-Universität zu Würzburg,' 1882.

eighth hour after coition. There is now less unanimity of action in point of time of the division of the several cells, so that it is fairly common to find embryos with five or seven segments. Here, again, four cells may be distinctly smaller than the remaining four, or all may be almost exactly equal in size. There is no difference to be detected in the character of the contents of the spheres.

4. The segments continue to divide with less and less regularity, so that the descendants of one primary segment become mingled with those of the other, there being much difference in size between the several segments.

5. The result of this continued activity is the formation of a solid "morula" of cells of varying size but similar character, and it is impossible to apply the term epiblast or the term hypoblast to any part of the embryo as yet.

The ovum when it leaves the ovary is enclosed within a sheath or protective investment, the zona radiata. As the ovum proceeds down the oviduct this protective investment is further strengthened by the deposition on its outer surface of a thick coat of some albuminous substance which is secreted by certain cells of the epithelial lining of the oviduct, and which forms a tough strong membrane which has an important influence on the future mode of development.

CHAPTER II.

The Formation of the Blastodermic Vesicle.

The Fourth Day (73rd to 96th Hours).

The most noticeable feature of this day's events is the commencement of a cavity within the morula, which cavity enlarges enormously.

What the object of this cavity is we can pretty safely infer, as also we can pretty safely conclude that the causes which bring it about originated in the organism in connection with the diminishing size of the ovum of the distant ancestral animal; but how this cavity is actually produced in the

embryo at the present day is a question of very great difficulty.

It can hardly be looked upon as a cavity comparable to the segmentation cavity of Amphioxus or Amphibia, or the Lamprey or Ganoids, for it has a fate different from that in any of those animals. The fate of the segmentation cavity of the above-mentioned animals is to disappear and take no part in the formation of the cavities of the adult.

The cavity of the blastodermic vesicle whose formation we are about to discuss never disappears, never, at any rate, in the rabbit, as part of it remains as the cavity of the alimentary canal of the adult. Part of it undoubtedly is comparable to the archenteron; possibly all of it is, for it becomes the gut-cavity of the adult.

The first cause which produces the cleft that subsequently enlarges into the cavity of the blastodermic vesicle may be a more active growth of the outer layer of cells. Undoubtedly there is after this time a more active growth of the cells of the outer layer. They increase much more rapidly than the cells of the inner mass.

It is not easy to explain why the energy which up to a certain time causes a solid morula should do so no longer. Why should not the morula steadily increase in size, but be still a morula? Although I believe that the subsequent vast increase in size of the blastodermic vesicle cavity is due to the diffusion inwards of fluid derived from the uterus, still this can hardly be the cause of the first starting of a cleft as in fig. 21. This seems to be best explained by the assumption of an increase in rate of growth of the outer cells over the inner cells of the morula. That such an increase does exist the following table provides evidence.

In a median section through an embryo:

Hour.	Number of cells cut through in outer layer.	Inner layer.
76th	19	27
82nd	24	24
83rd	27	25
100th	44	28

This, though rather a rough method of investigation, shows sufficiently well that there is a proportionally greater rapidity of increase of the cells on the outside over that of the inner cells.

Growth of the embryo must surely be dependent upon nourishment from without, when the bulk of the mass increases as it does from the 70th hour. It is the cells upon the outside of the morula which are in the most favourable position for the acquisition of such nourishment from the fluids of the uterus or Fallopian tube. No doubt during the early stages of segmentation the energy of division is derived from the nutriment —yolk, &c.—contained within the ovum itself. As this becomes used up the embryo will become more dependent upon external sources. This will mean that the externally placed segments will become more favourably placed for growth than the internally placed segments. May not this gradual exhaustion of intrinsic nutriment be a determining factor in the cessation of the increase of the embryo as a morula and causation of the first commencement of a cavity?

This may give rise to the first cleft, but in itself it can hardly account for the large increase in size of the blastodermic vesicle. The cells are so very delicate it is hardly conceivable that they could cause the great expansion of the very tough albuminous wall. It seems far more likely that the force which causes the expansion is due to an osmotic current being more rapid inwards than outwards, either simple or more probably assisted by the vital activity of certain cells of the embryo, as is supposed in the case of diffusion in intestine, and suggested by Heape in connection with the mole. Or the diffusion process may be a simple physical process, but the nature of the liquid after entering the cavity may be so changed by the activity of the cells as to render its diffusibility less when once inside than before its entrance from the uterus.

However this may be, the most noticeable fact of the development of the rabbit embryo during the fourth day is the commencement and enlargement of the cavity of the blastodermic vesicle.

Up to the moment of the beginning of the cavity there does not appear to be any pressure upon the walls of the embryo by zona radiata and albumen layer; in fact, the embryo may often be found to be slightly retracted from the zona radiata in the fresh state.

The diameter of the cavity within the zona radiata is, in the unsegmented ovum, about 0·11 mm., and up to the time that the cleft appears it has not greatly enlarged, measuring only about 0·12 mm.

On the appearance of the cleft the cells of the outer layer become pressed hard against the zona radiata and flattened, but no great increase in diameter of the outer border of the zona radiata is as yet recognisable, although the thickness of the zona radiata is diminished, apparently being of a compressible nature, and therefore becomes compressed between the pressure from within the blastodermic vesicle and the resistance afforded by the tough albumen layer from without. At this time, being in the very firm lower part of the Fallopian tube, the resistance afforded by the albumen layer is very likely aided by the walls of the Fallopian tube itself. In this condition the embryo usually passes into the uterus, although sometimes specimens may be found in the uterus (but very rarely) in which no cleft has as yet appeared. The embryos, I think, pass rather suddenly through the last 4 to 6 mm. of the Fallopian tube at some time between the 75th and 80th hours after coition.

No increase takes place in the thickness of the albuminous layer after entering the uterus, but by the stretching of it caused by the expansion of the blastodermic vesicle it rapidly thins. The zona radiata thins so much as to be hardly perceptible by the end of the fourth day.

Van Beneden's figures, 5, 8, 9, 10, 11, on pl. iv of his paper, taken from optical sections, represent very well the appearances presented by the embryos during these changes.

As van Beneden's figures represent optical sections, it is, I think, advisable to give a series of figures drawn from real sections, as none have hitherto been published.

Figs. 20 to 34 are all camera drawings, and each is magnified 465 times.

Figs. 20 to 25 show sections through the whole embryo, the subsequent figures through the embryonic disc, or a part of it only.

Fig. 20, and also fig. 16, are sections of silver nitrate preparations.

In both cases there is no difference between the cells at the surface and those towards the centre as regards their colouring or nuclei. Those inside are certainly more compressed than those on the surface. So also it frequently happens that a large segment may be so placed that although the greater part of it may be said to belong to the "inner mass," yet a small part of it may appear on the surface, as *x*. in fig. 20.

If such a specimen is examined in optical section, this individual cell might very likely give rise to such an appearance as van Beneden describes, and lead to the idea of an inner mass partially surrounded by an outer layer. The difference in colour which van Beneden describes is totally absent in real sections, and I can find no greater opacity of the inner mass in optical section than what can be equally well explained by the greater thickness through which the light must of necessity pass in viewing a sphere in optical sections. I am not able to offer any explanation of the condition figured by van Beneden in his second figure; I can only say that I have have not been able to find it.

Fig. 21 is a section through an unusually large specimen. This specimen was preserved in Perenyi. This specimen I believe shows the earliest stage in the formation of the cavity of the blastodermic vesicle (*C. BL.*).

There is as yet no evidence of an internal hydrostatic pressure, but the outer cells form a compact layer, and seem at one point to be as it were lifted away from the inner cells, leaving a slight cleft (*C. BL.*).

In fig. 22 this cleft has increased considerably. It must be noticed that it does not extend through more than about 240°.

Through the remaining 120° the inner mass is still as much part of the wall of the vesicle as the outer layer of cells.

In fig. 23 the cavity shows a further increase in size. The outer layer of cells (*O. L.*) now show signs of having become stretched, due, I believe, to the rapidly increasing hydrostatic pressure within the blastodermic vesicle. The walls of the vesicle are now, while living, firmly applied to the zona radiata and albumen layer. The space shown between the vesicle and the zone in the figure is due to reagents. The same remark applies to all the figures from the 22nd onwards. I have not been able to distinguish sharp lines of division between the outer layer cells in section after the commencement of the blastodermic vesicle cavity. In surface view there are certain lines of divisions, which, as van Beneden has shown, are very clearly brought out by silver nitrate.

In this fig. 23, which I believe to be a median section, there is a fairly clearly defined line marking the inner mass from the outer layer, which is less perceptible in fig. 22, and which becomes very distinct in later stages, such as in fig. 28.

It is now possible to distinguish clearly an inner mass as separate from the outer layer. This separation seems to be caused simply by the tension being more acute in the outermost of the segments of the mass *I. M.* in fig. 22, causing these segments to be more stretched than the remainder, because they are more directly united with the already separated and stretched cells *O. L.*

Lines of division between the segments of the inner mass can usually be found, but varying greatly in definition. I cannot say to what extent these several segments may be really divided. The inner mass is certainly connected in some way with the outer layer, but whether by direct protoplasmic union I am quite unable to say.

Fig. 24 presents no new characters excepting a tendency to flattening of the inner mass. In figs. 25, 26, 27, the inner mass is flattened out still more, so that it presents a lenticular form in section instead of a circular outline as it did in figs. 22 and 23. How does this change in form come about?

Van Beneden (p. 29) describes the process thus :—"La masse cellulaire endodermique s'aplatit à la face profonde de l'ectoderme et la surface de contact entre les deux feuillets s'étend peu à peu."

This is a quite correct account of the appearances but in that the description tends to suggest an inherent inclination on the part of the inner mass cells to flatten themselves; it is, I think, right to look for some other explanation.

The inner mass is attached in some way to the outer layer, and accordingly it seems only probable that this mass should be drawn out with the stretching of that part of the outer layer to which they are attached, during the expansion of the whole blastodermic vesicle by the increasing hydrostatic pressure within.

Figs. 22—27 are all sections of preserved specimens, and so mostly crumpled to a certain extent.

If, however, the angle subtended by the arc of the wall of the blastodermic vesicle to which the inner mass is attached is measured in optical sections of fresh specimens of ages corresponding to my figs. 22 to 27, it will be found that the angle is nearly constant. This angle is about 80° (see my diagram, fig. 41). Up to this time there is no indication of a separation into what one may call epiblast and hypoblast. There is a separation into outer layer and inner mass, but this I believe to be of no palingenetic importance whatever.

The separation I have tried to show is due to the individual circumstances of ontogeny entirely. So also, and as I shall endeavour to show in the next chapter, I believe the ultimate separation of epiblast and hypoblast is also devoid of all palingenetic influences such as any form of invagination, or epibole, which is usually spoken of as "gastrulation."

The several segments of the inner mass change their shape in response to the change of their individual environment. In figs. 20, 21 they are compressed and hence polygonal. As the pressure is removed during the growth of the cavity *C. BL.*, those towards the cavity become rounded (figs. 22—27), which character tends to become universal in figs. 28, 29, with excep-

tion of some which subsequently come under the influence of tension, to which I shall refer again.

CHAPTER III.

The Apparent Extension of the Hypoblast.

The Fifth Day (97th to 120th hours).

In the description of the shape and growth of the blastodermic vesicle, and the discussion which follows, I call the part of the blastodermic vesicle to which the inner mass is attached the upper pole, the part immediately facing this and furthest removed from it the lower pole. The zone midway between the poles I call the equator.

The part whereat the central portion of the inner mass is attached to the outer layer, that is to say that part where there are three layers of cells, I call the embryonic disc, but I do not thereby mean to say that only that area and none other takes part in the formation of the adult.

The events of the fourth day are concerned almost entirely with the enlargement of the embryo.

During the fifth day this enlargement continues, but other important developments now take place.

It is during this day that we can first detect a separation into the two well-known embryonic layers, epiblast and hypoblast, and as to the mode of origin of these layers I fear I cannot quite agree with the description and explanations of any former writers on the subject. As I shall point out in a future paper, I can see no necessity for the occurrence of any folding in or growing in of cells from without, usually known by the term "gastrulation," and most certainly I can find no evidence of it whatever. As far as I know, only one investigator has stated that he has found actual evidence for this in the case of the rabbit. This is Keibel, in his work "Zur Entwickelungsgeschichte der Chorda bei Saügern," 'Arch. für Anat. und Physiol.,' 1889. In this Keibel only found his "blastopore"

in one specimen, in which the albumen layer and zona radiata had been removed (thereby rendering it very likely that a tear may have occurred), and he was quite unable to find it in other specimens. If such an aperture really existed, it would certainly be perceptible in surface views of specimens stained with silver nitrate. Van Beneden makes no mention of such an aperture. I have hunted carefully but can find none, either in surface views of specimens treated with silver nitrate and other reagents, or in series of sections; in fact, I should be greatly surprised to find such an aperture at the time given by Keibel for its appearance. It seems to me quite useless to look for it, since it is supposed to represent a turning inwards, such as can be seen in Amphibian ova, for the Mammalian embryo at this age is utterly unlike the Amphibian embryo at the corresponding age, and therefore the conditions which lead to the apparent turning in as seen in Amphibia are hardly likely to be existent in the case of the Mammalian embryo.

It may be well to point out that this is not the view of so great an authority as Dr. Oscar Hertwig, who says, p. 90 of his text-book, when speaking of this spot, "Von dieser Stelle aus, nehme ich an, hat sich schon auf einen noch früheren Stadium das untere Keimblatt durch Umschlag eines kleinen Bezirks der einblätterigen Keimblase entwickelt."

The changes that occur during the fifth day affect both inner mass and outer layer.

The outer layer consists throughout the fifth day of flattened lenticular cells, whose boundaries are easily recognisable in surface views, and I have nothing to add to van Beneden's careful and accurate description of them. They are of very various shapes and sizes, the lines which mark their boundaries may be at any angle to each other. The boundaries may number six, five, or four, the most usual form being the hexagonal.

As the vesicle enlarges the cells become rather smaller, and become much less regular in outline, so that by the seventh

day the cells of the outer layer may be almost circular, or may have any number of sides curved or straight.

Changes in the inner mass.—These are very important during the fifth day.

Up to now the cells of the inner mass have been very uniform in character. During the process of flattening of the mass (a stretching out as above suggested) the individual cells have likewise become flattened, and each is somewhat lenticular in shape (figs. 27 and 28).

The inner mass presents an approximately circular outline when viewed from above or below, as in fig. 38, but here and there single cells (*HY. I.*) seem to jut out somewhat beyond the others. So we may say at this moment that the embryonic disc is several layers of cell thick at the centre, but thins out toward the periphery (figs. 26, 27).

The whole embryo is now spherical. Early on the fifth day, somewhere, as a rule, between the 96th and 100th hours, the cells which were noticed before as jutting slightly from the sides of the "inner mass" may now be seen to be quite separated from it, as in the diagram fig. 39, *HY. I.*, standing out clear and round. These are not easily seen in the perfectly fresh specimen, but are brought out beautifully by certain reagents, as, for instance, Flemming's strong solution, or a filtered mixture of one part Perenyi with one part picro-carmine; indeed, almost any reagent that stains slightly the cells and not the albuminous layer.

At the same time the cells of the centre of the inner mass have become more flattened, and can now be seen to form a patch of cells, nowhere more than two cells thick. That is to say, the embryonic area is composed of, in all, three layers of cells, the outer a definite membrane continuous with the general wall of the vesicle, and two other looser layers of cells slightly flattened but much thicker and rounder than the cells of the outer layer.

Beyond the periphery of this mass, a number of cells very much rounder may be seen scattered irregularly over the inner surface of the thin outer layer, extending over an arc of

about 60° from the upper pole in all directions (fig. 28, *HY. I.*).

From this moment the apparent changes for some hours seem to concern the innermost layer of cells of the embryonic disc, and the straggling cells.

The hypoblast, as a perfectly definite layer, is formed by the time that the blastodermic vesicle measures ·5 mm. in diameter, that is, by about the 102nd hour after coition. It is not, however, as yet by any means a continuous membrane, it is a network or a fenestrated membrane. For this reason in section it appears to be represented by isolated cells lying beneath the embryonic disc (v. fig. 29, *HY.*).

Certain cells can be detected in earlier stages which from their being more lenticular, and more inwardly placed, can probably be described as hypoblast cells (v. fig. 28, *HY.*). This stage is about the ninety-eighth to one hundredth hour, when the diameter of the blastocyst measures about ·36 mm. Also all the apparently—and in many cases I believe actually—isolated cells (*HY. I.* in figs. 28, 29, 37—40) can from the moment of their apparent wandering be termed hypoblast.

By the separation of the cells round the periphery of the inner mass, and certain others of the more inwardly placed of the inner mass into what we may call hypoblast, there are left cells in between the hypoblast and outer layer, which do not show the same tendency to be flattened or drawn out as the others. As is well known, this intermediate cell layer forms part of the epiblast, and so may be termed the inner layer of epiblast. To this I shall refer later.

I wish now to refer to the straggling cells (*HY. I.*) of the inner mass, and consider why they apparently wander round the inside of the blastodermic vesicle. That they arise from the inner mass I have no doubt.

I have explained above the flattening of the inner mass as due to being drawn out by the general expansion of the walls of the vesicle, and to this may also be due the original isolation of certain cells round the periphery of the inner mass as seen in figs. 28, 37, and 38.

If the cells of the inner mass do not multiply quickly enough, and if they are not connected together firmly, and if they adhere by some means or other to the outer layer, then, as the outer layer expands, there must be a tendency for the general separation of the cells of this inner mass, and this will be most apparent at the edges. That is to say, the centre, originally thicker than the edges, will become thinner, and at the periphery the edges will be drawn out to an irregular or regular fringe according to less or more regular local growth and expansion of the outer epiblastic layer.

If the spread of these is due to this cause, and if the growth is equal over the whole surface of the sphere, then, in a section taken through the centre of the sphere and the centre of the embryonic disc, the arc along which these isolated cells are found should subtend an angle equal to that subtended by the compact embryonic disc up till now; that is to say, an angle of about 80°. But on measurement at a rather later stage, 102 hours after coition, represented in fig. 39, the angle is found to be very considerably wider than 80°, in fact somewhere between 110° and 130° (the limit of these cells in question being very irregular), and by the eighth day an angle of 200°.

Hence it would seem that this does not account for the apparent growth round. If, however, we have any reason to suppose that the cells of the outer layer multiply more rapidly and thus allow the expansion of that part of the sphere to take place quicker around a zone bounded roughly by the edges of the compact embryonic area on the one hand and some other parallel line about the original equator on the other hand, we could still consider that the suggested cause is the correct one. I think we have, for these reasons:

The blastodermic vesicle of 96 hours is a sphere. The blastodermic vesicle of 120 hours is no longer a sphere, but is a body of such a nature that a horizontal plane taken through its longest diameter will not pass through the equator, but will be nearer the upper pole than the lower.

The circumference of this plane and of all planes taken parallel to it are, I think, as yet circles. There is nothing so far to indicate which will be anterior or posterior end of the embryo.

The blastodermic vesicle of about the 140th hour is in shape of the same character, but more markedly so.

In the blastodermic vesicle of the 175th hour sections taken parallel to the equator are now no longer circles, but are ovoidal. The fact that there are now a long and a short axis may be entirely due to the pressure upon the vesicle exerted by the walls of the uterus; but I want to point out more especially that the horizontal sections are not true ellipses, but have one end larger than the other. The large end corresponds approximately, but by no means always exactly, with the future posterior end of the embryo.

A median longitudinal vertical section of this stage is very instructive, for the asymmetry is very marked. One end, the future posterior end, is much more bulky than the anterior end. The embryonic area is always placed nearer to the anterior than to the posterior end.

Fig. 42 shows four stages, namely, at the 100th hour, the 125th, 140th, and 175th. *A.* is the more anterior, *P.* the more posterior end. The thick black line in each case is the outer layer of cells or epiblast; the thin black line represents those cells of the inner mass which become separated to form the hypoblast. It is quite clear that a very great change in shape as well as size has occurred between the 96th and 168th hours. How has this been produced?

I have argued that there is a hydrostatic pressure within the blastodermic vesicle causing it to expand. The pressure is sufficient to cause the stretching and flattening out of the cells of the wall of the vesicle, and also to cause the stretching and expansion of the very tough albumen layer. On the other hand, we know that the pressure is not so great as to rupture either the vesicle wall or the albumen layer; in other words, the vesicle wall and albumen layer are sufficiently strong to resist the hydrostatic pressure for, at any rate, some considerable time. The albumen layer becomes continually more

and more stretched, and quite late it does, as a matter of fact, rupture, but at a time (the ninth day) that does not concern the present question. No addition is ever made to the thickness of the albumen layer after the embryo leaves the Fallopian tube; it is an inanimate structure. The cellular wall of the blastodermic vesicle is, on the contrary, living, and capable of adding to itself at any part of its area. Like the albumen layer, it becomes greatly stretched and becomes very thin (v. figs. 21—29), and unless it received additional matter (i.e. multiplication of the cellular units) it would rapidly thin out altogether. After about the 100th hour the cellular wall ceases to get any thinner. Up to this moment we must suppose that the rate of increase of hydrostatic pressure has been in excess of the rate of addition of material to the cellular wall of the vesicle and so has stretched it, but from now there is no appreciable thinning out of this cellular wall (v. figs. 27, 28, 29, and 34).

This means, I believe, that the increase of cellular tissue just balances the increase of hydrostatic pressure, and so the vesicle grows in size, the thickness of the cellular wall remaining unaltered. Now there is no reason, as far as I can see, to doubt the albumen layer being equally tough on all sides of the embryo. The albumen layer is not secreted by the embryo, but is applied by the Fallopian tube.

Although preserved specimens and sections are not well adapted for this purpose, still my figures show that there is no regularity at all in these preserved specimens, such as a thicker part of the albumen layer being present over any one part of the embryo in stages up to the 96th hour (v. figs. 16, 20, 21, 22, 23, and 24), and in the fresh specimens the outer and inner limits of the albumen layer present in optical section true concentric circles. Therefore I do not think we can attribute any subsequent difference in thickness of the albumen layer to a difference in texture acquired by that albumen layer during its deposition. At a later stage we do find a difference in thickness occurring as a constant character.

The difference I find is as follows:

The most marked difference concerns the part of the albumen layer adjoining the embryonic disc. This is in the later stages very much thicker than elsewhere. Figs. 30 and 31 are portions of the upper and lower poles of an embryo taken from a rabbit of the one hundred and forty-fourth hour.

The albumen layer is at the upper pole about twice as thick as it is at the lower pole. This is a perfectly constant feature, and becomes more and more marked the later the stage.

Hydrostatic pressure exercises its influence equally in all directions. If it encounters less resistance in one direction than in another it will cause greater effects in that one direction. I have argued above that at any one moment (between one hundredth and one hundred and sixty-eighth hours) the hydrostatic pressure within the vesicle on the one hand, and the living cellular wall of the vesicle together with the non-living albumen layer on the other hand, are in a state of equilibrium. The blastodermic vesicle is always taut, but does not rupture. But the next moment the hydrostatic pressure has increased on the one hand, and the albumen layer has become thinner and the cellular wall has increased its material —and still the equilibrium is maintained. It seems to me to be clear that the degree of rapidity of increase of the cellular wall must be a factor in the resistance afforded to the hydrostatic pressure by the two walls of the vesicle.

If this is so, then it follows that at any area where the increase of cellular tissue is greatest, there the hydrostatic pressure will exert its greatest influence, and there the albumen layer will, as a consequence, be thinnest. This is, of course, dependent on the close attachment of the cellular layer to the albumen layer. I am bound to confess I do not find it easy to prove that there is no sliding of the albumen layer over the cellular layer; but, on the other hand, I see no evidence to suggest that there is such a sliding.

Accordingly, I take it that the great thickness of the albumen layer adjoining the embryonic disc over that adjoining the lower pole shows that there has been less stretching and

therefore less growth at the embryonic pole than at the lower pole.

It may be said that this is due to the fact of the cellular wall at this point being practically several layers thick. But it must be remembered that the cells of the inner cell-mass are very loosely arranged (figs. 28, 30, 35) at this time, and certainly do not give me the impression of being under great tension, as are the outer cells.

As far as the tension is concerned, I believe the outer layer has to bear it very nearly all, even in the embryonic disc. Sections show how very thin it is here.

Granted that they are only very equally stretched, it follows, I think, since the albumen shows not the same amount of stretching as elsewhere, that the multiplication of cells must have been going on more slowly in the embryonic region. Now from measurements of the albumen layer it will be seen that the thinnest place is not usually at the lower pole, but somewhere between the equator and the upper pole. This, then, marks out as an area a zone of more rapid multiplication of cells, that area which lies just outside of the embryonic disc.

Now we know that upon the eighth and many subsequent days there is a very great activity evinced by the cells of a zone immediately surrounding the embryonic disc; it is the zone called by Duval the ectoplacental area. If it is an area of very great activity upon the tenth day, of great activity upon the ninth and eighth days, when does it begin to be an area of activity? Why not upon the seventh or sixth, or even on the fifth day?

I believe that it does begin as early as the fifth day, and that it is to the presence of this area of more active cell division round the embryonic disc that the shape of the blastodermic vesicle is as I have described it to be (v. fig. 42), and that it is to this that the apparent growth round of the hypoblast cells is due.

The ectoplacental area is, as is well known, much more strongly developed all round the posterior end of the embryo

than anteriorly. Although present to a slight extent at first anteriorly, it never develops greatly. This means that the zone of special activity is at a later stage more intense posteriorly than anteriorly. So apparently it is at an earlier stage, and causes the greater bulkiness of the posterior end of the blastodermic vesicle of the seventh day, and causes the apparent throwing forward of the embryonic disc (v. "175 hr.," fig. 42).

Table of Measurements of Thickness of Albumen Layer at Different Parts of the Vesicle in Millimetres.

	Five Days.	Six Days Four Hours.
Embryonic area	·0060	·0058
Placental zone, *A.*	·0024	·0022
Placental zone, *P.*	·0030	·0024
Lower pole	·0036	·0034

To sum up the above section:

(1) There is no growth round of the inner mass cells over the surface of the outer layer cells in the sense of a migration. It is only an apparent growth round produced by the more rapid growth of a zone of the wall of the vesicle immediately surrounding the embryonic disc, in which zone the marginal cells of the inner mass lie.

(2) The presence of such a zone accounts in a great measure for the shape assumed by the blastodermic vesicle during the fifth, sixth, and seventh days.

(3) A zone of such a character undoubtedly exists during the eighth, ninth, and following days, giving rise to the ectoplacenta.

(4) Such a zone of activity accounts for the varying thickness of certain parts of the albumen layer.

To return for a time to the question of the growth round of the inner mass cells. It must be remembered that the cells never completely surround the cavity of the blastodermic vesicle. The lower pole is always, in the rabbit, one-layered only as long as it exists.

There is only one other alternative that will account for the apparent growth round of the inner mass on the inner wall of the blastodermic vesicle, as far as I can see, and that is actual active migration of the cells in question. Of course it is difficult to bring evidence to show that they have not migrated, since it is not possible, I fear, to follow the process in any one and the same specimen. At the same time I cannot find any evidence to prove that they have actually migrated.

If a cell does migrate, like an Amœba for instance, one would expect to find evidence of protoplasmic protuberances or pseudopodia. Of this I can find no trace. The majority of the cells seem at first (figs. 38, 39) to be quite isolated from each other, and to be approximately spherical, whether examined in the perfectly fresh condition or after treatment with various reagents. They are, it is true, slightly flattened on the side by which they adhere to the vesicle wall (fig. 28, *HY. I.*).

Certain of the cells here and there are connected by threads of protoplasm, but this, I think, is not a sign of pseudopodic activity, but merely indicates the final stage in division between the two cells. I have no doubt that these cells divide rapidly after a time, though I do not think much activity of division takes place during the first few hours after the apparent migration begins.

If one of these inner rounded cells is undergoing the process of division, then, as the wall on which it rests expands, the two dividing halves of the inner cell will be pulled apart, and a strand of protoplasm connecting the two cells may remain for some time.

Of course it is just possible, I suppose, that these rounded inner cells might migrate by means of a rolling motion consequent on "streams" of protoplasm within them, as do some protozoa. But is such a phenomenon known anywhere in the metazoan body? I cannot think we are justified in assuming this without evidence, for when examined in the fresh condition no such protoplasmic activity can I notice.

When the cells are so isolated as I believe them to be during

the 98th to 108th hours or thereabouts in the rabbit, I cannot conceive that the migration can be accomplished otherwise than by an actual migration, for which there is no evidence, or else by a process such as I have attempted to describe above. This I believe to exist. If the inner layer was not composed of isolated cells, but was a compact membrane, then it might creep round by means of its own interstitial growth, although I do not think it would in that case be a thin smooth membrane.

Such is the account of the growth round of the hypoblast in the mole. Heape makes no mention of any isolated cells at the edges; he says of the hypoblast, "it extends laterally by virtue of the multiplication of its cells, which at the same time become much flattened." It seems to me to be much more likely that the layer would be flattened if they were drawn out along with the epiblast cells; but I have no evidence at present whether the same cause can be attributed to the spread of the hypoblast in the mole as I suggest for the rabbit.

In fig. 42 the dotted lines radiating from the centre of the smallest blastocyst indicate the amount of growth of the several segments up to the one hundred and seventy-fifth hour.

Until the one hundredth hour I imagine the growth of all parts of the wall of the blastocyst to be equal.

From that moment there is a greater activity in an area around the embryonic disc, which causes the inner layer to be apparently carried further round the anterior of the blastocyst.

This zone of activity by the one hundred and fortieth hour has become more marked still, and now shows itself to be more intense posteriorly than anteriorly, which latter character is plainer still at the one hundred and seventy-fifth hour.

Eventually the activity culminates, upon the addition of resistance afforded by the walls of the uterus, in the production of the ectoplacental area, or part of it.

To this I have referred in another paper, and also to the hypothesis that this zone of activity, in the absence of a tough albuminous coat, results in certain other rodents in the production at once of a heaping up of cells—the träger.

In the embryonic disc region there is very much less activity

exhibited. The outer layer in that region is very much attenuated, and shows very little sign of activity. The inner layer consists all this time of rounded or ellipsoidal cells, and, like the outer layer of epiblast of that region, shows very little if any sign of activity.

So little activity of division does there seem to be in the inner layer of epiblast, that there is a distinct tendency for the several cells to become slightly separated as in fig. 30, which gives rise to the very irregular and speckled appearance of the embryonic disc of the one hundred and twentieth hour.

Very probably this is caused by the slight stretching of this region. It is more noticeable at the edges than towards the centre.

Whether there is any palingenetic meaning in this double-layered condition of the epiblast I have discussed in another paper. For the present, I think the right view to take of the condition is that derived from the study of the actual way in which the separation has originated, and to regard it as a consequence of ontogenetic circumstances only.

The Outer Layer of Epiblast.—This is by far the most active of the embryonic layers of the fifth day. It is in an active condition of growth during the whole of the day, and thereby allows of the expansion of the vesicle. The character of the cells seems to be just as it was during the latter part of the fifth day.

The Inner Layer of Epiblast.—This layer seems to be a region of rest throughout the whole of the sixth day. There is very little sign of cell multiplication. The cells are more or less circular in outline when viewed from above, and oval when seen laterally. They are rather scattered, and thus give rise to the speckled appearance noted above. They seem to be clearly separated from the outer epiblast above them and from the hypoblast below them, and since they are either quite separated from each other, or connected only by fine strands of protoplasm at certain minute spots, they are simply pulled apart by the expansion of the blastodermic vesicle, and are not individually stretched and flattened,

as are the cells of the outer epiblast and hypoblast, where they are more intimately connected one with another. In the latter case, that of the hypoblast, cells with a tendency to the features characteristic of both layers of epiblast are to be found.

CHAPTER IV.

Changes that occur during the Sixth Day (120th to 144th Hours).

If we may call any period of the development of an animal unimportant, it is to the period between the 120th and 144th hours of the development of the rabbit that this epithet might be applied.

During this day the blastodermic vesicle increases very greatly in size, and assumes very markedly the shape described in the last chapter as being characteristic of the later stages of the development of the vesicle, prior to its attachment to the walls of the uterus.

The blastodermic vesicle is no longer a sphere. It will be found by measurement to have longer and shorter equatorial axes, and a polar axis which is of less length than the shortest equatorial axis.

The following measurements are taken from specimens of the earlier part of the sixth day :

	Longest equatorial axis.	Short equatorial axis.	Polar axis.	Embryonic area.
No. 1. 5 days 5 hours . .	1·1 mm.	1·05 mm.	1·00 mm.	0·55 mm.
No. 2. 5 „ 5 „ . .	1·35 „	1·275 „	1·25 „	0·75 „

(1) Hypoblast of the Embryonic Disc.—This is now a continuous layer, sufficiently so as to show lines of demarcation between the cells when treated with silver nitrate. This continuous membrane extends a short distance beyond the periphery of the inner epiblast layer. The cells composing this membrane are completely flattened.

(2) Hypoblast beyond the Embryonic Disc.—The

scattered hypoblast cells have now become much more numerous, and are scattered more evenly over the portion of the wall upon which they are to be found. Many of them, possibly all of them, are now undoubtedly connected by more or less fine protoplasmic threads.

These scattered cells, although such conspicuous objects during the fifth day, are now extremely difficult to make out, and can very easily escape notice.

They are more numerous now nearer to the embryonic disc, and merge gradually into the continuous layer just described. They are now very much more flattened.

Towards the line of their outer limit they present more the characters of the former day, being fewer and rather rounded and more isolated.

Consideration of the Extent to which the Shape of the Cells of the several Layers may be attributed to Mechanical Causes.

At this time we find cells of two very different types, with cells showing all intermediate stages between the two.

The first type is the rounded, almost completely isolated cell, such as those of the inner layer of epiblast, or near the outer limit of the hypoblast; the second type is the flattened or stretched cell of the outer layer of epiblast or embryonic hypoblast, continuous with its neighbours around all its edges; and thirdly, forms of cells intermediate between these two types. How far can we hold this difference of form to be due to the environment of the individual cell apart from its own inherited tendencies?

Whether there is any cell in the embryo at this time quite separated from all others I am not certain. Of course they are all contiguous to one or more other cells, but possibly there is an actual protoplasmic union between one cell and another. This is certainly the case very frequently with the cells of the hypoblast, which at first sight seem to be quite separate.

In sections, and in surface views, there is no distinct connection to be made out between the rounded cells of the inner

epiblast layer. But when the embryonic disc is broken up in such a way as to scatter and tear apart the various cells, then there undoubtedly appear to be strands passing from some of these rounded cells to others of the same layer. At the same time I cannot say positively whether these strands are really connections between the cells in question, or whether they are fragments and shreds derived from the tearing apart of the hypoblast or outer epiblast layer, between which they are placed. But however this may be, the cells of the inner epiblast layer are, at the time I am speaking of, either isolated or else connected only at certain spots of small area. These are of the rounded type.

At the outer limit of the hypoblast there are also cells, some of which, I believe, may be quite isolated; others are connected to each other by a few strands of protoplasm. These approach very closely to the rounded type of cell. This is the type of cell which I believe to be the most natural, by which I mean the least influenced by its environment. This is the type of cell which first comes into existence in the segmentation of the ovum, when, within the protecting investments, the cells, at first uninfluenced by pressure or tension from without, or from each other, assume their natural or spherical contour. As the segmentation proceeds, the inner segments become the more compressed, and assume polygonal forms.

After the establishment of the cavity of the blastodermic vesicle, the outer cells, by the pressure from within increasing more rapidly than their rate of multiplication, are drawn out into thin plate-like cells.

These outer layer cells are from the first connected with each other by their edges, and form a continuous membrane, a condition without which in all probability the formation and enlargement of the blastodermic vesicle could not be produced.

As long as this tension within is maintained at a rate greater than the rate of multiplication of the cells, the cells retain their flattened condition.

What of the inner mass cells? Upon the removal of the pressure of the outer layer, the more outwardly placed of the

inner mass cells re-assume, at any rate on their free surfaces, the rounded contour which is natural to them.

As the blastodermic vesicle expands, the inner mass, which is adherent to the wall of the vesicle either by actual protoplasmic connections or otherwise, is drawn out into a lenticular shape.

I have tried to show that there is a zone of the wall of the vesicle which, by greater activity of multiplication of cells, admits of more rapid expansion of that part. Upon this zone rests the edge of the lenticular inner mass. The expansion of the zone is in direction radially from the embryonic poles. Hence the outermost cells of the inner mass, i. e. the cells at the edge of the lenticular mass, will tend to be separated more rapidly than the innermost. This will tend to isolate these cells from others of the inner mass. Let us suppose that all the cells of the hypoblast layer are dividing at a uniform rate. I think it is reasonable to suppose that the existence of strands connecting cells of this kind together have their origin in past cell divisions. Accordingly on this supposition the connecting strands will be more numerous and the nuclei nearer together, and the meshes of smaller area in the embryonic disc hypoblast than in the hypoblast outside that area. The hypoblast cells of the embryonic area will differ from those of the extra-embryonic area in this way :

(i) The embryonic cells will have more and shorter, and so presumably stronger, strands connecting them with their neighbours than will the extra-embryonic hypoblast cells.

(ii) The embryonic hypoblast cells will have connecting strands upon all sides, whereas the outermost extra-embryonic cells will have them upon one side only.

Is it possible for the flattening of the embryonic hypoblast cells to be due to their becoming stretched by the tension produced by the extra-embryonic hypoblast cells (with which they are in direct connection by means of the strands just mentioned) being removed in all directions by the rapidly expanding zone of the outer epiblast? If so, it is possible to account for all the shapes of the cells composing the embryo at this age.

As I have stated on a previous page, the hypoblast of the embryonic area is a network at first. Also, I believe that at first many of the outermost cells of the extra-embryonic area are really isolated. These will be under less tension than those near the embryonic pole, as they will, if they are connected at all with other hypoblast cells, have connecting strands upon one edge only. Hence these preserve for a longer period their rounded contour.

The ultimate conversion of the isolated cells into a network (or a series of networks) and of the networks into thin continuous membranes, and of thin continuous membranes into columnar membranes, would seem, therefore, to be but the result of increase of rate of multiplication over rate of expansion.

The inner layer of epiblast cannot be said to have come into existence as a layer until after the formation of the hypoblast. Until that moment it formed, together with the future hypoblast, the inner mass. It was impossible, except in as far as could be premised from their position, to say from their characters which would be inner epiblast cells and which hypoblast cells (v. fig. 28). What I believe takes place is this. Those cells of the lenticular inner mass which, being at its edges, are removed by the expansion of the wall of the vesicle, and those which are in direct connection with the cells so removed, become by virtue of their position the future hypoblast; the remainder become the inner layer of epiblast. That is to say, those cells of the inner mass which are not influenced by the expansion of the vesicle, as above described, and are accordingly upon that part of the wall, though not actually as yet part of it, which is least affected by the expanding forces, become the inner epiblastic layer.

Of all the cells, therefore, in the embryo at this time, these (the inner epiblast layer) are least affected by external causes. These cells are the more free to assume their natural shape, which I believe to be spherical, and are only slightly flattened between the two layers, outer epiblast and hypoblast.

CHAPTER V.

Changes that occur during the Seventh Day (145th to 168th Hours).

The Fate of the Outer Layer of Epiblast (or Rauber's Layer) in the Embryonic Disc.

The embryos have now grown to such a size as to cause them to respond more effectually to the impulses set up by contractile movements of the muscular walls of the uterus, and therefore we find them much further advanced along the uterine tube, and more scattered. They have not, however, taken up a permanent position as yet, for although this may occur in some cases during the last few hours of the seventh day, more usually it does not take place until the early part of the eighth day.

It will be best to describe the course of events in the three layers separately.

Outer Layer of Epiblast.—Very little need be said of the greater part of this layer, no change except such as has been described as occurring during the fifth and sixth days takes place. But special attention must be given to that part of the area which lies over the patch of inner layer of epiblast, i.e. embryonic disc.

Inner Layer of Epiblast.—During the early part of the seventh day the cells of this layer are just as described in the preceding chapter. They extend now over an area of about ·6 mm. The general outline of the mass is still circular. Each cell is distinct and rounded, with very large nucleus; and with nearly all stains that I have used they stain only lightly.

Early on the seventh day these cells show signs of greatly increased activity. They multiply, become pressed together, and now form a very compact layer at the same time as certain changes occur in the outer layer of epiblast.

The course taken by these two layers during the next few hours, and its significance, have been very differently described

by different authors; indeed, very opposite views have been held during many years. It is an interesting question from the extreme difficulty of the investigation and from the morphological problems connected with its solution. As regards the actual facts, there have been three quite distinct accounts. Most observers have noticed three layers at this stage: (1) an outer thin layer; (2) a middle thick layer; (3) an inner thin layer. These accounts are very briefly as follows:

(1) Van Beneden maintained that the inner layer is not epiblast at all, but is mesoblast, and that the outer layer becomes thickened over the embryonic disc area, and gives rise by itself to the epiblast of the embryo.

(2) Rauber, Lieberkuhn, and later Kölliker and others, hold that the outer layer is quite transitory, and that during the seventh day it splits up, degenerates, and disappears entirely, taking no part in the epiblast formation of the embryo. The epiblast, they hold, is wholly derived from the inner layer of ovoid cells, which van Beneden took to be mesoblast.

(3) Balfour and Heape contend that both layers persist as the epiblast of the embryo, but the two layers fuse together by the growth downwards of the outer layer cells in amongst the inner layer cells.

There can be no doubt now that van Beneden was wrong. The middle layer certainly forms part if not all of the permanent epiblast, and this is acknowledged by van Beneden himself ('Arch. Biologie,' vol. v).

It requires some careful examination to determine whether Rauber, Lieberkuhn, and Kölliker, on the one hand, or Balfour and Heape, on the other, were right, or whether all were wrong.

The surface views given by van Beneden (pls. v and vi) and Kölliker (pl. ii, figs. 13, 15, 16, and 19) are, I think, quite correct. The interpretation van Beneden put upon his figure he no doubt now admits to be wrong. The interpretation put upon them by Kölliker I believe to be correct, namely, that the large areas are cells belonging to the outer epiblast layer, and the smaller ones are cells belonging to the inner epiblast layer. I

have drawn a figure (fig. 36, Pl. 17) from a specimen aged six days five hours. This is very similar to Kölliker's aged six days. It is a silver nitrate specimen. This was drawn when the extreme upper surface was in focus. On focussing down it is possible to find small cell outlines under the large areas, but finer than those of the cells (*EP. I.*). On focussing still further down, the outlines of the hypoblast layer are visible as very fine, very wavy lines, forming as a rule more regular areas. The small cells (*EP. I.*) are present throughout the embryonic disc, but in some places are much more marked than others; that is to say, in some places the silver nitrate produces the characteristic black marks between the cells more strongly than in others. For this there must be a reason, and this seems to be that in some parts the small cells are at the surface (*EP. I.*); at others (*EP. O.*) they are covered by some other body, this other body being certain cells of the outer layer of epiblast (Rauber cells).

This is an intermediate stage. Earlier the outer layer is quite continuous over the whole of the embryonic disc, as shown in Kölliker's figs. 11 and 12, pl. ii (see also my sections of these stages, figs. 27, 28, and 30). In these the outlines of the outer layer are distinct as large polygonal areas. In the earlier stages the outlines of the inner layer of epiblast are not sharply defined in silver nitrate specimens, apparently because they are but loosely arranged. But in Kölliker's figs. 11 and 12, which represent specimens at the latter end of the sixth or beginning of the seventh day, the small cells, i. e. the inner layer of epiblast which now forms a more compact layer, show outlines which are visible below the outer layer cells, although faint.

I entirely agree with Kölliker that this outer layer (Rauber's layer) now becomes broken and, as it were, torn up into isolated cells or little patches of cells, and I think that the cause of this may be as follows. I have above given reasons for supposing that the cells of the embryonic disc region are comparatively inactive during the fifth day and first part of the sixth day. The outer layer of cells (Rauber's layer), I

pointed out, has been stretched to a very high degree of tenuity.

From the middle of the sixth day the embryonic disc area becomes an area of increased activity.

We have at this moment, therefore, an outer extremely attenuated membrane under high tension due to the hydrostatic pressure within. Each "cell" of this membrane represents an individual minute centre of protoplasmic activity. These are so drawn out that a few will extend over a considerable area.

Closely pressed against these is a layer of rounded or but slightly compressed cells, the inner epiblastic layer. Each cell of this layer also represents an individual minute centre of protoplasmic activity, and it will be seen that in any given area of the embryonic disc for one of these little centres of activity of the outer layer (Rauber's) there are three, four, or five of the little centres of activity in the inner epiblastic layer.

The hypoblast, although it will tend to press the inner layer of epiblast cells firmly against the Rauber layer, need not necessarily be affected by what I am going to describe, because at no time does the hypoblast, after its initial separation from the epiblast, appear to be at all firmly attached to the epiblast. Note the readiness in which it stands away from the epiblast in sections (v. all my figures, and those of Kölliker).

To return to the two epiblast layers. It is clear now that if the two epiblast layers of the embryonic disc acquire an increased activity, then if for any given area the inner epiblast contains, say, three times more cells than the outer epiblast, then the inner epiblast will increase its bulk, roughly speaking, three times more rapidly than the outer epiblast.

This might produce several effects. It might produce a heaping up of cells; it might produce an arching inwards of the inner layer of epiblast; it might produce an extension of the inner epiblast by sliding over the outer epiblast (or rather between the outer epiblast and hypoblast); or it might cause the

further stretching of the outer epiblast, or it might cause its rupture.

I believe it causes the rupture of the Rauber layer; whether the several fractures between the cells are sharp, or whether, as is possible, the cells are pulled apart so as to produce large meshes through which the inner layer cells come to the surface, I cannot say. If the latter case, although the Rauber layer as a membrane would be obliterated, still the continuity of protoplasm would not be broken; but I think, from consideration of all the appearances, the fractures are fairly clean.

We now come to the question of the fate of the Rauber layer cells. Kölliker says they disintegrate and disappear; Balfour and Heape say they pass into the inner layer of epiblast. I am fairly confident that the latter view is correct.

If my description and hypothesis are correct, the Rauber cells, as soon as they become broken up, are no longer under such a high degree of tension. If they have any vitality in them they will, as they grow, be able to assume other shapes than "squamous." They will become intermingled with the cells of the inner epiblast layer. Their nuclei will always form a knob, and thereby perform the part of the thin edge of a wedge to the whole cell, and so on recovering what we must allow was their more normal shape, lost only under pressure of the fluid within, will tend to become intermingled with the cells of the lower layer. No doubt some will get settled earlier than others, and often upon the eighth day it is possible still to detect a Rauber cell not yet accommodated. Many stages of this process, I believe, are shown in my figs. 32, 33, and 34. These are sections from the same specimen, which was taken from a rabbit at the 153rd hour, preserved in Flemming's strong osmic-aceto-chromic fluid, and stained with a weak borax carmine. In fig. 34 to the left is the thin layer of outer epiblast (formerly continuous with Rauber's layer alone) (*EP. O.*), lying between the albumen layer (*ALB.*) and hypoblast (*HY.*). To the right is the fused or fusing inner epiblast and "Rauber cells" or outer layer of epiblast. The greater part is stained only slightly, and contains numerous large rounded nuclei

(*EP. I.*). The outline of cells is hardly visible. Here and there, as though filling up interstices, are cells which stain much more darkly (*EP. O.*), and in the character of their nuclei appear more like the cells of the outer epiblast beyond the border of the embryonic disc. Some of these are only little wedge-shaped bodies on the surface (fig. 32, *EP. O.*), others pass right through; others seem to send flaps over the surface of the inner layer cells (fig. 33, *EP. OR.*). The last-named cell (fig. 33, *EP. OR.*) is very curious. It will be noticed there are several rather like it. These almost look as though they were being enclosed quite accidentally by the inner layer of cells. It seems to me quite possible for this to be very often the case. Take such a spot as that to which the line *EP. O.* runs in fig. 30; here, of course, the bend in the outer layer is in all probability artificial. But if a gap existed, as frequently happens, between the cells of the inner epiblast, then if at a neighbouring spot the outer layer became ruptured, the tension would be removed, and the Rauber cell might very well become enfolded quite passively, as I believe is taking place in fig. 33, *EP. O. R.* There is no reason why a cell thus enfolded should die, in all probability it would grow and multiply like any other cell of the region.

(It may be observed that of all the cells in the whole blastodermic vesicle at this time, none are so badly placed for nutriment [provided we allow that nutriment is all the time being received from the fluids of the uterus] as the cells of the outer epiblast layer over the embryonic disc. For they here lie between the thickest part of the albumen layer and the thick inner epiblast layer, which may account for the period of inactivity of this layer at this moment.)

I cannot leave this question without a short discussion of the morphological bearings of the events connected with the fusion of these two layers.

First of all, I wish to call attention to Heape's description of the mole's development. The mole is very like the rabbit in its developmental history.

Just after the separation of the hypoblast layer, Heape

describes the appearance of a cavity between the outer layer of epiblast and the inner layer of epiblast. It is hardly a cavity, for it is partially filled by very "stellate" cells. With the formation of this the inner layer of epiblast becomes arched inwards, a process termed "temporary inversion of layer" by Heape. Subsequently this arch flattens out again, and it and the outer layer cells and stellate cells between them all fuse together to form the permanent epiblast.

I have not studied the mole, but from Heape's description this seems to be an almost exact parallel to the process which occurs in the rabbit, with this exception: whereas in the rabbit the increase in activity of the inner layer of epiblast gives rise to a rupture of the outer layer of epiblast, in the mole one of the alternatives suggested above is taken, and the inner epiblast bulges inwards, leaving a loose space threaded across with "stellate" cells between it and the zona radiata. Subsequently on the expansion of the whole blastodermic vesicle the plate flattens out again and the "stellate" cells and other outer layer cells become intermingled with those of the plate and form the permanent epiblast. Heape regards this as a kind of inversion, and the stellate cells as "träger."

I cannot agree with Heape in considering the stellate cells he mentions as being equivalent to träger cells, and certainly I do not think that the "Rauber cells" of the rabbit are in any way connected with "träger"—but of this I shall say more in a later paper.

As regards the meaning of the fusion of the two layers, I do not see that it need necessarily have any morphological significance at all. It may be merely an accident of development. At the same time I cannot entirely neglect certain occurrences in another group of Vertebrates, and have discussed them in another paper.

By the union of the two layers the embryonic disc acquires a very much more distinct outline, which is now practically circular; its outline is considerably more regular than before the junction just described has taken place.

Hypoblast.—(1) Hypoblast of embryonic area. This seems to have become in very slight measure changed. It is now undoubtedly a continuous membrane in the region of the embryonic area. This condition seems to extend a distance from the embryonic area equal to about the diameter of the embryonic area, beyond which it becomes a network and passes insensibly into—

(2) The straggling cell portion of hypoblast. This part of the hypoblastic layer retains its irregularly scattered condition of the sixth day, but certain features may be remarked upon which were unnoticed before.

The cells are more thickly scattered about; they are more irregular, having entirely lost their rounded form, and are more flattened. Many in all parts may be seen to be connected together by fine filamentous strands, not only in the close proximity of the embryonic hypoblast, but also near its periphery.

Again, the outer limit of this zone is much more marked, and is, in fact, now rendered very plain indeed. It forms a well-marked edge, very irregular it is true, but an almost if not quite continuous edge. Along this edge the cells are slightly crowded, and rather elongated in the equatorial plane of the vesicle. What I mean may be made out from fig. 40, and an idea of the general history of events connected with the development of this part of the hypoblastic layer may be derived from the four figures 37—40.

The extent of area covered by the two parts of the hypoblast is now rather more than half the whole area of the inside of the wall of the blastodermic vesicle.

Another point of interest may be noticed. There is a strong tendency for this line of limit to be thrown into small folds or waves, as shown in fig. 40, Pl. 17.

These two characters may be observed thoughout the area under discussion. If an embryo of this age is cut in two, and the cut edge examined under a high power, these characters are seen very clearly. That is to say, the cells forming this limiting line are themselves rather more rounded or " hog-

backed," and the joining strands are curved and even arch away from the epiblast, and in some cases undoubtedly the cells themselves seem to stand away from the surface.

It is, however, quite possible that the sinuosity of the line and arching away may be the result of reagents, as I have not examined this edge in a fresh specimen with success.

EXPLANATION OF PLATES 13—17,

Illustrating Mr. Richard Assheton's paper, "A Re-investigation into the Early Stages of the Development of the Rabbit."

List of Reference Letters.

A. Anterior end of blastodermic vesicle. *ALB.* Albuminous layer acquired in Fallopian tube. *C. BL.* Cavity of blastodermic vesicle. *EM. D.* Embryonic disc. *EP. I.* Inner layer of epiblast. *EP. O.* Outer layer of epiblast. *EP. OR.* Cell of outer layer of epiblast becoming "accidentally" included in the inner layer of epiblast. *HY.* Hypoblast of embryonic disc. *HY. I.* Hypoblast of region beyond embryonic disc. *I. M.* Inner mass of cells of blastodermic vesicle. *L.* Larger of the first two segments. L^2. Supposed second generation of layer of first two segments. L^3. Supposed third generation of layer of first two segments. *O. L.* Outer layer of cells of blastodermic vesicle. *P.* Posterior end of blastodermic vesicle. *P. B.* Polar body accidentally enclosed. *PL. S.* Sinuous protoplasmic junction between two hypoblast cells. *S.* Smaller of the first two segments. S^2. Supposed second generation of smaller of first two segments. S^3. Supposed third generation of smaller of first two segments. x., x^1. Deceptive appearances suggesting a van Beneden blastopore. *Z.* Zona radiata.

All the figures excepting Figs. 37, 38, and 41 have been drawn with the help of a camera.

All those figures of which the magnification is 465 times were drawn with Powell and Lealand's $\frac{1}{8}$ apochromatic oil immersion; the others with Zeiss or Reichert's lenses.

PLATE 13.

FIG. 1.—Fertilised ovum. Rabbit 24½ hours. × 165.

FIG. 2.—Ovum in two segments, from same rabbit as above. 24½ hours. × 165.

FIG. 3.—Ovum in two segments, showing great difference in size, from same rabbit as above. 24½ hours. × 165.

FIG. 4.—Ovum in two segments, from rabbit 24 hours. Polar bodies separated. × 165.

FIG. 5.—Ovum in two segments, from rabbit 25½ hours; drawn after mounting in Canada balsam. × 165.

FIG. 6.—Embryo in four segments, from same as preceding. 25½ hours. × 165.

FIG. 7.—Embryo in five segments. 27½ hours. × 165.

FIG. 8.—Embryo in eight segments. × 165.

FIG. 9.—Embryo in eight segments. × 165.

FIG. 10.—Embryo in seven segments. × 165.

FIG. 11.—Embryo in eight segments, showing internally-placed polar body. 39 hours. × 165.

FIG. 12.—Embryo of 47th hour, showing contrast in size of the several segments (seventeen segments). × 165.

FIG. 13.—Isolated segments of same specimen (47th hour). × 165.

FIG. 14.—Embryo (27½ hours) in seven segments.

PLATE 14.

FIG. 15.—Embryo (66½ hours), showing great difference in size of segments.

FIG. 16.—Section of a specimen preserved in silver nitrate ¼ per cent.; stained picro-carmine, and cut in paraffin. 72 hours. × 465.

FIG. 17.—Embryo, showing contrast in size of segments.

FIG. 18.—Section of embryo (72 hours) preserved in Perenyi; stain, borax carmine.

FIG. 19.—Another section of the same embryo. Both showing what might be a van Beneden blastopore, but on opposite sides!

FIG. 20.—Section through rabbit embryo of the 47th hour. Cut in paraffin. Preserved ½ per cent. silver nitrate 2 minims, water and sunlight ½ hour; no other stain. × 465.

PLATE 15.

FIG. 21.—Section through rabbit embryo of the 77th hour. Removed from Fallopian tube. Preserved in Perenyi; stained borax carmine; cut in paraffin. This seems to be an unusually large specimen. × 465.

FIG. 22.—Section through rabbit embryo of the 80th hour. Removed from uterus. Preserved in Perenyi, and stained in borax carmine; cut in paraffin. × 465.

FIG. 23.—Section through rabbit embryo of the 83rd hour. Removed from uterus. Preserved in Perenyi, and stained in borax carmine; cut in paraffin. × 465.

FIG. 24.—Section through rabbit embryo of the 80th hour. Removed from the same uterus as Fig. 22. Preserved in Perenyi, and stained picro-carmine; cut in paraffin. × 465.

FIG. 25.—Section of embryo of rabbit of the 80th hour. Taken from uterus (same as Figs. 22 and 24). Preserved in Perenyi, and stained in borax carmine, and cut in paraffin. × 465.

PLATE 16.

FIG. 26.—Section of the embryonic disc of rabbit of 96th hour. Taken from the uterus and preserved in Perenyi, and stained in aniline blue black; cut in paraffin. × 465.

FIG. 27.—Section of embryonic disc of rabbit of 96th hour. Taken from the uterus. Preserved in silver nitrate $\frac{1}{4}$ per cent. 25 minims; water and sunlight 2 hours; alcohol; borax carmine; cut in paraffin. × 465.

FIG. 28.—Section of the embryonic disc of a rabbit of the 100th hour. Taken from the uterus, and preserved in Perenyi. Stained in borax carmine, and cut in paraffin. × 465.

FIG. 29.—Section through the embryonic disc of a rabbit embryo of the 103rd hour. Preserved in Perenyi; stained borax carmine. × 465.

FIG. 30.—Section through a portion of the embryonic disc of a rabbit embryo of about the 140th hour. Preserved in Perenyi; stained picro-carmine. The hypoblast is now nearly a perfect membrane in the embryonic area. × 465.

FIG. 31.—A portion of the lower pole of the same section. × 465.

FIGS. 32, 33, and 34.—Portions of sections of the embryonic disc of a rabbit of the 153rd hour. Preserved Flemming strong formula; stained in borax carmine. The fusion of layers is taking place. × 465.

Fig. 35.—Portion of a section of the embryonic disc of a rabbit of the 125th hour. The hypoblast here is seen as consisting of cells with very conspicuous central portion, but these are connected by fine and apparently discontinuous strands—really a network. × 465.

PLATE 17.

Fig. 36.—Surface view of a portion of the embryonic area of a rabbit of about the 150th hour. Prepared with nitrate of silver. This corresponds to the sections Figs. 32—34. The shading is diagrammatic, and represents only my interpretation of the areas as defined by the silver nitrate lines, which are drawn by camera.

Fig. 37.—A diagram of the embryonic area of the rabbit of the 96th hour.

Fig. 38.—A diagram of the embryonic area of the rabbit of the 100th hour.

Fig. 39.—A surface view of a portion of the wall of the blastodermic vesicle of the rabbit of the 103rd hour. Seen from within. Preserved Flemming; stained borax carmine. × 350.

Fig. 40.—A portion of the wall of the vesicle, showing the termination of the inner layer at the 144th—150th hours. × 350.

Fig. 41.—A diagram to show the constancy of the angle subtended by the inner mass during the earlier stages of the growth of the blastodermic vesicle.

Fig. 42.—Camera drawings of the blastodermic vesicles of a rabbit of the 100th, 125th, 140th, and 175th hours, as seen from the side, arranged so as to show the supposed variation in rate of expansion of the different zones under the influence of—(1) increase of hydrostatic pressure within; (2) resistance (mechanical) of cellular wall of vesicle and albuminous layer, which are supposed to be nearly constant factors; and (3) the vital activity of the cellular wall of the vesicle, which by hypothesis is supposed to vary along certain zones and areas.

On the Phenomenon of the Fusion of the Epiblastic Layers in the Rabbit and in the Frog.

By

Richard Assheton, M.A.

With Plate 18.

In my paper upon the early stages of the development of the rabbit I have given evidence in support of the views held by Balfour and Heape concerning the fate of the outer layer of epiblast over the embryonic disc of the rabbit embryo of the seventh day. The two layers of epiblast gradually fuse together, and cells from each take part in the formation of the permanent epiblast.

In the description I have given of the process, and in the attempt I have made to explain how the fusion is brought about, I have regarded the phenomenon as being entirely accidental and of no morphological importance.

It is, however, only right to point out that there is a fusion of two epiblastic layers in certain Amphibians which seems to have a deeper meaning, and to which in some way the condition in the rabbit may be comparable.

As far as I know, there is no account published of this phenomenon, and I am not aware that anyone else has noticed it. Therefore, it seems to me, a short account of the facts as they appear in young embryos of Rana temporaria may be of some interest.

For the purpose of following the fusion of the two epi-

blast layers in the frog, it is best to examine the sections unstained.

In Rana temporaria the epiblast is from a very early period divided into two layers—an outer called the epidermic layer, and an inner called the nervous layer. If a section is taken, say, transversely though the neural plate of a tadpole about the time of the folding up of the neural folds, a section is obtained of which fig. 1 is a drawing.

The two layers are seen to be very sharply and distinctly divided, the outer or epidermic layer of epiblast (*E. EP.*) is a single cell in thickness. The cells are much more deeply pigmented than are the cells of the inner or nervous layer of epiblast (*E. NE.*) which forms a much thicker layer. The cells of this layer are very closely packed in the region of the neural plate through which this section is taken; so much so as to render it impossible at this stage, at any rate, to distinguish the boundary of the cells—if, indeed, there are any distinct boundaries. The nuclei are large, but are only seen with difficulty without staining. Some indication of the boundaries of the cells is to be seen in the slight increase of pigment along certain lines.

In fig. 2, which is taken at a slightly later stage, but before the neural folds have completely closed, the cells of the epidermic layer may be seen to have become much elongated, their inner borders being no longer truncated, but mostly pointed, and seem to be growing into the mass of nervous epiblast.

In fig. 3, which is from a section of a tadpole of about 3 mm. to $3\frac{1}{2}$ mm., in which the neural tube is now completely closed and separated off from the skin, the appearance of the darkly pigmented cells is very remarkable and instructive.

I can see no reason to doubt that the darkly pigmented cells of the epidermic epiblast, seen to be elongated in fig. 3, have by this time elongated and passed right through the mass of nervous cells (or nuclei) and spread out into fine filaments on the further side of the nervous layer. I cannot say for certain whether these fine filaments anastomose or not, but, however

that may be, they seem to form that which may, as a whole, be regarded as a reticulum, which is comparable to the myelospongium of His.

The outlines between the cells of the nervous layer are quite imperceptible in unstained specimens of this stage. The nuclei of the nervous layer are only seen with great difficulty.

The boundaries of the epidermic layer cells can in most cases be readily perceived, owing to the deeper pigmentation of the cells of this layer. The processes (*PR.*) are sharply and clearly defined, and are heavily loaded with pigment.

The bodies of the epidermic epiblast cells remain as yet lining the interior of the neural canal, although there is certainly a tendency for the nuclei in some of them to move more inwards. Also there seems to be a tendency for the epidermic cells to become pressed apart by the nervous cells. This is more marked at this stage in the spinal cord than in the brain, as may be seen in fig. 4.

I think we may take these appearances as a conclusive proof of the occurrence of an intimate fusion of the two epiblastic layers in the frog.

Observe the condition of the auditory vesicle in fig. 3. Here the walls of the vesicle are composed entirely of the light-coloured elements. There is no trace of the dark, deeply-pigmented strands such as are to be seen in the central nervous system. This is a further piece of evidence that the dark strands in fig. 3 are derived from the epidermic layer. For, as is well known, in the frog the nervous layer alone gives rise to the auditory vesicle—so it is a most significant fact that the dark strands should be entirely absent in this case.

After this stage (3—5 mm.) it is extremely difficult to trace the fate of the epidermic and nervous cells respectively. Up to now, the fact of the greater pigmentation of the former has rendered the inquiry easy.

The origin and meaning of the pigmentation is obscure, but its presence in the frog seems to be due to two causes, separate at any rate chronologically.

Firstly. Pigment is present in the unfertilised ovum as a

superficial layer covering the upper pole. Hence for a long time we find the superficial layer of cells after segmentation to be more deeply pigmented than the more internally situated segments.

Secondly. Pigment seems to be in some way connected with the actual protoplasmic activity, as it appears internally wherever division of cells takes place.

So also I believe the intensely black appearance of the processes of the epidermic epiblast which I have been describing is in some way connected with their intense activity just evinced by their growth inwards—which seems to be very rapid, and rather sudden.

When at a later period certain groups of nerve cells show a similar intense activity, there is a similar deposition of pigment in and around their processes, as, for instance, in the development of the ganglion habenula as shown in fig. 5. This pigment in each case becomes greatly lessened after the period of intense activity of growth has passed by.

The Question of Early Separation into Neuroblastic and Spongioblastic Elements.

Although the evidence to be drawn from the figures accompanying this paper is far from being conclusive, yet I think it tends very strongly towards the inference that the epidermic layer of epiblast in the frog gives rise to the spongioblastic elements, and the nervous layer to the neuroblasts.

His has shown how the spongioblastic network precedes the development of neuroblasts and nerve fibres and forms an irregular network with angular processes by no means unlike the dark strands in my fig. 3. These processes are not at all like the processes of nerve cells, which are always more tapering and less knotty. Further, at this stage there cannot be found any trace of definite nerve-fibres. These do not appear until later, till the tadpole has attained a length of about 6—6½ mm.

I can only interpret these figures as showing the conversion of the epidermic layer of epiblast into a supporting

framework in between the cells of the nervous layer. It is quite possible that we ought to regard the nervous layer of epiblast as comparable to the germinal cells of His rather than to the fully-developed neuroblasts.

If this is correct, we then must conclude that in the frog there is a very early separation of the neuroblastic from the spongioblastic elements.

Comparison between Rabbit and Frog.

Is it then possible that the condition in the rabbit has its parallel here in the frog? It is true that the epiblast is double only over a certain area of the embryo in the rabbit, whereas in the frog it is double throughout.

In the frog the nervous layer soon becomes much thickened along the future dorsal surface of the embryo, and over the rest of the embryo the nervous layer becomes reduced to a layer of one cell only in thickness, like the epidermic layer.

Now, although it is extremely difficult to trace the history exactly, I am almost sure that the area over which the inner layer of epiblast cells in the rabbit is found, corresponds to that area in the frog over which the nervous epiblast remains thick, or becomes thicker—i. e. to the neural plate.

In the frog the whole of the neural plate does not become folded up to form the neural tube, but the outer lateral portions of the anterior part remain outside of the tube, giving rise to the ganglia of the anterior cranial nerves.

I have endeavoured elsewhere to bring evidence to show that the epiblastic wall of the anterior part of the body of the rabbit embryo includes more than the double-layered part of the embryo, i. e. more than the so-called "embryonic disc."

Whether the "embryonic disc" goes to form the neural tube and ganglia of anterior cranial nerves as in the frog, or whether it forms only the neural tube, I have no evidence to offer.

The embryonic disc is precisely in the same position relative to the primitive streak as is the neural plate to the blastopore of the frog.

I am well aware that the epiblast is not at first double in all

mammals. For instance in the opossum, according to Selenka, it is a single layer. But it also is not always double in the Amphibia. In Triton it is at one time only a single layer of one cell in thickness. Why there should be this early differentiation into spongioblastic and neuroblastic elements in one and not in another so comparatively closely allied animals it is not easy to guess. Possibly it may be that, since the spongioblastic elements of the nervous system are the first to show activity of growth in the nervous system (His, and above), then in those animals in which, owing to various individual causes, the epiblast is many-layered, the outermost layer of "cells" or centres of activity being, by reason of its external position more favorable to processes of respiration and so in a condition more favorable to active growth, it will be this layer of epiblast that will take on itself the earliest phase in the further development of the nervous system.

Although I think the rabbit's condition can be quite well explained without reference to the above, on the other hand there may be some deeper meaning in it, for which reason I have thought it best to make notice of the condition in the Anura.

To make this parallelism complete and certain it is necessary to show that in the rabbit the cells derived from the outer layer give rise to the spongioblastic tissue, and those derived from the large inner layer cells to the neuroblastic tissue. This I cannot do. After a while the cells which have originated in each of the layers become equally active, but I have as yet been unable to trace their fate respectively.

DESCRIPTION OF PLATE 18,

Illustrating Mr. Richard Assheton's paper, "On the Phenomenon of the Fusion of the Epiblastic Layers in the Rabbit and in the Frog."

List of Reference Letters.

AU. Auditory vesicle. *E. NE.* Nervous epiblast. *E. EP.* Epidermic epiblast. *NE.* Neuroblast. *PI.* Stalk of pineal gland. *PR.* Processes of epidermic layer cell. *T. E.* Band of nerve-fibres passing from ganglion habenula to the ventral side of the brain.

Fig. 1.—Transverse section through a portion of the neural plate of a frog embryo (Rana temporaria) during the process of folding up of the neural plate. Unstained. × 165.

Fig. 2.—Transverse section through corresponding region, but at a slightly later period, of a frog's embryo. Unstained. × 165.

Fig. 3.—Transverse section through the corresponding region after the separation of the neural tube from the skin. Tadpole, 3½ mm. Unstained. × 165.

Fig. 4.—Transverse section through the spinal cord of the same specimen as Fig. 3. Unstained. × 165.

Fig. 5.—Transverse section through ganglion habenula of a frog tadpole of 13 mm., showing deeply pigmented neuroblastic processes. Aniline blue-black. × 350.

On the Causes which lead to the Attachment of the Mammalian Embryo to the Walls of the Uterus.

By

Richard Assheton, M.A.

With Plate 19.

I.

The First Attachment of the Rabbit Embryo to the Walls of the Uterus.

By the end of the eighth day, if not actually attached to the walls of the uterus, the embryos have become definitely located, and their presence is made evident from the exterior of the uterus by a bulging in the wall opposite the mesometrium. Frequently by this age they are actually attached to the uterus, and cannot easily be extracted without damage.

It must also be noted that the embryos by this time no longer lie anyhow in the uterus, but when definitely located the position of the embryo to the uterus is such that the embryonic area is always towards the mesometric wall of the uterus.

This is a very important fact. It is probably necessary for the development of the rabbit that it should be thus situated. It would be very awkward if the embryo became fixed in any other position. The shape of the uterus at this stage, and the shape of the blastodermic vesicle at this stage, are so beautifully adapted one to the other as to render any other position almost impossible. The blastodermic vesicle by this time is a slightly elongated body whose lower pole is semicircular in transverse section, while the upper pole is much flattened. If

a piece of uterus is blown out with water, then hardened, and a transverse section cut, the cavity will be seen to be bounded by a semicircular wall on the abmesometrial side, and, by reason of the two largely developed "placental" lobes, a very flattened wall on the mesometrial side.

Fig. 1, Pl. 19, is a figure of the section of the uterus during the early days of pregnancy, or in such part of the uterus in which no embryo is present during rather a later stage in the earlier part of pregnancy. *M.* is the mesometrium; *C.* is the cavity of the uterus.

The general external outline of the uterus is circular; so is the inner muscular system, but the latter is eccentrically placed to the outline of the uterus.

Within these muscular coats, which form the most resisting part of the uterus, comes the soft connective tissue and epithelial lining of the uterus. The loose connective-tissue layer seems to be the most yielding, and, as a consequence of the blastodermic vesicle within the cavity of the uterus, the folds or lobes, as they appear in transverse section (*PL. L.*, *PP. L.*, *OP. L.*), diminish in size, and as the body within increases in size, one by one they begin to disappear. The obplacental lobes have quite disappeared by the middle of the eighth day, and the periplacental lobes can no longer be described as lobes or folds after the beginning of the ninth day. The placental folds being the largest, and also upon the less expansible side of the uterus, become much flattened but never entirely disappear.

About the time that the blastodermic vesicle becomes definitely located, its shape is in transverse section as fig. 4.

The lining of the uterus is seen in fig. 1 to be thrown into folds. When the blastodermic vesicle (fig. 4) is inserted into the cavity, the folds become pressed out to a greater or less extent: thus the folds called obplacental are entirely obliterated (*OP. L.*, fig. 2), the periplacental are nearly obliterated, but the placental lobes being very much larger, and also being supported by a larger mass of muscular tissue, are hardly compressed at all.

The cavity of the uterus is thus bounded on the mesometrial side by an almost straight line, while the opposite wall is circular. A body having the shape shown in fig. 4, in passing down the tube by virtue of the continual slow contractions of its walls, could hardly fail to become so fitted as to lie with its flatter surface against the flatter wall of the uterus.

Since the flatness of one surface of the blastodermic vesicle is caused by the embryonic disc and changes connected therewith being upon that surface, it follows that in the rabbit the embryo always comes to lie up against the mesometrial side of the uterus, which is, under the circumstances, by far the most favorable position for its future development. For in this position it will be less exposed to the tension which must necessarily arise upon the opposite side in the subsequent expansion of the uterus by the accumulation of fluid within the blastodermic vesicle.

The blastodermic vesicle does not by any means become attached with the centre of its flat surface always exactly adjoining the median cleft between the two placental lobes. It may sometimes be a little to one side or the other, but it is never very far out.

As the blastodermic vesicle expands, it eventually fills up the whole cavity of that section of the uterus in which it happens to be. With further expansion it necessarily exerts a pressure upon the walls of the uterus, and this pressure is made apparent without by a swelling, or protuberance, occurring upon the side of the uterus away from the mesometrium.

The first visible sign of the commencement of the formation of the placenta—or, at any rate, of those structures which eventually cause the placenta to come into existence, appears very shortly after the appearance of this swelling. I allude to the papillæ and protuberances of epiblast.

I said just now the first visible sign because I believe that the outgrowths are due really to a continuation of those causes which have given to the blastodermic vesicle its present form and shape, and described in a former paper.

The immediate cause of the origin of the papillæ seems to be

the pressure which now is applied to the vesicle from without by reason of the resistance offered by the elasticity of the walls of the uterus to the internal hydrostatic pressure.

First of all, let us consider what is the nature of these papilla-like growths.

Sometimes during the eighth day, it may certainly be as early as the seventh day four hours, when the uterus is swollen very considerably by the pressure of the contained blastodermic vesicle, here and there it may be noticed in transverse section that over all the lower surface of the vesicle certain of the epiblast cells are no longer so much flattened, but the nuclei appear rounded instead of oval in section, and the protoplasmic part of the cell much more distinct and granular—altogether more comfortable-looking (v. *a.*, fig. 5). A little further along, at *b.*, may be seen a couple of such nuclei in a mass of granular protoplasm. At *c.* a group of three or four, or more, of such nuclei in a mass of granular protoplasm. Outside this may be seen the torn remnants of the much attenuated albumen layer. The portion of the walls of the vesicle here figured was part of the lower pole of the blastodermic vesicle of an embryo of seven days four hours. Although I did not succeed in taking it out of its place in the uterus unhurt, it was, nevertheless, not yet attached to the walls of the uterus at any point. It may be noted that here there is no trace of an inner layer or hypoblast.

In fig. 6, another piece of the wall of a vesicle of about the same age cut in situ in the uterus, this piece shows a portion of the side of the blastodermic vesicle, or, at any rate, a portion not so far removed from the embryonic pole as that drawn in fig. 5. Here in fig. 6 the same features are to be seen in the epiblast as described for fig. 5. There is here, however, a well-developed hypoblastic layer.

Fig. 8 is a section of the upper part of the wall of the vesicle, of that part which closely adjoins the embryonic disc. This specimen is from an embryo from the same rabbit as that from which fig. 5 was drawn, and almost exactly the same size. Here it will be noticed that not only are the cells

here and there large and granular with round nuclei, but the whole of the epiblastic layer is now composed of thickened cells, almost columnar with rounded nuclei, and for a given length there are many more nuclei than there were at that region during the sixth and seventh days. In fig. 9 a piece of the same region a few hours later is shown, and here the epiblast is seen to be so thickened as to be actually several cells thick,—in fact, a proliferation of cells is taking place outwards.

Figs. 10 and 11 are still later stages of the same area.

I believe that to understand the first outgrowth of these papillæ, both the small ones scattered over the lower pole, and the area nearer to the embryonic disc, the great change that the embryo has now undergone in the physical and mechanical conditions must be taken into careful consideration.

During the fifth, sixth, and seventh days I described in my previous paper the growth of the walls of the vesicle as being due to the hydrostatic pressure within it, together with the multiplication of the cells of the walls of the vesicle, support being rendered to the delicate wall of the vesicle by the albumen layer. The cells of the wall (the epiblast) are very much flattened because of the tension produced by the hydrostatic pressure.

The hydrostatic pressure is sufficient to keep the cells always taut, and any increase in size of a cell owing to growth, or any aggregation of cells by multiplication, is prevented by their being flattened out by the internal pressure as soon as formed. Thus all the cells are uniform in thickness, and extra activity of one part shows itself by extra expansion of that arc of the vesicle.

What happens, however, when the walls of the uterus, by reason of the great size now attained by the blastodermic vesicle, affords supports to the walls which was hitherto wanting? It must decrease the ratio of hydrostatic pressure to the rate of growth of the cell wall.

If the amount that the cells are stretched is constant when the hydrostatic pressure is to the rate of growth of the cell

walls as, say, 10 is to 10, then when the hydrostatic pressure (P) is to the rate of growth (R) of the cell walls as say 8 : 10, it follows that the cells will not be so much stretched as when P : R : : 10 : 10.

The actual pressure within the vesicle no doubt does not diminish, more probably it increases, but the relation of P : R is altered by the fact that additional resistance is now added without by the walls of the uterus. At any rate, it upsets the ratio formerly existing.

The result, I believe, is that now the rate of growth of the cells of the wall is as compared with the rate of increase in size of the blastodermic vesicle greater than it was before, so that when a cell "grows" and "divides" it no longer becomes at once stretched out, but forms a rounded granular cell, or group of cells, as in figs. 5 and 6. The cells which are for the time inactive will remain flattened, for elasticity is not an attribute of protoplasm.

This is the case at the lower pole of the blastodermic vesicle and at the lower sides. In the region near the embryonic disc (where it has all along been assumed that there is a more active growth) it is found that almost every cell has evinced signs of activity, for here the whole area has become thickened, and the cells far more closely packed than they were, and almost columnar instead of being flattened (fig. 8).

It is, I believe, usual to describe the first attachment as occurring between the ectoplacenta of the embryo and the placental lobe of the uterus. This I am convinced is not an accurate statement. The first actual attachment is between the lower parts of the blastodermic vesicle and the periplacental and obplacental folds.

The exact course of the procedure I am doubtful about, but I believe it to be a combination of at least two main causes, but it may involve more; or possibly it is wholly due to only one of the two I am about to mention.

The first attachment is effected by means of the "papillæ," or thickened spots of epiblast of the lower pole of the embryo already described (figs. 5 and 6, *a.*, *b.*, *c.*). In fig. 7 one of

these thickened spots is shown to be on the point of effecting an attachment. It may be noticed to be wedge-shaped in section; it is a little blunt cone. Practically each papilla assumes this shape, and is being pressed against the epithelium lining the uterus. In this figure (and almost any number might be drawn showing the same characters) the papilla certainly looks as though it were piercing the epithelium by reason of the pressure from within the vesicle.

Of course the actual hydrostatic pressure will be the same at *a.* as it is at *e.*, but nevertheless a greater pressure will be exerted on the uterine wall at the apices of any knobs on the wall of the vesicle than at the area between them if the uterine wall is in a state of tension, which undoubtedly it is at this time.

If we consider that in all probability the uterine epithelium is a softer material than the muscular and connective tissue outside it, it is all the more probable that the softer (if it is softer) uterine epithelium will give way between the two.

I think it quite possible that this may be the only necessary cause, and by this means the "papilla" reaches the capillary system of blood-vessels in the uterine connective tissue, a point of the utmost importance to the welfare of the embryo.

On the other hand, although I have no doubt that the additional pressure which exists at the points of these knobs is of much importance in that it causes very close contact between the uterine epithelium and parts of the wall of the blastodermic vesicle, yet it is more than possible that the breaking down of the uterine epithelium at those points is not due to mechanical pressure alone, but to a chemical or a physiological process, such as absorption of the uterine cells by the vital activity of the cells of the knobs. But the possibility should not, I think, be lost sight of that the first breaking through of the uterine epithelium at these points may be entirely due to mechanical pressure alone.

A point to which special attention must here be drawn is

that the moment after the uterine wall comes to take the place of the albumen layer as a support, then the greater part of the expansion of the combined vesicles must be that part furthest removed from the embryo, because it is here that the uterus wall is infinitely less resistent, and with it will go that part of the vesicle to which it is attached.

On the Importance of the Albumen Layer and Zona radiata.

Those who have followed my account of the development of the blastodermic vesicle of rabbit between the eightieth hour and the time of attachment of the vesicle to the walls of the uterus at about the 170th hour, must have seen how important a part the albumen layer plays in producing the form actually assumed by the vesicle. I pointed out how that the thin cellular wall of the blastodermic vesicle would be itself quite able to withstand the hydrostatic pressure within ; and, further, that it is not until the vesicle has attained such a size as to stretch the walls of the uterus as to cause them to afford the support necessary to prevent the bursting of the vesicle, that the albumen layer was lost. By this time the albumen layer has become exceedingly thin, and the disappearance of it is brought about by its rupture. Traces of it may be found curled up and crumpled several days later. Once burst, its function, as far as I can judge, is at an end.

Now it has almost certainly at least one other very important influence upon the development of the rabbit. By its presence until the eighth day it absolutely prevents the cellular tissue of the blastodermic vesicle from coming into close contact with the cellular tissue of the uterus. The embryo up to the eighth day is as free probably from the protoplasmic influence of the mother, as is the egg of a bird after it has been covered with a thick calcareous shell. There is also, it will be remembered, another coat to the ovum, the zona radiata, which is present from the very first, and has a similar effect to that which the albumen layer has, but being less thick its effect is more evanescent. The two coats may be considered as one.

As regards the rabbit. I have shown in considerable detail how many events are, or at least may be, explained by external influences (such as albumen layer, hydrostatic pressure, pressure of uterus, rupture of albumen layer) acting in conjunction with a simple steady force or energy, the primary centre of cell multiplication.

In doing so, it will be remembered that in connection with the actual forms assumed, and phases passed through, in the rabbit, the presence of the albumen layer, the size of the cavity of the uterus, and even the shape of the walls of the uterus, had important consequences ascribed to them.

If my line of argument has been correct, the effects produced by the albumen layer and the size and shape of the uterus must be very different or absent altogether in forms in which these conditions are different or absent. As regards the size of the ovum itself, there is very little difference amongst mammals.

Let us examine the case of a rat,—a rodent, and so not very distantly removed genetically from a rabbit. And yet how different is the form assumed in the earliest stages of development! Do the conditions differ from those in the rabbit, and if so, how?

They differ in these respects:

(i) There is no zona radiata or albumen layer.

(ii) The diameter of the uterus is very much smaller, and the lumen proportionately smaller still.

(iii) The walls of the uterus are of a more uniform thickness.

In the rabbit the zona radiata is so quickly covered by the albumen layer that it cannot be said to have in itself much effect as a support or protective coat.

In the rat there is no zona radiata or albumen layer. The ovum develops freely in the cavity of the uterus unprotected by any coat. It would seem to pass very rapidly down the Fallopian tube. Robinson found one early stage of segmentation; from this he figures the mesial section, which shows parts only of twelve segments, so we may conclude that his specimen was a fairly early stage of segmentation—comparable, perhaps, to that of the rabbit of the forty-eighth hour.

The segmented ovum of the rabbit lies within a thick, tough spherical coat, and is spherical. The ovum of the rat is not thus placed within a spherical mould, and can therefore take other forms, which from the conditions would seem to be necessarily disc-like or oval.

The blastodermic cavity ("Dottersackhöhle" of Salenka, "vitelline cavity" of Robinson) would seem to be produced by a hydrostatic pressure from within, as in the rabbit, but owing to there being no resistance of albumen layer the walls of the cavity of the vesicle offer less resistance. Thus there is less tension, and this has the effect of producing a shape of no symmetry except such as is given to it by the walls of the uterus in which it lies.

In the rabbit an early tendency to rapid growth of the area immediately surrounding the embryonic disc gives rise to an expansion of that part of the vesicle; so, also, the same tendency to a rapid growth occurs in the rat round the corresponding area, but with very different results. A heap of cells accumulates, which gives rise to the irregular mass growing among the cavities of the uterus, and usually called Träger. After a while the blastodermic vesicle becomes fixed in the walls of the uterus, and the condition then is similar to that in the rabbit, i.e. the necessary resistance is supplied, and a more or less spherical swelling appears upon the uterus, in which the embryo develops in safety.

As regards the actual fixing of the rat embryo, this is no doubt primarily due to the pressure exerted between the irregularities of the walls of the uterus, the "träger" (and other external layer cells of the embryo), this pressure being brought about by—

(i) Multiplication of cells of träger and general growth of embryo.

(ii) Hydrostatic pressure within the blastodermic vesicle.

Thus we see that what corresponds to the träger in the rabbit becomes obvious in the rat at a very early period; whereas in the rabbit its presence (essentially as an area of increased energy) is marked by the continued expansion of the vesicle, as described in a former paper.

Then, again, let us look at the conditions of the mole. Here is an animal, an insectivore, which is considerably removed genetically from a rabbit. Yet here we find that the development is extremely like that of the rabbit. It is true that there is a slight approach to the inversion condition, but only slight; and I think that what appears to be an inversion for a short time is very possibly in no way connected with the inversion such as that of the house mouse, field mouse, rat, or guinea-pig. The separation figured by Heape ('Quart. Journ. Micr. Sci.,' 1883, Pl. 29, figs. 24 and 25) seems to be due to quite other causes, namely, temporary and rather sudden acceleration of growth of the embryonic disc itself, and not of the area just beyond it. For the cells which become separated from the epiblast, and which Heape compares with the träger, afterwards again unite and fuse with the epiblast and form together the epiblast of the embryonic disc; whereas in all other forms any cells which have once become separated as "träger" never participate in the formation of the permanent epiblast. I should say these cells correspond rather to the outer layer of epiblast in the embryonic disc in the rabbit, and that in the mole the representatives of the träger cells would be those which at this moment (Heape, figs. 24 and 25) form part of the wall of the vesicle beyond the region of the embryonic disc, as in the corresponding region of the rabbit.

Until the time of this temporary bending in of the embryonic area, the development of the mole is exactly comparable to that of the rabbit. The ovum segments and forms a spherical morula. A blastodermic cavity and vesicle are formed almost exactly comparable to the rabbit, and the hypoblast is formed in the same way; the blastodermic vesicle is a spherical bladder-like body; and may not the reason of this be that the ovum develops up to a certain point under quite similar conditions, that is to say, surrounded by a thick protective covering of zona pellucida and "mucous layer" (v. Heape)? This mucous layer develops rather differently, being applied according to Heape in the uterus, and not in the Fallopian tube.

The coat, however, is very much less firm, and offers apparently less resistance than that of the rabbit, for, later, after the vesicle has filled the cavity of the uterus the walls very soon become moulded into the shape of the cavity of the uterus (v. Heape, Pl. 28, figs. 8 and 9).

Of course it is necessary to take into consideration the fact that the actual lumen of the uterus in the mole is very much less than in the rabbit, and that therefore the developing vesicle will be influenced by the resistance offered by the muscular coat of the uterus at a time when the vesicle is much smaller than in the case of the rabbit.

Now to consider another case where we have pretty complete records, i. e. the guinea-pig.

During the early segmentation stage the ovum of the guinea-pig is surrounded by a zona radiata. We find that in the development of the early stages the embryo assumes just the same form as in the mole or rabbit; but after the embryo has reached the uterus the zona is ruptured, and from this moment the embryo is naked and goes through phases which are certainly more like those of the rat than those of the rabbit or mole. It can hardly be necessary to point out that whereas at first the conditions were similar to those of the rabbit or mole, from this moment they resemble more closely those of the rat.

In this question I have only gone into any details in the case of the rabbit. But still, although I can only judge of the other forms from either rather superficial personal examination or from the writings of others in which this point has not been given any special prominence, I think I may be allowed to make a few general remarks upon such other forms in which marked divergence of shape and structure occurs in the method of formation of the blastodermic vesicle and germinal layers.

Amongst the Carnivora, from observations on the dog by Bischoff and Coste, and on the cat by Fleischmann, it seems clear that the condition most nearly resembles the rabbit.

The ova are in all these cases invested with a firm coat,

either zona pellucida or albumen-layer, and more probably both. These are present until as late a period as in the rabbit. There is no inversion in either case. The shape is somewhat different in both cat and dog. The vesicles are spherical until about the time that the resistance of the uterine walls is encountered, after which time they assume a much more oval shape. Whether this is due chiefly to the effect of the walls of the uterus, or whether due in part to a modification in intensity or of the extent of the träger area, it is impossible to say without careful investigation.

Amongst the Ungulates we have descriptions of the deer by Bischoff, the sheep by Bonnet and Coste, the pig by Keibel.

Although the literature on the point I am now discussing is very scanty for this group of animals, we know two facts. Firstly, that the vesicle, at first spherical, grows to an enormous length—as much as 130 mm. in the pig (Keibel), or the whole length of the uterus in cases where there is only one embryo. Also we know there is no inversion.

They are surrounded by a zona radiata or some other investment, but it would seem to be very thin. Bischoff says there is no albumen-layer in deer.

As regards inversion, the Ungulates, as far as at present known, behave like the Carnivora and rabbit. But after a certain point the vesicle lengthens enormously. It seems probable that this lengthening is due partly to the large cavity in which the vesicles lie. All the Ungulates of which we have any record are large. The lumen of the uterus in the pig is enormous as compared with the lumen in the uterus of a rabbit.

Also there seems to be evidence to show that the zona radiata or albumen layer is thinner, and offers therefore less resistance, and accordingly has less effect in resisting the pressure of the walls of the uterus, which, it must be remembered, actually lie in contact with each other.

We must also suppose that although the pressure is sufficient to prevent the vesicle from retaining an approximately spherical form, it is not as yet sufficient to cause a fixation of the em-

bryo to the walls of the uterus. What actually brings about the fixation in the embryo in Ungulates I do not know. It would seem possible, from the frequency of a cotyledonary placentation, that a number of spots of pressure arise at considerable intervals amongst the folds of the uterus, and so bring about local areas where the rate of growth of the cellular wall is in excess of the rate of expansion of the elongated vesicle.

In connection with the elongation of the blastodermic vesicle, I may draw attention to a fact in the development of the rabbit.

It follows from what I have said, that if the albumen-layer in the rabbit was less thick, or absent, its development would be very different. So it happens that after the rupture of the albumen-layer there is a tendency for the vesicle to elongate during the eighth and ninth days. The normal shape of the vesicle upon the ninth day is shown in fig. 3.

It is important for the development of the space (*C. BL.*) that after the rupture of the albumen layer the passages (*UT.*) should be closed; otherwise, unless the thin wall of the blastodermic vesicle is very much stronger at that point than hitherto, it could not withstand the pressure from within, which must now be considerable, and would rupture, and the liquid would escape and the swelling (*C. BL.*) would collapse. In the rabbit, no doubt, the mucous membrane of the approximated walls of the uterus at this point (*UT.*) is thrown into folds and pressed together by becoming a little bent inwards, and by this means tends to block the passage, but also, no doubt, the horns of the vesicle (*H.*) also help to obliterate and plug up the cavity at these points.

Not unfrequently it occurs that either one of the horns (or both) extends through this narrow passage (*UT.*) and is prolonged (the albumen layer having ruptured by now) for a short distance along the cavity of the uterus. Sometimes a horn seems to be prolonged for a distance of as much as 34 mm., but the greater part of this is non-cellular (*Z.*), the origin of which I have not traced.

Summary.

Section 1.

(i) The blastodermic vesicle of the rabbit becomes first attached to the walls of the uterus by its lower pole.

(ii) This attachment of the lower pole is regarded as the result of a mechanical pressure of certain spots or knobs of thickened epiblast of the blastodermic vesicle upon the epithelium of the uterus (the pressure being the hydrostatic pressure within the vesicle), whereby the uterine epithelium is pierced and the knobs of epiblast become embedded in the connective tissue below.

(iii) These knobs of epiblast, as also the thickening along the träger region, are regarded as being the direct result of a destruction of the equilibrium between the rate of increase of the hydrostatic pressure within the blastodermic vesicle and the rate of growth of the cellular wall of the vesicle, under which conditions the epiblast had hitherto remained practically at a constant measure of thickness.

The destruction of the equilibrium is brought about by the additional pressure put upon the expanding blastodermic vesicle by the resistance of the uterine walls. This is a continuation of the same series of forces which in my former paper were supposed to account for the peculiar shape of the blastodermic vesicle and apparent growth round of the hypoblast.

(iv) In the träger region the attachment is effected in a rather different manner. Here the activity of the epiblast is greater than at the lower pole, so that here the thickening is of a more general and more rapid character, which results in a somewhat extensive and irregular area instead of isolated knobs of thickened epiblast. Also the soft tissue immediately underlying the epithelium of the uterus is here very extensive.

Thus the conditions are not such as to cause a perforation of

the epithelium. Instead of this, the epiblast of the embryo becomes very thick and amounts to almost a loose proliferation of cells, which cells become eventually pushed into the irregularities and glands of the placental lobes.

Section 2.

(i) The primary cause of "inversion" is the fact that the embryonic area is at one time a region of less activity, and is surrounded by a zone of greater activity in connection with the future formation of placenta and a space within which the embryo can develop unaffected by pressure external to itself.

(ii) The occurrence of "inversion" is determined by the production of a heaping-up of cells at an early stage by this zone of greater activity and so forcing the embryonic area inwards.

(iii) Inversion is prevented by causes which impede the heaping-up of tissues around the embryonic area.

(iv) Foremost among these preventing causes is the presence of an investing coat, either zona radiata, or albumen layer, or mucous coat, &c., which—

(1) By hindering a close connection between the cells of the blastodermic vesicle and the uterine walls;

(2) By affording a strong support to the walls of the blastodermic vesicle—

allow the blastodermic vesicle to assume, under the expanding influence of the hydrostatic pressure within, shapes due more to its own inherent tendencies and less to the effect upon it of the pressure of the uterine walls.

(v) The thicker and more lasting these coats are, the more marked are the intrinsic characters of the blastodermic vesicle, and the longer deferred is the impression of characters due to the physical effects of the uterine walls.

For instance, both the rabbit and the dog have investing coats. In the rabbit it is far thicker, so in the rabbit it is found that the vesicle assumes a shape which can be best accounted for by intrinsic causes, while in the dog, where it is much thinner, the uterus seems to be the more potent

factor in moulding the shape of the vesicle. In the rabbit the vesicle attains a far greater size than in the dog before becoming attached to the uterus, though this may, no doubt, also be partly owing to the far greater resistance offered by the uterus of the dog.

(vi) When the lumen of the uterus is large, and where the investing coat is present, but delicate, the blastodermic vesicle may grow to a great length, and possibly the development of villi and consequent placental attachment may be brought about by local developments of regions of pressure.

(vii) Even in the rabbit, after the rupture of the albumen layer the blastodermic vesicle may become extended along the cavity of the uterus to a length of over 20 mm.

EXPLANATION OF PLATE 19,

Illustrating Mr. Richard Assheton's paper "On the Causes which lead to the Attachment of the Mammalian Embryo to the Walls of the Uterus."

List of Reference Letters.

a. Epiblastic papilla. *ALB*. Albumen layer. *b*. Epiblastic papilla. *C*. Cavity of uterus. *c*. Epiblastic papilla. *C. BL*. Cavity of the blastodermic vesicle. *e*. Wall of the blastodermic vesicle. *EC*. Ectoplacental cells. *EL*. External longitudinal muscle layer. *EMB*. Embryonic area. *EP*. Epiblast. *H*. Horn of the blastodermic vesicle. *HY*. Hypoblast. *IC*. Internal circular layer of muscle-fibres. *M*. Mesometrium. *MES*. Mesoblast. *P. L*. Placental fold of the uterine mucous membrane. *OP. L*. Obplacental fold of uterus. *PP. L*. Periplacental fold of uterus. *UT*. Cavity of the uterus. *UT. EP*. Epithelium of the uterus. *Z*. Non-cellular continuation of horn of vesicle attached to the remains of albumen coat.

Fig. 1.—Transverse section of the uterus of a rabbit before any distension has been caused by the presence of an embryo.

Fig. 2.—Transverse section of the uterus of a rabbit on the seventh day after fertilisation. The distension is chiefly at the expense of the weaker

wall, i. e. the obplacental folds. At this moment the location of the embryo can only with difficulty be recognised from without.

Fig. 3.—A diagram illustrating a longitudinal section of the blastodermic vesicle and uterus of a rabbit about nine days after fertilisation. The epiblastic papilla and ectoplacental area are omitted. The cavity of the uterus is seen to be obliterated by means of the horns of the vesicle (*H.*).

Fig. 4.—A transverse section of the blastodermic vesicle about the time that it becomes finally located.

Fig. 5.—Section of the lower pole of the blastodermic vesicle of a rabbit. Seven days four hours. × 465.

Fig. 6.—Section of the side of the blastodermic vesicle of about the same age. × 465.

Fig. 7.—Section of the lower pole of the blastodermic vesicle of a rabbit (after attachment) and of the uterine epithelium. One of the wedge-shaped papillæ is seen to be piercing the epithelium. × 465.

Fig. 8.—Section of the ectoplacental area of the blastodermic vesicle of about the same age as that of which Fig. 5 is a section. × 465.

Fig. 9.—Section of ectoplacental area later than Fig. 8. × 465.

Fig. 10.—Section of ectoplacental area still later. × 465.

Fig. 11.—Section of ectoplacental area about the time of its attachment to the placental lobes. × 465.

The Primitive Streak of the Rabbit; the Causes which may determine its Shape, and the Part of the Embryo formed by its Activity.

By

Richard Assheton, M.A.

With Plates 20—22.

The following pages are offered as a contribution towards the elucidation of the mode of formation and the function of the primitive streak in the rabbit.

Although it is necessary to make incidental remarks upon the formation of the mesoblast and notochord, I have not given here a full description of the results of my own observations upon those points, but I hope to be able to do so, and to discuss the results of former authors upon this and other animals, in a later communication. In the present paper I have confined my remarks almost entirely to the rabbit, and to my own suggestions towards an explanation of certain phenomena connected with the structure we call the primitive streak in that animal.

Of recent years the theory of concrescence has been again brought into great prominence in attempts to account for the growth in length of the Vertebrate embryo. The present paper will, I hope, tend to show that there is no trace of such an occurrence in the rabbit, and that the growth in length of the embryo can quite as well—and to my mind infinitely more easily—be accounted for by a process of addition of new cellular units between the pre-existing embryo and an area of rapid cell-production. I have attempted to show which part of the embryo is to be regarded as the "pre-existing embryo," and which as being due to the activity of the area of rapid cell-production—the primitive streak.

I have also tried to explain how the changes in shape of the primitive streak are brought about.

I begin with a short account of this area of activity (which I shall speak of as the secondary area or centre of cell-production—as compared with the process known as the segmentation of the ovum, which I call the primary centre of cell-production) which adds certain details of interest to the accounts already given of the rabbit primitive streak by other authors, notably by Hensen, Kölliker, and Rabl.

The Earliest Signs of the Formation of the Secondary Area of Proliferation.

Until the middle of the seventh day the embryonic disc is circular in outline. After the fusion of the inner and outer layers of epiblast, the outline of the embryonic disc becomes very much sharper. This state continues until about the middle of the seventh day, when, although the anterior periphery of the embryonic disc retains this character, the arc of the hindermost quadrant becomes very much less distinct, and is no longer a semicircle.

On measuring the embryonic disc very soon after this, it is found to have long and short axes, the long one usually parallel to the long axis of the vesicle, the short to the short axis of the vesicle. The lengthening has been in the antero-posterior direction. The anterior end presents much the same shape and features as before, except that its periphery describes an arc of a larger circle than before. It is still sharply defined. The posterior end is, if anything, more ragged, and is undoubtedly at this moment thinner than the rest of the embryonic area.

The measurements are as follows:

Blastodermic vesicle,	long axis . . .	4·1 mm.
„ „	short axis . . .	3·85 „
„ „	polar axis . . .	3·25 „
Embryonic area,	long axis . . .	1·35 „
„ „	transverse axis . .	1·25 „

This ragged border I believe to be the effect of the setting up of an area of increased activity of cell-production either

upon, or just within the posterior border of the previously circular embryonic disc (see *PS.*, Pl. 20, fig. 2).

Whether this activity concerns in the first instance both the cells derived from the outer layer of epiblast and those derived from the inner layer of epiblast equally, I cannot form at present a definite opinion.

Very soon the centre of this ragged area becomes thickened, and the embryonal area has now the appearance shown in fig. 3. The original outline of the embryonic disc is fairly distinct, but of course slightly larger than in the earlier stage (fig. 1). This thickening, which is due to a greater accumulation of cells at this point, would seem to be the result of a greater activity of cell multiplication at this spot.

As to the cause of this sudden increase of activity I have no suggestion to make. It is, of course, quite possible that the activity was present from the first, and that it is only owing to changed conditions that it now becomes evident as a heaping-up of cells. To give a strictly epigenetic explanation some such cause ought to be adduced, but for the present I am not able to suggest any, and so must ascribe it to a palingenetic cause.

It is not possible to point out the exact boundaries of this area of increased activity, but probably if that portion of the epiblast which is distinctly thickened is taken to be the area of increased activity, I think the error will not be great. Up to now, and for some considerable time to come, there is no change noticeable in the condition of the hypoblast.

The darkened area *PS.* in fig. 3 may be taken as the area of increased activity. The posterior border of this area is semicircular, the anterior border is conical. The darkest part of this area is about the position of the posterior border of the original embryonic disc.

Fig. 4 is a slightly later stage. The anterior portion of the embryonal area still shows a circular darker part, which I take to correspond approximately to the original circular embryonic disc of the sixth day. The secondary area of activity is of the same nature as in the preceding figure, but its anterior conical part is slightly more pronounced. On either side of it there are

two parts which are very much less opaque, but still more opaque than the extra-embryonic part of the blastodermic vesicle.

The specimen of which fig. 4 is a drawing was cut into a series of transverse sections, that of which fig. 4 A is a drawing, into a series of sagittal sections. Fig. 17, Pl. 20, is a very nearly median sagittal section of the specimen represented by fig. 4 A. In this drawing *A.* is the anterior end, and *P.* the posterior.

The epiblast (*EP.*) of the embryonic disc is thickened throughout. The under or inner surface of the epiblast is even along the anterior half, while along the posterior it presents a very irregular, and towards the extreme posterior end, broken edge.

The hypoblast (*HY.*) is separate all along, and forms a continuous layer. It is specially thickened at the two points where it underlies the extreme anterior and posterior ends respectively. There are now between the hypoblast and the epiblast, towards the posterior end, certain scattered cells, some apparently entirely, others only partially separated from the epiblast. These are the first mesoblast cells which make their appearance.

The figs. 13—16 on Pl. 21 are from transverse sections of the specimen shown in fig. 4. They are drawn on a larger scale, and, taken together with section 17, demonstrate pretty accurately the structure of the specimens figs. 4 and 4 A.

Fig. 13 is taken through the anterior part of the specimen along the line 13 in fig. 4. The central portion only is given in fig. 13 (see fig. 13, *A.*). This section carries out the evidence derived from the longitudinal section (fig. 17), and shows that the under or inner surface of the epiblast is quite smooth anteriorly.

There is no median thickness of the hypoblast, and no sign of mesoblast cells. In fact the anterior region of the embryonal area has not greatly altered from the condition of the circular embryonic disc upon the day previous. The posterior end, however, is very different. Fig. 16 passes through the specimen (fig. 4) along the line 16—that is to say, through the very densest portion of the area.

Here we see the epiblast enormously thickened, and a certain

number of cells seem to be lying separate or forming a loose network between the epiblast and hypoblast. These are mesoblast cells.

As the sections pass more posteriorly this primitive streak knob becomes smaller and ends rather abruptly, as seen in the sagittal section, fig. 17. But forwards the thickening of the epiblast is continued much further, getting less very slowly, as figs. 14 and 15 show, which are taken along the lines 14 and 15 in fig. 4. This narrow band of thickened epiblast is seen as the irregular lower surface of the epiblast in fig. 17. It extends very nearly halfway, along the length of the embryonal area.

I think it is pretty clear that this is part of the same area of proliferation as that through which fig. 16 passes, as will appear more certainly later on. Hence the most anterior point to which this reaches is a point of very great importance. This is marked *A. PS.* in figs. 3 and 4.

The proliferation of cells is so rapid at the posterior end as to cause an eminence in transverse section (fig. 16; evident also in fig. 17). The three sections, figs. 14, 15, 16, differ only in amount of proliferating area and accumulation of cells proliferated. This I take to mean a difference only in intensity. Excepting in intensity there is no difference suggested by the structure of the secondary area of activity in the three sections.

The secondary area of proliferation is now fully established, and has changed in shape from an ill-defined spot in fig. 2 to a more and more elongated area in figs. 3 and 4.

In fig. 5 it has still further lengthened. Figs. 21, 22, 23 are sections taken across this specimen at corresponding spots to figs. 14, 15, 16 respectively, and must be compared one with the other. It may be noticed that there is here extremely little difference in the extension transversely of the area of proliferation. The cells, which appear to be entirely separated, extend further from the middle line on each side, but the area of fusion itself (between epiblast and mesoblast) differs very little. This is especially noticeable in the most

posterior of the sections, namely, figs. 16 and 23. The actual area of proliferation is about the same.

In the anterior sections, if there is a difference, it is that in the more advanced specimen (fig. 5) the sections show a smaller extent of proliferating area than in the younger specimen.

In specimen fig. 5 there is now a very slight groove along the surface of this area, so slight as to be scarcely recognisable in sections fig. 22 (*P. GR.*).

There is another point of interest. Up to this moment the extreme anterior end of the secondary area of proliferation has almost shaded away imperceptibly into the epiblast of the future neural plate, at any rate it has always been the most slender part of the structure. Now it has become a very well-marked spot—it forms almost a knob—and is henceforth recognisable as what other authors have called "Hensen's node." Fig. 21 passes through the centre of this.

Fig. 6 is a later stage still. In this the secondary area of proliferation has attained about its maximum length, and at this moment shows the greatest degree of distinction into different parts: (i) hindermost, a broad area of proliferating surface, its posterior border forming an arc of a circle, while anteriorly it tapers off into (ii) the middle region, a long narrow strip of proliferating area deeply grooved along its length; more deeply grooved anteriorly and less posteriorly (fig. 26). This again passes abruptly into (iii) the most anterior part of the primitive streak or Hensen's node, a very much thickened and compressed region of short extent (vide section, fig. 25).

This stage is the height of development of the primitive streak. The secondary area of proliferation is at this stage a perfectly typical primitive streak, with well-developed primitive groove.

In the preceding figure (fig. 5) the groove is very shallow and wide, and easily recognisable in the whole specimen. In fig. 6 the groove is very deep and narrow, and is only with difficulty seen in surface views as its walls are nearly approximated, and it appears when seen by transmitted light as a

narrow dark line instead of a wide lighter line when seen under similar conditions at the earlier stage.

In all the stages described hitherto there has been no fusion of the hypoblast with the proliferating epiblast. In stage fig. 5 at the extreme anterior end of this area, where there is a special thickening of the primitive streak, the hypoblast is in very close contact (see figs. 20 and 21).

In this stage (fig. 6) the fusion is complete, and anteriorly the hypoblast in the middle line and the epiblast and mesoblast are continuous in the mass spoken of as Hensen's node.

Figs. 24, 25, 26, and 27 are sections taken along the lines 24, 25, 26, and 27 in fig. 6.

A section taken more anterior still than fig. 24 corresponds to figs. 13 and 19.

The last three sections (figs. 25, 26, and 27) should be compared with figs. 21, 22, and 23, and figs. 14, 15, and 16. Here again it will be noticed that there is no great increase or diminution of extent in transverse section of the area of active proliferation. The most posterior parts differ hardly at all except in the spreading of the separated cells (mesoblast).

It must be noticed that there is now a region hitherto not to be found. The section drawn (fig. 24) passes through this region, which is just anterior to the primitive streak.

The section through the middle region (fig. 26; compared with figs. 22 and 15) differs in one particular only (besides extension of mesoblast). Instead of the area of proliferation appearing at the surface on a level with the rest of the outer layer, it lies at the bottom of a deep groove. The sides of the groove are not "fused" with mesoblast; it is only the floor of the groove that is participating in the addition to the mesoblast.

Fig. 25, the section through the anterior region of this area of proliferation, shows an intensified form of the state in the preceding stage (fig. 21). Here the groove suddenly ceases, and even in most instances gives rise to an eminence. This eminence so overhangs the groove posteriorly as to give rise to

a lumen in a section through its posterior half, as shown at *P. GR.* in fig. 25.

The origin of this groove—the primitive groove—I shall discuss in a later paragraph.

The presence of this groove necessitates that the thickened epiblast of the neural plate anteriorly, and the thickened epiblast of the floor of the groove posteriorly, should be at different levels. In this it is possible that any intrinsic growth of the neural plate may tend to produce an overlapping, and so give rise to what has been termed a notochordal canal.

The secondary area of proliferation having now attained its maximum length and most complicated form, it henceforth tends to return to its original shape, and becomes less and less like a typical primitive streak.

In fig. 7 no very great change has taken place as regards the area in question, with the exception that the primitive groove is not nearly so deep or narrow. It resembles more closely in transverse section the stage of fig. 5. Also the layer of mesoblast cells on either side of the primitive groove are thicker now than in fig. 6.

Fig. 8 shows the area still at its greatest length, and figures of sections taken through the anterior end and the middle are given on Pl. 19, figs. 30 and 31, and should be compared with figures of the corresponding sections from the earlier stages.

The most marked difference is the entire absence of the groove. Compare fig. 31 with fig. 26.

The apparent canal into Hensen's node has gone, and the outline of the embryonal area adjoining this region has changed shape. In fig. 4 the outline is arched away from the primitive streak. In fig. 5 it is less arched, in figs. 6 and 7 it is nearly straight, in fig. 8 it is becoming again curved. From this moment the length of the secondary area of proliferation becomes less.

In fig. 10, in which five protovertebræ are visible, this area has diminished to about one third of its former length.

Figs. 32, 33, and 34 are three sections through such a speci-

men. The most posterior, fig. 34, is in no way different from fig. 27, except that the proliferating area may be slightly broader. A similar remark may be offered with regard to the most anterior section, fig. 32, namely, that except the area of proliferation may be slightly broader, it very closely resembles fig. 25.

In both figs. 32 and 34 the mesoblastic plates on each side of the fused area are thicker than in figs. 25 and 27.

The section that passes through the middle of the length of the primitive streak presents the most noteworthy appearance. In this, fig. 33, the breadth of the area of fusion is very much greater than in any of the specimens previously described. There is no groove; in fact, there is, in the place of a groove, actually an eminence (*P. S.*). The cells proliferated are much more numerous and more crowded within a given space as compared with fig. 31, and still more so as compared with fig. 26.

The total length of this secondary area of proliferation is now only about one third of what it was when at its maximum length as in fig. 6. Thus it has apparently become shorter and thicker.

In an older stage, when there are as many as twelve protovertebræ formed, the foundations of the most bulky portion of the body have been laid down. The secondary area of proliferation is now less conspicuous. Its length is not much more than one fifth, if so much, of its greatest attained length.

In stages much later than this it is not possible to observe it in surface views, as such parts as remain are then placed at the extreme tip of the tail, after the complete development of which it disappears.

To sum up—the secondary area of activity arises as a small spot excentrically placed to the primary centre of activity. It increases in magnitude, then becomes elongated, and very much reduced in breadth towards the centre of its length, and is deeply grooved. Rather suddenly, after attaining its greatest length, the groove disappears entirely; the area becomes much shortened, and thicker at the spot where it had been so thin. Now, instead of a groove, there

is a ridge along the median line of the area; it still further shortens and diminishes in size, but does not finally disappear until the last segment has been formed (or until sufficient material has been added to allow of the formation of the requisite number of segments).

The above description draws attention to the most noteworthy features of the primitive streak itself, from the moment of its first appearance. At no time during its existence, or before its existence, is there any trace of any form of concrescence such as Duval has described for the Avian primitive streak. Is it possible to account for the changes as seen in the rabbit by a consideration of the conditions under which the blastoderm of the rabbit is placed?

The following pages contain what seems to me a likely explanation.

The Process of Elongation of the Primitive Streak.

In the exercise of its functions the secondary area of activity is essentially centrifugal in its results. While it is, so to speak, spread out flat upon a plane (as during the stages represented in figs. 3—10), the most noticeable feature of its action is the production in every direction of a sheet of mesoblast, of almost circular outline. When after ten days or so it becomes a freely-growing knob, it produces a mass of cells, the outline of which still is circular, the area itself being now also circular in outline.

If the nature of the area, as shown by its action and by the form assumed when perfectly free, is to be radially symmetrical, why does it assume a linear form? Why does it become distorted? Is it an ontogenetic distortion, or is it connected directly with phylogenetic causes?

I believe the actual elongation, or rather all the changes it undergoes, including the grooving, is due entirely to the ontogenetic influences, and has no recapitulatory meaning. In one sense it may be said to be due to the phylogeny; but only in that, unless the mammalia had been descended from animals which had large yolked eggs, the ontogenetic development of a

rabbit would not, in all probability, have been upon the lines on which it is—provided a rabbit could ever have been evolved under other circumstances. What I mean is: a bird or a reptile has a large egg because the embryo obtains its nourishment from yolk, the presence of which causes the egg to be so large.

A mammal does not require the yolk as a nourishment, and therefore the egg is small. A mammal is enabled to develop within the body of its mother because, we presume, originally the embryo was protected within the membranes of a large egg within the oviduct of the mother, and it was only by the substitution, no doubt very gradual, of (i) placental nourishment instead of the yolk, and (ii) fluids exerting considerable pressure on the uterine walls instead of yolk, albumen, and shell, that it was possible for a mammal to dispense with a large-yolked egg and to be developed under the conditions in which we now find it.

Thus it is to the phylogeny that the conditions under which development takes place to-day are due. Now these conditions were imposed upon the development at a comparatively very recent period in the evolution of the rabbit.

So when we find that certain forms assumed by certain centres of activity, which centres of activity themselves undoubtedly date back to infinitely earlier epochs than the date of these superimposed conditions, are due entirely to the conditions of this day, we must be very careful in drawing any morphological conclusions from those forms assumed. If, for instance, I can show that the changes in form of the primitive streak from a nearly circular spot to a linear expression, and its groove which appears during part of its existence, are due to these present conditions alone, then to draw any conclusions such as frequently are drawn—as, for example, the theory that the line-like primitive streak and its groove represent the lips and aperture of an elongated ancestral gastrular mouth, is impossible.

If the blastodermic vesicle, at the time that the secondary area of activity is established, became an object freely suspended in some fluid, and was under no influence of internal hydro-

static pressure, it seems probable that the immediate effect of this rapid proliferation of cells at one spot would be to produce at first a mass of cells or a knob, as in reptiles, and then a wrinkling of the thin wall of the vesicle, and eventually a projection or outgrowth. This is, indeed, what takes place a little later, when, by reason of the attachment to the uterus of the walls of the blastodermic vesicle immediately surrounding the embryo, the circumstances are such as to fulfil these conditions of equilibrium. The result at this time is that a definite projection and rolling over is effected—the tail fold.

But as yet the blastodermic vesicle is unattached and lies freely within the cavity of the uterus, but is continually expanding in size as described before, owing to the combined effect of increasing hydrostatic pressure and multiplication of the cells of its walls.

I believe that the conversion of the almost circular spot in fig. 2, or pyriform area in fig. 3, to the linear expression of figs. 6 and 7, and back again to the pyriform area of fig. 10, is intimately connected with two facts:

(i) Growth of the embryo taking place from different centres.

(ii) Expansion of the whole area, due in this case (rabbit) to the hydrostatic pressure within the blastodermic vesicle.

In a former paper, "A Re-investigation into the Early Stages of the Development of the Rabbit," I ascribed the apparent growth round the hypoblast as being due to its being carried round by the outer wall, due to the combined effect of the greater area of special activity around the embryonic disc, and increasing hydrostatic pressure within. May not a similar explanation be given to account for the apparent growth outwards of the primitive streak mesoblast from the primitive streak? In this case the area of special activity is much more concentrated and vigorous than in the former, and is therefore itself evident as an area of rapid growth. All the cells towards its periphery will be as liable to be removed by the expansion of the walls as the outer layer cells, as with them the inner mass cells towards the periphery are, according to my hypothesis, apparently removed in the former case.

Having drawn attention to this possibility, I must leave the consideration of the mesoblast for the present.

What are the conditions under which the blastodermic vesicle exists during the lengthening of the primitive streak and the formation of the deep primitive groove?

In the previous paper mentioned above, I have accounted for various changes in shape of the blastodermic vesicle, as well as the apparent growth of one layer over another, by pointing out how these changes could be produced by increasing hydrostatic pressure within, together with more rapid cell-division in one part, and less rapid cell-division in another part.

So, I believe, the same principle holds good with regard to the lengthening of the secondary area of proliferation into a streak, and to the production of the median groove.

The blastodermic vesicle is still expanding rapidly by reason of the increasing hydrostatic pressure. The walls of the vesicle are thin except at one point, the embryonic disc, where the wall is thick and compact. Somewhat suddenly, a spot at the posterior edge of this embryonic disc becomes extremely active—the secondary area of proliferation. This gives rise, as we have seen, to a mass of cells produced very rapidly at one spot (fig. 3), forming a second compact disc.

We have now two very distinct compact masses in the outer layer or epiblast, one formed by the presence of the inner layer of epiblast and its fusion with the outer layer, the other by the very rapid proliferation at one spot caused by the appearance of the secondary growing point or primitive streak. While this is proceeding, the whole blastodermic vesicle is still expanding from the hydrostatic pressure within. Now what effect, if any, will this hydrostatic pressure have in regard to these two compact masses? In the first place there will be a tendency for them to part. There will be a tendency to part from another cause, namely, the counteracting effect of their respective growths; but this would tend to produce a space (i. e. mass of cells) between the primary growing point and the anterior border of the secondary growing point (i. e. anterior end

of primitive streak). This takes place, no doubt, and is a very conspicuous feature at a later stage, as described later, but it does not account for a lengthening of, and within, the secondary area of proliferation itself.

The lengthening of the, at first, circular patch into a streak, i. e. the change from fig. 11 to fig. 12, I believe to be due almost entirely to the expansion of the blastodermic vesicle by the hydrostatic pressure within.

The expansion of the vesicle is quite sufficient to allow of this. It is not easy to make very satisfactory measurements in support of or against this view. If we take a specimen about the stage shown in fig. 3, we find the secondary area to measure not less than ·38 mm. antero-posteriorly, and the diameter of the whole vesicle about 4·5 mm. to 5 mm.

If we take another specimen in which the secondary area has attained its maximum elongation, we find the area to measure about 1·08, which is rather less than three times the length it was in stage fig. 3. The diameter of such a blastodermic vesicle is about 10 mm. to 12 mm., which is from rather under two and a half to rather over two and a half times the length it was in stage fig. 3.

I may, perhaps, make my meaning clearer by reference to figs. 11, 12, Pl. 20.

In fig. 11 the point *M* is the centre of the primary area of cell-production, i. e. the centre of the embryonic disc. The point *N* is the centre of the secondary area of cell-production. The grey represents the circular embryonic disc; the white represents the proliferating area of the primitive streak.

Really this diagram represents no actual stage. Fig. 3 is very near it. If my account of the course of events is correct, there never could be a stage which would be exactly represented by this diagram, although it theoretically represents the condition.

Now the tendency of each of these two areas considered separately would be to expand equally in all directions, from the centre *M* in the one case, and *N* in the other. But these two areas are not separate, they even overlap, and therefore

they must have some disturbing effect upon each other; and the line along which that disturbance will be most marked will be along a line drawn from the centre of the one to the centre of the other—from *M* to *N*.

I have argued before that while the increasing hydrostatic pressure within the blastodermic vesicle causes its expansion, yet a more rapid expansion of one part of the wall than another may be brought about by the more rapid cellular growth of that part of the wall. In other words, areas of weakness are produced upon which the hydrostatic pressure takes more effect than elsewhere.

So at this stage must there not be a line of weakness between the two points *M* and *N* in diagram 11, an area along, at any rate, that portion in which the influences of both centres are at work, in which there will be less resistance offered to the expansive force of the hydrostatic pressure than elsewhere? It seems to me that this is extremely likely, and that a figure such as that represented in diagram fig. 12 must be the result.

The anterior half or portion of the secondary area of proliferation will be drawn out as indicated in the diagram by the separation of the black dots; the posterior portion, being uninfluenced by the primary area, will not lose its radial symmetry.

Similarly the embryonic disc will be influenced, and its hinder portion drawn out as indicated by the black crosses on the two diagrams, its anterior border not losing its radial symmetry. It must be remembered, however, that the secondary area of proliferation is in its function of cell-production more vigorous now than the primary area, and becomes still more vigorous for the next two days.

Also the visible line of the primitive streak does not even at this comparatively early stage represent the whole of the effects of the secondary area of proliferation. No doubt the epiblast immediately surrounding it owes its origin to the energy of the area in question, but to what extent it is not easy to determine. At any rate it is probably not less than that part beneath which primitive streak mesoblast lies. So that a more accurate figure of the conditions of the two areas of activity respectively

would probably be such as I have drawn in fig. 43, on Plate 22, to be explained presently. I conclude, therefore, that the lengthening of the primitive streak is due to the expansion in an antero-posterior direction of the most anterior part (half?) of the secondary area of cell-proliferation due to the conditions under which the embryo is developing.

May not the primitive groove be also due to ontogenetic conditions?

It will be noticed that it does not exist during the early stages of the formation of the primitive streak (vide figs. 1—4 A, and the sections of these).

It will be noticed also that it is at its greatest development at the time the primitive streak reaches its greatest length, and from that moment it becomes shallower and very rapidly disappears altogether (vide fig. 8 and sections). It is deepest and most distinct along that part of the streak which is thinnest, and gradually shallows and disappears towards the thicker posterior end of the streak.

The sections through the stages in figs. 4, 5, and 6 show that the groove commences at the same time as the spreading of the mesoblast.

The mesoblast is a reticulum connected with the area of proliferation, and is seen to spread out from both sides of the drawn-out proliferating area.

I have explained above how this spreading out of the mesoblast may be due to the general expansion of the walls of the vesicle of this region by the increasing hydrostatic pressure. If the proliferating area produces cells more quickly than they can be removed laterally by the expanding walls of the vesicle, a heap of cells will be the result at this spot, or if the proliferating area is linear a ridge will be formed. If the cells produced exactly balance those removed, the surface will remain flat; there will be no ridge.

But what will happen if the cells produced along the proliferating area are removed more rapidly than they are produced? If the deficiency of production is very great, then probably the cells—few in number—will be torn apart and removed as

isolated cells. But if the deficiency is only slight, surely the result will be that the meshes of the reticulum of mesoblast will be drawn out into larger meshes, and the connecting filaments become finer.

It seems to me that this must mean that a tension between cell and cell must be produced, and not only between cell and cell of the reticulum, but between the reticulum on each side of the primitive streak and the actual proliferating area itself. Is it not possible for this tension to produce such a groove as that shown in figs. 22 and 26?

Posteriorly there is no groove. Here the supply of cells is greater than the removal, and a heap is formed; but along the narrowest part of the proliferating area the conditions favour the formation of a groove as I have suggested, for not only is the proliferating area very attenuated, but lateral dragging of the mesoblast is in the same direction along a considerable length.

It follows that upon the above explanation of the lengthening of the primitive streak and formation of the groove any increase in activity of the proliferating area might lead to the obliteration of the groove, and even perhaps a cessation of the increase in length of the streak.

Also any modification which either prevented the increase of hydrostatic pressure within the vessels, or which prevented the portion of the wall containing the embryonal area from being affected by the increase of hydrostatic pressure, would bring about the obliteration of the groove and cause the cessation of lengthening of the streak. In the rabbit it seems probable that the latter event occurs.

During the stages represented by figs. 1 to 5, that is during the formation of the greater length of the primitive streak, the blastodermic vesicle lies freely in the uterus. About the age represented by fig. 7 the papillæ are appearing by which the lower pole becomes attached to the obplacental portion of the uterus. The ectoplacental area is thickening, but is still quite smooth and quite free to slide over the surface of the placental lobes; but between that time and the time represented by fig. 8, the ectoplacenta has become irregular, the albumen

layer has ruptured, and the ectoplacental region is firmly attached to the placental lobes.

From this moment the upper pole of the blastodermic vesicle can only be expanded by the hydrostatic pressure as much as the placental lobes and the thick mesometrial wall of the uterus will allow it. As is well known, this portion of the uterus expands now very little and slowly; by far the greater part of the swelling of the blastodermic vesicle concerns only the ab-embryonic pole of the vesicle and the obplacental lobe of the uterus.

In this way a very large part, if not all, the tension is rather suddenly removed from the actual embryonal area and immediately surrounding tissues, and this is exactly coincident with the attainment of the maximum length of the primitive streak and with the rather sudden obliteration of the primitive groove.

No doubt to all appearances the primitive streak of a bird is at the time of its greatest development extremely like the primitive streak of a mammal of the type of development such as we find in the rabbit.

If we accept Duval's description for the bird, and also the explanation I have offered above for the rabbit, as being both correct, we are bound to conclude that it is really only a coincidence that the secondary area of cell-production in each case should assume a linear form. Such a coincidence is unlikely, though of course not impossible.

An Attempt to determine which Portion of the Embryo is derived from the Cells proliferated from the Primitive Streak.

Those who have followed my description of figures will have noticed that, according to the account, there is one part of the embryo laid down as the immediate result of the segmentation of the ovum, and that the most conspicuous part of this is a circular patch—the embryonic disc. Subsequently a renewed activity of cell-production takes place upon the posterior border of this embryonic disc, giving rise to new tissue, and

continues active till the formation of the extreme end of the tail is completed.

Is it possible to determine approximately which parts of the embryo are formed by the two centres of growth respectively? Evidence may, I think, be brought to show that the primitive streak in the rabbit is the growing point of the whole of that part of the animal which is situate posterior to the head, while from the primary centre of activity is formed the part of the embryo anterior to the first protovertebra. In other words, the secondary centre of growth is responsible for the metamerically segmented part of the animal.

During the later stages of development the line of demarcation between the two—which perhaps is never absolutely definable—becomes less and less distinct. For instance, the heart, which is formed distinctly in the region of the primary centre of growth, becomes located more posteriorly; while parts of the nervous system, owing to the more rapid growth of the neural tube, become moved forwards, so as to bring into the primary region portions whose origin has been due to the secondary area of activity.

If we regard the secondary area of proliferation or primitive streak simply in its functional capacity, neglecting for the moment all preconceived ideas of its morphological or recapitulatory meaning, and consider it only as we find it in the rabbit embryo, it seems to me that we are bound to describe it as the visible expression of an area of intense protoplasmic activity, which is continuously budding off cells, the most conspicuous of which are those from its lower surface, the primitive streak mesoblast cells. These cells, according to my hypothesis, are rapidly removed from this area, being carried away by the expanding wall of the blastodermic vesicle, to which they are closely approximated. This part of the wall is enabled to respond to the expanding influence of the hydrostatic pressure within, by itself receiving rapid additions of cells from the proliferating area.

On this hypothesis we may take the outline of the primitive streak mesoblast as indicating also the outline of epiblast

derived from the primitive streak, at any rate for the stages up to about the one drawn for fig. 6. At this time mesoblast cells are separated from the hypoblast, both at points where there is already existing primitive streak mesoblast, and at points where up to now there has been no mesoblast, e. g. under the part of the embryonal area in front of the primitive streak. After this stage it is extremely difficult to determine in many places what the origin of the mesoblast cells has been. As, however, this involves the question of the formation of the whole mesoblast, I must leave the further discussion for another paper.

I must now refer again to the figures of the embryonal area (figs. 3 to 6). I have given above my explanation of the change in shape, and of the process of elongation of the proliferating area.

Has the whole difference in length between the embryonal areas (figs. 5 and 6) been due to the elongation of the primitive streak itself?

In the following paragraphs the measurements given are taken from measurements made upon photographs of the specimens, drawings of which are given in figs. 3, 4, 5, 6, 8, and 10. The measurements, reduced to their natural magnitude, are given in millimetres.

It is evident that growth has taken place somewhere, for the embryonal area in fig. 6 measured 1·72 mm., while fig. 5 measured but 1·38 mm. It is due hardly at all to lengthening of the primitive streak area, as the primitive streak area of fig. 6 measured 1·02 mm., and that of fig. 5 measured 1 mm. Of course one must not suppose that the primary area of growth has ceased to produce effects. Its effects hitherto have been to produce a circular patch. If we suppose it still to exercise a like influence the diameter of the head plate will have increased anteriorly the same as it has from side to side.

Fig. 5 measures transversely ·94 mm. Fig. 6 measures transversely 1·1 mm. Therefore we may say that at least ·16 mm. of the length may have been due to growth of the anterior

portion. So by adding to 1·38 mm. (length of fig. 5) the sums of ·02 mm. due to lengthening of the primitive streak and ·16 mm. the amount due to growth of anterior end, we have ·16 mm. still to account for to produce the length shown in fig. 6.

Where has this extra length been acquired? In transverse section it is evident that there is now a region which was not to be found in the preceding stage.

A section through this region is given in fig. 24. The most noteworthy feature is the notochordal thickening. A few sections further forward this thickening is quite absent. It is surely legitimate to ascribe the appearance of this new region as being the result of cells budded off from the front end of the primitive streak. This becomes much more evident in the later stage (fig. 8). In this the total length of the embryonal area is about 3·11 mm.

Fig. 6 measured 1·72 mm. There has therefore been an increase of 1·39 mm. The proliferating area measured the same as in fig. 6, namely 1·2 mm. There is at the anterior part of the embryonal area a very well marked circular area. The neural groove along this area is narrow, while the neural groove along the embryonal area between the above-mentioned circular area and the anterior end of the primitive streak is broad and shallow. The junction between the two corresponds almost exactly with the circumference of the circular area at the point *Z*.

Does this anterior circular patch represent either exactly or approximately the area of influence of the primary centre of activity? I wish to point out the possibility of all that part between the anterior end of the primitive streak and the posterior border of the circular anterior area (namely that part of the embryonal area which is marked by the shallow, broad, neural groove) having been formed entirely by the activity emanating from the primitive streak area, or, to be more exact, the anterior and antero-lateral parts of that area of proliferation; the parts of the embryonal area in front of this region having been formed chiefly as the result of the activity

of the primary centre of growth (the most anterior portion entirely from the primary centre, the most posterior possibly from both).

The circular area measures transversely 1·2 mm. Therefore we may suppose that at least ·1 mm. of the increase in total length of the whole embryonal area may be due to the activity of the primary centre. This leaves ·97 mm. to be accounted for.

If we measure 1·2 mm. (the total length assumed to be due to the primary area of activity) from the anterior end we come to a spot *Z*. The space between this spot *Z*. and the anterior end of the primitive streak, *A. PS.*, measures ·97 mm. This seems to me to be evidence that this space, *Z*. to *A. PS.*, is the area which has been added between the stages fig. 6 and fig. 8. The question remains, where have the cells composing this portion of embryonal area originated? They must have arisen either (i) from an area of rapid proliferation in front, or (ii) from a general area of proliferation in situ, or (iii) from a general area of proliferation behind. Absolute proof is, as far as I can see, impossible, but I will take each alternative separately, and give my reason for believing that the latter only can be the true solution.

(i)—(*a*) From the very first moment of the development the effect of the primary centre of activity is in the ontogeny of the rabbit to produce an embryo of a radially symmetrical figure, whereas the area in question between *Z*. and *A. P. S.* shows no sign of a radial symmetry.

(*b*) By the time that the area in question is developed, and while it is being developed, there is no sign of one spot from which all pre-existing structures could obtain material.

For these reasons I think growth does not take place in front.

(ii)—(*a*) Growth of the area in question is, comparatively speaking, rapid, but there is no sign of the rapid multiplication of undifferentiated cells, every cell within this area is distinctly either epiblast, hypoblast, or mesoblast.

(*b*) This area continues to grow rapidly in length, and the "mesoblastic somites," which are a characteristic feature of

this area, are continually being added on posteriorly, showing that the differentiation is at any rate taking place from before backwards.

(*c*) If this is a centre of growth there should, under the circumstances, be some sign of a radial disposition of tissue, which there is not.

For these reasons I think we cannot conclude that there is any positive proof of a rapid addition of new cell material, although an arrangement of the mesoblast cells into mesoblastic somites undoubtedly takes place in situ.

(iii) Since, therefore, we cannot find probable proof of growth of new segments either anteriorly or in the area in question itself, and since we do find an area immediately behind the area in question and continuous with it in which there is an undoubted rapid proliferation of cells (which are all similar and connected, and only become epiblast, hypoblast, or mesoblast according to which region they may adjoin), I think it may be considered that there is very good evidence indeed that the growth of the embryo of the area in question has taken place by the addition of indifferent cells from this area —the primitive streak.

Although such measurements as the above are useful in support of my contention, they are not satisfactory, because every specimen of exactly the same stage is by no means always exactly the same size.

A very useful landmark is supplied by the band of tissue, or rather an area which gives rise at first to the mesoblast forming the pericardium, and rather later the endothelial lining of the heart. This area is clearly marked as a circular band in fig. 10, *PC*.

In this stage the neural tube, owing to its more rapid growth, has become folded forwards so as to hide the anterior part of the pericardial band *PC*.

In fig. 28, Pl. 20, which is a median sagittal section of this specimen, it is seen at the point *PC*.

In fig. 9, an earlier stage, before the neural tube has become thrust forward, this band is seen to follow the contour of the

neural tube (*NP.*). So also, but less distinctly, in the earlier stage (fig. 8).

In the stages earlier than this it is not perceptible in surface views, but may be easily recognised at its first appearance in stages like figs. 4 and 4 A.

At this time—its first appearance—it shows itself as a slight tendency to a more rapid growth of the hypoblast immediately underlying the anterior and lateral edges of the embryonal area.

Figs. 17 and 18 show the anterior edge cut at *PC.*

Sections 13 and 14, if continued to the edges, would show the same slight thickening of the hypoblast.

Figs. 35—38, Pl. 21, show the subsequent history of this thickening.

Fig. 35 is a transverse section through the lateral edge of the anterior part of the embryonal area of a stage between those represented in figs. 4 and 5. It is in front of the primitive streak area. There is as yet in this region no mesoblast. The hypoblast (*HY.*) shows, however, signs of increased activity, and is thicker than before. This is specially the case at the edge of the thickened epiblast.

Fig. 36 is a slightly older specimen, still rather younger than fig. 7. Here the hypoblast (*HY.*) is thickened over the whole area, extending under the thickened epiblast, but more especially so at the edge of the epiblast.

A certain number of cells are to be seen lying between the epiblast and hypoblast, marked *PC.* in my drawing. These I believe to have been budded off in situ from the hypoblast (*HY.*). Cells are budded off from the hypoblast all over this area of the anterior part of the embryonal disc, but more thickly at this region round the edge of the thickened epiblast than elsewhere. Fig. 37 is a section from the same region of a later stage, a stage with two mesoblastic somites (vide fig. 9).

There seems to be here still a slight proliferation of cells from the hypoblast of this region, but not so great as before. The cells formerly budded off, which we can call mesoblast, have become arranged so as to leave a slight cavity between

them (*PC.*). This cavity gives rise to the pericardial cavity, the inner thick wall to the muscular wall of the heart, and peritoneal lining of the cavity around the heart; the outer thinner wall to the peritoneal lining of the rest of the pericardial cavity. The cells, which seem now to be budding off from the hypoblast of this spot (marked *END. H.*), give rise, I believe, to the first of the cells which, in fig. 38, are seen to be forming into a tube, which is the endothelial lining of the heart (*END. H.*).

Fig. 38 is from an older stage, an embryo with seven mesoblastic somites, a little older than fig. 10. Although it is not possible to give a decided opinion, I am inclined to think that at this stage cells are still being budded off from the hypoblast to form the endothelial lining of the heart.

At the same time I must mention that at an age intermediate between figs. 37 and 38 I have found a stage in which a mass of cells lies between the *PC.* cells and the hypoblast cells, showing no trace of a budding off either from the hypoblast or the mesoblast mass marked *PC.*

In fig. 38 the endothelial cells may be seen to be attached to both the pericardial cells and the hypoblast cells, though the latter give one the impression of being concerned in the production of the cells in question rather than the former.

This band, originating as a thickening of the hypoblast at a time when the embryonal area is hardly at all affected by the primitive streak activity, seems to be of great use in preserving the outlines of the embryo due to the primary centre of growth.

Now no part of the pericardial thickening seems to be affected in the growth of the embryo caused by the secondary centre of activity, the primitive streak, excepting that it is absent posteriorly. Where it is present, that is, anteriorly and laterally, it forms almost an accurate "segment" of a circle, the circumferential boundary of which extends through about 270°, or three quarters of a whole circle.

From this I argue that all the parts of the embryo which are formed within the outer peripheral boundary of the pericardial

band may be ascribed to the activity of the primary centre of growth (segmentation of the ovum) ; all posterior to this due in the main to the secondary centre of growth (primitive streak). Referring again to fig. 8, it will be seen that this again indicates the point *Z.* as the line of demarcation between the two areas. This spot is marked by a very distinct difference in the character of the neural groove, which is seen in the later stages also. It is at this spot that the first protovertebræ are formed (vide fig. 9).

As development proceeds, the fore-brain, whose outline is in figs. 8 and 9 concentric with that of the pericardial band, becomes thrust forward (vide fig. 10), so as when viewed from above to be no longer concentric. So also there is a similar thrusting forward of the whole of the dorsal portion of the embryo, including the protovertebræ, and the pericardial band no longer serves as a landmark of the same character.

The Shortening of the Primitive Streak.

Although the primitive streak becomes very much shortened, I am not at all sure that its area is diminished during the process.

The hinder end of the streak is about the same width throughout its existence up to such a stage as fig. 10, and somewhat later, but the elongated anterior portion is extremely narrow. After the shortening the anterior part is almost as wide as the posterior part. So I doubt whether there is any diminution of area during the contraction.

After the cessation of tension has been effected by the close attachment of the surrounding walls of the blastodermic vesicle to the uterus, the result of growth must be soon to cause pressure of the nature of a thrust in the tissues which are most actively growing. This, we know well, brings about a very considerable thrust in the direction of the longitudinal axis of the embryo, causing the head and tail folds.

There is also the tendency that has all along existed for the secondary area of proliferation to be radially symmetrical. This, together with the pressure which is known to exist in the direction of the longitudinal axis, seems to be quite sufficient cause to

bring about the compression of the primitive steak, without assuming a conversion of it in situ, as it were, into the embryo.

On Pl. 22 will be found six diagrams which illustrate my conception of the lines of growth between the stages of figs. 1 and 8 of Pl. 20.

Fig. 40 represents the embryonic pole of the blastodermic vesicle before there is any visible sign of the appearance of the secondary area of cell-production. The grey central area is the embryonic disc. The vesicle is expanding approximately equally in all directions; this is indicated by the concentric circles round the embryonic disc. The dotted line is an imaginary line drawn outside the embryonic area. All parts of the vesicle outside this line are only slightly affected by the subsequent origin of the secondary area of cell-production, as seen by the next diagrams.

In fig. 41 the secondary centre of activity has become established, its centre being about the spot marked with a cross in the posterior region of the embryonic disc (grey). The effect is as yet slight, leading to a little more rapid expansion of that part of the embryonic disc indicated by the ellipticity of the lines of growth. This represents the stage of fig. 2, Pl. 20.

In fig. 42 the activity of the secondary area of cell production has become more intense. It is very concentrated, and is now marked by a heaping-up of cells. The outline of this actual thickening must not be taken as being the boundary of the part of the wall of the vesicle due to the secondary centre of activity, for the outermost cells produced thereby will no doubt be stretched and flattened.

Fig. 43 represents a stage intermediate between figs. 5 and 6 on Pl. 20. The primitive streak is at its greatest development. The anterior part of the embryonal area, which owes its existence to the primary centre of activity alone, is shown to have its posterior borders distorted as explained in the earlier part of this paper. It is practically the same condition as that illustrated by fig. 42, but more pronounced. The outline of the part of the wall of the vesicle due to the secondary centre of activity is de-

rived from the outline of the primitive streak mesoblast, which upon my hypothesis would approximately mark this area.

Immediately in front of the anterior end of the secondary area of activity (i.e. in front of the front end of the primitive streak) there will be a lesser tendency to expansion, and accordingly a heaping-up of cells proliferated from the front end of the primitive streak, forming the thickening known as "Kopffortsatz." From this moment the increased intensity of action of the secondary area of cell-production over the primary is shown by the fact that the outline of the former increases its diameter four times by the stage illustrated by fig. 8, whereas the latter's increase is scarcely perceptible.

Fig. 44 illustrates the conditions of fig. 8. The two centres of growth have become further removed from each other by the interposition of new cell material proliferated chiefly by the secondary area. In other words, growth in length of the embryo has now very markedly taken place.

This growth in length is represented in the diagram by the area within the curves of which the one marked 1 is the outermost. The region of the embryonal area due to the primary activity has recovered from its temporary distortion, and has regained its radial symmetry to a great extent. This can be detected in the series of drawings figs. 1—8.

The secondary area of activity also, upon the diminution of the tension to which it had been subjected, tends also to assume a more radial form (fig. 45, with which fig. 10 may be compared). Ultimately the counteracting effects of the two centres cause each to become tilted over, so that instead of lying in the same plane they lie in different planes parallel to each other, and at right angles to the original common plane. In fact they assume their natural positions.

On this explanation it is clear that most, if not all, of the ectoplacental region is really derived from the primitive streak, which may perhaps account for its much greater activity than that part of the blastodermic vesicle which arises directly from the primary centre of activity.

EXPLANATION OF PLATES 20—22,

Illustrating Mr. Richard Assheton's paper on "The Primitive Streak of the Rabbit; the Causes which may determine its Shape, and the part of the Embryo formed by its activity."

List of Reference Letters.

A. Anterior end. *AM.* Amniotic cavity. *A. PS.* Anterior end of primitive streak. *END. H.* Endothelial lining of heart. *EP.* Epiblast. *HN.* Hensen's node. *HY.* Hypoblast. *M.* Centre of primary area of cell-production. *MES.* Primitive streak mesoblast. *MES. HY.* Hypoblastic mesoblast. *N.* Centre of secondary area of cell-production. *NCH.* Notochord. *NG.* Neural groove. *NP.* Neural plate. *P.* Posterior end. *PC.* Pericardial band. *P. GR.* Primitive groove. *P. PS.* Posterior end of primitive streak. *PS.* Primitive streak. *Z.* Point which marks approximately the boundary between the results of the primary centre of growth and that of the secondary centre of growth.

PLATE 20.

Fig. 1.—Surface view of the embryonic disc of a rabbit embryo of the 150th hour. × 18.

Fig. 2.—Surface view of the embryonic disc of a rabbit embryo, at the earliest moment at which the primitive streak activity is perceptible as a thickening. Age about 158 hours. × 18.

Fig. 3.—Surface view of the embryonal area of a rabbit embryo, in which the primitive streak is very evident. Age about 168 hours. × 18.

Fig. 4.—Surface view of the embryonal area of a rabbit embryo, in which the process of lengthening of the primitive streak is well advanced. Age about 168 hours. × 18.

Fig. 4A.—Surface view of the embryonal area of a rabbit embryo, slightly more advanced than the preceding. Age about 168 hours. × 18.

Fig. 5.—Surface view of the embryonal area of a rabbit embryo, in which the primitive streak has become greatly elongated and is grooved slightly. Age about 172 hours. × 18.

Fig. 6.—Surface view of the embryonal area of a rabbit embryo, in which the primitive streak has attained its maximum length, and is most deeply grooved. This figure has unfortunately been drawn slightly larger than the photograph from which it was copied, on which the measurements in the text were made. The error is about $\frac{1}{15}$. Age about 180 hours. × 18.

Fig. 7.—Surface view of the embryonal area of a rabbit embryo, in which the primitive streak has become much shallower. Growth in length of the embryo has now taken place. Age about 188 hours. × 18.

Fig. 8.—Surface view of the embryonal area of the embryo of a rabbit. The primitive groove has entirely disappeared. The ecto-placental region is now firmly attached to the placental lobes of the uterus. Age about 192 hours. × 18.

Fig. 9.—Surface view of embryonal area of the embryo of a rabbit. Age about 196 hours. × 18.

Fig. 10.—Surface view of the embryonal area of the embryo of a rabbit. The primitive streak has now become compressed to its former dimensions; the mid-dorsal portions of the embryo have become thrust forward, and the head-fold has commenced to be formed. Age about 200 hours. × 18.

Fig. 11.—Diagram to illustrate the relative positions of the two areas of cell-production.

Fig. 12.—Diagram to illustrate the effect produced upon the two areas of cell-production by the increasing hydrostatic pressure within the blastodermic vesicle.

Fig. 17.—A sagittal section through the specimen of which Fig. 4A is a drawing. It is taken along the line 17. × 100.

Fig. 18.—A median sagittal section through a specimen intermediate between Figs. 5 and 6. × 72.

Fig. 28.—A median sagittal section through Fig. 10. × 54.

Fig. 39.—A horizontal section through a portion of the sheet of mesoblast surrounding the primitive streak. The portion drawn was a lateral portion. × 350.

PLATE 21.

Figs. 13, 14, 15, 16.—Transverse sections through specimen Fig. 4, along lines 13—16. × 175.

Figs. 19, 20, 21, 22, 23.—Transverse sections through specimen Fig. 5, along the lines 19—23. × 175.

Figs. 24, 25, 26, 27.—Transverse sections through the specimen Fig. 6, along the lines 24—27. × 175.

Figs. 29, 30, 31.—Transverse sections through a specimen similar to that drawn in Fig. 8, along lines corresponding to those marked 29, 30, 31, in Fig. 8. × 175.

Figs. 32, 33, 34.—Transverse sections through the anterior end, the middle, and posterior end of the primitive streak of a specimen slightly older than that of which Fig. 10 is a drawing. × 175.

Fig. 35.—A transverse section through the edge of the embryonal area of a specimen rather older than Fig. 4, on a level corresponding to the line 13. × 175.

Fig. 36.—A transverse section through the corresponding region of a specimen like Fig. 7. × 175.

Fig. 37.—A transverse section through the corresponding region of a specimen like Fig. 9. × 175.

Fig. 38.—A transverse section through the corresponding region of a specimen rather older than Fig. 10. × 175.

PLATE 22.

Fig. 40.—Diagram showing lines of growth, i. e. expansion of blastodermic vesicle of the embryonal area and surrounding parts of a rabbit embryo, corresponding to Fig. 1, Plate 20.

Fig. 41.—A similar diagram of a slightly later stage, corresponding to Fig. 2 at the time of the first appearance of the secondary centre of growth, which is placed eccentrically to the centre of the primary area of growth.

Figs. 42, 43, 44.—Similar diagrams illustrating the increase in importance of the secondary centre of growth. These correspond to Figs. 3 or 4, between 5 and 6, and to 8.

Fig. 45.—A portion of a similar diagram of the posterior end only of the embryonal area of a specimen, corresponding to Fig. 10, or a little later.

On the Growth in Length of the Frog Embryo.

By

Richard Assheton, M.A.

With Plates 23 and 24.

In some previous papers on the development of the rabbit I have attempted to show that there are two main centres of growth, each in itself tending to produce a radially symmetrical form; but since these two centres of growth are situated eccentrically to each other the resulting embryo is cylindrical, and subsequently bilaterally symmetrical.

I endeavoured to show that no concrescence occurred in the rabbit, and that no theory of concrescence was necessary to account for the facts.

I wish now to indicate the manner in which two centres of growth can also bring about the corresponding results in the frog embryo, without any concrescence of the dorsal lips of the blastopore.

In a paper in this Journal Dr. Robinson and I discussed the question of the formation of the archenteron in the frog, and came to the same conclusion as Moquin-Tandon (in Anura) and Houssay (in Axolotl), that the archenteric cavity was due to a splitting amongst the cells in situ, and not to an invagination or overgrowth of surface cells.

Recently Jordan, and Umé Tsuda and Morgan have made very interesting communications upon the subject.

The former author, after an able summing up of the evidence on both sides, concludes (p. 331), "The evidence thus far adduced

for invagination is, to say the least, inconclusive;" and on the other hand (p. 332), "There is not a shred of evidence to show that the large cells at first surrounding the mouth of the blastopore are not subsequently pushed in by the ingrowth of ectoblast cells. No positive evidence whatever exists to prove either the impossibility of invagination or the likelihood of no invagination. I find it difficult to gather the reasons that have influenced Houssay and Robinson and Assheton to adopt the view that invagination does not occur."

Jordan then describes (pp. 333–4) "ocular evidence that the small cells around the lips of the blastopore are actually infolded."

Morgan, after a description of interesting experiments after the method of Roux upon the living egg, says, "The statement of Robinson and Assheton that no portion of the archenteron in the anura is formed by invagination is certainly incorrect, as I hope to show in a later paper."

Of course this latter statement must depend upon the exact meaning to be attached to the word archenteron.

My own conception of the term archenteron is that cavity which in the embryo is supposed to represent the digestive cavity of a hypothetical ancestral "gastrula," no matter how this cavity was brought about.

If, however, by archenteron is meant any subsequent prolongation of this cavity, such as would represent a post-gastrula condition ancestrally, then certainly such a statement was inaccurate.

When Dr. Robinson and I made the statement referred to, we regarded as archenteron part of the cavity which I now consider to represent a post-gastrula condition. In other words, I agree with Morgan and others to a certain extent as regards the growth over of the dorsal lip of the blastopore, and consider only the most anterior part of the gut cavity of the frog's embryo at the time of the closure of the blastopore as being formed by a splitting, and as representing the true archenteron.

Accordingly, in my opinion, the sentence (referred to above) by itself accurately describes the facts, but in the context in

which it stood I admit that I now think it inaccurate. My reasons for so thinking I will now proceed to give.

Again, Morgan and Umé Tsuda say that we "apparently at the outset have orientated the embryo wrongly, for they state the segmentation cavity has a roof which ultimately becomes the anterior wall of the gastrula; for the anus which marks the posterior end of the embryo appears at the opposite side of the ovum,—that is, on the floor of the segmentation cavity."

I cannot understand their objection to this paragraph.

Figs. 7 to 11 on Pl. 24 are all placed with what we conceive to be the dorsal surface (*D.*) directed towards the top of the plate.

As regards the ocular evidence of an invagination spoken of by Jordan, it is a pity that more details are not given of the observations.

Is it possible to trace a cell, or a spot on the surface some distance from the lip of the blastopore, to gradually approach and fold over the edge and so disappear, or do only cells actually on the edge seem to be affected by the process?

What is the cause of the invagination? I can quite well imagine that individual cells at the edge may, by multiplication of their neighbours or themselves, be pushed over the edge, as also might cells on the inner edge appear to be pushed outwards, if we could see that edge. The splitting theory still seems to me to be the more probable for the commencement of the archenteric cavity and its extension forwards.

But, as I shall point out a little further on, there is undoubtedly an apparent overgrowth, and I think certainly an actual overgrowth of the lower pole cells by the dorsal lip of the blastopore, together with the lateral and ventral lips, as they are formed at a later period. This process, however, should not, I think, be compared with the process of gastrulation so called, or formation of primitive archenteron, but should be considered to be intimately connected with the growth in length of the embryo. In other words, to follow the same line of argument that I have used in the description of the rabbit embryo, the

formation of the primitive archenteron is by a process of splitting, and is the direct effect of the primary centre of growth; whilst the continuation of the cavity produced by an overgrowth is the direct effect of the secondary centre of growth, producing the elongation of the animal.

The splitting process in the frog corresponds in results to the invagination process of Amphioxus, while the overgrowth of certain parts of the white pole of the ovum of the frog by the dorsal, and subsequently lateral and ventral lips of the blastopore, together with the continuation of this process in the formation of the tail, corresponds to the elongation of the gastrula in Amphioxus, by means of what Hatschek called the polar cells.

I shall now attempt to explain what I believe to be the actual method in which the splitting is brought about.

The frog's egg segments, as has been described by many observers, more rapidly at one pole than the other. This is, I think, universally supposed to be due to the greater accumulation of yolk granules at the "lower" pole, which thereby hinder the segmentation activity at that pole.

If we admit that "yolk" determines the inequality of the process known as segmentation, we must admit it also in the case of each cell. If it is true of the segmented ovum, it is equally true of the unsegmented ovum. To say that yolk being more plentiful in one part of a cell than in another hinders the activity of the protoplasm, is the same as saying that a cell divides into two parts, which in magnitude are in inverse ratio to the purity of the protoplasm contained. In other words, the result of a simple process of cell division, such as we see in the segmenting ovum, is two cells equally balanced as regards protoplasmic energy.

Fig. 15 on Pl. 24 is a diagram of a vertical section of the unsegmented ovum of the frog.

The circles 1 to 7 represent diagrammatically what I imagine to be the distribution of yolk, as determined from a consideration of the segmented ovum.

The space No. 1 is that region in which segmentation is most retarded, and so presumably the region in which yolk is

most abundant. The space No. 2 contains less yolk to a given area than No. 1, No. 3 less than No. 2, and so on.

For the sake of simplicity we may regard the outer space only. This may be supposed to contain protoplasm of a uniform degree of purity. Accordingly division of this space will be such as to produce two spaces whose areas are equal. This is about the spot marked by the line (*a*), and will represent the third furrow of segmentation, that is the first horizontal furrow.

Similarly, the next horizontal furrows will be about the spots *b b*, the next at *c c c c*, the next at *d d d d d d d d*, and so on; always resulting in a balance of protoplasmic energy on each side of the furrow.

In this way the frog's egg becomes segmented more and more rapidly in the upper hemisphere than in the lower. For a considerable time there is an almost complete absence of horizontal furrows in the lower hemispheres.

This point is very well seen in Umé Tsuda's figures IV, V, of Plate 24, 'Quart. Journ. Micr. Sci.,' vol. xxxv, part 3.

Another effect is that as segmentation proceeds there is a continual increasing disparity in size between the cells of the black pole and those of the white. Whereas at first the superficial area of the cells of the extreme upper pole bears to the superficial area of the cells of the extreme lower pole the ratio of 1 to 2, at the time of the commencement of the blastopore it bears the ratio of 1 to 5.

In this way there is a gradual apparent creeping of small (black) cells over the surface of the egg—though in reality it is conversion of large cells into smaller *in situ*, as, I believe, is now generally accepted.

My diagram fig. 15 gives the idea of no segments in the white or lower hemisphere of the ovum. This is because it deals only with horizontal furrows.

The segmentation energy may be said to produce its effects along the area of least resistance. Is it not possible that the commencement of the archenteron may be a continuation of this same process?

The effect up to now has been to produce a fairly sharp line of demarcation between small and large cells upon the surface at the point × in diagram, fig. 15.

On the supposition that this diagram represents fairly accurately the distribution of yolk, it is clear that as this line advances it encounters greater and greater resistance. May not a time come when it will find the path of least resistance to be inwards and backwards, as in diagram 16?

Diagrams 15 and 16 are inaccurate for later stages of segmentation, because they do not show a segmentation cavity.

Fig. 12 is a more accurate representation of a completely segmented egg.

A. is the black upper pole (the anterior wall of the future embryo); *P.* is the white lower pole (posterior end of the future embryo); *sg.* the segmentation cavity.

The letter *A.* points to the smallest cells of this stage, *y. p.* to the largest.

There is a gradual merging of the one into the other, not along the surface, for here the line is much sharper, but along the cells to which *y.* and *x.* are directed.

My idea is that the continuation of the segmentation process is the conversion of, first, the cells *y.*, then the cells *x.* into smaller ones, and in this way a layer of small cells will be produced lying up against the mass of much larger cells *y. p.*

This layer I have indicated in the fig. 12 by the dotted line.

If a section of an embryo of the stage in which the blastopore is nearly complete is examined, it will be seen that there is such a layer of cells along the floor of the segmentation cavity.

Fig. 21 is an outline camera drawing. Such details as are shown were not drawn by camera. The smallest cells are those forming the lip of the blastopore.

The point *a.* represents the at present most anterior limit of the archenteron. More anteriorly, however, following the lines *a a.*, *a a.*, there is what I take to be a differentiation of the yolk-cells, that is a splitting up into smaller cells, which cells, upon the splitting hypothesis, will eventually form the

roof of the archenteron, and come to lie up against the epiblast now forming the roof of the segmentation cavity, as shown diagrammatically in figs. 12 and 13.

The differentiation as seen at the point *a a*. must, on this hypothesis, be considered to be the effect of the direct continuation of the process of differentiation on the surface of the ovum (whereby the epiblast is separated), that is a direct continuation of the process of segmentation. This line bounded above by small cells, below by large cells, constitutes a line of separation or a split, which is, I believe, the first commencement of the archenteron, and is a result of the primary centre of activity, comparable to the events of the first five days in the development of the rabbit, or to the formation of the gastrula in Amphioxus. But although corresponding in effects, the only really homologous feature is the presence of a primary centre of activity, or process of segmentation of the egg; the actual directive agencies being in each case cœnogenetic and entirely different.

The conversion of the narrow slit into a spacious cavity is to be considered to be due, at any rate in part, to the effect of the secondary centre of activity, to which I shall refer again.

I must now refer to the experiments made by Roux and Schultze and Morgan and Umé Tsuda upon the developing egg by following natural or artificial spots, which experiments I have myself repeated during this spring.

It is impossible to repeat these experiments without becoming convinced that there is a change of relative position between certain spots on the ovum,—for instance, the dorsal lip of the blastopore, and the most inferior spot upon the white pole of the ovum. These two spots, as seen from without, undoubtedly approach one another before the complete formation of the circular blastopore. But the question to what extent this approximation is carried, and whether by a concrescence of the lateral lips of the blastopore, or by a rolling under of the white pole, or by a growth over the upper lip without concrescence, is answered differently by the several observers.

My own suggestions are as follows.

The Secondary Area of Cell Production.

I think that every one will agree that after the closure of the blastopore, the embryo grows in length by the proliferation of cells at the spot which formerly formed part of the lips of the blastopore.

There is very little doubt that rapid growth at this spot takes place before the final closure of the blastopore ; the question is, when does this growth begin?

Again, it must be remembered that growth of the embryo as a whole, derived from the rapid multiplication of the cells in this area, is growth in length. It is the secondary area of growth comparable to the secondary area of growth or primitive streak of the rabbit.

If there is such a growth backwards of the blastoporic lips before their closure, there will then be a portion of the future gut cavity of the embryo that will have been formed, not by a splitting nor by an invagination, but by a growth backwards of the blastoporic lips.

Amphioxus, after the completion of the process of invagination, begins to grow in length. According to Hatschek's account this was largely due to the activity of two pole cells. Recently, however, Wilson has stated very clearly that these pole cells are "a myth." They never exist at any time, but the posterior region of the larva of later stages "is rapidly growing, and numerous mitoses may be observed in all the cells in the region of the mesenteric canal."

Now although the process of invagination produces the double-layered condition of the embryo of Amphioxus, and at the same time the cavity of the archenteron, yet it is only the anterior part of the archenteron that is formed in this way.

There is a posterior point of the archenteron which is formed, not by invagination, but by growth of the blastoporic lips. This must be so, whether we accept Hatschek's or Wilson's description of the secondary growing point.

The exact line of demarcation between the two parts I have no means of showing. It is not easy to say at what moment

the secondary growing point becomes a functionally active area in Amphioxus. It is possible for it to become established as soon as a blastoporic lip is formed, and not before, because a characteristic feature of this secondary area of proliferation is that it should produce cellular units to all existing cellular layers.

I have in a previous paper tried to locate this line of demarcation in the rabbit. The moment of origin of the secondary area of proliferation in the rabbit is fairly well marked. Of Amphioxus I cannot speak. Can we find it in the frog? The frog is by no means so simple as the rabbit, but is more amenable to experiment than is Amphioxus.

The frog differs in one respect from Amphioxus, which is of importance in reference to the question now under discussion. In Amphioxus the blastoporic lip is formed at the same moment apparently at all points of its circumference. In the frog it is formed at one point first, namely, at the future dorsal region, and many hours elapse before the lip is formed ventrally. Hence it is possible for the secondary area of proliferation to become established much sooner dorsally than ventrally.

In my account of the frog given above, the production of the split forming the primitive archenteron, which I believe to represent the process of gastrulation of Amphioxus, although no invagination occurs, is to be considered, like the invagination process in Amphioxus, as the result of the primary area of proliferation, and of itself would tend to the production of a radial symmetry. The very moment this split begins, a portion of the blastoporic lip is thereby formed.

If my supposition is right, that the secondary area of proliferation may be established as soon as there is a mass of cellular tissue in connection with all the primary layers, it is clear that possibly the secondary area of proliferation may start immediately upon the formation of the dorsal lip of the blastopore, and not delay until the whole blastoporic rim is completed.

Accordingly, on this view, there will be an extension of gut

cavity anteriorly by means of a splitting, the result of the primary area of activity, and posteriorly by means of a growth backwards of the dorsal lip of the blastopore, the result of the secondary area of activity, comparable to the corresponding parts in the rabbit, formed previous to the eighth day, and upon and subsequently to the eighth day respectively. In the rabbit and in Amphioxus the lining of the archenteron of the primary area is completed before the secondary area of proliferation has become established, but in the frog afterwards; and so the linings of both parts of the gut cavity are formed together.

Many actual experiments and observations have been made upon the eggs of the frog with the object of demonstrating the mode of formation of the blastopore, and the relative position of the blastopore when it has a completed margin to the originally black and white poles of the unsegmented ovum. Such attempts have been made with varying results by Roux, Schultze, Hertwig, Morgan, and Umé Tsuda, and although the experiments described are in many cases contradictory, yet there seems to be no doubt that the dorsal lip of the blastopore does overgrow a portion of the whiter side of the embryo prior to the completion of blastoporic lip ventrally.

I have myself made similar experiments in repetition of Roux, and I am quite convinced that this overgrowth does occur to a certain extent, but I am equally sure that it is incorrect to assert that the neural plate is formed entirely upon the lower (white) pole of the ovum.

The dorsal lip overgrows the white segments, at any rate apparently, but so do the lateral and ventral lips as they are formed. It is only because the dorsal lip is formed first that this part seems to overgrow the white pole to so large an extent.

The overgrowth is a part of the same process which produces the lengthening of the embryo. If the whole blastoporic lip could in the frog be formed at once the embryo would, I suspect, change rapidly from a sphere to an oval, as does the embryo of Amphioxus (v. Hatschek, figs. 30 and 34).

In the frog the dorsal lip cannot of itself grow outwards and so produce an oval embryo until the rest of the blastoporic lips are formed. Unless it remains inactive it must follow the contour of the ovum. That it does not remain inactive I have convinced myself, and therefore I agree with the above-mentioned authors that a portion of the white area passes out of view of the observer by becoming hidden by the advancing dorsal lip of the blastopore.

Experiments in marking Parts of the Ovum.

General Remarks.—I find—

(i) That it is impossible to fix the egg in any one position so as to prevent with certainty the rotation of the ovum within the vitelline membrane without injuring or distorting the ovum.

(ii) That, accordingly, any fragment which exudes from the ovum through the aperture made in the vitelline membrane when pricking the ovum in order to mark one spot is useless as a landmark.

(iii) A scar upon the ovum itself, fixed to the ovum and within the vitelline membrane, is the only mark which can be relied upon for drawing conclusions as to the relative rate of growth, and a change of position at different points upon the surface of the ovum.

(iv) A severe injury by pricking naturally produces much abnormality of development; whereas a very slight injury, although admirable for a short observation, is apt to recover and so get lost and obliterated after many hours.

Some of my own experiments I will now briefly describe. Outline figures are given upon Pl. 23.

Figs. 1*a*—1*d* show the results of an experiment. Here the puncture was very small, and made midway between the two horns of the developing blastoporic lips (fig. 1*a*). Three hours and a half later the blastoporic lips were completely marked. By this time (fig. 1*b*) the mark was distinctly closer to the dorsal lips of the blastopore. Three hours later the mark had approached the dorsal lip still nearer, but the ventral

lip of the blastopore had gained a little upon the mark. After a lapse of eight more hours the blastopore was very much smaller, and the mark was found partly covered by the dorsal lip. In this (fig. 1*d*) the ventral lip had gained much more upon the mark than had the dorsal lip.

Figs. 2*a*—2*d* are figures of a specimen which had a natural mark upon the white pole of the ovum. The drawings were made at 5.30, 8.30, 10.40 p.m., and 8.30 a.m.

I thought the scar was part of the embryo, but upon the blastoporic rim reaching it the scar became partly scraped off on to the rim.

Both this specimen and the last show that the apparent overgrowth of the dorsal lip of the blastopore is much more marked at first than afterwards.

This is well illustrated by figs. 4*a*, 4*b*. In this specimen a mark was made after the complete formation of the blastopore (fig. 4*a*) near the centre of the unenclosed yolk. Fig. 4*b* is the same specimen seventeen hours afterwards. I do not know to which lip the mark was approximated.

Figs. 3*a*—3*g* show a similar apparent overgrowth of the dorsal lip, and also that this overgrowth is greater during the earlier period of blastopore formation.

Figs. 5*b*, 5*c*, and 5*d* are from embryos which have completed the closure of the neural plate. All these were, at the moment of the first signs of the blastoporic lip, pricked near to the margin between the black and white at the point most distant from the commencing blastopore, and equidistant with the latter from the equator of the ovum. Fig. 5*d* shows the scar a little to the left of the spot where the blastopore has closed.

Fig. 5*b* shows the scar upon the side of the embryo about its middle, both dorso-ventrally and antero-posteriorly.

Fig. 5*c* shows the scar upon the ventral edge of an unclosed blastopore. In this specimen the injury was very severe, a large mass (*exo.*) exuded, and, as always follows in such a case, an abnormal embryo was formed.

Fig. 5*a* shows the spot near which they were all pricked.

Ten embryos were pricked in the centre of the lower pole of

the ovum in the blastula stage before any trace of the dorsal blastoporic lip could be detected. Of these when preserved, at which time the normal ones were from 4½ mm. to 5 mm. in length, seven showed no trace of the injury externally, and seemed to be quite normal. One showed no injury, but was rather abnormal in shape. Two failed to develop beyond the blastula stage.

Another specimen was pricked, as shown in fig. 6*a*, on both sides of the blastopore, on one side upon the lip, on the other a slight distance away from the line where the lip was apparently about to develop. Fig. 6*b* was drawn ten and a half hours afterwards.

After the blastopore had closed I was unable to detect the injuries.

There is no doubt that one must be very cautious indeed in drawing conclusions from injuries made upon eggs. This is especially so with injuries made upon the more active part of the embryo, i.e. the more deeply pigmented cells. A very inconsiderable injury is sufficient to produce an abnormality. Three slightest punctures possible upon the rim of the blastopore equidistant from each other are sufficient to prevent the closure of the blastopore, while one only, if at all severe, will have the same effect.

This clearly must be the case, as the closure of the blastopore is an effect of increase of bulk of certain parts of the walls of the embryo, and if this increase in bulk is, through pricking these walls, prevented or delayed by the letting out of matter, and thereby obviating the necessity for the blastoporic lip to advance, the blastopore does not close; and so also injuries to the white pole or yolk plug by allowing the escape of material from that area, and thereby diminishing the bulk of the part of the embryo that can be covered by the advancing lips of the blastopore, hastens the closing of the blastopore.

My own experiments are in part confirmatory, but mostly contradictory to those of Roux. They are upon the whole confirmatory of those performed by Morgan and Umé Tsuda. Roux asserts that the dorsal lip of the blastopore passes over

the lower pole of the ovum through at least 170°. The ventral lip according to him does not advance at all.

Morgan and Umé Tsuda consider that the ventral lips and lateral lips advance, but not to so great an extent as the dorsal lips. They also notice the "first overgrowth of the dorsal lip of the blastopore is more rapid than the later growth; that is, the approach to the points of injury is faster at first."

I quite agree with the latter authors that "it seems . . . . . most probable that the blastopore does not start at the equator of the egg, but some distance below that circle."

Now my experiments do not give evidence of an overgrowth by the dorsal lip of more than 60° or 70° from the moment of the first commencement of the dorsal lip, and to the closure of the blastopore. More probably, I think, the apparent overgrowth is even less.

According to Roux the overgrowth is at least 170° to 180°.

If Roux is right in both his suppositions, namely, that the dorsal lip moves over the white pole, and to an extent of 180°, I cannot understand how the last remaining portion of the blastopore to remain open should show so white a piece of yolk plug. This piece of yolk plug is as white as any part of the surface of the ovum of the frog ever is. There is a considerable amount of variation in the pigmentation of the unsegmented ovum. It is extremely rare in England to find eggs in which there is deficiency of pigment over an area subtended by so great an angle as an angle of 120°. An area where there is almost an absence of pigment is much more restricted. Very frequently the less pigmented area extends over a much smaller arc. On Roux's supposition, the part which remains longest uncovered ought to be grey, if not quite black. It is, as far as I have observed it, an almost invariable rule to find the yolk plug at its latest stage intensely white.

I have only once seen embryos which in the gastrula stage showed a darkened blastopore, and these were from eggs which in the unsegmented stage were so intensely pigmented

that the lower pole was only slightly lighter in colour than the upper, and this for an area not greater in extent than that subtended by an angle of 50°. Yet in these the blastopore, though very dark, was quite as light as the lightest part of the unsegmented ovum.

If the centre of the blastopore at the moment it is in the stage represented in fig. 20 is not either the lower pole, or some spot extremely close to the lower pole, of the unsegmented ovum, the intense whiteness of this spot must have been produced by a disappearance of pigment previously existing. Is there any evidence of this? I cannot think of any. On the contrary, there is evidence of increasing pigmentation, as, for instance, in the epiblast-cells as they form upon the surface, in the cells of the splitting archenteron, and even in the white cells themselves. If the final position of the blastopore is, as Roux supposes, at the equator, and at a spot removed 170° from the spot of the first commencement of the blastopore, surely the uncovered part of the surface would be dark, if not black, and certainly not intensely white.

At the moment the definite outline of the ventral lip of the blastopore is formed the anus of Rusconi thus fashioned is not of uniform tint. The dorsal part of the area is much lighter in colour than the ventral part, fig. 19. When, however, it has diminished to the condition of fig. 20, it is of uniform tint and intensely white. This is accounted for by the more rapid closure of the ventral lip from this moment, as my experiments and those of Morgan and Umé Tsuda demonstrate, as also may be seen by examination of sections as described in a former paper (Robinson and Assheton).

Except for a slight advance of the dorsal lip of the blastopore my experiments do not support Roux's.

According to Roux, injuries made at the point *x* in fig. 5 *a* ought to have appeared upon the dorsal side in the medullary folds a little way anterior to the blastopore. Instead of which one was in the median line ventral to the blastopore, one was laterally placed on a level with the blastopore, and one laterally placed but far forwards. So, again, if the lips of the

blastopore concresce as Roux assumes, then marks made upon the lips should show upon the dorsal surface somewhere along the neural folds. In no case did I ever find this to occur. As the result of such injuries, I either found the scar upon the lateral lip of the blastopore when completed, or else as in figs. 6 *a*, 6 *b*, further removed from the blastopore but in the same relative positions.

I never found a defect in the neural plate except when the dorsal, or near the dorsal lips of the blastopore, was injured. This spot is unfortunately at the same time the most interesting to injure, and the most delicate, and most liable to produce abnormalities which prove very little. There is certainly no need to assume a concrescence, as the facts can be equally well accounted for by other means, which to my mind agree far better with the development of other Vertebrates than does the concrescence theory.

In other words, I believe that as in the rabbit, so in the frog, there is evidence to show that the embryo is derived from two definite centres of growth, the first, and phylogenetically the oldest, being a protoplasmic activity which gives rise to the anterior end of the embryo (= gastrula stage); the second, which gives rise to the growth in length of the embryo: which centres of growth occupy the same relative positions in location and in sequence of time, and probably to each are due the same parts of the embryo.

In the rabbit the area is a spot which assumes for a time a linear form, but is unaffected by its change of shape in the functions it has to perform.

So in the frog, although at first crescentic, then circular, then linear, and ultimately a knob, its function is precisely the same as in the rabbit, and is unaffected by the change in form. From the moment of its first appearance it performs its one function—that of adding on new cellular units to the previously existing embryo.

One difference of effect is that owing to the manner of its coming into existence, one portion arising before the other, that portion—the dorsal—becomes functional before the

ventral, and so the dorsal part of the embryo is developed more quickly than the ventral.

I believe the true way of regarding this area of secondary proliferation, both in the rabbit and in the frog, is as a single area, whether circular, annular, or linear, whose sole function is the addition of cellular units to the posterior end of the previously existing embryo. Its form is the result of secondary or ontogenetic causes.

The exact line of demarcation is not easy of determination. Very careful marking of the dorsal lip might give it as far as the nervous system is concerned, but organs, no doubt, change their relative position somewhat as they develop. The brain is certainly thrust somewhat forwards. I brought forward evidence to show that in the rabbit this point was about the level of the first mesoblastic somite. It is, at any rate, possible that metameric segmentation may be directly due to this process of elongation. It seems always to be closely connected with it. If so it may be due to this, that the anterior mesoblastic somite of the frog is the smallest, and each for succeeding five or six becomes longer dorso-ventrally than its preceding neighbour.

For upon my suppositions of the non-concrescence of the blastoporic lips, and of the unity of the nature of this area of secondary proliferation, then, since the first part of this proliferating area to be formed is that part adjoining the dorsal surface, those parts in the mid-dorsal line, e. g. neural plate, will be the first, and at first the only part of the embryo to receive additions from the proliferating area. As the lateral lips of the blastopore are formed, more and more of the lateral plates of mesoblast will receive additions, so that in this way it is possible that the gradual increase in size of the first six mesoblastic somites in the frog may be connected with the gradual development of the area of proliferation.

Every one is agreed that there is a certain part of the neural plate formed on an area anterior to the first commencement of the blastopore lip. The point in discussion is to what extent does this pre-blastoporic formation exist?

Morgan and Umé Tsuda conclude that all except "the thickness of the medullary folds" round the dorsal lip of the blastopore is formed by the growth of the lip.

Roux shows the same in his figure.

Pflüger, however, thinks it possible that a considerable length of the anterior part of the nervous system is formed in this black hemisphere, and with Pflüger I quite agree on this point.

I find that the neural plate in normal embryos at the time it becomes visible on the surface extends through fully 170°, if not more, while the distance through which the dorsal lip of the blastopore travels I cannot make out to be more than 70° at the most; that is, from a spot a little below the equator to the lower pole, or perhaps a little beyond it.

Figs. 7 to 14 represent diagrammatically the views put forward in this paper. Fig. 7 is the fully segmented frog's egg, the white pole placed to the right of the paper; the black pole or roof of the segmentation is placed to the left, as representing the future anterior end of the embryo. All the others, 8—14, are arranged similarly. Fig. 8 represents the stage at which the dorsal lip of the blastopore has become established.

Up till now there has been but one general centre of growth. From this moment the secondary centre of growth is in existence, and we have now the commencement of the conversion of an embryo radially symmetrical into an embryo bilaterally symmetrical. As yet only the dorsal part of this secondary area of proliferation is in existence, and accordingly the dorsal part of the embryo is developed more rapidly than the ventral, as the annexed figure 9 shows. In this figure the ventral part of the secondary area has just been completed, and now the whole of the secondary area of proliferation, the homologue of the whole of the primitive streak of the rabbit, is complete, and new material is added to ventral and lateral and dorsal parts of the embryo, as diagram fig. 10 illustrates. The shape now rapidly changes, and the radial symmetry is lost and the bilateral symmetry acquired, fig. 11.

The neural plate is indicated in fig. 11 by the continuous

line; the dotted line represents only approximately the supposed division between the parts of the embryo derived from the primary and secondary areas of proliferation respectively. The subsequent fate of the secondary area of proliferation (or primitive streak) I have, with Dr. Robinson, described and discussed in a former paper. The three figures 8, 9, and 10 represent my views of the extent to which the white pole becomes overgrown by the dorsal lip of the blastopore. The neural plate is indicated as in fig. 11 by the continuous line. The extreme anterior end of this part of the epiblast has been obtained by subtracting the amount due to overgrowth from the total amount observed when definitely established.

It does not necessarily follow that the distance through which the edge of the dorsal lip of the blastopore advances represents the total growth in length due to that part of the secondary area of proliferation. In order to advance, the lip of the blastopore has to exert pressure upon the "yolk plug," causing it to be forced inwards. It thus follows that an equal force must be exerted in the other direction. What effect this has will depend upon the strength of the resistance offered by the anterior wall of the embryo.

From the fact that the archenteron is a slit in the stage represented by figs. 8, 21, and 17, and is a spacious cavity in the stage represented by figs. 9, 13, and 19, it seems likely that the growth of the blastoporic lip is rendered evident, not only by the amount of white yolk plug covered, but also by the arching up of the dorsal roof of the archenteron. But, on the other hand, the arching up may be in great part, if not entirely, due to its own interstitial growth; though I do not think this is likely, for it seems to me to require the thrusting energy of the blastoporic lip to account for the obliteration of the segmentation cavity.

If Schultze's idea of the apparent overgrowth of the white yolk plug being due to a rolling inwards of the white pole were correct, ought not the ventral end of the segmentation cavity to become obliterated before the dorsal? But it is the dorsal part that first disappears.

DESCRIPTION OF PLATES 23 & 24,

Illustrating Mr. Richard Assheton's paper "On the Growth in Length of the Frog Embryo."

Complete List of Reference Letters.

A. Anterior end. *a a*. Small cells in floor of segmentation cavity. *bl. d*. Dorsal lip of blastopore. *bl. v*. Ventral lip of blastopore. *D*. Dorsal surface. *exo*. Exovate. *P*. Posterior end. *sg*. Segmentation cavity. *y*. Dorsal lip of blastopore. *x*. Floor of segmentation cavity. *z*. Yolk-cells, which will be overgrown by dorsal lip of blastopore. *y. p*. Yolk-plug. ×. Spot at which the blastopore commences.

PLATE 23.

Fig. 1.—*a*—*d*. Ovum pricked in centre of white between the developing lips of the blastopore.

Fig. 2.—*a*—*d*. Course taken by a natural mark on the white pole.

Fig. 3.—*a*—*g*. A mark was made near the centre of the white pole.

Fig. 4.—*a*, *b*. Ovum was marked in centre of blastopore when first outlined.

Fig. 5.—*a*—*d*. Three embryos marked at the cross in 5 *a*. In *b* the mark was on the side; in *c*, ventral to blastopore; in *d*, in which it was very indistinct, at the side of the blastopore.

Fig. 6.—*a*, *b*. This embryo was marked at the sides of blastopore.

PLATE 24.

Fig. 7.—Frog embryo before appearance of blastopore. The white (or posterior) pole is placed to the right of the observer.

Fig. 8.—Frog embryo at the time of the commencement of the blastopore, which is shown as a dark crescentic groove. The dorsal cap represents that part of the epiblast which will form the anterior part of the neural plate.

Fig. 9.—Frog embryo at the moment of the completion of the ventral lip of the blastopore. The dorsal lip has grown over the white pole through an arc of 50° to 60°. The dotted line indicates that part of the embryo supposed to be derived from the secondary area of proliferation. The arrows indicate from which portion of the rim the respective parts have been formed.

Fig. 10.—Frog embryo at the time of first appearance of neural plate, visible only in sections. The blastopore is much reduced. The ventral lips

have closed more rapidly than the dorsal. Dotted line and arrows indicate same features as in Fig. 9.

Fig. 11.—Frog embryo when the neural plate is a conspicuous object externally, and is deeply grooved. The embryo has become very distinctly elongated, owing to the growth due to the secondary area of proliferation. This was drawn with a camera. The dotted line and arrows represent my own interpretation of the facts as in the preceding figures.

Fig. 12.—A section of the same stage as Fig. 8, semi-diagrammatic. The dotted line indicates the location of the split amongst the cells, whereby the archenteron is supposed to have originated and become prolonged forwards.

Fig. 13.—A diagram of a stage intermediate between Figs. 9 and 10. The portion of the gut cavity indicated by the dotted line represents that part which I suppose to be formed by the splitting amidst the yolk-cells. The future continuation of this slit is represented by a prolongation ventralwards of the dotted line. The posterior part of the roof of the gut cavity, marked with small dots, is that part formed by the active growth of the dorsal lip of the blastopore, which is shown by the diagonal shading.

Fig. 14.—A diagram of a later stage, such as Fig. 11. In this the segmentation cavity has become entirely obliterated. The secondary area of proliferation is completed and active all round the blastopore.

Fig. 15.—A diagram to show the sequence of the horizontal furrows during segmentation of the frog's egg.

Fig. 16.—A diagram to show in which direction the process of segmentation will incur least resistance, on the supposition that the yolk is distributed as indicated by the intensity of the shading.

Figs. 17—20.—Figures of the frog's egg during the formation of the blastopore, to show which part of the surface of the ovum forms the yolk-plug. Each figure is arranged in the same position.

Fig. 21.—A section of a frog's egg of a stage intermediate between Figs. 8 and 9. The ovum was drawn with camera.

APR 26 1895

On the Variation of the Tentaculocysts of Aurelia aurita.

By

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University College, London.

With Plate 25.

It was a suggestion from Professor Weldon that led me to examine a large number of specimens of the ephyræ and adult stage of Aurelia aurita for the purpose of finding out the variation in the number of tentaculocysts, and if a variation occurred among the ephyræ to see how far it affected the adults.

All the specimens were collected and preserved at Plymouth by the officials of the Marine Biological Association, and I sincerely thank the director, Mr. Edward J. Bles, for the loan of so many specimens.

The ephyræ are divided into two sets; the first collected during the spring of 1893, the second specially obtained for me during the spring of 1894.

The ephyra of Aurelia normally has eight arms, each bearing a tentaculocyst, four perradial bundles of gastric filaments, and four mouth lappets.

The first table gives the numerical variation of the tentaculocysts of 359 specimens collected in 1893.

Table I.

The Numerical Variation of the Tentaculocysts of 359 Ephyræ collected in 1893.

Number of tentaculocysts.	Number of specimens.	Percentage.
Six	4	1·1
Seven	8	2·2
Eight (normal)	278	77·4
Nine	22	6·1
Ten	18	5·0
Eleven	12	3·3
Twelve	14	3·9
Thirteen	3	0·8

It will be seen from this table that no less than 81 specimens (22·6 per cent.) are abnormal in possessing more or less than eight tentaculocysts, and that the range of variation extends from six to thirteen tentaculocysts. There are only 12 specimens (3·3 per cent.) with less than eight tentaculocysts, and the remaining 69 specimens (19 per cent.) are above the normal number.

Table II.

The Numerical Variation of the Tentaculocysts of 1156 Ephyræ collected in 1894.

Number of tentaculocysts.	Number of specimens.	Percentage.
Five	1	—
Six	6	0·5
Seven	34	3·0
Eight (normal)	883	79·1
Nine	75	6·7
Ten	61	5·4
Eleven	35	3·1
Twelve	17	1·4
Thirteen	3	0·2
Fourteen	1	—

The second table shows in detail the variation of the tentaculocysts of 1156 specimens collected in 1894. On

comparing it with the first table it will be seen that the percentage of abnormal specimens is nearly the same. In the first set 22·6 per cent., and in the second set 20·9 per cent. of the ephyræ are abnormal.

The decrease is mainly due to a falling off in the number of specimens with twelve tentaculocysts amounting to $2\frac{1}{2}$ per cent.

By taking a larger number of specimens the range of variation has extended from five to fourteen tentaculocysts, but only one specimen of each of the two extremes has been found.

The ephyra with five tentaculocysts (fig. 1) has four perradial arms, equal in size; but the fifth is interradial and about half the size of the other arms.

The variation in the number of tentaculocysts does not affect the other organs of the body, which may vary independently of one another.

Two specimens have only three bundles of gastric filaments instead of the normal four; both have four mouth lappets; but one (fig. 2) of them has six and the other seven tentaculocysts. Six specimens have six bundles of gastric filaments and six mouth lappets; three possess eleven tentaculocysts (fig. 3) and the others have twelve.

A few curious abnormal growths of the arms were also observed. One specimen (fig. 4) has a perfect double arm with two tentaculocysts, like two arms united together. Another specimen (fig. 5) shows a bifurcation of an arm, each branch terminating with a tentaculocyst.

Perhaps the most interesting monstrosity is that which occurs in a specimen (figs. 6 and 7) with a large outgrowth on the aboral side of the umbrella. The outgrowth has two arms, and one of them bears a tentaculocyst. There are also seven other arms, with tentaculocysts, in the normal position and a vacant place for two more.

Adult Aurelia.

The adult specimens of Aurelia were also collected at Plymouth during the summer of 1894, and belong to the

same generation as the ephyræ taken in the spring of that year.

The umbrella of these specimens varied from $1\frac{1}{2}$ to $2\frac{1}{2}$ inches in diameter. The tentaculocysts of 383 specimens were examined, and the number possessed by each specimen is recorded in Table III.

TABLE III.

The Numerical Variations of the Tentaculocysts of 383 Adult Aurelia collected in 1894.

Number of tentaculocysts.	Number of specimens.	Percentage.
Six	2	0·5
Seven	18	4·7
Eight (normal)	296	77·2
Nine	33	8·6
Ten	16	4·1
Eleven	10	2·6
Twelve	7	1·8
Thirteen	0	—
Fourteen	0	—
Fifteen	1	—

There are 87 specimens (22·8 per cent.) with a variation in the number of tentaculocysts, 20 having less than the normal number and 67 showing an excess.

On comparing the abnormal number of tentaculocysts of the adults with those of the ephyra stage, it will be seen from the percentages that there is only a slight difference. The ephyræ have 22·6 per cent. abnormal in 1893, and 20·9 per cent. in 1894; the adults show 22·8 per cent. It is clear from these figures that the abnormal ephyræ do not appear to suffer from their abnormality, but are able to reach in safety the adult stage. The figures also show a slight increase of abnormal forms in the adult stage. This may be due to an insufficient number of adult specimens; the small number is due to their scarcity at Plymouth.

On comparing the 359 ephyræ taken in 1893 and the 383 adult specimens taken in 1894, it will be seen that the per-

centages of abnormality are very close for nearly the same number of specimens. It is probable that if a thousand adults could have been obtained at Plymouth the percentage of abnormal forms might have been closer than 2 per cent. of the ephyræ taken in 1894. The adult specimens were taken at random out of large jars; and it is interesting to note how close the percentage of abnormality of each complete hundred comes to the mean abnormality. The first hundred showed 23 per cent., the second 22 per cent., and the third hundred 24 per cent. of abnormal forms. An examination of the specimens does not show that any particular position on the margin of the umbrella is favoured either by an increase or decrease of the tentaculocysts.

Eighteen specimens possess seven tentaculocysts, and in eleven of these the missing tentaculocyst is a perradial one, and in seven it is adradial. The presence of an extra tentaculocyst may either affect the symmetry of a single quadrant or one half of the umbrella, and in a few cases by being very close to another not even upset the symmetry.

Ten specimens with nine tentaculocysts show that the extra tentaculocyst is in one quadrant of the umbrella, and thirteen specimens have one half of the umbrella containing five tentaculocysts about equal distances apart, and the other half possessing the normal four. Five specimens have eight tentaculocysts occupying their normal positions, and an extra one only separated from a normal one by a few marginal tentacles. When the tentaculocysts exceed nine in a specimen their position is by no means constant, and a different arrangement occurs in almost every specimen. In some the tentaculocysts are about equal distances apart, and in others one half of the umbrella contains the greater number.

A few specimens have three tentaculocysts very close together, and usually separated by a few marginal tentacles.

One specimen of an adult Aurelia has fifteen tentaculocysts with the normal number of genital pouches and arms. This exceeds the maximum number reached among the ephyræ. None of the adults have, however, either thirteen or fourteen

tentaculocysts. Their absence is probably due to the examination of an insufficient number of specimens.

A variation in the number of tentaculocysts does not interfere with the other organs of the body. There appears to be a correlated variation between the number of genital pouches and buccal arms, and eight specimens show it.

One specimen has three genital pouches, three buccal arms, and nine tentaculocysts.

Three specimens have three genital pouches, three buccal arms, and each one has traces of a fourth genital pouch and a fourth arm. Two have eight tentaculocysts, and the other has ten tentaculocysts.

One specimen has five genital pouches, five buccal arms, and eight tentaculocysts.

Three specimens have six genital pouches, six buccal arms; two have eleven tentaculocysts, and one has twelve tentaculocysts.

I have not given drawings of these specimens, as somewhat similar forms have already been figured by Ehrenberg (1) and Romanes (2). Mr. Bateson, in his recent book, 'Materials for the Study of Variation,' gives abstracts and figures from the papers of Ehrenberg and Romanes. He also gives a table which shows that 26 specimens (1·49 per cent.) out of 1763 adult Aurelia, washed ashore on the Northumberland coast in 1892, have more or less than four genital pouches. The Plymouth specimens show 2·08 per cent. with an abnormal number of genital pouches.

It may be difficult to notice in a few years what effect the numerical variability of the tentaculocysts has upon the adult Aurelia. The variation shows a tendency for an increase of the tentaculocysts, since the specimens displaying an increase are about three times as numerous as those possessing a diminished number. Whether this will change the present characteristic features of the species or not can only be found out by examining the ephyræ and adults at long intervals of time, and comparing the results with previous records.

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1. Ehrenberg, C. G., 1834.—'Abh. K. Ak. Wiss.,' Berlin, pp. 199—202, plates.

2. Romanes, G., 1876.—'Journ. Linn. Soc. Zool.,' vol. xii, p. 528; vol. xiii, p. 190, plates xv, xvi.

3. Bateson, W., 1894.—'Materials for the Study of Variation,' pp. 426—429, fig. 128.

DESCRIPTION OF PLATE 25,

Illustrating Mr. Edward T. Browne's paper "On the Variation of the Tentaculocysts of Aurelia aurita."

Fig. 1.—Ephyra with five arms. Aboral view. × 25.

Fig. 2.—Ephyra with six arms, three bundles of gastric filaments, and four mouth lappets. Aboral view. × 35.

Fig. 3.—Ephyra with eleven arms, six bundles of gastric filaments, and six mouth lappets. Oral view. × 25.

Fig. 4.—Ephyra with a perfect double arm, seven tentaculocysts, four bundles of gastric filaments, and four mouth lappets. Aboral view. × 40.

Fig. 5.—A portion of the umbrella of an ephyra, showing a bifurcation of an arm. × 40.

Fig. 6.—Ephyra with an outgrowth of two arms from the aboral side of the umbrella. Aboral view. × 35.

Fig. 7.—A lateral view of Fig. 6. × 25.

On the Structure of Vermiculus pilosus.

By

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With Plates 26—28.

In 1892 (3a) I published a short description of an interesting new Oligochæte I had found on the sea-shore near Weymouth. Since then I have obtained more material, which I have worked at in Professor Lankester's laboratory at Oxford, and am now able to give a more complete and accurate account of its anatomy.

The worm, which I have called Vermiculus pilosus, was discovered in the rich black mud underlying the sandy surface of the shore about halfway between Weymouth and Portland. It lives there in considerable numbers with various species of Pachydrilus and Tubificids, especially Heterochæta costata, Clap., from which it is quite indistinguishable to the naked eye. Unlike the Enchytræids, it does not seem to venture to the surface amongst the decaying seaweed and in the tidal pools, nor is it so often found underneath large half-buried stones as other Tubificids, Heterochæta for example. Fig. 1 represents it natural size; it is from about 4 to 6 cm. long, and of a dull reddish tinge. The body is soft, and its movements are slow. Under a low power of the microscope it can be distinguished at once, after a little practice, being much less transparent than any of its associates. This opaqueness, caused chiefly by the structure of the body-wall, the network of blood-vessels, and the enormous number of cœlomic

corpuscles, renders it very difficult to make out the details of its anatomy in the living animal.

The prostomium (figs. 2 and 6, *pr.*) is rather large and conical. Each segment, from the second, is provided with two dorsal and two ventral bundles of setæ (fig. 2, *l. d. s.* and *l. v. s.*), placed in a transverse section nearly at the four corners of a square. Each bundle contains from two to five setæ, generally three. All the setæ, both dorsal and ventral, are alike S-shaped in outline, with a thickening about one third of the way from the distal end, which is bifurcate (fig. 3); they are of the ordinary Tubificid furcate type.

On close examination in a favourable light it is seen that the whole worm is clothed from head to tail in a more or less dense furry covering of hair-like processes, closely set, extremely fine, and apparently of cuticular origin (fig. 4, *pcl.*). These hair-like structures are not cilia; they do not move, and are not protoplasmic. Resembling the so-called "sense-hairs" or palpocils found on the prostomium and first segments of most aquatic Oligochætes, they are probably homologous with these, but developed to an extraordinary degree. The cuticle itself (fig. 4, *c.*) presents no peculiarity. The epidermis (figs. 4 and 7, *ep.*) is formed of regular more or less cubical cells, with large oval or round nuclei. Below the epidermis is a narrow layer of circular muscles (fig. 7, *c. m.*) within which are the longitudinal muscles (fig. 7, *l. m.*). In the transverse section the contractile fibrils are of different heights, being cut through at various distances from the middle of the cells which bear them. Along each side, about halfway between the dorsal and ventral bundles of setæ, the layer of longitudinal muscles is interrupted by a row of cells forming the lateral line (fig. 7, *l. l.*). Dr. Hesse has recently shown (**4**) that these are the cells to which belong the circular muscular fibres; my preparations support this view. Lining the inside of the body-wall is the cœlomic epithelium, composed for the most part of large vesicular cells (fig. 7, *c. ep.*).

The cœlomic corpuscles, which as above stated are very numerous, are spherical and coarsely granular (fig. 28, *c. c.*).

The vascular system is very peculiar. In fig. 5 the main blood-vessels have been represented somewhat diagrammatically, omitting for the sake of clearness the complex fine network found all over the first ten segments. A large longitudinal dorsal vessel (fig. 5, *d. v.*), reaching from the brain to the last segment, gives off on either side in front of each septum from the 2nd to the 10th segment a lateral dorsal vessel (fig. 5, *l. d. v.*), which soon branches out over the body-wall, reuniting below into two lateral ventral vessels which enter the longitudinal ventral vessel (fig. 5, *v. v.*). One of the lateral ventral vessels, the smaller, enters the ventral vessel about the middle of the segment (fig. 5, *m. l. v. v.*); the other, and larger, runs into it towards the hinder end of the segment (fig. 5, *p. l. v. v.*). In my preliminary note I stated that there were a pair of "hearts" in the 10th segment; this was a mistake. Indeed, the fact that there is no direct communication between the longitudinal dorsal and ventral vessels is the most striking thing about the blood-system of Vermiculus. The manner in which the lateral dorsal communicate with the lateral ventral vessels by means of the cutaneous network is shown in fig. 9. In the post-genital region only small vessels are given off at each segment to the body-wall and to the intestine.

The dorsal longitudinal vessel from the third to the last segment is dilated in front of the region where each pair of lateral vessels is given off into a small bulb or swelling, round the posterior constricted edge of which are ranged large cells (often as many as eight or nine), forming a system of valves (figs. 8 and 10, *v.*). Each cell is attached at its posterior end only, while its main body can swing backwards and forwards; it contains a number of bright fatty granules and a small spherical nucleus (figs. 8 and 10). The lateral dorsal vessels increase in size from the 2nd to the 10th segments; from the 3rd segment to the 10th they have an increasing number of dilatations and constrictions, each provided with valves similar to those in the longitudinal vessel (figs. 5 and 10). The vessel in the 10th segment, which is by far the largest, has as

many as five such sets of valves; the number of cells round each constriction diminishes the farther it is from the longitudinal vessel. It is this vessel (of the 10th segment) which supplies the blood to the ovisac, into which it passes directly from the 11th segment. Both the longitudinal and these lateral dorsal vessels are contractile.

The non-contractile longitudinal ventral vessel runs straight up to the 3rd segment, where it divides and joins again, forming a ring. From the anterior border of this ring are given off three vessels, which join the terminal dorsal branches under the brain (fig. 5). The ventral system has no valves.

The circulation of the blood (which is red) and the action of the valves are as follows: The blood is propelled forward in the dorsal longitudinal vessel by the contraction of its walls; it is helped in its course by the position of the valves, which prevent its returning, for when the flow is forwards they assume the position *a'* (fig. 11), leaving a clear passage; when the blood tries to return they take up the position *b'* (fig. 11), meeting in the middle and closing the way. Occasionally the return flow is too strong, and the valves are forced right back to the position *c'* (fig. 11), when they more or less completely shut off the communication with the side branches. The course of the blood-stream in these branches is from the longitudinal vessels outwards to the body-wall. As already mentioned, coming off from the lateral branches there is over the whole surface of the body-wall of the first 10 segments underlying the cœlomic epithelium a fine network of blood-vessels, which must form a very efficient respiratory system. Thus we see that, in the main, the course of the blood is up the longitudinal dorsal vessel, down the anterior lateral vessels, spreading over the body-wall to be aërated, and returning to the ventral vessel, in which it will flow backwards. Thence it probably reaches the dorsal vessel again by means of the small branches given off to the intestine, where nourishment is no doubt absorbed, and so brought into the general circulation. As will be seen later, excretion probably takes place from the ventral vessel by means of the nephridia which cling closely to it.

The alimentary canal is simple, and quite similar to that of Tubifex. A short way behind the mouth (fig. 2, *m.*) is a muscular pharynx in the 2nd segment (fig. 6, *ph.*), followed by a slender œsophagus stretching through the 3rd and 4th segments (fig. 6, *œs.*), and from the 5th segment backwards a straight intestine covered with brown chloragogen cells (fig. 6, *int.*). The anus is terminal. The pharynx is provided with irregular masses of glandular cells. From the pharynx backwards the alimentary canal is lined by ciliated columnar epithelium, outside which is a thin layer of circular and longitudinal muscles. In the wall of the intestine is a system of blood-capillaries forming a plexus similar to that described in the Enchytræids and other Tubificids.

The cerebral ganglia form a bilobed brain, deeply cleft in front and situated in the 1st segment (fig. 6, *br.*). The structure of the ventral nerve-cord is somewhat exceptional. On examining a transverse section taken between the ganglia (fig. 16), it is seen to consist of an outer covering of cœlomic epithelium (fig. 16, *c. ep.*) enclosing the nerve-cord proper and three strands of muscular fibres, of which the middle one (fig. 16, *m. n. c. m.*) is the most important. The nerve-cord itself is formed of two circular strands of ordinary nerve-fibres, on the dorsal surface of which run three neurochordal fibres. Owing to their delicate structure, these enlarged fibres are so difficult to see in specimens preserved in corrosive and stained with borax-carmine that at first I thought they were altogether absent. They are, however, plainly visible in sections of specimens preserved in Lindsay-Johnson fluid and stained with paracarmine (fig. 15). A transverse section taken through a ganglion (fig. 15) shows that the ganglionic cells extend on the under surface of the cord, and rise up at the sides, actually overlapping the muscular strands (fig. 15, *g. c.*). In a section taken at the point where the nerve-cord is passing through the septum (fig. 14), we see that muscle-fibres pass from the septum underneath the median muscular strand overlying the cord (fig. 14, *m. s.*), while the lateral strands communicate with those which descend on either side to the body-wall.

The nephridia of Vermiculus are no less peculiar than are its other organs ; they are situated in Segments 6 to 9 inclusive, and all the segments behind the 11th till near the tail end. It is interesting to observe a nephridium opening into the 11th segment in the adult. The general shape of the nephridium is shown in fig. 25. Stretching back from the funnel (fig. 25, *n. f.*) we have a narrow duct which passes through the septum, and gradually swells into a pear-shaped mass (figs. 25 and 28, *p. s. m.*), which is slightly pigmented, contains a large number of granules, and in which the canal is much convoluted (fig. 28, *nph. c.*). The main body of the nephridium (fig. 25, *b. n.*) is a more or less irregularly lobed mass lying by the side of, and spreading over the ventral blood-vessel, to which it closely adheres (fig. 32). Although the right and left nephridia in the same segment mingle over the blood-vessel, their canals do not communicate. From the main body starts a canal to the exterior (fig. 25, *c. ext.*), which opens on the surface in front of the ventral setæ by a minute pore (fig. 25, *nph. p.*). The internal funnel is small and somewhat flattened, but has its dorsal lip expanded into a large, flat, ciliated process of oval shape (figs. 26—29, *c. p.*) Delicate waving cilia are set round the edge of the funnel and its process (figs. 26 and 27, *ex. ci.*) ; on the lower or inner surface of the flat process alone arise numerous long cilia, forming a flame-like structure with its free end pointing into the canal (figs. 26, 27, and 29, *fl.*). These long cilia beat together with a peculiar quick motion, forming rapidly moving undulations which, starting from the rim of the process and travelling towards the tip of the flame, give the whole structure, with its transverse waves, somewhat the appearance of a tennis racket with its cross strings. The nephridiostome is formed of two cells with oval nuclei (fig. 29, *n. f.*) ; the rest of the organ consists of a mass of cells with roundish nuclei, and without distinct cell-outlines, pierced through and through by the delicate nephridial canal (fig. 30). Except for the few short blind diverticula given off, especially from that part of the canal which leads to the exterior (fig. 25, *c. cxt.*), it does not branch,

and although much convoluted, appears to be continuous throughout. Here and there this canal enlarges into an ampulla, in which waves a flame-like bunch of cilia pointing towards the external aperture (figs. 28, 31, and 32, *c. a.*). No cilia are found anywhere else but in these ampullæ, which are not very numerous.

The gonads consist of a pair of testes on the anterior wall of the 10th segment (fig. 12, *t.*), and a pair of ovaries on the anterior wall of the 11th segment (fig. 12, *ov.*). The sperm-ducts are rather short tubes opening by means of widely opened shallow ciliated funnels into the 10th segment (fig. 12, *sp. f.*). A short narrow duct passes from behind the funnel through the 11th septum, rapidly changes into a thick tube for about half its course, then narrows again and opens into a median chamber below the nerve-cord (fig. 12, *sp. ch.*). This cavity, which may be called the median spermiducal chamber, opens to the exterior towards the posterior end of the 11th segment (fig. 2, *m. p. sp.*) by an irregular longitudinal aperture. The openings of the sperm-ducts into the chamber are situated very near together, close to the nerve-cord (fig. 22, *sp. p.*)

Fig. 24 represents a median longitudinal section through the spermiducal chamber; it shows how the nerve-cord (*n. c.*) and the ventral blood-vessel (*v. v.*) pass over it. From the structure of its wall with the cuticle, the epidermal cells (*ep.*), the longitudinal and circular muscle-fibres (*c. m.* and *l. m.*), the median chamber is obviously a direct invagination of the body-wall. Moreover, that this is really the case is proved by the fact that in the young worm the sperm-ducts come separately to the surface (fig. 23, *sp.*), and there is then no sign of a median pore. The gradual formation of the median chamber can be traced from its earliest beginning.

The sperm-duct, except the narrow terminal region, is ciliated. The structure of the wide region is shown in a longitudinal section in fig. 17, where it is seen that its large size is chiefly due to the covering of cœlomic epithelium cells (*c. ep.*), which have become modified into long columnar cells

with granular protoplasmic contents. Under this is a thin layer of circular and a few longitudinal muscles (*c. m.*), followed by the ciliated lining epithelium of the duct (*w. of sp.*) composed of small granular cells. In the narrow region of the sperm-duct the cœlomic epithelium is not modified (fig. 18, *c. ep.*), while the lumen is bounded by columnar epithelium, the cells of which are very deep when the duct is not expanded (fig. 18, *w. of sp.*). There is no special penial apparatus, and no prostate.

The oviducts appear to be reduced to a mere depression on each side of the ventral edge of the 12th septum (fig. 12, *ovd.*).

The spermathecæ are two pear-shaped sacs (fig. 12, *spth.*), opening in the median ventral line by means of a common median pore immediately below the nerve-cord at the anterior end of the 10th segment (fig. 2, *spth. p.*). Fig. 20 shows a transverse section of an adult worm in this region passing through the median pore (*spth. p.*), at which open both the right and the left spermathecæ (*spth.*). It is interesting to note that in the young worm a similar transverse section (fig. 21) shows that here the two spermathecæ (which have all the appearance of being direct invaginations of the epidermis) arise distinctly from right and left points some distance from each other (fig. 21, *spth. p.*); it is only later that the necks of the two organs approximate and finally open by a common aperture. This may easily be confirmed by surface views of the living worm. The structure of the wall of mature spermathecæ is shown in fig. 19. Outside is the ordinary cœlomic epithelium (*c. ep.*), followed by a layer of scattered longitudinal and circular muscles; finally we have lining the interior of the organ a thick epithelium, with large oval nuclei ranged with their long axis parallel to the surface. The lumen of the neck of the spermathecæ has a pronounced constriction and enlargement (fig. 12), as in many Tubificidæ. Spermatophores have not been observed.

In the mature worm there are two large sperm-sacs and one large ovisac. By cutting longitudinal sections of individuals

of varying ages the development of these organs can be traced, and the result has been diagrammatically represented in fig. 13. The spermatozoa are shed at an early stage of development into segment 10, and the anterior septum of this segment soon bulges out, forming a sac—the anterior sperm-sac (fig. 13, *a. sp. s.*). Later on this sperm-sac pushes its way across segment 9, through its anterior septum into segment 8. The hinder wall of segment 10 also bulges out, forming the posterior sperm-sac (fig. 13, *p. sp. s.*) The ova are shed into segment 11. The hinder septum of this segment forms the ovisac (fig. 13, *ov. s.*), which ultimately pierces as many as seven or eight septa behind it.[1] The posterior sperm-sac also enlarges, enters the ovisac, and grows back through five or six segments. It is within the space between the wall of the ovisac on the outside and the wall of the posterior sperm-sac within, that the blood-vessels run which supply nourishment to the ova (fig. 5, *v. ov.*); it is also, no doubt, through this space that the ova escape back again to the 11th segment when ripe.

The clitellum extends over segments 10—12, and part of segment 13.

Summary and Conclusions.

The chief characters of Vermiculus pilosus are therefore the following:

Four bundles to each segment of furcate setæ, generally three per bundle.

A dense covering of hair-like processes.

A vascular system, containing red blood, and composed of a dorsal and a ventral longitudinal vessel communicating by means of lateral vessels which branch on to the body-wall. The absence of hearts or commissural vessels. An elaborate system of unicellular valves in the longitudinal and transverse dorsal vessels.

[1] The ovisac pierces the septa at a point nearer to the dorsal blood-vessel than is represented in the diagram.

A brain deeply cleft in front, and a nerve-cord bearing muscular strands of considerable size.

A compact nephridium, the funnel of which is peculiarly modified.

A pair of testes in the 10th, and a pair of ovaries in the 11th segment.

Two short sperm-ducts, without prostate, opening into a median chamber, which itself opens to the exterior on the 11th segment by a large median pore. Oviducts rudimentary.

Two pear-shaped spermathecæ, opening by a common median ventral pore on the 10th segment.

An anterior and a posterior sperm-sac, and an ovisac.

A clitellum extending over the 10th, 11th, 12th, and part of the 13th segments.

Many of these characters place this little worm in a very isolated position. The dense covering of "sense-hairs," although perhaps of no great morphological importance, is quite unique amongst the Oligochæta. The absence of commissural vessels again distinguishes it, I believe, from all its allies, except the smallest and lowest, such as Æolosoma; nor is it usual among the Tubificidæ, to which Vermiculus is doubtless related, to find strong muscular strands on the nerve-cord.

As for the nephridium, the ciliated process of the funnel is no doubt of the same nature as the long ciliated processes on the nephridiostome of Nereis (3);[1] all such structures appear to be specialised processes of the funnel-cells themselves. The presence of cilia in special ampullæ, and their absence in the rest of the nephridial canal, is a character which may, I think, prove to be common to all the so-called Microdrili. Such ampullæ are certainly present in other Tubificids I have examined, and in the Enchytræids, to the nephridium of which latter the compact excretory organ of Vermiculus bears no little resemblance. Professor Vejdovsky (6) has described a Planarian, Microplana humicola, in which the nephridia

[1] I once observed in an Enchytræid long ciliated processes on the nephridiostome, exactly like those of Nereis above referred to.

terminate in flame-cells, while the canal itself is provided with "flames" at intervals along its course; it seems not improbable that the flame-like cilia of the nephridiostome of Vermiculus represent the "flame" of the terminal cell, and the cilia of the ampullæ represent the "flames" distributed along the course of the canal in the Planarian. Possibly the arrangement found in the large nephridia of the Earthworms, described by Benham (1), in which whole tracts are ciliated, is derived from some such system as we find in Vermiculus and the lower Oligochætes by the extension of the cilia over a great length of the canal.[1]

The late development of the median spermiducal chamber is another of those characters quite peculiar to our worm. What the function of this chamber may be it is difficult to conjecture; perhaps it acts as a sucker during copulation; the disposition of the muscles would favour this supposition. However, it must be noticed that besides the formation of the spermiducal chamber, the apertures of the genital organs themselves show a distinct tendency, as it were, to unite in the middle line. This is clearly seen in the case of the male pores, which come close to each other; but more especially in the case of the spermathecal pores, which become actually confluent. As a striking contrast, we may compare such a form as Heterochæta, in which, as described by Benham (2), the sperm-ducts and spermathecæ open above the ventral setæ!

The fact that Bothrioneuron, described by Stolc (5), possesses a median male pore might suggest a close relationship between the two worms, but they differ in other particulars most markedly from each other. The setæ, nervous system, and nephridia of Bothrioneuron are all very different from those of Vermiculus; it possesses commissural vessels, a prostate, a complicated system of genital setæ, and no spermathecæ.

On the whole, it must be concluded that Vermiculus stands

[1] Since this was written, Professor A. G. Bourne has in this Journal (vol. xxxvi, 1894, "On Moniligaster grandis") described somewhat similar undulating bundles of cilia, which, however, are attached at both ends, not hanging freely in the canal as the cilia do in the ampullæ described above.

very much by itself; the shape of its setæ, and above all the situation of its gonads, place it in the family Tubificidæ, but its more intimate relationships remain obscure for the present.

March 30th, 1894.

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EXPLANATION OF PLATES 26—28,

Illustrating Mr. E. S. Goodrich's paper, "On the Structure of Vermiculus pilosus."

List of Reference Letters.

a. sp. s. Anterior sperm-sac. *b. n.* Main body of the nephridium. *br.* Brain. *b. v.* Blood-vessel. *b. w.* Body-wall. *c.* Cuticle. *c. a.* Ciliated ampulla. *c. c.* Cœlomic corpuscle. *c. ep.* Cœlomic epithelium. *c. ext.* Canal to the exterior. *ci.* Cilia. *cl.* Clitellum. *c. m.* Circular muscles. *c. n.* Cell of the nephridium. *c. p.* Ciliated process. *cœl.* Cœlom. *d. v.* Dorsal longitudinal vessel. *ep.* Epidermis. *ep. cl.* Epidermis of clitellum. *ep. spth.* Internal lining of the spermatheca. *ex. ci.* External cilia. *fl.* Flame-like cilia. *g. c.* Ganglionic cell. *int.* Intestine. *l. d. s.* Left dorsal bundle of setæ. *l. d. v.* Lateral dorsal vessel. *l. l.* Lateral line. *l. m.* Longitudinal muscles. *l. v. s.* Left ventral bundle of setæ. *l. v. v.* Lateral ventral vessel. *m.* Mouth. *m. l. v. v.* Middle lateral ventral vessel. *m. n. c.* Muscles on the nerve-cord. *m. n. c. l.* Lateral strand of muscles. *m. n. c. m.* Median strand of muscles. *m. p. sp.* Median pore of the spermiducal chamber. *m. s.* Muscles of the septum. *n. c.* Nerve-cord. *n. f.* Nephridiostome. *nph. c.* Nephridial canal. *nph. p.* Nephridiopore. *œs.* Œsophagus. *ov.* Ovary. *ovd.* Oviduct. *ovs.* Ovisac. *pcl.* Palpocil or sense-hair. *p. l. v. v.* posterior lateral ventral vessel. *ph.* Pharynx. *pr.* Prostomium. *p. s. c.* Post-septal canal. *p. s. m.* Post-septal mass. *p. sp. s.* Posterior sperm-sac. *r. v. s.* Right ventral body of setæ. *set.* Seta. *sept.* Septum. *sp. p.* Spermiducal pore. *sp. ch.* Median spermiducal chamber. *sp. f.* Spermiducal funnel. *spth.* Spermatheca. *spth. p.* Spermathecal pore. *t.* Testis. *v.* Valve. *v. ov.* Vessel to ovisac. *v. v.* Ventral longitudinal vessel. *w. of sp.* Wall of sperm-duct. *w. of v.* Wall of blood-vessel.

Fig. 1.—Adult Vermiculus pilosus, drawn natural size.

Fig. 2.—View of the anterior part of the body, slightly twisted so as to display the dorsal and ventral setæ and the median genital apertures.

Fig. 3.—Enlarged view of a seta. Z. apoch. 4 mm., oc. 4, camera.

Fig. 4.—Small portion of the body-wall, drawn from the living to show the covering of hair-like processes. Z. apoch. 4 mm., oc. 4.

Fig. 5.—Semi-diagrammatic view of the vascular system from the dorsal surface; from the living.

Fig. 6.—View of the brain and anterior part of the alimentary canal; from the living.

Fig. 7.—Portion of a transverse section of the body-wall in the region of the lateral line. Z. apoch. 4 mm., oc. 4, camera.

Fig. 8.—Longitudinal section through the longitudinal dorsal vessel, passing rather to the side, so that the lumen does not appear continuous. Z., ob. D, oc. 4, camera.

Fig. 9.—Portion of the vascular system, showing the manner of communication between the lateral dorsal and the lateral ventral vessels; from the living.

Fig. 10.—Small portion of the dorsal blood system in the region of the 10th and 11th segments, greatly enlarged to show the valves; from the living. Z., oil imm. 2 mm., 4 oc.

Fig. 11.—Diagram of the three positions taken up by the valves: a', when the flow is forwards; b', to stop the flow backwards; and c', when they are forced back.

Fig. 12.—Diagram representing the genital organs in situ; from the living and sections.

Fig. 13.—Diagram representing the development of the sperm-sacs and ovisac in an immature specimen, as seen in a solid longitudinal section; chiefly from sections.

Fig. 14.—Transverse section of the nerve-cord passing through a septum. Z., ob. D., oc. 3, camera.

Fig. 15.—Transverse section through a ganglionic region of the nerve-cord. Z., ob. D, oc. 3, camera.

Fig. 16.—Transverse section of the nerve-cord between two ganglia. Z., ob. D, oc. 3, camera.

Fig. 17.—Longitudinal section of the thick region of the sperm-duct. Z., ob. D, oc. 4, camera.

Fig. 18.—Longitudinal section of the narrow region of the sperm-duct. Z., ob. D, oc. 4, camera.

Fig. 19.—Section through the wall of the spermatheca. Z. apoch. 4 mm., oc. 4.

Fig. 20.—Transverse section of an adult worm passing through the median spermathecal pore. Z., ob. A, oc. 3, camera.

Fig. 21.—Tranverse section of a young worm passing through the spermathecæ. It has been necessary to combine two sections to show this clearly. Z., ob. A, oc. 3, camera.

Fig. 22.—Transverse section of an adult worm passing through the median spermiducal chamber and its pore. Z., ob. A., oc. 3, camera.

Fig. 23.—Transverse section of a young worm in the same region, showing the absence of a median chamber. It has been necessary to combine two sections. Z., ob. A, oc. 3, camera.

Fig. 24.—Longitudinal section through the median spermiducal chamber.

Fig. 25.—General view of a nephridium, seen from below; from the living. Z., ob. D, oc. 4.

Fig. 26.—View of the nephridial funnel from below; optical section from the living. Z. apoch. 4 mm., oc. 8.

Fig. 27.—View of the nephridial funnel from the side; optical section from the living.

Fig. 28.—View of the post-septal mass of the nephridium, reaching through the septum to the funnel; from the living. So far diagrammatic that the whole course of the canal is represented, although it could not be followed out for certain in all its parts. Z., ob. D, oc. 8.

Fig. 29.—Section through the nephridiostome and post-septal canal, showing the nuclei of the funnel cells. Z. apoch. 4 mm. oc. 4, camera.

Fig. 30.—Small portion of section through the body of the nephridium, showing the canal and the nuclei of the nephridial cells. Z. apoch. 4 mm., oc. 4, camera.

Fig. 31.—Small portion of the living nephridium, showing the ciliated ampullæ. Z., oil imm. 2 mm. oc. 4.

Fig. 32.—Main body of the nephridium attached to the ventral longitudinal blood-vessel; from the living. Z. apoch. 4 mm., oc. 4.

On the Mouth-parts of the Cypris-stage of Balanus.

By

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With Plate 29.

The buccal mass of all ordinary Cirripedes consists, as is well known, of three pairs of jaws, together with the labrum and palps. The form and disposition of these mouth-organs in the various genera of the Thoracica has been well investigated by Darwin, and the differences observed between the different forms in respect to these parts have been utilised for purposes of classification. The morphological significance of the jaws has, however, never been satisfactorily ascertained, and the current views are all practically based upon suppositions, the actual development having been traced in no case.

Darwin (Nos. 1 and 2) regarded the frontal filaments seen in the larva as the first pair of appendages, and taking the eyes to mark the first segment of the head, and believing that the prehensile antennules arose within the fronto-lateral horns, and that the three pairs of jaws arose in front of the Nauplius appendages, regarded the mandibles of the adult as the appendages of the fourth segment of the typical Crustacean, and the two pairs of maxillæ as the fifth and sixth respectively.

The eyes, however, are now no longer regarded as modified appendages; the frontal filaments, too, have been more correctly regarded as sense-organs, and the fronto-lateral horns have been proved by Claus and others to be glandular processes of the carapace.

Kröhn, Willemoes-Suhm, and Lang (Nos. 3, 7, and 9) have proved that the prehensile antennules of the Cypris-stage in the Thoracica arise from the first pair of Nauplius appendages (antennules), and Delage (No. 10) has shown the same for Sacculina.

With reference to the fate of the remaining Nauplius appendages, Metschnikoff, Willemoes-Suhm, and Lang (Nos. 3, 7, and 9) believed that both pairs were lost, and the first-mentioned supposed that the mandibles and two pairs of maxillæ were all new structures formed inside a fourth pair of appendages seen behind the third pair of Nauplius appendages.

Claus, on the other hand, supposed (No. 8) from the analogy of other Crustacea that the mandibles of the Cypris-stage arose from the third pair of Nauplius appendages, the latter not being lost like the second pair, but greatly reduced in size. This observer did not, however, succeed in proving his point, and I shall attempt in the following remarks to show that this view is the correct one.

The Cypris-stage, as is well known, possesses a well-defined buccal mass. This, on account of its concealment within the carapace, its small size, and delicate nature, as well as the close packing of the component parts, is difficult to make out satisfactorily.

Darwin found in the Cypris-stage of Lepas australis "all the masticatory organs of a Cirripede in an immature condition."

Pagenstecher (No. 4) describes the mouth-organs of the Cypris-stage of Lepas pectinata after fixation as consisting of imperfect lobes and papillæ without bristles or teeth.

Willemoes-Suhm (No. 7) did not succeed in satisfactorily separating the mouth-parts in Lepas fascicularis, but describes the buccal mass as consisting of "three parts all very rudimentary."

Claus (No. 6) also describes three pairs of gnathites in an undetermined Cypris-stage. These had the form of simple outgrowths; the mandible was largest and connected with the labrum by a finger-shaped palp referable equally to the upper

lip or to the mandible; the two hinder pairs Claus describes as giving the impression of a single pair of appendages.

Of the two species of Balanus the Cypris-stage of which I have carefully examined, B. perforatus, on account of its minute size, is much less favourable for purposes of investigation than B. balanoides. Repeated dissections of the pupa of the latter form have enabled me to form a tolerably good idea of the constitution of the buccal mass.

Viewed from behind (fig. 3), a pair of appendages (mx^2.), clearly identical in position and relations with the lower lip, or second pair of maxillæ of the adult, is recognisable. These are attached on each side behind along an oblique straight line, and are somewhat broader at the base than at the apex. Like the second maxillæ of the adult, they are closely applied to one another in the middle line. Seen in side-view (fig. 8) they are somewhat lanceolate in shape, the apex generally projecting somewhat forwards; the hinder margin is convex, while the anterior is somewhat sinuous. Like the remaining mouth-parts, and like those of Lepas, as described by Pagenstecher, they are simple lobes devoid of teeth or bristles. At the junction of these appendages with the first pair of maxillæ may be seen a conical chitinous process (figs. 1, 3, 4, 6).

The first maxillæ (mx^1.) are situated externally to the second, and project somewhat behind the latter (fig. 1, cf. also fig. 11). They are similarly convex externally, but are simply concave on the inner side; they lie in planes inclined outwards at a greater angle than the second maxillæ (cf. fig. 11). Like the remaining mouth-parts they are broadest externally, narrowing towards the centre. They do not attain quite the vertical height of the second maxillæ (fig. 1).

The mandibles are large, and occupy the outer part of the buccal mass (figs. 1—3), being found externally and anteriorly to the first pair of maxillæ (figs. 1—3; cf. also fig. 11). The main portion of the mandible passes distally into an inwardly-curved process with a somewhat truncated end, giving the appendage much the form of the adult mandible or first maxilla, though, as already remarked, no teeth or hairs are present.

Anteriorly the basal portion of the mandible gives off a somewhat inwardly-curved palp-like process (figs. 1—5, *palp.*). This has all the appearance of being an integral portion of the mandible, and is filled by a mass of developing muscle continuous with that of the rest of the appendage, and running, together with the muscle of the two pairs of maxillæ, to the anterior part of the thorax (fig. 1). The anterior margin of the basal portion of the mandible (figs. 1 and 5) is apparently very short, though this is difficult to ascertain definitely, since in teased preparations the buccal mass commonly tears away from the head immediately in front of the palp; it is, thus, easy to see why Claus regarded the palp as belonging equally to the mandible or labrum. The evidence, too, afforded by the muscle-supply must be taken with caution, as Nussbaum (No. 11) describes a muscle supplying the mandible in the adult Conchoderma as having its origin in the palp. The evidence, however, afforded by the immature Cypris-stage to be described immediately appears to clearly show that the palp belongs to the mandible.

The three pairs of gnathites, according to Claus, are situated beneath a labrum; the labrum, however, as a definite prominence, is exceedingly small at this stage in Balanus, and projects as a very minute lobe between the distal ends of the palps. The site of the future labrum is, nevertheless, recognisable as a broad area in front of and between the mandibles and their palps. The jaws are directed ventrally, so that this area is to be described as situated anteriorly to, rather than below the jaws.

Some stages of an undetermined species of Balanus obtained from Messrs. Sinel and Hornell in Jersey in the spring of the present year (1894) were specially valuable as throwing light on the question of the fate of the Nauplius appendages, and on the homologies of the jaws of the adult. The antennules of the Nauplius, as already pointed out, have long been known to give rise to the prehensile antennules of the Cypris-stage, and these, according to Darwin, can often be recognised in the adult. The fate of the antennæ of the Nauplius has not been

traced. Fig. 12 shows the position of the base of the antenna and its socket in the last Nauplius-stage of the Balanus in question. The socket is seen to lie at the side of the labrum, just behind that of the antennule; the innermost part of the base of the mandible projects as a pivot, which fits into a slight indentation in the side of the labrum. The origin of the mandibles is well behind the mouth and attachment of the labrum. As the Nauplius prepares for the moult which transforms it into the Cypris-stage the body contracts, and many of the structures belonging to this latter stage can be seen beneath the cuticle. If the larva be now carefully dissected out of the cuticle, or be examined just after the moult, it shows peculiar features in which it approaches the Nauplius-condition, and which are not found in the perfect Cypris-stage. Figs. 9 and 10 show the larva in this stage. The edges of the carapace have not yet met in the mid-ventral line, and allow good views of the ventral surface of the animal. The prehensile antennules can be seen at the anterior end of the labrum, which, in most cases, is still recognisable, often as a well-developed structure. The sides of the labrum are indented, as in the Nauplius-stage, by the remnants of the antennæ, which are now undergoing histolysis and absorption, but are easily recognisable. Behind these can be seen the mouth-parts of the Cypris-stage already well-formed. These consist of the mandibles with their palps, and the first and second pairs of maxillæ. In the more advanced stages the labrum and antennæ are small (fig. 11) or have practically disappeared, so that the larva has attained the condition (so far as these organs are concerned) seen in the perfect Cypris-stage. A series of stages were obtained showing the gradual reduction of these parts. A highly important feature is that the mandibles, clearly forming with their palps a single appendage, can be detected on the site of the mandible of the Nauplius, behind the antennæ and labrum. The labrum shows in some cases more or less clear indications of the median and lateral lobes found in the Nauplius at the distal extremity. It is clear, then, that the palps can neither be regarded as equivalent to the antennæ, nor to the lateral lobes

of the labrum of the Nauplius, but belong to the mandibles. Judging by analogy the palp will represent the ramus of this appendage, the mandible proper being the gnathobase.

Of the two pairs of maxillæ, the first pair are indicated after the first moult undergone by the Nauplius by a row of bristles which has been termed the extra-maxillary arc (No. 12). At a later stage this becomes a small foliaceous appendage (fig. 13) provided with a number of bristles, and already correctly regarded by Claus as an early condition of the "outer maxilla" (No. 8). Inside this the first maxilla of the Cypris-stage clearly arises (fig. 13). The second pair of maxillæ appear later, simultaneously with the six pairs of cirri; they arise, as described by Claus, just in front of the first pair of the latter, and are clearly serially homologous with the thoracic appendages. Further details as to the origin of the two pairs of maxillæ will be given on a future occasion.

It may be regarded as tolerably certain from what has been said above that:

(1) The antennæ of the Nauplius become definitely lost with the moult resulting in the production of the Cypris-stage.

(2) The biramous mandibles of the Nauplius become reduced at the same time to the small mandibles, the ramus being probably preserved in the form of the small palp.

(3) The first pair of maxillæ arise behind the mandibles, and at a later date, as a small pair of foliaceous appendages.

(4) The second pair of maxillæ arise still later just in front of the first pair of thoracic legs (cirri).

The mandibles, first maxillæ and second maxillæ are accordingly developed consecutively in the order named. They cannot be regarded as parts of a single or fourth pair of appendages as Metschnikoff maintains, neither can the two pairs of maxillæ be regarded as outer and inner parts of a single pair of appendages as Claus suggests. The mandibles of the Nauplius are not lost as many authors have maintained, but give rise, as Claus supposes, to the mandibles of the Cypris-stage. The jaws represent, then, the third, fourth,

and fifth pairs of appendages of the typical Crustacean series.

This conclusion is quite in harmony with that of Nussbaum, as deduced from his beautiful anatomical investigations of the adult Cirripede, "Die Anordnung der Musculatur spricht dafür, dass jeder der drei Kiefer aus einem Beinpaare hervorgegangen ist" (No. 11).

In the fate of the mandibles of the Nauplius and in the constitution of the head of the adult the Cirripedia thus conform to what is recognised as typical among other Crustacea.

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EXPLANATION OF PLATE 29,

Illustrating Mr. Theo. T. Groom's paper, "On the Mouth-parts of the Cypris-stage of Balanus."

Fig. 1.—Side view of buccal mass of Cypris-stage of Balanus balanoides. *Mandible.* Mandible with (*palp*) its palp. *mx*^{1}. and *mx*^{2}. First and second maxillæ, showing chitinous process between their bases. *bucc. m. musl.* Muscles supplying buccal mass. *Thoracic muscles.* Muscles filling up the first segment of the thorax.

Fig. 2.—View of same from in front. Letters as in Fig. 1. Also, *adductor.* Adductor muscle of carapace.

Fig. 3.—View of same from behind.

Fig. 4.—Similar view, the form of the appendages being shown by transparency.

Fig. 5.—Mandible and its palp.

Fig. 6.—View of first maxillæ from outside.

Fig. 7.—View of same from side.

Fig. 8.—View of second maxillæ from side.

Fig. 9.—Side view of an immature Cypris-stage of an undetermined species of Balanus from Jersey. *adductor.* Adductor muscle of carapace. *ant*^{1}. Antennule. *ant*^{2}. Antenna. *caudal app.* Caudal appendage. *compd. eye.* Compound eye. *frl. aperture.* Aperture of fronto-lateral gland. *mx*^{1}. First maxillæ. *mx*^{2}. Second maxillæ. *palp.* Palp of mandible.

Fig. 10.—Ventral view of similar larva. Lettering as in Fig. 9. Also, *ant*^{1}. *muscles.* Muscles to antennule. *cp. edge.* Edge of carapace.

Fig. 11.—Ventral view of mouth-organs of a larva in a somewhat more advanced stage. The labrum is now greatly reduced, and the sites of the antennæ barely recognisable.

Fig. 12.—View of labrum and bases of antennules, and antennæ with their sockets (*ant*^{1}. *sock.*, *ant*^{2}. *sock.*) of the last Nauplius-stage of a species of Balanus from Jersey. *mandible sock.* Socket of mandible.

Fig. 13.—View of the developing maxillæ (*mx*^{1}. and *mx*^{2}.) of the Cypris-stage, as seen in a similar Nauplius. The small setigerous first maxillæ are external, while the two pairs of pupal maxillæ are underneath the cuticle; the first pair of these has the tip slightly withdrawn from the external maxillæ, inside which they were formed.

A Study of Coccidia met with in Mice.

By

J. Jackson Clarke, M.B.Lond.

With Plate 30.

Nearly all that has been published on Coccidia of mice is contained in the writings of Eimer[1] and Th. Smith.[2] Ludwig Pfeiffer,[3] in reference to what these authors have said on this subject, concludes: "Unsern frühern Auseinandersetzung nach, handelt es sich hier um das Schwärmercystenstadium einer Coccidie: das dauercystenstadium ist unbekannt." In order to supply data which may fill the gap thus indicated, I venture to submit the following observations.

A white mouse, kept in a place previously occupied by rabbits, was found dead. Dissection revealed the presence of large numbers of Coccidia in every part of the alimentary tract beyond the cardiac opening of the stomach. There was no other abnormal feature. Sections of the intestine showed an altered condition of the epithelium, but although decomposition had not begun when the tissue was fixed I could not, apart from the encapsuled parasites collected in the lumen of the gut, have been certain that epithelial infection by Sporozoa was present, so soon after death do some of the intracellular protozoa lose their characters.

The contents of the intestine were spread on a clean slide,

[1] Eimer, 'Ueber die Ei- oder Kugelförmigen sogenannten Psorospermien, &c.,' Würzburg, 1870.

[2] Th. Smith, Washington, 'Journal of Comp. Med. and Surg.,' 1889.

[3] L. Pfeiffer, 'Protozoen als Krankheitserriger,' 1891, p. 57.

placed on damp blotting-paper in a Petri's dish,[1] and kept at room temperature (May—June).

The ripe free parasites were uniformly much smaller (averaging 18 × 13 μ) than the Coccidium oviforme of the rabbit (averaging 32 × 22 μ). They were also of a more rounded shape. When first examined the capsules of the free parasites were completely filled by granular protoplasm, having the usual dense, round, central body; they were examined on successive days for a fortnight, and I may briefly indicate the results by saying that with the modification in size previously alluded to they underwent the same changes as does C. oviforme of the rabbit when placed under similar circumstances. Thus on the sixth day most of the parasites had subdivided (fig. 2) into four granular spheres, of which some possessed a clear oval corpuscle placed as far as possible from the point of meeting of the segments (fig. 3). On the eighth day the subdivisions (sporogonia) had assumed an oval form, and some presented a capsule and a differentiation into spores and granular residual matter (nucléus de reliquat). Many of the spores showed the dumb-bell form described by Leuckart in C. oviforme of the rabbit (see fig. 5), and in one or two cases I was able to see the division of the C-shaped body into two comma-shaped spores, as described by Balbiani. This arrangement of lasting spores is, as in the case of the rabbit's Coccidium, often departed from, and appearances such as are depicted in fig. 6 occur frequently.

In order to have material for histological study, and to test the time required for the manifestation of the disease, a young healthy mouse, whose fæces were found to be free from Coccidia, was fed with bread-sop containing some of the material taken from the Petri dish after twenty-two days' exposure to air.[2]

[1] This consists of two shallow glass dishes, one of which is larger than the other, and when inverted forms a cover which excludes dust from the smaller one.

[2] I have given the days on which the various appearances were first noted, and I do not wish to convey an impression that on any given day all the

No change was noticed in the animal until six days after the parasites were administered. On this day its coat was noticed to be rough, the abdomen was distended, and the thighs somewhat drawn up towards the belly, so that the animal's gait was stiff. The stools were softer than normal. The next day blood [1] was noticed about the animal's anus, and this sanguineous discharge contained coccidia of the same dimensions as those described above. On the seventh day the animal was distinctly ill and suffering, so it was killed. After death the stomach was found to be distended, the contents consisting chiefly of parasites, many of which showed the formation of sickle-spores [2] as shown in fig. 8. The large and small intestine contained practically little beside ripe Coccidia. The stomach, intestines, and portions of the liver and kidneys were fixed in saturated solution of corrosive sublimate and hardened in the usual way. After embedding in paraffin, sections were cut and stained in Ehrlich's acid hæmatoxylin and eosin (Grübler's wasserlöslich).

Histological Examination.

In the stomach the glands of the cardiac end contained free Coccidia, many of which were encapsuled and filled with small swarm-spores as shown in fig. 8; but the most striking feature consisted in masses of minute bodies exactly resembling the swarm-spores within such capsules, but stained and lying free on the surface of the mucous membrane, and distending the ducts

coccidia presented the same appearance. On the contrary, up to nineteen days after the parasites were placed in the moist chamber, nearly all the phases could be observed at the same time; but on the date last named all the parasites were subdivided into sporocysts, each of which comprised two spores and a nucleus de réliquat, as the residual body has been termed by Aimé Schneider.

[1] This phenomenon is of interest in connection with an observation recorded by Hess ('Schweitz Archiv für Thier.,' Zürich, 1892, vol. xxxiv, p. 125), to the effect that the "Rothe Ruhr" of cattle is associated with the presence of a coccidium.

[2] Compare R. Pfeiffer, 'Beiträge zur Protozoon-Forschung,' Berlin, 1892.

of the glands. In the latter situation the accumulated spores, devoid of any containing capsule, had an appearance which recalled the well-known Sarcosporidia—a resemblance which will be referred to again in a subsequent part of this paper. That these small free bodies were swarm-spores derived from the coccidia was shown conclusively, I think, by a close examination of the epithelial cells against which they lay. Many of the cross-sections of the pyloric glands had the appearance shown in fig. 9, where some of the lining cells contain one or several minute bodies, many of the same size and appearance as the small free bodies lying in the lumen of the gland.

From the minute intracellular parasites which lay in the cell-protoplasm a gradation of forms could be traced up to the encapsuled swarm-sporing parasites; but in the stomach the full-sized intracellular parasites were very few in number compared with those about to be described in the intestine. This was probably due to the majority of the parasites having subdivided into swarm-spores without the formation of a capsule, a process which occurs in C. oviforme in the acute disease, and which will be described immediately in the small intestine in the mouse.

Small intestine.—Sections taken from various parts all presented similar features. The epithelial cells of the glands of Lieberkühn were almost every one infected, and in the majority of them the parasites were spherical with numerous "corps albuminoides de réserve," and a small central body which stained with eosin, and in the lumen of the gut lay the encapsuled free parasites.

Sections of the small intestine showed that there the epithelial cells were as much infested as in the stomach, but the parasites presented a somewhat different aspect, as is shown in fig. 10, which represents a cross-section of one of the crypts. The epithelial cells show signs of having proliferated, being in places three deep instead of forming a single layer, and the sickle-shaped swarm-spores are wanting. Most of the intracellular parasites are in the shape of granular spheres, the granules staining with eosin; but some, such as

the one marked *a*, have larger granules[1] staining deeply with hæmatoxylin. One parasite (*b*) has surrounding it a thick capsule, and is in the same phase as that shown in fig. 1. The granules and the central body took the eosin and not the hæmatoxylin of the stain. Other parasites were larger than this, but still devoid of a capsule (*c*), and represented, I believe, an intermediate phase between the common form (*b*) and the phase represented by the bodies *d* and *e*, which measured respectively 34 × 19 μ and 35 × 22 μ. The marginal part is represented as seen in optical section, and is marked by a close-set series of short rods which stained a deep blue with hæmatoxylin. The lighter dots in the bodies represent the surface rods seen out of focus, and the series of darker dots in their inner parts are represented as seen in focus, though they are placed on the surface of the parasites. I have met with parasites in this phase in the liver and intestine of rabbits suffering from acute coccidial disease, and as I have nowhere seen a full account of this particular phase, I may introduce two figures (11 and 12) taken from sections of a rabbit's liver. These short rods I believe to be of the nature of chromosomes, and the parasites in this phase to be almost wholly nuclear in constitution. I once believed them to be wholly nuclear, until lately, at the suggestion of Professor Marcus Hartog (Cork), I looked for a layer of protoplasm, which I have found to be present, but so thin is this layer that it is difficult to make out. Some of the modes of subdivision of this phase of the parasite are illustrated in fig. 10, *d*, and figs. 11 and 12.

The large intestine contained innumerable coccidia both within the epithelial cells and free, but I could find none in the phase of sickle-formation as in the stomach, nor were there any with peripheral rods as in the small intestine. The liver and kidneys showed no abnormal features.

I have not yet been able to procure Eimer's original paper, but it is freely abstracted by Leuckart.[2] I have been able to

[1] These granules, like those staining with eosin, are in some cases "corps albuminoides," i. e. stored food material.

[2] Leuckart, 'Parasites of Man,' Hoyle's translation, 1886, pp. 197 and 219.

conclude that the parasites of Eimer (Eimeria, A. Schneider) are the same as those described in this paper. The chief grounds for this conclusion consist in the round form Eimer refers to in many of his parasites, and to their dimensions, 18 μ × 18 μ and 26 μ × 16 μ, approaching more nearly to the parasites described above than to C. oviforme in the rabbit, where the intestinal parasites average, I have found, 32 μ × 22 μ.

In order to test the transmutability of C. oviforme into Eimeria, I fed two young white mice whose excrement I had found to be free from coccidia with food mixed with the dung of a rabbit containing spore-ripe C. oviforme. The result of these experiments was negative.

A third experiment gave a positive result. A very old white mouse, whose fæces had been found to contain no coccidia, was given food mixed with some material which had been taken from the cæcum of a rabbit, and kept for two months in a moist chamber. There were many spore-ripe coccidia in this material. Five days after the administration of the parasites the mouse died, and its alimentary tract was found to be filled with coccidia like those in the other mice referred to above.

I may now conclude these observations by indicating the conclusions to which they point.

Conclusions.

1. That the Sporozoa described above as they were found in white mice belong to the Coccidiidea, and, like C. oviforme, to the Disporeæ.

2. That they are probably identical with the Sporozoa described by Eimer in the intestines of mice, and named Eimeria by Schneider.

3. That Eimeria is probably only a variety of C. oviforme, and may be but a modification of C. oviforme (although two out of three experiments made with a view of solving the point gave negative results) determined by the

smaller dimensions of the epithelial cells of the intestine of the mouse as compared with those of the rabbit.

4. That the appearance of large numbers of swarm-spores in the gastric glands of the mouse is very similar to that presented by the Sarcosporidia, and suggests that the latter is but one phase of a Sporozoon which may have in other phases a form resembling that of the Coccidia. A similar conclusion to this has been arrived at by L. Pfeiffer as the result of comparing Klossia with the Sarcosporidia.

October 3rd, 1894.

EXPLANATION OF PLATE 30,

Illustrating Mr. J. Jackson Clarke's paper, "A Study of Coccidia met with in Mice."

Fig. 1.—Ripe Coccidium, as seen with $\frac{1}{12}$ o. i. immediately after removal from the body of the mouse.

Fig. 2.—Coccidium after six days in the moist chamber.

Fig. 3.—The same after the same length of time.

Fig. 4.—The same after eight days.

Fig. 5.—The same, 10th day.

Fig. 6.—The same, 12th day.

Fig. 7.—Section of a duct of a cardiac gland (mouse's stomach).

Fig. 8.—Encapsuled parasite subdivided into sickle-shaped swarm-spores, examined fresh from the mouse's stomach.

Fig. 9.—Cross-section of duct of a pyloric gland.

Fig. 10.—Cross-section of a gland of the small intestine of a mouse, showing two large naked intracellular parasites with close-set peripheral bars of chromatin. The darker points on the surface of the parasite appear to mark the commencement of subdivision.

Fig. 11.—Naked and free coccidium from a section of a rabbit's liver, showing the mode of subdivision of a naked parasite with peripheral chromatin bars.

Fig. 12.—The same, showing another phase of subdivision.

Observations on Various Sporozoa.

By

J. Jackson Clarke, M.B.Lond.

With Plates 31, 32, and 33.

Certain problems propounded in the last few years demand for their solution a closer study of the intimate structure of the sporozoa than has hitherto been found necessary. The variations of nuclear form presented by these organisms call for especially close examination. For some of the higher members of the group this work has been well begun by Wolters,[1] who examined Clepsidrina blattarum, Monocystis magna and agilis in Lumbricus agricola, and Klossia in the kidney of snails. The description of the nuclear changes in the common gregarines of the earthworm is particularly complete, and since I have been able to confirm many of Wolters's observations it may be of interest if they are briefly reviewed here. He found in Lumbricus agricola both Monocystis magna and agilis constantly present, and almost to the exclusion of other species. In both, at every stage, a distinct nucleus was present. In the former it was relatively large and oval, in the latter of a rounded form. Under compression the nuclear membrane ruptured, but the contents did not escape, and when the compression was removed the nucleus tended to return to its primitive form. Thus the nucleoplasm appeared to be of a solid structure. In M. agilis this was also observed. The nuclear membrane was found to

[1] Max Wolters, "Die Conjugation und Sporenbildung bei Gregarinen," 'Archiv für mikroskop. Anat.,' p. 99, 1891.

be strong and sharply defined, and in the young parasite there was a single large nucleolus. Later the outline of the nucleus had become irregular and the nucleoli more numerous. In M. agilis, and in one instance in M. magna, Wolters encountered what he has named the flame-nucleus, a condition in which the nuclear membrane has disappeared, and the nuclear substance is prolonged at various points into the protoplasm of the body of the protozoon. As soon as the nucleolus has broken up into subdivisions the parasites are ripe for conjugation. It was found that after the syzygium was formed the nucleus of each parasite moved to the periphery, became elongated, and soon exhibited a typical nuclear spindle with the chromatin now massed together at the middle of the spindle. The chromosomes were very small. The division of each nucleus took place, and half of each nucleus was extruded as a polar body. Meanwhile the surfaces by which the parasites adhered to each other became altered in such a way that instead of the sharply marked line of division previously seen throughout a complete series of sections of a syzygium, there was at one part of the applied surfaces a communication through which the two parasites fused together. In each parasite the polar body was extruded on the surface opposite this communication, towards which, after the polar bodies had been formed, the two nuclei moved, and having reached the spot at the same time they fused together. After this a nuclear spindle was to be seen in each half of the syzygium, and could be distinguished, by its position close to the area of communication, from the spindles which were concerned with the formation of the polar bodies. Thus it appeared that the conjoined nuclei had undergone division, and that the daughter nuclei were again subdividing. The resulting two nuclei moved towards the periphery in each half of the syzygium, and there formed two spindles. This Wolters found to be the case in complete series of sections. These spindles were smaller than those of the polar bodies. By repeated subdivision these peripheral spindles and resulting nuclei increased in numbers and became surrounded by protoplasm constituting the sporogonia, which arranged themselves

peripherally, and also in spaces extending from the periphery into the remains of the body substance of the parasite.

The single nucleus of the sporogonia subdivided into eight, and meanwhile the sporogonia became surrounded by capsules constituting sporocysts (pseudo-navicellæ), the substance of which split up into eight crescentic spores, each of which contained a nucleus. Such are the chief results obtained by Wolters with regard to the Monocystides, and the thoroughness of the work and the beautiful drawings by Nussbaum, at whose instigation the work was undertaken, go far to establish the conclusions arrived at.

I will now detail some of the features I have obtained by examining the seminal vesicles of Lumbricus agricola, taken in the month of May. I have not had the opportunity of making such complete serial sections as Wolters, and in so far my criticism must be incomplete; still, the observations recorded below may be found of some interest. In this place it is advisable to describe the methods employed. Wolters found, and I have had the same experience, that Flemming's fluid did not give good results with gregarines. Wolters's results are chiefly based on the examination of sections of material fixed in saturated solution of picric acid. I have employed a method more commonly adopted, and which I have found most satisfactory, not only for gregarines, but for Coccidium oviforme and for animal tissues in general. Small portions of the tissue are placed for twenty-four hours in Foa's reagent, i. e. a mixture of equal parts of a saturated solution of corrosive sublimate in normal saline solution and a 5 per cent. solution of bichromate of potassium or Müller's fluid. Then the material is transferred for twenty-four hours to running water, and afterwards placed on successive days in 30, 60, and 90 per cent. alcohol. After that they are placed in absolute alcohol, and after saturation with chloroform are embedded in paraffin, care being taken that the bath does not reach a temperature higher than 50° C. The sections were cut with a Minot's microtome, and fixed on the slide with albumen and glycerine. After the usual process they were

stained with Ehrlich's acid hæmatoxylin diluted with distilled water, and when they had assumed a brownish pink colour were transferred to a bath of tepid tap water and left for at least two hours. Then for two or three minutes they were stained with a solution[1] of Grübler's water-soluble eosin, dehydrated, cleared by xylol, and mounted in the usual way.

With regard to Wolters's description of polar bodies, I can only say that structures which are explicable only as of this nature are of frequent occurrence in M. agilis. They resemble flattened nuclei, and are placed beyond the surface of the parasite and lie between it and the capsule. A nucleus is usually to be seen within the parasite close to such bodies, and frequently remains of a spindle can be made out passing from the nucleus peripherally towards the polar body, as with Wolters I am inclined to regard it.

I have not been able to give sufficient time to the investigation to place me in a position to criticise Wolters's description of the fusion of the nuclei after extrusion of the polar bodies. I have been able to confirm Wolters's view of the origin of sporogonia in the main, but some modifications are, I think, required of the process as described by Wolters, who would appear to say that every nuclear division after the fusion and re-separation of the original nuclei proceeds by regular mitosis. Against this, such appearances as I have sketched in Pl. 31, fig. 1, may be objected. The drawing represents a syzygium of M. agilis. It was surrounded by a connective-tissue capsule. About the middle of each half of the syzygium is a mass (*a*) which I think can only be regarded as nuclear. These masses are composed of fine granules, most of which are coloured purple[2] by hæmatoxylin. Amongst these are coarser granules, which give with the same reagent a deep blue reaction characteristic of chromatin in both animal and vegetable cells. These chro-

[1] This was obtained by dropping a few drops of a strong alcoholic solution into a watch-glass filled with distilled water.

[2] This is Ehrlich's metachromatic reaction. "Metachromatisch d. h. in Einer dem angewöhnten Farbtone abweichenden Nüance farben," Ehrlich, 'Gesammelte Mittheilungen,' 1891, p. 2.

matic granules are arranged in lines (*b*) which radiate out into the substance of the parasite, in the body of which numerous similar granules can be seen, and they are often joined together by achromatic filaments. Besides these granules numerous typical spindles (*c*) and nuclei are to be seen, placed for the most part at the periphery of each part of the syzygium. I regard the granules as a phase of mitosis, and the purple granules of the nuclear bodies as chromatin in a modification previous to that in which karyokinetic activity begins. Wolters seems at one time to have held a similar opinion to mine in reference to the radiating strings of granules, and to have relinquished it, I think, on insufficient grounds. "Um diese Spindeln sah ich bei präparaten welche durch Flemmingsche lösung abgetodte waren, viele stark färbende Körnchen in der Substanz vertheilt, ebenso hier und da, auch weit ab von den Spindeln, in den Syzygiten. Ich war geneigt dieselben als chromatische Substanz anzusprechen. Spätere Untersuchungen an Hoden, die ich mit Umgehung dieser Lösung abtödtete und härtete, zeigten nichts davon, sodass ich von meiner ansicht zurück gekommen bin, ohne eine befriedigende Erklärung dieser Körnchen geben zu können." In a second drawing, Pl. 31, fig. 2, of part of the periphery of another couple of M. agilis I have represented some of these granules (*a*) on a larger scale. In this case some of the deep blue granules were surrounded by material which was coloured by the eosin of the stain. On comparing these with the sporogonia (*c*) lying at the periphery a close similarity of structure was observed, the body of the sporogonium staining in the same tone with eosin as the material investing the chromatic granules. The nuclei of the sporogonia all stained of the same deep blue as the granules, and they presented a great variety of form. In some mitotic processes seemed to have begun, and the same holds good for the sporogonia of Mon. magna, as is shown at (*b*) in the lower of the two in fig. 3. I am able to confirm Wolters's description of the formation of sporocysts and spores. Whilst speaking of sporocysts I may mention that I have encountered in the seminal vesicles

of Lumbricus agricola prismatic pseudo-navicellæ exactly like those described by Bosanquet[1] in the body-cavity.

The observations made by Wolters on Clepsidrina blattarum are, unfortunately, scanty. Much remains to be done with regard to the earlier phases, both of the poly- and the mono-cystides. More importance attaches to Wolters's description of certain phases of Klossia helicina. This parasite is of great interest from the position it holds between the Gregarines and the Coccidia. From the fact that conjugation has not been observed, I, like L. Pfeiffer,[2] should be inclined to place it with the latter group. First described by Kloss[3] of Frankfort, it has since been described by A. Schneider and by L. Pfeiffer (loc. cit.). To the latter author I am indebted for many beautiful preparations of the parasite in Helix hortensis and Succinea Pfeifferi. Pfeiffer has arrived at some interesting conclusions based on the study of this parasite. Of these the more important are—1st, the phenomenon of multiple[4] infection, as many as fifteen parasites being found within a single epithelial cell; 2nd, that when multiple infection occurs only one of the parasites reached maturity; and 3rd, that the size attained by the parasite is determined by the size of the epithelial cells of the kidney of the species of snail infested by the parasite, though in all cases the sporogonia have the same dimensions, the number of sporogonia varying with the size of the parasite from which they are derived.

The general features of Klossia I have never seen better than in some common grey slugs which I examined in July, 1892. The slugs were found in a hollow in the rocks below the falls of the river Shin, in Sutherland. With them were

[1] W. C. Bosanquet, 'Quart. Journ. Micr. Sci.,' 1894, No. 143, p. 421, fig. 19.

[2] L. Pfeiffer, 'Protozoen als Krankheitserriger,' 1891, p. 72.

[3] Hermann Kloss, 'Senkenbergische Abhandlungen,' vol. i, 1855-6.

[4] L. Pfeiffer compares this with what occurs in the Sarcosporidia, the microsporidia in Coccidium salamandræ, and in the Coccidia of the kidneys of he goose and the dog.

numerous examples of Helix hortensis. The kidneys of the snails and the slugs were all alike infested by the sporozoa, but only in the slugs did I find swarm-sporing side by side with the ordinary mode of reproduction by sporocysts. One of these parasites, as seen in a teased preparation of a slug's kidney, is shown in fig. 4. The nucleus (*a*) was large and oval, and showed a single large nucleolus (*b*). In fig. 5 is represented a cell (*a*) as seen in a section, and containing a parasite subdivided into four sporocysts (*c*) (others were present out of focus), each of which contains six crescentic spores (*d*). In some of these a single nucleus could be made out. The spores were very large, averaging 12 μ in length. All the sections of the kidneys of several slugs showed a marked infection, and in most of the sections swarm-sporing was well seen. Fig. 6 shows an example of this. A much hypertrophied cell (*a*) contains a large sporing parasite, and six smaller parasites (*e*). The sickles (*b*) are very large, 20 μ in length. Some of them are undergoing farther subdivision.[1] The capsule of the parasite has ruptured, and at the point of rupture are some free sickles, which also are undergoing farther subdivision (*c*). The appearance of swarm-sporing in a fresh teasing is shown in fig. 7, where some detached sickles (*b*) are present. The colour reactions in these sections were not good, probably owing to the slugs having in the first instance been placed in Scotch whiskey, so that they will not serve as a basis for comparison with Wolters's descriptions; but since I have been able to find in Coccidium oviforme all this author encountered in Klossia, and also many additional features, I will pass to the consideration of this more familiar parasite. For examination I chose a highly infected liver in which the lesions were still in process of evolution. Fig. 8 shows an average appearance of a portion of the epithelial lining of a cyst as big as a small pea. All the larger parasites show signs of nuclear activity. The chromatin, in nearly every instance, gave a typical deep blue reaction to acid hæmatoxylin. The most abundant form

[1] The large sickles formed in swarm-sporing would thus appear to have the equivalents of sporogonia, not of spores.

in which Coccidium oviforme usually presents itself in sections is the spherical granular body represented in fig. 9 to the left. The granules (*b*) stain slightly with eosin, but remain transparent. They have, no doubt, the same signification as the Gregarina corpuscles[1] in the higher Sporozoa, i. e. they serve as stored food. The "dauerform" (L. Pfeiffer) of the parasite is represented in the same drawing to the right. In it the granules have disappeared, and the nuclear body (*a*), which is round in the spherical-granular phase (*a'*), is oval in the encapsuled parasite. In neither case, however, does the nucleus give the reaction of chromatin, but is stained by the eosin. One other phase of the parasite may be mentioned in passing. This is a small, dense, spherical body, devoid alike of granules and of nuclear body, and staining throughout without eosin. Sometimes a parasite possessing a thick oval capsule is found to have broken up into sickle-shaped swarm-spores, but the rule is that when the parasite multiplies whilst still within the body of the host, it has either no capsule at all or only the delicate so-called primordial capsule. It is to the changes which lead to subdivision of the parasite within the host that I wish to direct attention. R. Pfeiffer[2] first described swarm-sporing, but chiefly in fresh specimens, and without any detailed account of nuclear processes. L. Pfeiffer (loc. cit., p. 45) described distinct hæmatoxylin-stained nuclei in the process of swarm-sporing, and says, "Eingehendere Untersuchungen sind hier noch sehr nöthig, dar der mehr oder weniger akute Krankheitsverlauf von Massgebenden einfluss ist auf die Vermehrungsweise des zugehörigen Parasiten."

Among the parasites in actively extending lesions of the rabbit's liver some present a distinct "geflammte Kern," like that shown in fig. 10 (*c*). Such nuclei take only the eosin of the stain, or at most a slight tinge of purple at their edge. Most

[1] The chemical nature of these bodies is not yet determined. They dissolve n alkalies and mineral acids, and are not fat, nor do they contain lime. See Max Wolters, loc. cit.

[2] R. Pfeiffer, 'Beiträge zur Protozoenforschung,' Berlin, 1891.

of the nuclei which are preparing for subdivision show distinct radiating processes, which stain deep blue with acid hæmatoxylin. Such a nucleus is shown in fig. 11 (*a*). The food-granules in such parasites have become diminished in numbers, and in some cases stain more deeply with eosin. The next stage is shown in fig. 12, where the nucleus has subdivided into two parts with the formation of a typical spindle. Wolters was unable to observe spindle-formation in Klossia, but from the identity of some of the phases of nuclear structure in C. oviforme, and in Klossia as described by him, it is probable that in both as also in Gregarines, spindle-formation takes place. In this way two or three (fig. 1, *a* and *a'*) nuclei are formed. Fig. 13 shows the subdivision of a separated portion of the nucleus. A typical spindle (*a*) is present, and the chromosomes are like those of the Gregarines, extremely small. In many instances the subdivision of the nucleus appears to take place more rapidly, particles of nuclear matter being detached to the periphery along single achromatic filaments. The appearance results in a structure almost identical with that figured by Wolters in Klossia (loc. cit., pl. vii, figs. 10 and 11). When the peripheral nuclei are large the resulting subdivisions of the parasite may be termed sporogonia, for they undergo farther subdivision in the formation of sickles (see fig. 8, *b*). When the peripheral nuclei are small, sickles are formed immediately, as a comparison of figs. 14 and 15 suggests. Fig. 14 shows within a capsule (*c*) the optical section of a parasite with a central nuclear mass (*a*) and small peripheral nuclei (*b*). Fig. 15 shows a collection of sickles (*b*) within a cell (*a*). Sometimes the peripheral arrangement of chromatin takes the form of a zone of fine granules, as is shown in fig. 16, *a*. The peculiar behaviour of chromatin in Sporozoa may be illustrated by the fact that sometimes capsules are met with containing sickles stained only with eosin, though in perfectly similar capsules close to them the sickles have single nuclei well stained with hæmatoxylin. With regard to the phase of Coccidium oviforme, marked by the presence of peripheral bars of chromatin which I mentioned in a previous

paper, I may add that it arises as a modification of the peripheral distribution of chromatin already described, but here the particles are rod-shaped, and at certain stages are connected with a central nuclear mass by a beautiful arrangement of achromatic lines, as shown in optical section in fig. 17, where within the host-cell (*a*) the central nuclear mass (*b*) is connected by achromatic filaments with nuclear rods (*c*) at the periphery. When once this system of peripheral rods is constituted, the subdivision of the parasite by in-dippings of the surface would appear to be the rule. Fig. 18 shows in a surface view an example of such a parasite in which subdivision has proceeded to some extent. Some of the parasites with peripheral rods are of considerable size, and show evidence of having changed their form by active movement. The arrangement of the deep blue rods is then often most complex; this is shown in fig. 19, where the large parasite (*a*) touches the basement membrane on one side of a dip between two papillæ, and on the other the nucleus of a cell, so suggesting a previous conjugation of two parasites. It also has two dark bodies on its surface near its base. These bodies have an appearance quite similar to the polar bodies referred to in gregarines.

The general arrangement of chromatin in the swarm-sporing process in Coccid. oviforme thus agrees with Wolters's description of Klossia, and also with what L. Pfeiffer (loc. cit., p. 31) observed in coccidia: "Rasch folgen auf diese anflockerung des primären Kernes eine Reihe von Kerntheilungen wie sie die Bilder in fig. 12 wiedergeben. Die zahlreiche Tochterkerne liegen an dem Mantel der Parasiten Kugel dicht an und oft in schöner geometrischer Anordnung." As far as I am aware, the description of nuclear spindles in coccidia is here given for the first time. What may be the more minute features of the nuclear figures, i.e. with regard to the attraction-spheres, remains yet to be shown. The different behaviour of the parasites with peripheral rods of protoplasm as compared with such as the one shown, fig. 14, may perhaps prove to throw light on direct nuclear division described by Arnold, as compared with the more commonly observed

indirect division of cells. During the subdivision of parasites such as the one shown in fig. 18, when acid hæmatoxylin is used alone, bright crimson particles of chromatin are sometimes visible in the interior of the segments.

The process of nuclear division is not limited to the larger parasites. Some of the smaller (6—8 μ) intra-cellular coccidia present nuclei which are dividing indirectly, see Pl. 32, fig. 21, *a*. The chromatin is, as a rule, not arranged into chromosomes which can be separately distinguished, but is arranged in what under a magnification of 1000 diameters appear to be masses of material which stain purple or blue with hæmatoxylin. Such small parasites have no large food-granules, and in proportion as the nuclei multiply the cytoplasm and the chromatin increase in amount, giving rise to bodies such as those shown in Pl. 32, fig. 21, *b*, and in Pl. 31, fig. 19, *b*.

Achromatic filaments are distinctly recognisable in many of the parasites in this modification. The usual termination of the process is the formation of sporogonia which contain several minute nuclear masses embedded in eosin-staining protoplasm. The sporogonia subdivide into sickles of the ordinary form. Not infrequently, however, sickles are formed directly, i. e. without a sporogonium stage. Again, the growth of this non-granular form of coccidia may pass beyond the average size (fig. 19, *a*), and the chromatin becomes minutely subdivided and arranged at the periphery, thus producing the phase marked by peripheral rods, as shown in Pl. 32, fig. 21, *d*. Returning to the larger granular coccidia, I would here explain that regular mitotic figures, such as Pl. 31, fig. 12, are extremely few in number. Not so infrequent are typical but slightly irregular spindles, in which all the chromatin of the original nucleus is concerned. Examples are given in Pl. 32, fig. 20, *a*, *b*, and *i*. The whole of the chromatin may become active without the formation of a spindle, as shown in figs. *c*, *k*, and *l*. In the parasite represented in fig. *k* the peripheral collection of chromatin (2) gave a metachromatic (crimson) reaction, whilst the other (1) extremity stained dark blue. In the greater number of the parasites the main mass of the nucleus

remains unchanged for a time, the first indication of karyokinetic activity being the separation of hæmatoxylin-stained chromatin particles joined by achromatic filaments (figs. *d*, *e*, and *h*). When this is the case the unchanged part of the nucleus stains but faintly with hæmatoxylin alone, and when eosin after staining is used assumes a red colour. Sometimes the whole of the nucleus stains deep blue with hæmatoxylin at first without any appearance of nuclear filaments. In such cases the nucleus has the appearance shown in fig. *f*. Irregular mitoses are abundant. Such are shown in figs. *f* and *g*. In such mitoses the first portions separated from the main nucleus frequently give the crimson reaction to hæmatoxylin. Nothing is more striking than the growth of chromatin in coccidia. In the final multinucleate condition the coccidia possess more than a hundred times the amount of chromatin they had at the commencement of the process.

Once more reverting to the parasites with peripheral chromatin rods, it should be observed that in a few cases, which can only be found by patient search, the rods are replaced by fine granules of chromatin, as shown in fig. *m*. Others of the parasites with peripheral rods appear to break up into an immense number of extremely minute sickles without previous subdivision into segments. Finally, with regard to the segmentation of the parasites in the phase marked by peripheral rods of chromatin, it is sometimes seen that the subdivisions possess sickle-shaped outlines like the one seen in optical section in fig. *n*. It is to be observed, however, that such crescentic segments are not homologous with single sickles, but that each peripheral chromatin rod is the potential nucleus of a spore.

I would now turn to mention, and it can be but too briefly, L. Pfeiffer's recent work on the Myxo-, Sarco-, and Microsporidia.[1] In these groups Pfeiffer has added much to bio-

[1] See L. Pfeiffer, 'Protozoen als Krankheitserriger,' 1891, and 'Untersuchungen über der Krebs,' 1891.

For a general account of the Sporozoa and the groups included, together with figures of most of the important forms, see Lankester's 'Zoological Articles' (A. and C. Black), article "Protozoa."

logical knowledge, and has closely studied the effects of the parasites on their hosts,—or, in other words, their pathological effects. Since Dr. Pfeiffer has most kindly given me many beautiful preparations of Sporozoa belonging to this and other groups, I have been able to study, and, I may add, to confirm his results.

With regard to the Myxosporidia, the description of the epidemics they have caused from time to time in some of the rivers of Germany forms a most interesting part of Dr. Pfeiffer's work, and is worthy of the close attention of pisciculturists. Barbel, pike, and perch were chiefly attacked. "The sick barbel present a striking appearance from the presence of discoloured tumours in the skin, and of deep crateriform ulcers on the head, the hinder part of the body, and the tail: the ulcers have a widely infiltrated base." Fig. 22 shows part of one of Dr. L. Pfeiffer's sections of a myxosporidial tumour of a barbel. The growth is alveolar in structure, the alveolar walls being composed of fibrous tissue. The contents of the alveoli consist solely of parasites. In the upper part of the figure is a portion of the periphery of a reticulated parasite from which sporocysts have separated; at the edge of the parasite is a nuclear spindle. The thread-cells of the sporocysts are stained deeply. Some of the sporocysts are devoid of definite characters; whether they are young forms, or residua after the escape of the single amœboid spores, is a question I have not been able to determine. L. Pfeiffer shows that these tumours begin as an infection of striped muscle-fibres, and some of the preparations demonstrate this point most distinctly, the young parasites in all stages of existence being visible within muscle-fibres at the periphery of the tumour.

Writing of the effects of Sarcosporidia, Leuckart has said, "Although the tubes occasionally occur in immense numbers close to one another, so that the flesh looks as if half of it consisted of psorosperm tubes, yet they seem usually to cause no special uneasiness. In many cases, however, the phenomena of paraplegia, retarded respiration, and even suffocation, are

observed as associated with the presence of the tubes, and may with some probability be referred to this cause."[1]

L. Pfeiffer has demonstrated the nature of these fatal cases, showing that they are the result of the escape of the sickles from the muscle-fibre in which they are developed, and their entrance into the surrounding muscle-fibres, in which they appear as groups of minute round cells, and growing to a certain size constitute a new Miescher's tube, which, unless the host acquires a greater resisting power, again ruptures and liberates its swarm-spores.

I have been able, both in Dr. Pfeiffer's preparations and in others of my own, to confirm these observations. Pfeiffer has further shown that an emulsion of Sarcosporidia injected into rabbits causes an intense inflammatory reaction and toxic phenomena, and this is in keeping with what is seen in the progressive infection of muscle referred to above. The free spores seem to have a marked attraction for leucocytes (chemiotaxis), and thus the process has been termed by Pfeiffer "myositis sarcosporidica."

When this progressive infection is limited to a certain region, instead of inflammatory changes tumour formation is seen. Thus in horses L. Pfeiffer has found that tumours included by Kölliker in a group termed "Muskelknospen" are determined by sarcosporidia. Some of Dr. Pfeiffer's sections show this in, I think, a most conclusive manner. The growth possesses a stroma of fibro-cellular tissue, in which three zones may be described: an inner containing dense foci of degenerated material surrounded by giant-cells, &c.; a middle zone containing numerous Meischer's tubes, from which the sickle-spores are escaping; and an outer zone containing many muscle-fibres infected by Sarcosporidia.

One effect of a chronic progressive infection by Sarcosporidia has been most exquisitely shown by the same observer. On the œsophagus of sheep, white cysts, of which the largest are as big as horse-beans, are sometimes encountered. The larger (older) cysts are provided with a fibrous capsule. Dr. Pfeiffer's

[1] Leuckart, Hoyle's (1886) translation, p. 202.

sections show that the capsule is formed by a reactive inflammatory process, and that between its fibres lie compressed muscle-fibres (fig. 23, *a*). Those muscle-fibres which are embedded in the deeper, i.e. older part of the capsule, are distended by young sarcosporidia, which present a definite nucleus and a clear protoplasm (fig. 23, *b*). Next to the capsule come large spaces (fig. 23, *c*), filled with sickles. The spaces result from the distension of muscle-fibres and the consequent separation of the fibrous fasciculi of the capsule. It may be noted that some of the sickles in the spaces close to the capsule are subdivided into segments. I may add that whilst the sickles contain particles of chromatin throughout the whole of their substance, the youngest parasites have a definite nucleus and a clear protoplasm. L. Pfeiffer has thus shown that progressive inflammatory changes, cyst- and tumour-formation may be determined by Sarcosporidia.

Finally Dr. Pfeiffer's sections of the muscles of frogs show that like the Myxo- and Sarco-sporidia, the Microsporidia occur within muscle-fibres. Fig. 22 shows the remains of a muscle-fibre containing some of these parasites. One group of spores are not yet liberated from the parent cell, and present a central nuclear spot and an unstained peripheral region. The free spores are minute (2 μ) bodies, most of them slightly curved, so that they present the strongest possible resemblance to a collection of vibrios.

I cannot close this article without expressing my warm thanks to Dr. L. Pfeiffer for the liberal manner in which he has answered my request for specimens and material.

London; December 27th, 1894.

EXPLANATION OF PLATES 31—33,

Illustrating Mr. J. Jackson Clarke's "Observations on Various Sporozoa."

Fig. 1.—Section through a syzygium of Monocystis agilis, × 500 diams. From the nuclear masses, *a*, in the central part of each half of the syzygium numerous moniliform threads, *b*, of chromatin are directed towards the periphery, where are many small nuclei and nuclear spindles, *c*.

Fig. 2.—Section through part of the periphery of a syzygium of Monocystis agilis, × 1000. From left to right are (1) the connective-tissue capsule, *a*, (2) sporogonia, *c*, (3) part of the body of the parasite with gregarina corpuscles, nuclear spindles, *b*, and particles of chromatin, *a*, round some of which a covering of protoplasm has collected.

Fig. 3.—Two sporogonia of Monocystis magna, × 1000 diams. The nucleus of the lower of the two shows achromatic filaments, *b*, joining the particles of chromatin. The body, *a*, of the structure stained with eosin.

Fig. 4.—A free example of Klossia from the grey slug, × 1000 diams. Fresh specimen. Nucleus, *a*, nucleolus, *b*. × 1000.

Fig. 5.—An epithelial cell in the kidney of a grey slug containing a parasite subdivided into sporocysts. Some of the sickles contain a single nucleus. There are no residual bodies. × 1000 diams. From a section.

Fig. 6.—A cell, *a*, from the kidney of a slug containing a parasite in the process of swarm-sporing. There are six smaller parasites, *e*, in the protoplasm of the cell. *b*. Large sickle-shaped sporogonia. *c*. The same dividing. *d*. Undivided central part. × 1000 diams.

Fig. 7.—A free Klossia showing swarm-sporing with some sickles, *b*, detached. Fresh teasing. × 750 diams.

Fig. 8.—Part of the epithelial lining of a coccidial cystadenoma of a rabbit's liver. *a*, *a'*. Parasites with two and three nuclei respectively. *b*. A parasite subdivided into sporogonia. × 1000 diams.

Fig. 9.—A round granular and an oval encapsuled coccidium. *a*, *a'*. Nuclear body (red). *b*. Cytoplasm. *c*. Definitive capsule. 1000 diams.

Fig. 10.—An intra-cellular coccidium with a "Geflammte-Kern" at *a*. × 1000 diams.

Fig. 11.—An intra-cellular coccidium, the nucleus, *a*, of which shows radiating bars of chromatin. × 1000 diams.

Fig. 12.—Intra-cellular coccidium, the nucleus, *c*, of which is dividing by regular mitosis. *b*. Cell-protoplasm with food-granules. *a*. Host-cell. × 1000 diams.

Fig. 13.—A coccidium which shows, besides the main nucleus, a nuclear spindle. × 1000 diams.

Fig. 14.—An encapsuled coccidium (capsule, *c*) with peripheral particles of chromatin, *b*, which are connected with a central nuclear mass, *a*, by achromatic filaments. × 1000 diams.

Fig. 15.—An epithelial cell, *a*, of the rabbit's liver filled with nucleated sickle-spores, *b*. × 1000 diams.

Fig. 16.—An intra-cellular coccidium with numerous minute chromatin granules at *a*, the periphery.

Fig. 17.—An intra-cellular (host-cell, *a*) coccidium showing close-set peripheral rods of chromatin (*c*) connected with a central nuclear mass (*b*) by achromatic filaments. × 1000 diams.

Fig. 18.—A coccidium with peripheral chromatin rods seen in a surface view, and showing the mode of subdivision. × 1000 diams.

Fig. 19.—Part of the epithelial lining of the rabbit's cystadenoma showing large free parasite with peripheral rods and granules of chromatin, and at *x* a multinucleated parasite. × 1000 diams. The free parasite is seen in surface view.

Fig. 20.—*a*. Granular coccidium with a slightly irregular nuclear spindle.

b. A parasite with a nucleus similar to that shown in Fig. *a*, but with granules of chromatin at the left half of the spindle.

c. Granular coccidium, the nucleus of which has become altered so that it presents a collection of granules of chromatin.

d. Granular coccidium, of which the outer part of the nucleus is separated in an irregular spindle (1), whilst the central part remains unchanged (2).

e. A similar condition to that shown in Fig. *d*.

f. and *g*. Irregular mitoses.

h. A condition similar to that shown in Figs. *d* and *e*. The central unaltered part (2) of the nucleus stained with eosin; the peripheral part (1) (irregular spindle) stained with hæmatoxylin.

i. Irregular spindle.

j. Elongated nucleus stained densely with hæmatoxylin.

k. Irregular mitosis. Left extremity of the chromatin band stained blue, right extremity crimson. Acid hæmatoxylin alone.

l. Irregular mitosis, granules stained purple.

m. A large parasite in a phase equivalent to that with peripheral arrangement of rods, the latter replaced by granules of chromatin.

n. Large parasite with peripheral rods undergoing segmentation, one of the segments crescent-shaped. Optical section.

21.—A portion of one of the papillary processes of a coccidial tumour seen in section. *a*. Young parasites with division of nuclei. *b*. Larger but still not granular coccidia with dividing nuclei. *c*. Capillary vessels and basement membrane.

Fig. 22.—Section of a myxosporidial muscle-tumour of a barbel. From a preparation made by Dr. L. Pfeiffer. × 1000 diams.

Fig. 23.—Section of a sarcosporidial cyst from a sheep's œsophagus. From a preparation made by Dr. L. Pfeiffer. × 1000 diams.

Fig. 24.—Microsporidia in frog's muscle. From a preparation made by Dr. L. Pfeiffer. × 1000 diams.

A Revision of the Genera and Species of the Branchiostomidæ.

By

J. W. Kirkaldy.
(From the Laboratory of the Linacre Professor, Oxford.)

With Plates 34 and 35.

The opportunity of examining a number of species allied to the well-known Cephalochordate Amphioxus lanceolatus was afforded to me by Professor Lankester, who had for some time collected material for the purpose of revising the genus, and suggested that I should go over the specimens in his possession, and prepare a series of drawings showing the chief characteristics of the species. The drawings have been executed by Mr. Bayzand from rough ones prepared by me.[1]

The material examined by me is as follows:

1. Various collections from the Zoological Station, Naples (A. lanceolatus).

2. Eight specimens from Ostend sent to Professor Lankester by Professor van Beneden of Liège (A. lanceolatus).

3. A collection of about one hundred and fifty specimens from the South Australian coast, presented to the Oxford Museum by Professor Baldwin Spencer of Melbourne, Victoria (H. Bassanum).

4. A collection of about ninety specimens from Torres Straits, lent to Professor Lankester by Professor Haddon of Dublin, by whom they were collected (H. cultellum).

[1] The present memoir must be taken as superseding a note published by me in the 'Reports of the British Association,' 1894, Oxford meeting.

5. Four specimens from Ceylon collected by Mr. Haly, and lent to Professor Lankester by Professor Haddon (H. cingalense).

6. Eight specimens from the coast of Brazil, presented to Professor Lankester by Professor van Beneden of Liège (A. caribæus), by whom they were collected.

7. Twelve specimens from the Bahamas presented to Professor Lankester by Professor Alexander Agassiz, by whom they were collected (A. Lucayanum).

8. Five specimens from Ceylon, forwarded to Professor Lankester by Professor Good Brown, of the National Museum, Washington, U.S.A. (H. cingalense).

9. Ten specimens from the coast of California forwarded to Professor Lankester by Professor Good Brown. Cooper's type-specimen of A. californiensis was among these.

10. In addition to the above-mentioned material which I have been able to study, and in many cases to examine, by means of transverse sections in Professor Lankester's laboratory at Oxford, I have been allowed by Dr. Günther to examine the specimens of another species (A. Belcheri), of which only five exist, in the British Museum.

I am desired by Professor Lankester to express his great obligations to Professors Spencer, Haddon, van Beneden, Agassiz, and Good Brown for their kindness in communicating their specimens to him.

For the nomenclature of the various structures of the Branchiostomidæ already in use the reader is referred to Professor Lankester's memoir (No. 7), and to the recently published work by Dr. Arthur Willey (No. 15).

The characters to which I have, at Professor Lankester's request, given attention, and have found to be important for the purpose of dividing the Branchiostomidæ into genera, subgenera, and species, are as follows. I enumerate the characters approximately in the order of importance which it has been found necessary to assign to them.

1. Præoral cirrhi uniformly connected to one another by a low web, or per contra separated into two ventrad and two

laterad groups, the ventrad groups distinguished by a very high web.

2. A caudal expansion of the median dorsal and ventral fin-ridge present or absent.

3. An unsegmented "urostyloid" caudal region, one seventh the length of the whole animal, present, or per contra only a minute projection of the notochord beyond the last myotome.

4. A single, or per contra a double post-atrioporal cæcum to the atrial chamber.

5. Only the right, or per contra both metapleura continuous with the ventral portion of the snout or rostral fin.

6. The præoral gustatory groove (groove of Hatschek) superficial and shallow, or per contra strongly marked.

7. Fin-ray spaces present or per contra absent in the median ventral fin.

8. Fin-rays developed in the fin-ray spaces of the median ventral fin, or per contra absent.

9. Right metapleur terminating behind the atriopore similarly to the left metapleur; median ventral fin unconnected with metapleura; or per contra right metapleur continued without break into the median ventral fin.
thus, its posterior continuation.

10. Number of tentacles to the oral sphincter (intra-buccal cirrhi) twelve, ten, or sixteen.

11. Gonads developed on both sides of the body, or per contra only on one side.

12. Number of myotomes throughout the body; number of myotomes between snout and atriopore, atriopore and anus,

[N.B.—In counting the myotomes I have reckoned that coincident with the atriopore as belonging to the anterior or præatrioporal group; and similarly I have reckoned the post-anal group as commencing behind the posterior margin of the anus. In many specimens the anus is elongated antero-posteriorly, so as to extend under as many as three separate myotomes.]

13. Proportional height of the median fin.

14. Form of the anterior (rostral) and posterior (caudal) expansions of the median fin.

15. Number and myotomic position of the gonad pouches.

The valuable diagnostic character afforded by the total number of the myotomes, and of the number in the three groups known as præatrioporal, præanal, and post-anal, was first made use of by Sundevall and later adopted by Günther (No. 5), whose essay is up to the present time the most important on the taxonomy of the Branchiostomidæ. During the past year, however, a very important memoir has appeared by Andrews (No. 1) on a Branchiostomid from the neighbourhood of the Bahamas, which presented so many novel features as compared with the forms already known that it was necessary to place it in a new genus, to which Andrews gave the unfortunate name Asymmetron. Before the appearance of Andrews' memoir Dr. Arthur Willey had, at Professor Lankester's suggestion, undertaken the examination of Professor Haddon's collection of Branchiostomidæ from Torres Straits, and the result was a short memoir (No. 14) in which some important characters of B. cultellum, Peters (of which the entire collection consisted), are pointed out; and the significant suggestion is made that the median ventral fin of the Branchiostomidæ is not truly a median structure, but is the continuation of the right metapleuron. The two papers of Willey and of Andrews made it desirable to re-examine as far as possible all the species of Branchiostomidæ, in order to ascertain whether the peculiar features as to the unilateral character of the gonads, continuity of metapleur and ventral median fin, absence of ventral fin-rays in the one case and of ventral fin-ray chambers in the other, described by these authors, obtain in other forms which had not been looked at by their original describers with these questions in mind. The nature of my work is thus explained. Among the characters made use of, I have added to those introduced into consideration successively by Sundevall, Günther, Willey, and Andrews, only one, viz. the number of the cirrhi or tentacles lying on the inner face of

the oral sphincter (intra-buccal cirrhi), and this I have not determined in every species. Possibly other more skilful observers may be able to render some of the structures examined by me available for specific and generic characterisation, but my own attempts to make use of the position of the atrio-cœlomic funnels, of the skeleton of the præoral cirrhi, of the form of the branchial bars (in section), and of the disposition of Müller's renal papillæ, were unsuccessful.

The drawings given of B. Belcheri, Bassanum, californiense, and caribæum are the first which have been published of those species.

The Branchiostomidæ are the sole family of known forms comprised in the branch Cephalochorda of the phylum Vertebrata. Retaining the name Vertebrata for the great phylum to which some recent writers have proposed to apply the name Chordata, Professor Lankester recognises three distinct branches or lines of descent within that phylum, for which in 1877 ('Quart. Journ. Micr. Sci.,' vol. xxvii, p. 450) he proposed the names Urochorda (the Tunicates), Cephalochorda, and Craniata. To these three diverging branches Bateson has proposed to add a fourth, the Hemichorda, to comprise the forms known as Balanoglossus. We may begin the systematic characterisation of the Branchiostomidæ by a definition of the Cephalochorda.

Branch CEPHALOCHORDA, Lankester, 1877.

Leptocardii, Müller. 'Abhandl. k. Akad. Urss.,' Berlin, 1844, p. 204.
Myelozoa, J. Geoff. St.-Hilaire. 1852.
Acrania, Haeckel. 'Gen. Morphol.,' 1866.
Cirrhostomi, Pharyngobranchii, } Owen. { 'Lectures on Compar. Anatomy,' 1846.
Entomocrania, Huxley. 'Proc. Zool. Soc.,' London, 1876, p. 58.
Cephalochorda, Hatchett Jackson. 'Forms of Animal Life,' 1888, p. 437.

Vertebrata exhibiting the distinctive vertebrate combination

of the four characters indicated by the terms notochord, pharyngeal gill-slits, tubular myelon, and myelonic eye.

The notochord is large and unconstricted, is continued through the region of the head, and projects beyond the first myotome into the snout or rostrum. The longitudinal musculature of the body-wall is divided by membranous septa into a series of well-marked myotomes. There is no marked enlargement of the anterior region of the myelon as brain, but a slight dilatation of its cavity anteriorly. The myelon terminates anteriorly short of the termination of the notochord; there is no special protective skeleton (cranium) for the anterior portion of the myelon. The vascular system is devoid of a specialised "heart." Minute tubular nephridia, arranged serially, are present. Existing forms exhibit a deep-seated asymmetry, masked by a secondary superficial symmetry; they are of small size, and probably are degenerate representatives of symmetrical bilateral ancestors of more fully elaborated organisation.

Family (unica) Branchiostomidæ.

Amphioxidæ, Gray. 'Synopsis Brit. Mus.,' 1842, p. 150.
Amphioxini, Müller. 'Abhandl. k. Akad.,' Berlin, 1844.
Branchiostomidæ, Bonaparte. 'Cat. metodico Pesci Europ.,' 1846.
Amphioxoidei, Bleeker. 'Enum. sp. Piscium Archip. Ind.,' 1859.
Cirrostomi, Günther. 'Cat. Fishes Brit. Mus.,' vol. viii, p. 513, 1870.

Cephalochorda of minute size, and laterally compressed elongate form—acutely terminated, both anteriorly and posteriorly. The integument is raised dorsally into a median plate or ridge forming the dorsal fin, which is uninterrupted throughout the length of the entire body. Two similar plates or outstanding ridges—the metapleura—exist on either side of the anterior two thirds of the body, which is bluntly triangular in section: the superior angle supports the median fin; the infero-lateral angles support the two lateral plates or metapleura. A median ventral fin is present in the hinder third of the body; in some species it is continuous with the right metapleur, which is also continued in front of the mouth to

join the ventral portion of the rostral fin. The left metapleur is not continued forward into the rostrum, nor more than a short distance behind the atriopore. The gill-slits are numerous and developed on both sides of the pharynx: they do not in the adult present any numerical relation to the metamerism of the myotomes; they open into what is in the embryo a median ventral groove, which subsequently becomes closed in as a canal extending over the anterior two thirds of the ventral wall, and opening by a pore (the atriopore) posteriorly. By later enlargement this canal expands into a considerable cavity (the atrium), separating a lateral region of the body on either side (the epipleura) from the axial region; by similar growth it is extended posteriorly within the body as the single or double post-atrioporal cæcum or cæca of the atrium. The gonads are developed in either or both of the epipleura. A præoral muscular hood is developed in front of the mouth, provided along its circular margin with numerous tentacles, supported by a cartilaginoid skeleton; there is one median unpaired tentacle in the median ventral line, and from ten to twenty (according to age and species) on either side.

A single circular area of pigment—the eye-spot—is developed on the inner surface of the median anterior termination of the hollow myelon. A single laterally placed olfactory pit may be present on the left side of the snout, consisting simply of a tubular depression of the epidermis by which it is brought into continuity with a slight upgrowth of the terminal wall of the myelon. The anus is placed on the left side of the ventral fin.

A forwardly projecting cæcum (the hepatic cæcum) is developed on the right side from the alimentary canal at the point where the pharyngeal perforations cease.

The oral sphincter (buccal apparatus) is provided with a series of long sensory intra-buccal cirrhi, which project backwards into the pharynx.

The gonads are developed in a numerous series of cœlomic pouches, corresponding in number and position to the myotomes of the mid-region of the body. There are no genital ducts.

The mid-ventral surface in front of the atriopore is traversed by numerous longitudinal pleats, which permit of the expansion and contraction of this area so as to alter very considerably the volume of the viscera and cavities of the body.

A cartilage-like tissue is developed as skeletal support for the præoral cirrhi, and for the median and lateral (metapleural) fin-plates. In the median dorsal fin, and usually, but not always, in the median ventral fin, this tissue takes the form of separate fin-rays, each contained in a lymph-space or fin-ray chamber; in the metapleura it forms a continuous unsegmented band with a related canal-like lymph-space.

I find it desirable to recognise in the family Branchiostomidæ two genera—Branchiostoma and Asymmetron. The genus Branchiostoma is divided by me into sub-genera—Amphioxus and Heteropleuron.

Genus I.—Branchiostoma.

Branchiostoma, Costa. 1834.
Amphioxus, Yarrell. 1836.

Præoral tentacles or cirrhi, forming a single series united to one another by a uniformly low intertentacular membrane.

A median ventral tentaculum impar present, the rest symmetrical.

Dorsal and ventral median[1] fins expanded in the caudal region to form a lancet-shaped so-called "caudal" fin, within which the axis of the body terminates.

Fin-ray chambers, with or without enclosed fin-rays, present in the præanal portion of the ventral fin.

Infra-rostral fin continuous with the right metapleur; the left metapleur dying out before reaching the infra-rostral fin.

Atrial chamber produced behind the atriopore into a single tapering cæcum, reaching as far as the anus.

Præoral tentacles provided with numerous sensory papillæ.

[1] Although morphologically the ventral median fin is possibly a portion of the right metapleur, it is convenient to retain the name "ventral median fin" for that continuation of the right metapleuron which lies between atriopore and tail terminus in the mid-ventral line.

Gustatory groove of Hatschek (on the right side of the roof of the præoral hood) shallow.

Sub-genus 1.—Amphioxus.

Amphioxus, Yarrell.

Both metapleura terminate immediately behind the atriopore, and overlap the median ventral fin. The ventral fin-ray chambers form a single median series, each containing a pair of fin-rays, excepting the more anterior and more posterior chambers.

Gonad pouches developed on both right and left epipleura.

1. Amphioxus lanceolatus, Pl. 34, fig. 1.

Limax lanceolatus, Pallas. 'Specil. Zool.,' x, p. 19.
Branchiostoma lubricum, Costa. 'Cenni Zool.,' Napoli, 1834, p. 49.
" " " 'Frammenti Anatomia Comparata,' fas. i, Napoli, 1843.
Amphioxus lanceolatus, Yarrell. 'British Fishes,' 1836, p. 462.
Branchiostoma lanceolatum, Gray. 'Cat. Brit. Mus. Fish.,' vol. vii, p. 150, 1851.

Myotomes, maximum number 62, minimum number 58, most frequent 60 (J. W. K. 50 specimens); myotome formula (præatrioporal, præanal, post-anal) 36, 15, 10 (cf. Lankester); 35, 14, 12 (cf. Andrews); 35, 14, 11 (J. W. K.).

Dorsal fin of moderate height, namely, one seventh of the height from the crest of the fin to the free edge of the metapleur at the mid-point of the animal's length.

Snout or rostral fin small and pointed, not marked off from the dorsal fin. Caudal expansion of the dorsal and ventral fin long and lancet-shaped with sharp angles.

Ventral fin with a variable number of median fin-ray chambers, in each of which are two paired fin-rays (excepting those at either end of the series). Thirty-four to forty-one pairs of ventral fin-rays present.

Oral sphincter placed in a line drawn vertically from the apex of the anterior angle of the seventh myotome.

Intra-buccal tentacles of the oral sphincter are twelve in number. Oral hood large; tentacles of the oral hood twenty-one in small specimens to forty-one in the largest specimens. Olfactory pit present.

Gonad pouches twenty-three to twenty-nine on the right side, and twenty-one to twenty-eight on the left side (in fifty specimens, J. W. K.); usually twenty-six on each side.

Average length of fifty specimens (sent from Naples as well-grown) 4·8 cm. (J. W. K.); maximum length 5·8 cm.

Distribution.—Mediterranean Sea, English Channel, North Sea, coast of Norway.

2. Amphioxus californiensis, Pl. 34, fig. 4.

Branchiostoma californiense, Cooper. 'Nat. Wealth of California,' 1868, p. 498.

Myotomes, maximum number 73, minimum number 69, most usual 71 (in ten specimens, J. W. K.). Myotome formula 45, 17, 9 (J. W. K.); 44, 19, 8 (J. W. K.); 44, 16, 9 (Cooper's type-specimen, J. W. K.).

Dorsal fin of moderate height; series of dorsal fin-ray chambers extending from the eye-spot to the anal myotome. Rostral fin very small. Caudal expansions of the dorsal and ventral fins long and shallow. Ventral fin with fin-ray chambers and paired fin-rays. Anterior extremity of notochord dipping downwards. Oral hood and circlet of tentacles relatively small in size. Gonad pouches, thirty-one right and thirty-one left.

Oral sphincter underlies the apex of myotome 4. Maximum length of ten specimens 7·4 cm. (J. W. K.).

Distribution.—Coast of California.

Remarks.—This species comes nearest to the B. elongatum of Sundevall, the specimens of which are lost. It may be recognised by the relatively small size of the cephalic region; the oral sphincter is placed far forward (myotome 4), and the anus is placed far backward (myotome 62).

3. Amphioxus caribæus, Pl. 34, fig. 5.

BRANCHIOSTOMA CARIBÆUM, Sundevall. 'Ofvers. vet. Akad. Forsk.,' vol. x, 1853, p. 12.

Myotomes, maximum number 61, minimum number 59, most usual number 60 (in eight specimens, J. W. K.). Myotome formula 37, 15, 9; 38, 14, 7; 37, 15, 8 (most usual).

Dorsal fin low—one eighth of the height from the crest of the fin to the free edge of the metapleur at mid-region of body. Fin-ray chambers commence in front of the eye-spot, and extend to myotome 55. Rostral fin marked off from the dorsal fin by a shallow notch, and terminating bluntly in front. Caudal fin small and shallow. Ventral fin low, with fin-ray chambers and paired fin-rays. Oral sphincter underlies the apex of myotome 5, and is provided with twelve intrabuccal cirrhi. Præoral tentacles and gonad pouches as in A. lanceolatus.

Maximum length among eight specimens 4 cm. (J. W. K.); according to Sundevall 5·1 cm.

Distribution.—East coast of the United States and of South America; West Indies.

Remarks.—This species stands very near to A. lanceolatus. It is distinguished from that form chiefly by the slight development of the caudal fin and the shortness of the post-anal region.

4. Amphioxus Belcheri, Pl. 35, fig. 8.

BRANCHIOSTOMA BELCHERI, Gray. 'Proc. Zool. Soc.,' 1847, p. 35.

Myotomes, maximum number 65, minimum number 63 (in four specimens examined in the British Museum (J. W. K.). Myotome formula 37, 14, 14 = 65 (according to Günther); 38, 17, 10 = 65 (J. W. K.); 37, 16, 10 = 63 (J. W. K.).

Rostral fin well marked, and separated from the dorsal fin by a depression. Notochord dipping downward before its anterior termination. Inferior lobe of the caudal fin relatively large.

Other characters apparently as in A. caribæus and A. lanceolatus.

Maximum length among four specimens 5 cm. (J. W. K.).

Distribution.—Coast of Borneo (Sir Edward Belcher); Prince of Wales Island, Torres Straits (Dr. Coppinger).

Remarks.—The only known specimens of this species are preserved in the British Museum, Natural History.

Sub-genus 2.—Heteropleuron.

Heteropleuron, J. W. K., sub-genus nov.

The left metapleur terminates immediately behind the atriopore; the right is directly continued without interruption into the median ventral fin.

Ventral fin chambers with or without fin-rays.

Gonad pouches limited to a single series, which is developed on the right epipleur.

Intra-buccal cirrhi sixteen in number.

1. Heteropleuron Bassanum, Pl. 34, fig. 6.

Branchiostoma Bassanum, Günther. 'Report Zool. Coll., H.M.S. "Alert,"' 1884, p. 31.

Myotomes, maximum number 78, minimum number 70, usual number 75 (in 50 specimens, J. W. K.).

Myotome formula 43, 16, 12; 44, 14, 17; 45, 17, 15; 45, 16, 14 (usual).

Dorsal fin shallow. Rostral fin large, and marked off from the dorsal fin by a dip. Caudal fin long and low, the superior and inferior angles entirely removed (as compared with A. lanceolatus). Ventral fin with fin-ray chambers and paired fin-rays. Præoral cirrhi from 31 to 33 (in specimens examined). Olfactory pit present. Oral sphincter underlies apex of seventh myotome. Intra-buccal cirrhi sixteen in number. Gonads 26—31, only present on the right epipleur.

Maximum length of 50 specimens 4·3 cm. (J. W. K.).

Distribution.—Bass's Straits, Australia.

Remarks.—The specimens of H. Bassanum sent by Pro-

fessor Baldwin Spencer were in an excellent state of preservation. They all show a greater compression of the body from side to side, i. e. less thickness, than do similar specimens of Amphioxus lanceolatus.

Although the gonad pouches develop on the right epipleur, they extend when ripening across the median line of the body, so as to be visible through the left epipleur.

2. Heteropleuron cingalense, n. sp., Pl. 35, fig. 7.

Myotomes, maximum number 64, minimum number 61 (in eight specimens, J. W. K.).

Myotome formula 39, 17, 6; 39, 17, 8; 39, 16, 8; 38, 17, 8.

Dorsal fin low (one eighth of height from crest of fin to edge of metapleur at mid-body). Rostral fin small, not marked off from dorsal fin. Ventral fin with fin-ray chambers and paired fin-rays. Oral sphincter underlies the apex of the fourth myotome. Intra-buccal cirrhi not determined. Gonad pouches twenty-five, only present on the right epipleur.

Maximum length of eight specimens 3 cm. (J. W. K.).

Distribution.—Coast of Ceylon.

Remarks.—Four of the specimens examined by me were collected by Mr. Haly, and supplied to Professor Lankester by Professor Haddon. Three of these gave the myotome formulæ 39, 17, 8; one (that figured) gave 39, 17, 6. Five specimens were lent to Professor Lankester by Professor Good Brown from the United States National Museum. Of these, one appears not to belong to this species at all, and, in fact, belongs to the sub-genus Amphioxus. The remaining four presented the myotome formula 39, 16, 8; 38, 17, 8; and 37, 15, 9. From these facts I think it is clear that there is a Cingalese Heteropleuron distinct from H. Bassanum, from which it differs chiefly in its smaller number of myotomes. Whether the specimen of Amphioxus included amongst the specimens sent from the United States National Museum indicates a distinct Cingalese species of the sub-genus Amphioxus, or is referable to A. Belcheri, I prefer to leave an open question.

3. Heteropleuron cultellum, Pl. 34, fig. 2.

Epigonichthys cultellus, Peters. 'Monatsbericht der k. Preuss. Akad. der Wiss.,' Berlin, 1876, p. 322.
Branchiostoma cultellum, Günther. 'Report Zool. Coll. H.M.S. "Alert,"' 1884, p. 32.
" " Willey. 'Quart. Journ. Micros. Sci.,' vol. xxxv, 1894, p. 361.

Myotomes, maximum number 56, minimum number 50, usual number 52.

Myotome formula 32, 10, 10; 32, 12, 8; 33, 10, 10; 34, 11, 10; 32, 10, 10 (usual number in thirty specimens).

Dorsal fin of great height, especially in the anterior region, where it is more than one third of the total height from fin crest to metapleur edge. The rostral fin is short and deep, and is not marked off by a notch from the dorsal fin. The ventral fin presents fin-ray chambers, which do not, however, ever contain fin-rays, either single or double. (Willey first observed this, and I can confirm him.)

The caudal fin is lancet-shaped, but not strongly marked.

The oral sphincter underlies the angle of myotome 6; there are sixteen intra-buccal cirrhi. The præoral tentacles are from forty-one to forty-three in number (in the specimens examined). The notochord is slightly depressed in the rostral region, and, instead of tapering to its anterior extremity, is expanded to form a club-like termination. Posteriorly the notochord projects beyond the last myotome more than it does in other species of Heteropleuron or Amphioxus, and terminates bluntly. The gonad pouches are from seventeen to twenty in number, forming a series on the right epipleur. The maximum length shown by specimens in Haddon's collection was 3·5 cm. (Willey).

Distribution.—Torres Straits, Australia.

Remarks.—This species differs more from the other species of Heteropleuron than any of them do from one another, and differs in features which remove it further than they are from

the sub-genus Amphioxus. It would be almost justifiable to place H. cultellum in a distinct sub-genus on account of the absence of fin-rays from the ventral fin-ray chambers, the great depth of the dorsal fin, the swollen anterior knob-like termination of the notochord, and the considerable tract of terminal notochord projecting posteriorly beyond the last myotome.

Dr. Arthur Willey (loc. cit.) was the first to draw attention to the unilateral character of the gonadic pouches in H. cultellum, and, in fact, to all the points here noted, excepting the number of the intra-buccal cirrhi. Dr. Willey states that the præoral tentacles are devoid of the projecting sensory papillæ which occur in all species of Amphioxus and Heteropleuron. I find in well-preserved specimens where the epithelium is still present that the sensory papillæ are clearly developed.

Dr. Willey was unable to find an olfactory pit in this species, and I have not found one.

Genus II.—Asymmetron.

Asymmetron, Andrews. 'Johns Hopkins University Circulars,' June, 1893, vol. xii, p. 104.

Præoral tentacles grouped into ventrad and laterad series by the presence of a very high intertentacular membrane uniting the tentacles of the two ventrad groups, the lateral series having a low intertentacular membrane like that of the whole series in Branchiostoma: a median free ventral "tentaculum impar" between the two ventrad groups of high-webbed tentacles.

Dorsal and ventral median fins expanded some distance in front of the caudal extremity, and contracted again along the terminal seventh of the body, so as to leave a narrow caudal or urostyloid process, and no "caudal" fin. Myotomes not developed in the urostyloid process, which is, however, traversed by the notochord and nerve-cord. No fin-ray chambers or fin-rays present in the ventral median fin.

Right metapleur continued without break to join the ventral

median fin (as in Heteropleuron); left metapleur dying out immediately behind the atriopore.

Infra-rostral fin in direct continuity with both the right and the left metapleura. Atrial chamber extending behind the atriopore as two laterally paired cæca. Præoral tentacles devoid of sensory papillæ. Gustatory groove of Hatschek (on the right side of the roof of the præoral hood) very deep and pit-like.

Species unica.—A. Lucayanum, Pl. 34, fig. 3.

Asymmetron Lucayanum, Andrews. 'Johns Hopkins Univ. Studies,' 1893, p. 213.

Myotomes, maximum number 69, minimum number 63, usual number 65—66.

Myotome formula 43, 8, 12; 44, 9, 13; 45, 10, 14; 46, 10, 12; usual formula 44, 9, 13.

Dorsal fin of moderate height (one seventh of length from crest of fin to edge of metapleur at mid-body). Rostral fin very slightly developed, either above or below the notochordal rostrum, which, however, is of unusually great length. Ventral fin devoid of fin-ray chambers, and of fin-rays; a few irregular spaces apparently represent the fin-ray lymph-spaces of other genera. Oral sphincter underlies the angle of myotome 8. Ten intra-buccal cirrhi are present. From twenty-one to twenty-nine præoral tentacles were counted. The nine most ventrally placed are separated into two groups of four by a single free median ventrad tentacle; the two sets of four ventrad tentacles right and left of the tentaculum impar present a high intertentacular membrane, by which tentacle 2 (counting the impar as tentacle 1) is united to tentacle 3, 3 to 4, and 4 to 5: the remaining tentacles on either side corresponding to the numbers 6, 7, 8, 9, 10, &c., are free from one another excepting for a low basal connection as in other Branchiostomidæ. All the præoral tentacles are smooth and destitute of projecting sensory villi or papillæ. Gonad pouches twenty-six to twenty-nine, in a single series on the right epipleur.

Maximum length observed in 12 specimens 1·9 cm. (J. W. K.).

Distribution.—Off the Bahamas, pelagic (?).

Remarks.—Several interesting points in the structure of this species are detailed in Mr. Andrews' account (loc. cit.). Attention may especially be drawn to the following in addition to those which have been indicated as generic and specific characters. The anus occupies a nearly median position, the ventral fin being here deflected to the right, occupying thus more nearly its true morphological position as right metapleur. The first pair of nerves arise below the eye-spot; in place of the second pair there is a single nerve, which, with the first pair, supplies the rostrum.

In young specimens the urostyle-like process is not developed, and there is a terminal caudal fin.

The terminal branches of the rostral nerves are furnished in all Branchiostomidæ with cellular end-organs; these are of especially large size in A. Lucayanum.

Incertæ Sedis.

1. Amphioxus elongatus, Sundevall. 'Ofvers. vet. Akad. Förhand.,' 1852, p. 147.
   Branchiostoma elongatum, Sundevall. 'Ofvers. vet. Akad. Förhänd.,' 1853, p. 12.

Myotomes 79. Formula 49, 18, 12. Dorsal fin low. Caudal fin small. Oral cirrhi wanting. No eye-spot. Length 6 cm.

Distribution.—Coast of Peru.

Remarks.—This species is described as above by Sundevall, but the description does not enable us to determine whether the species belongs to the sub-genus Amphioxus or Heteropleuron, or to the genus Asymmetron, or to a distinct genus. The apparent absence of oral tentacles is very probably due to alcoholic shrinking.

Eigenmann (3) records the capture of a number of Branchiostoma from San Diego Bay, California, which he is disposed to refer to this species, but from his description it appears probable that they belong to the species A. californiensis.

2. Branchiostoma pelagicum, Günther. 'Challenger Reports,' vol. xxxi, 1889, p. 43.

Myotomes 67. Myotome formula 36, 16 (?), 15.

Dorsal fin low; caudal fin paddle-shaped; ventral fin without fin-rays; oral cirrhi wanting; notochord projects beyond the myotomes posteriorly. Nerve-cord with eye-spot; gonads in two series, twenty-six (?) on either side.

Length 1 cm.

Distribution.—A single specimen taken in the tow-net near Honolulu.

Remarks.—Of this species only the single specimen above described is known. After Dr. Günther's description and figure were published it was examined by Professor Lankester by means of transverse sections, but the state of preservation was such as to render any satisfactory observations impossible. The specimen had been stained strongly in carmine before it came into Dr. Günther's hands, and mounted under a cover-glass which had greatly compressed it.

Tabular Enumeration of the Genera and Species of Branchiostomidæ.

Genus I.—Branchiostoma, Costa.

Sub-genus Amphioxus.

1. A. lanceolatus, Yarrell (Pallas).
2. A. californiensis, Cooper.
3. A. caribæus, Sundevall.
4. A. Belcheri, Gray.

Sub-genus Heteropleuron.

1. H. Bassanum Günther.
2. H. cingalense, J. W. Kirkaldy.
3. H. cultellum, Peters.

Genus II.—Asymmetron, Andrews.

1. A. Lucayanum, Andrews.

INCERTÆ SEDIS.

1. Branchiostoma elongatum, Sundevall.
2. Branchiostoma pelagicum, Günther.

Note.—Whilst the three species of Heteropleuron seem to be distinctly characterised, it is very questionable whether more than two species of the sub-genus Amphioxus should be recognised, namely, A. lanceolatus (including A. caribæus and A. Belcheri) and A. californiensis.—E. RAY LANKESTER.

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EXPLANATION OF PLATES 34 & 35,

Illustrating Miss J. W. Kirkaldy's paper, "A Revision of the Genera and Species of the Branchiostomidæ."

All the figures are drawn of the same absolute size, so as to facilitate a ready comparison of the proportions of each species. A line placed above each pair of figures gives the usual length of adult specimens of the species indicated. With the exception of fig. 1, which is copied from originals drawn from living specimens at Naples by Professor Lankester, the drawings have been made from spirit-preserved specimens. In the views from the ventral surface the præoral hood has been represented as expanded, as it would probably appear in life, but in the side views (excepting in fig. 1) the actual condition of contraction of the hood seen in the spirit specimens has been more closely followed, and accordingly the side view is not in this region precisely coincident with the ventral view. The ventral mid-surface is represented as distended as it is in life, so that in the profile views it projects below the metapleur. It would be as easy as it is desirable to have drawings from the life of the South Australian Heteropleuron Bassanum and of Amphioxus californiensis.

Fig. 1.—Ventral and profile views of Amphioxus lanceolatus, Pallas, copied from Lankester (No. 7) with the addition of a tentaculum impar to the oral hood. The animal is represented as seen in life in a basin of water lying on its back (upper figure) or on its side (lower figure), with the præoral hood and tentacles expanded, the atrial chamber distended, and the atriopore widely open.

Fig. 2.—Ventral and profile views of Heteropleuron cultellum, Peters. Præoral hood contracted in the profile view.

Fig. 3.—Ventral and profile views of Asymmetron Lucayanum, Andrews.

FIG. 4.—Ventral and profile views of Amphioxus californiensis, Cooper. In the profile view the præoral hood is represented as much contracted as it is in spirit specimens.

FIG. 5.—Ventral and profile views of Amphioxus caribæus, Sundevall. In the profile view the præoral hood is incompletely expanded.

FIG. 6.—Ventral and profile views of Heteropleuron Bassanum, Günther.

FIG. 7.—Ventral and profile views of Heteropleuron cingalense, Kirkaldy. Præoral hood in the profile view only partially expanded.

FIG. 8.—Ventral and profile views of Amphioxus Belcheri, Gray. In the profile view the præoral hood is not nearly so fully expanded as in the ventral view.

Sedgwick's Theory of the Embryonic Phase of Ontogeny as an aid to Phylogenetic Theory.

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In a recent number[1] of this Journal there appeared a paper by Mr. Adam Sedgwick on the significance of the embryonic phase in development, which embodies a principle which, if true, seems to me well fitted to throw light on some obscure problems in morphology.

It is not the object of the present essay to discuss the correctness of Mr. Sedgwick's views, but rather, assuming them to be true, to point out some of their consequences.

These views may be briefly stated as follows. Making a broad survey of the facts of ontogeny, we find that there are two main types or phases of development—the larval and the embryonic. In the former case the immature organism pursues a free life, engaging in the struggle for existence; in the latter case the developing animal is shut off from the influence of external conditions, either inside an egg-membrane or in the uterus of the mother; but in both cases it is relieved from the necessity of having to seek its own living, since nourishment is provided for it either in the shape of food-yolk or fluid nourishment exuded from the uterine walls.

In many cases the whole course of the ontogeny of an animal

[1] "On the Law of Development commonly known as von Baer's Law; and on the Significance of Ancestral Rudiments in Embryonic Development," Quart. Journ. Micr. Sci.,' April, 1894.

is embryonic, but in every case larval development is preceded by a longer or shorter period of embryonic development.

The whole interest of the science of Embryology lies, of course, in the fact that features observed in both types of development seem inexplicable except on the assumption that they are reminiscences of structures possessed by the ancestors of the animals in whose development they appear. Such traces of the history of the race are to be found in the vast majority of larvæ; in embryos they are likewise to be found, though here they are less prominent, as is seen by comparing the development of two allied forms, in one of which the larval type prevails, and in the other the embryonic. Now Mr. Sedgwick's theory of the relation of the two types to one another is that that portion of embryonic development in which ancestral features are observable represents a larval stage passed over inside the uterus or egg-membrane and modified in consequence. Thus the chick during the first four or five days of its existence is to be regarded as an immensely modified larva.

If this view be true it follows that, however modified the record of ancestral history contained in the larval development may be, the embryonic record of the same history can never rise above it in value.

It was until lately customary to assume, explicitly or implicitly, that there was an inherent tendency for the ontogeny of the individual to be a summarised repetition of the phylogeny of the race. In proof of this statement we may adduce Balfour, who in his 'Text-book of Comparative Embryology' (vol. ii, p. 298), says, "Unless secondary changes intervened this record [of ancestral history] would be complete;" and Bateson,[1] in his discussion of the ancestry of the Chordata, commits himself to a similar position. That there can be no such general tendency is, however, shown by the fact that in

[1] "The Ancestry of the Chordata," W. Bateson, 'Quart. Journ. Micr. Sci.,' 1886. "Development within an egg-shell as involving a less complicated struggle with enviromental forces, is less subject to variation than that in the open sea, and consequently is more likely to preserve ancestral features."

bud ontogeny there is no trace of anything which can be interpreted as ancestral structures, and that some most striking and recent changes, such as the loss of limbs in snakes or the reduction of the toes of the ostrich to two, are not recorded in embryology, i. e. the organ concerned shows from its inception the adult arrangement.

How then does the theory we have adopted account for the retention of ancestral characters by larvæ?

So far as we can judge by comparative anatomy, the stimuli to evolution (in the sense of change of structure) have been two, viz. (1) change of environment and habits, and (2) increased or decreased demands on the working of certain organs. As we therefore pass along a series of genetically connected animals, we should find, pari passu with the environment and the functional demands of the organism, the structure changing. If these stimuli commenced to act from the beginning of free life, then each individual adult in the chain would show from the beginning the modified structure belonging to it; but if these stimuli were deferred in their operation till the animal had attained a certain size, then what was before a uniform life-history would become differentiated into two periods—a larval during which the ancestral habits were retained and the structures corresponding to them, and an adult in which new habits were assumed and structure correspondingly modified.

An illustration will make this clear: if young flat-fish when they emerge from the egg were at once to adopt the adult mode of life, then that most interesting larval stage, in which they are bilaterally symmetrical, would be missed out in their development.

Thus we see as a race of animals progressed from point to point in evolution, it would tend to develop a trail of larval stages, each grade of development surmounted being represented by a new larval stage intercalated in the ontogeny. This process, however, could not go on indefinitely; there would soon arise the tendency for the earlier larval stages to be passed over whilst still in the egg-membrane, and so a

portion of the development would become embryonic, and so subjected to the various modifying influences which are connected with this type of ontogeny. Therefore it follows, as the first important deduction from Sedgwick's theory, that in seeking to obtain a basis for phylogeny, most importance must be attached to animals which show long larval histories.

Balfour with his usual sagacity has, so to speak, instinctively anticipated this conclusion. Although he points out that "the favourable variations which may occur in the free larva are much less limited than those which can occur in the fœtus," he says that there is "a powerful counterbalancing influence tending toward the preservation of ancestral characters, in that larvæ are compelled at all stages of their growth to retain in a functional state such systems of organs at any rate as are essential for a free and independent existence" ('Comp. Emb.,' vol. ii, p. 299).

The objection, alluded to in Balfour's statement, that larvæ as well as adults have been subjected to the modifying influences of their environment, will readily occur to most minds. Let us consider whether it is possible to approximately estimate the nature and amount of such influences; and, first of all, let us consider what is meant by secondary larvæ.

Balfour imagined that secondary larval forms might be produced by a diminution in the food yolk, and consequent earlier commencement of free existence (loc. cit., p. 300). There is no evidence to suggest that such a change has ever taken place; all the facts point in a contrary direction. We shall see that food yolk produces the most diverse distortions of development; the developmental processes of free larvæ are, on the other hand, remarkably uniform. Secondary larvæ must be regarded as having arisen owing to the young adults having taken to a new mode of life; the best instances of this are perhaps the aquatic larvæ of the may-flies and dragon-flies. We have the strongest reason for believing that the immediate ancestors of insects were terrestrial animals, and the aquatic larvæ mentioned show their secondary character by the fact that their respiratory organs are modified from organs adapted

to air breathing. Their "tracheal" gills, instead of like all other gills bringing the blood into close proximity to the water, bring their blood first into contact with air contained in a system of closed tubes, and then this air into contact with the water.

In the case of ordinary larvæ, the probability of modifications due to adaptation to the environment cannot be denied. If, however, Sedgwick's hypothesis is correct that "larval history is constructed out of ancestral stages," or, in other words, that the larva retains ancestral characters because it retains the ancestral mode of life, then the environment has remained to a large extent constant (at any rate in the commonest case, that of pelagic larvæ), and the changes they are likely to have undergone, instead of being, as Balfour supposed, unlimited, will be comparatively few in number.

Of these changes reduction in size is the most important. The passage to the adult state is often accompanied by the loss of larval organs, and great changes in those which are retained, necessitating in some cases the complete destruction of their constituent cells, and their reconstruction from rudiments which have retained the embryonic condition (histolysis). It is, therefore, clearly to the advantage of the larva to grow no larger than necessary before it undergoes metamorphosis. Correlated with this loss of size is the frequent disappearance of all traces of segmentation, since this is probably to be regarded as essentially the same phenomenon as vegetative reproduction, only held in check by the individuality of the whole. Metameric series of organs are represented only by those members which are absolutely necessary. Another change which larvæ are prone to undergo, is the acquisition of transparency. What results this carries in its train will be mentioned below. Finally, the occurrence of long spines is a widespread phenomenon, though what their precise use is it would be rash to surmise. Possibly they are of a protective nature.

Let us now apply these principles in a concrete case, for example the larvæ of the Crustacea.

The characteristic larva of the Entomostraca is the well-

known Nauplius, which shows no signs of segmentation. We have however from comparative anatomy, strong reason for believing that the ancestor of the Crustacea was segmented, and that it was probably related to the Polychæta. How is this apparent contradiction to be explained? We answer that the Nauplius retains the ancestral habits of Crustacea, and with this a certain necessary amount of ancestral structure; but it has diminished in size and external segmentation has disappeared. Since in the ancestor locomotion and prehension of food were effected by one or two pairs of anterior appendages only, we have these alone represented in the Nauplius, though the ancestor doubtless possessed in addition a series of segments bearing undifferentiated parapodia-like appendages. The complete disappearance of these is a mark of the high specialisation of the larva; if we compare the various families of the Entomostraca with one another, we find that in the primitive group of the Branchipoda, the Nauplius shows indications of a postoral segmentation; whereas in the highly specialised Cirripedes and Ostracods we get a specialised Nauplius. In the former case this is brought about by the outgrowth of great spines, in the latter by the precocious appearance of the adult bivalve shell, and in neither instance is there a trace of segmentation.

The larva of the Malacostraca the Zoæa, has been a great puzzle to morphologists. It is quite impossible to regard some of the peculiar features, such as the suppression of the thoracic segments and their appendages, as ancestral, and the question has been raised by Claus,[1] whether it has any phylogenetic significance at all.

Applying Sedgwick's principle, we explain the Zoæa as representing a later ancestral stage than the Nauplius, in which some of the Nauplius appendages had become exclusively masticatory and others exclusively tactile in function; the main locomotor function had been, so to speak, passed on to the two or three pairs of maxillipedes, which are

[1] L. Claus, "Zur Kenntniss d. Malakostrakenlarven," 'Würz. Naturw. Zeitschrift,' 1861.

always large and biramous, whilst at the same time some of the most posterior segments have been modified to form a powerful jointed "tail." The thorax retained its primitive character, and is accordingly suppressed in the larva; though here in comparing the Zoææ of the various groups we meet with a precise parallel to the case of the Nauplii. The Zoæa of the undifferentiated Schizopod possesses only one pair of maxillipedes, and even they are short and somewhat foliaceous, but it shows a distinct segmentation of both thorax and abdomen. Still more instructive are the larvæ of the lower families of the Decapods, the Sergestidæ and Penæidæ. Taking Penæus for example, we find that it escapes from the egg-membrane as a Nauplius: it gradually changes to a Zoæa with two pairs of maxillipedes and the thorax distinctly segmented and with rudimentary appendages; this passes into a form with thorax well developed and all its appendages biramous—the so-called Mysis-stage, closely resembling the adult Schizopod,—and from this it passes to the adult state. On the other hand, in the highly specialised Brachyura we find a highly specialised Zoæa, in which the thoracic segments are totally suppressed and the thorax prolonged into great spines, the Mysis-stage is dropped but a new "Megalopa"-stage is introduced, which strongly recalls the Macrura, and may be taken to indicate a Macrurous ancestor. The existence of the Megalopa and Mysis stages, the significance of which is obvious, affords the strongest reason for maintaining the ancestral significance of the Nauplius and Zoæa stages; in doing so one merely follows the universal rule of science, i. e. reasoning from the known to the unknown.

Turning now to the embryonic type of development, let us examine the causes which are likely to modify a course of development which is primitively larval. First we must discriminate between various kinds of embryonic development. There is, in the first place, the type in which the organism is confined within the egg-membrane and supplied with nutriment by means of yolk stored up in its cells. Secondly, we have cases in which the embryo, still remaining in the egg-membrane, is retained in the body of the mother, the egg being

closely applied to the uterine wall, from which nourishment is obtained, and the yolk having consequently in large measure disappeared. Thirdly—a much rarer case,—a number of eggs are enclosed together in a capsule and only one develops; the eggs destined to be eaten being known as yolk cells. As a less extreme case we have the eggs all developing up to a certain stage, but only a few surviving. This condition is seen in Prosobranch Mollusca. Lastly, we may mention those cases in which the uterus or other brood-pouch of the mother is used, so to speak, as a nursery for the larvæ, the embryos escaping from the egg-membrane, and passing the earlier part of their existence as free-swimming organisms inside the brood-pouch.

Taking the first case, which is by far the commonest, the disturbances of development which are found in it are due to two main causes—yolk and the egg-membrane. It is owing to the cramping influence of the latter that external differentiation of form is to a large extent lost. The gastrula of Asterina gibbosa, for instance, is almost spherical, contrasting thus with the common form of Echinoderm gastrula, which is more or less elongated. Where the egg is enclosed in a roomy capsule, on the other hand, as in the Pulmonata, this is less frequently the case; for instance, we have the velum of Limnæus and Planorbis. Mere disuse will not suffice to account for the disappearance of external organs, as in this case all traces of ancestral history ought to disappear in internal as well as external organs, and this is not the case.

The presence of food yolk exercises the most distorting influence on development. To Lankester[1] is due the credit of first laying emphasis on this. In treating of the development of Mollusca he points out that the question whether the endoderm is represented by many or few cells, and whether, consequently, these are invaginated to form the gut, or whether the ectoderm grows over them, is entirely determined by the amount of yolk present. Balfour, who had almost at the same time instituted a

[1] "On the Invaginate Planula or Diploblastic Phase of Paludina vivipara," E. Ray Lankester, 'Quart. Journ. Micr. Sci.,' vol. xv, 1875.

similar comparison between the segmentation of the eggs[1] of vertebrates, subsequently put forward the thesis,[2] based on a comprehensive survey of the facts of embryology, that the rapidity of segmentation of a given region of the ovum is inversely proportional to the amount of yolk contained in it.

The general effect of the presence of yolk, therefore, when massed specially in the endodermic end of the ovum, is to impede cell division, and render processes of development which depend on folding (e. g. invagination) impossible.

There is, however, another manner in which yolk can be accumulated, and that is in the more central portion of the ovum, instead of at one end. This is characteristic of the Arthropoda. When it is comparatively moderate in quantity, as in the case of Lucifer, segmentation and invagination can proceed normally, though the number of cells composing the blastosphere is small. When it is somewhat greater in quantity, as in Branchipus, segmentation at first proceeds normally, but soon the inner yolky ends of the blastomeres fail to be governed by the rapidly increasing nuclei, and segmentation only affects the outer layer of the egg, the inner ends of the first formed blastomeres fusing together to form a central yolky mass. In most Crustacea the yolk is so large in quantity that only superficial segmentation is possible from the beginning. Invagination of this outer layer to form the gut still occurs in some cases, the yolky mass being pushed before it; but, since the yolk is eventually absorbed by the endodermic cells, even this soon ceases to be possible, and we reach eventually a condition in which the segmentation and first processes of development recall to a certain extent those found in telolecithal eggs when the yolk increases to such an extent as to prevent segmentation at the endodermic pole at all (meroblastic eggs). In the scorpions and insects segmentation in its earlier stages is totally suppressed, and represented merely by the multiplication of nuclei; and in the later stages segmentation only occurs where developing organs require it, and thus a mimetic meroblastic segmentation is produced.

[1] 'Quart. Journ. Micr. Sci.,' 1875. [2] 'Comp. Emb.,' vol. i, p. 121.

The second type of embryonic development, viz. that in which the egg is applied to the uterine wall, is characterised by the reduction of the food yolk, so that the segmentation reverts to the total type. Sharply marked traces, however, of the former presence of yolk remain. The gorging of the endoderm with yolk has rendered the archenteron functionless, and as it still remains functionless when the plan of absorbing nutriment from the uterus through the general surface is introduced it is deferred in development, and in fact the destiny of the first products of segmentation is totally different in Mammalia from what it is, for example, in Echinoderms.

In the third type of embryonic development, in which the ovum is enclosed in a capsule with a number of yolk cells, the most weird changes are produced in development. We read of a complete separation and subsequent reunion of the blastomeres for instance; this type is almost confined to the Platyhelminths, and is alluded to here only for the sake of completeness, and to show how few traces of ancestral history the development of these animals affords. One family only, the Dendrocœla, lay their eggs singly, and in this case we have a large amount of food yolk present; yet Platyhelminths have bulked largely in many phylogenetic speculations.

Lastly, we have those few cases in which the developing animal escapes from the egg-membrane, but remains in the uterus or brood-pouch. In these cases we have comparatively little interference with the normal course of development. The early stages occur in a perfectly regular manner, and we have, in fact, free-swimming larvæ within the brood-pouch; it is only in the later stages that they commence to absorb fluid from its walls. It is necessary to emphasise this type of development, though it is comparatively rare (Brachiopods, Paludina, and Amphiura squamata) because if it is confounded with the foregoing types, its totally different characteristics would seem quite inexplicable.

I ought perhaps to mention that the earlier stages of Amphiura squamata are described as being abnormal by

Russo,[1] but neither the figures nor the methods of this author are calculated to inspire much confidence. These earlier stages are very difficult to obtain, but I have strong reason to suspect from those which I have seen, that the earlier development follows the ordinary Echinoderm type.

Having thus rapidly reviewed the principal disturbing factors in embryonic development, we can employ our knowledge in attacking one of the most vexed questions in morphology, viz., the significance of the mesoderm and its contained cavity the cœlom.

Is the former to be regarded as a differentiated portion of the gut-wall, and the latter as a portion of the enteric cavity, or is the cœlom to be regarded as a mere enlargement of the cavity of the gonad as Hatschek[2] has suggested?

In all Annelids and all Mollusca (Paludina and Cephalopods excepted) the mesoderm first appears as two symmetrically situated large cells—the primary mesoblasts. In Paludina, Echinodermata, Sagitta, Brachipoda, and Amphioxus, it arises as one or more pouches of the gut. Now, leaving out of account Anthropods, Vertebrates and Cephalopods, where the development has been complicated by the enormous amount of yolk present, we find that of the other groups the Echinoderms have by far the most prolonged larval development. They are unique amongst the Cœlomata in the fact that the blastosphere is a free-swimming larva, and that consequently the development of both endoderm and mesoderm takes place during their free-swimming life. Here, then, we may on our hypothesis expect to find ancestral structure preserved, and here we find that the cœlom is developmentally a part of the archenteric cavity.

No Annelid or Molluscan larvæ commence free life so early; most of them may be ruled out at once for the purposes of this comparison, since the disturbing presence of yolk shows itself plainly in the fact that the endoderm is represented by a few large spheres, and the production of a pouch has become

[1] Achille Rosso, "Embryologia d' Amphiura squamata," 'Rendiconti della Società Reale di Napoli,' tome vii (2nd series).

[2] 'Lehrbuch der Zoologie,' 1891, B. Hatschek.

an impossibility. A few Annelid eggs have, however, very little yolk, and the larvæ commences a free life in the gastrula stage.[1] Even here, however, if the blastosphere be compared with that of an Echinoderm, one is struck at once by the comparatively small number of cells it has, and one understands why the mesodermal rudiment should be represented by a single cell. The small number of cells is doubtless due to the comparatively large quantity of yolk, even if it be fairly uniformly distributed. This is probably, however, not the only reason why the cœlomic gut pouch is not found in the Annelid larva. If we compare Echinoderm larvæ with one another, we find that the blastocœle or segmentation cavity and the cœlom vary inversely with regard to one another. Thus in the creeping larva of Asterina the cœlom is very spacious, and the blastocœle reduced to a mere slit; in the pelagic larva of Asterias, on the other hand, the blastocœle is exceedingly large, and the cœlom has the form of two narrow tubes the lumen of which is in parts occluded. A similar comparison can be made between the ordinary Tornaria larva of Balanoglossus and Bateson's larva. The reason of this difference is not far to seek. It is to the over-development of the blastocœle with its contained jelly that pelagic larvæ owe that transparency which is so invaluable to them; hence the great development of the blastocœle in pelagic larvæ and consequent feeble development of the cœlom.

Now whatever may be the functions of the cœlom in Echinoderms, in Annelids its main functions are excretion, and the production of the sexual cells. Of these the first is performed in the Trochophore (the characteristic Annelid larva) by the so-called protonephridium, and the second has, of course, no place in larval economy. Hence, if we regard the Trochophore as bearing somewhat the same relation to the Echinoderm larva as the Zoœa of the crab does to that of Penæus, we see why the cœlom should have been entirely suppressed and the mesoderm represented only by a few large cells.

[1] Compare figures given in Korschelt and Heider's 'Lehrbuch der Vergleichende Embryologie.'

Thus the cœlom appears to be, phylogenetically, simply a differentiated portion of the archenteron; when the lumen of the latter is small, and its walls are composed of only a few large cells, the mesodermic walls of the cœlom are represented by a single large cell on each side.

A little consideration will throw light on the reason why what we see to be probably the primitive mode of development should appear in Paludina Brachiopods and Amphioxus. In all these cases the yolk is exceedingly small in quantity and uniformly distributed; in the first two cases we have a pseudo-embryonic development—the fourth type mentioned above,—and of course in this case there are no pelagic conditions to require a suppression of the cœlom. Amphioxus has a long larval history. Sagitta, on the other hand, pursues a true embryonic development within the egg-membrane; but the yolk appears to be quite uniformly distributed, and hence its primitive character.

A second vexed question which naturally follows directly on that of the origin of the mesoderm, is the origin of the endoderm, and consequently of the gastrula itself. Echinoderm development suggests the idea that the ancestral form of metazoon was a sphere of ciliated cells, and that the archenteron arose through the specialisation of a portion of the surface of the sphere to fulfil digestive functions and its invagination into the anterior. This is the view adopted by Korschelt and Heider;[1] and it is, of course, the famous gastræa hypothesis of Haeckel.[2] On the other hand, contrary opinions have been put forward by Metschnikoff,[3] Lankester,[4] and Sedgwick.[5]

[1] 'Lehrbuch der Vergleichende Embryologie,' vol. i, p. 81. Heider showed experimentally that carmine granules were swept by the cilia to the posterior end.

[2] Haeckel, 'Studien der Gastraea Theory,' Jena, 1877.

[3] El. Metschnikoff, "Spongiologische Studien," 'Zeit. für wiss. Zool.,' Bd. xxxii, 1879.

[4] E. Ray Lankester, "Notes on Embryology and Classification," 'Quart. Journ. Micr. Sci.,' vol. xvii, 1877.

[5] A. Sedgwick, "The Development of the Cape Species of Peripatus, pt. iii," 'Quart. Journ. Micr. Sci.,' vol. xxvii, 1887.

Metschnikoff starts, like Haeckel, from the blastosphere or blastula as an ancestral form; he supposed it, however, to have become filled up by cells wandering in from the periphery. In the midst of these a digestive cavity was later developed, and finally the mouth was formed by the specialisation of the area through which food was taken in. Lankester, starting from the same form, supposes the inner ends of the cells of the blastula to have become differentiated so as to be specially digestive in function, and later that they became separated off as a special layer. The cavity of the blastosphere was thus the digestive cavity, and food at first taken in over the whole surface, was later taken in only at one point, and thus a mouth was formed.

Sedgwick, on the other hand, is inclined to start from a protozoon in which cell territories were non-existent, though many nuclei were present. He supposes that the gut originated as a digestive vacuole, and that the nuclei acquired a definite arrangement with regard to this vacuole and other organs, and thus tissues were constituted. Cell territories, in so far as they exist in the adult, he regards as due to secondary rearrangement of the protoplasm.

We have already pointed out that Echinoderm development tells strongly in favour of the view supported by Haeckel and Korschelt and Heider, and that Echinoderm development, from its almost exclusively larval character, is of the very greatest importance in deciding such a question. Its evidence is by no means solitary; the statement may be made that, in all Cœlomata without exception, when the yolk is feebly developed and evenly distributed we find the embryo pass through a blastula stage which is converted into a gastrula by invagination (cf. Leucifer among Crustacea, Polygordius and Serpula and many others in Annelids, Paludina and Chiton in Mollusca, Amphioxus and Cyclostomes in Vertebrata, &c.). The groups which constitute the chief support of Metschnikoff's theory are Sponges and Cœlenterata. We may leave the first entirely out of account, as it is quite possible that they constitute a distinct phylum to the rest of the Metazoa. In many Cœlenterates we start from a blasto-

sphere, which is in some cases a free-swimming larva. This blastospere, however, becomes filled up by cells which wander in from the external layer; in most cases this seems to take place from one end of the somewhat elongated blastosphere, and in Aurelia this process is replaced by invagination. Now the important point to notice in these larvæ is that during their free-swimming life the gut is functionless; and this accounts for the fact that it is represented by a solid rudiment. A precise parallel to the difference between the endoderm of the Cœlenterate and Echinoderm larvæ may be found amongst the larvæ of the Ectoproct Polyzoa. In the pelagic larva of Membranipora (Cyphonautes), which has a long free-swimming life, we find a perfectly well-developed gut with mouth and anus; on the other hand, in the larva of Alcyonidium we have a stomach of yolky cells, an almost occluded œsophagus, and no intestine, whilst in that of Bugula the whole mesoderm and endoderm is represented by a solid mass of cells. These larvæ are developed from yolky eggs and take in no nutriment during their free life. I hold, therefore, that Heider[1] is perfectly justified in his statement that the ancestor of the Cœlenterata was "a ciliated, oval, free-swimming form, in which by invagination at the posterior end an archenteron was formed."

Lankester's view finds its chief support in the development of Geryonia. In fact, this form is the only known one in which such a process as he supposes to have taken place in the blastula ancestor appears in the ontogeny. Are there any reasons for regarding the development of Geryonia as specially primitive? I think we may fairly say none; but that on the contrary it shows manifest signs of its secondary character; the egg is yolky, and the development proceeds directly to the medusa form, the hydra form being suppressed. The most conclusive argument, however, against Lankester's hypothesis is that on his assumption the cavity of the blastosphere is identical with the cavity of the future gut. Now all recent investigations have gone to show that the blastocœle is the rudiment of the

[1] 'Lehrbuch der Ver. Emb.,' vol. i, p. 81.

blood system, and has no connection with the gut whatever. In many Cœlenterates the stage of the hollow blastosphere is missed out, and segmentation results in a "morula" of which the external layer is the ectoderm and the rest endoderm. I think we must imagine that in the development of Geryonia the shortening process has gone one step further, and that as a result of segmentation we reach at once the stage of the hollowed-out planula.

Sedgwick's hypothesis was suggested from a study of the embryos of Peripatus capensis. Their developmental history is, however, the very last place where one ought to seek for indications of the ancestral meaning of the earlier stages —at any rate if Sedgwick's own hypothesis as to the significance of the embryonic phase be correct. All species of Peripatus so far as is at present known are oviparous; in Peripatus Novæ-Zelaniæ, however, the eggs are large and yolky, and the development conforms to the ordinary centrolecithal type so characteristic of Arthropods—the peculiarities of which we have described above. In Peripatus capensis nutriment is supplied by the wall of the oviduct, and the yolk has in large measure disappeared, at any rate its more solid portions; but the development still bears the impress of centrolecithal segmentation, i.e., in the imperfect definition of the blastomeres. It is obvious one might with equal justice expect to find information as to the character of the ancestor of Metazoa in the eggs of mammals.

Let us now briefly rehearse the conclusions to which the foregoing discussions seem to point. The earliest well-marked larval stage which we have discovered is the blastula—a sphere of uniformly ciliated cells. This "animal Volvox," as Huxley[1] calls it, may be regarded as a protozoon colony, not in the sense of consisting of independent units any more than does Volvox, but rather in the sense of being built up by the repetition of a unit as a result of what Lankester[2] calls "eumerogenesis," just as is the colony of a Hydromedusan. At first all

[1] 'Anatomy of Invertebrates,' p. 678.
[2] Art. "Hydrozoa," 'Encycl. Brit.'

elements in the blastophere were alike in structure and function. Later, however, coincidently with its acquiring the capacity for moving in a definite direction, a change would take place: the form first became elongated, and it is interesting to observe that the free-swimming blastulas of both Echinocyamus and Eudendrium have this form; then the cells at the posterior end, being least favorably situated with regard to promoting the locomotion of the colony, and best situated for seizing food particles, since they are in a kind of backwater from the eddies produced by the ciliary motion of the rest, would become specially digestive; increase in their number could only take place coincidently with invagination if the form of the colony were to be preserved and at the same time the digestive cells were to remain in contact with the surrounding medium—and thus we have the archenteron formed. The cells at the anterior end, on the contrary, are in the best position for receiving stimuli from the outer world; and here we should expect the first sense-organ to appear, and it is just at this spot that we find the larval sense-organ of Conatula with its associated nervous tissue, and the still more primitive sense-organ of the Echinocyamus larva, this latter consisting of a thickened patch of ectoderm bearing stiff cilia, which take no part in locomotion. In the same place the apical plate or larval brain of the Trochophore is found, also bearing cilia or more probably sense hairs.

Metschnikoff's[1] great objection to regarding invagination as the primitive method of forming the endoderm was that the blastopore sometimes became the mouth and sometimes the anus. Sedgwick's suggestion, however, that mouth and anus were differentiated from a slit-like blastopore, seems to answer this difficulty. That a slit-like opening can be represented by two independent perforations is shown by Echinoderm development. Thus in Holothurians the larval mouth by a shift of position becomes the adult; in Asterids and Echinids, on the other hand, it is represented by a totally new perfora-

[1] "Vergleichend Embryologische Studien. (3) Über die Gastrula Einiger Metazoen," 'Zeit. für wiss. Zool.,' Bd. xxxvii.

tion. No one can suppose that the ancestral form gave up its old mouth and developed a new one; the change was one of size and relative position only. We must assume that the original mouth was a wide one, and that part is utilised by the larva and aborted in the adult.

The cœlom, as we have seen, arose as a specialised portion of the gut.

It is to be observed that the history we have just sketched is in accordance with that rule which seems to hold in all cases where we can by means of comparative anatomy show with reasonable probability that evolution has occurred, viz. that new organs never arise de novo, but by the differentiation of older organs. This rule seems, however, to me to be violated by supposing that either archenteron or cœlom arose as a split in a solid mass of cells. The history affords also an explanation of that rigorous separation of primary and secondary body cavities, the blastocœle or hæmocœle, and the cœlom, which all recent research has tended to emphasise. The first is, in fact, morphologically inside and the second outside the primitive blastosphere.

Lastly, the conception of the primitive metazoon as a colony of Protozoa is in accordance with that repetition of similar parts on which Bateson[1] has laid so much stress as one of the most marked characteristics of living things. We should recall also the high individuality acquired by colonies of Siphonophora, Polyzoa, and Ascidians.

[1] 'Materials for the Study of Variation,' W. Bateson, Cambridge, 1890.

The Anatomy of Alcyonium digitatum.

By

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With Plates 36—39.

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Preface.

The work that is recorded in these pages has occupied a considerable portion of my time for the past two years, and was entirely conducted in the morphological laboratory at the University of Cambridge and the Marine Biological Association's laboratory at Plymouth. In some respects it is not so complete as I had hoped it would be. My original intention was to publish with the account of the anatomy of the species a description of its development and spermatogenesis, but unforeseen difficulties presented themselves, and I have decided to postpone the publication of the results I have already obtained until they are more complete. The development of Alcyonium can only be studied in the months of December and January, when the investigations are frequently interrupted or delayed by storms and rough weather. I have experienced very great difficulty in keeping the young embryos alive when I have been successful in effecting artificial fertilisation of the ova.

If I am successful this winter in obtaining the stages in development that I have not hitherto obtained, I hope to publish an account of the maturation and fertilisation of the ovum and the development in the course of next year.

As I felt that the determination of the chemical constitution of the homogeneous substance composing the bulk of the mesoglœa required considerable skill and time, which I did not possess myself, I entrusted this portion of the work to Mr. W. L. Brown, of St. John's College, Cambridge, and his paper, which accompanies this, is a valuable addition to our knowledge of the chemical constitution of the Cœlenterate tissues.

Section I.—History of Investigations.

The common "dead men's fingers" of our coasts was considered by the ancients to be one of those substances formed by the churning of the sea waves. It received its name Alcyonium probably from Alcyone, daughter of Æolus and

Enarete, who threw herself into the sea for grief on the death of her husband, Ceyx, in a shipwreck.

In the middle of the last century a great controversy arose as to the nature of the so-called zoophytes, of which Alcyonium was one. Peysonnel (1727), de Jussieu (1742), Reaumur (1742), Ellis (1754), and others, all maintained that they were animals, notwithstanding the vigorous opposition of the great Linnæus, who considered them to be partly animal and partly vegetable in nature, of Baster and others. It is not necessary, however, to give an account of this controversy here, as the reader will find a most interesting account of it in the first edition of Johnston's 'Zoophytes.' In the course of the controversy, however, de Jussieu paid a visit to Honfleur to study these forms in the living state, and the result was (19) that he produced for the first time a description and some fairly good figures of the polyps of Alcyonium digitatum. He says, "Nous avons soin de tremper dans nos bocaux une branche de chacun des ces plantes en particulier, et nous fumes surpris au premier aspect d'apercevoir, sans secours d'aucun instrument, de petits insectes qui avoient chacun pour loge une des petites cellules formées dans le tissu de ce qui nous paroissoit la feuille d'une plante."

It is to be noted that in this memoir de Jussieu uses the term "polype:" "J'appelle et dans la suite j'appellerai en général polype, une famille d'insectes de la nature des vers plus ou moins longs, dans les uns desquels la tête et dans les autres le corps sont ou environnez ou parsemez de cornes, qui servent aux uns connue de mains pour prendre des choses dont ils font la nourriture, et outre cet ouvrage tiennent encore lieu aux autres de pieds pour se mouvoir."

It is difficult to say, however, whether he or Reaumur was the first to apply the word polype to the Cœlenterate individual. The following passage in Reaumur's great work (34) is interesting as showing the reason for its adoption. Writing of Hydra he says, "De concert avec M. de Jussieu qui en avoit observé aux environs de Paris et fait dessinez une espèce du même genre, mais plus grande et d'une autre couleur je leur imposai

le nom de polype parceque leurs cornes nous parurent analogues aux bras d'animal de mer qui est en possession de ce nom."

The term was subsequently adopted by Trembley (36), and used by him in his classical work on 'Hydra.'

At the commencement of the present century, however, the name Alcyonium was used to include a considerable number of marine fleshy, gelatinous, and spongy zoophytes, which are now known to belong to widely different groups of the animal kingdom.

The description given by Ellis (8) of the genus is as follows:

"Animal, plantæ forma crescens.

"Stirps fixa, carnosa, gelatinosa, spongiosa, vel coriacea; epidermide cellulosa poris stellatis s. osculis pertusa, hydras tentaculatas oviparas exserentibus."

Baster (1) describes the genus quite briefly as follows:

"Die Alcyonia zyn zagte dog vaste zeegewassen die als een middelsoort tussen de Kruidige (Herbacea) en Hoornagtige (Keratophyta) entmaken."

Included in the genus, as defined by Ellis, were a large number of Ascidians, sponges, and probably also some Bryozoa and Hydroids. In the systems of Müller (1776) (31), Fabricius (1780) (10), Berkenhout (1795) (2), Turton (1809), Bosc (1807) (3), and others, we find the same conglomeration of animals included under the name Alcyonium.

It must not be supposed, however, that because these naturalists included all these different forms of animals under the one generic name they were necessarily ignorant of more easily discerned characters of Alcyonium digitatum; it was rather their want of knowledge of the anatomy of the Ascidians, Bryozoa, &c., which led to the confusion.

Ellis's (7) description of his figure runs as follows:—"A piece of Alcyonium manus marina cut perpendicularly through the middle to show that it is formed of tubes, which branch out into others, each ending on the surface in a starry opening of eight rays; in each of these openings is a polype-like figure or sucker with eight claws, fastened to the inside of the tube at its lower part by eight fine, tender filaments, by

which it can raise or sink itself at pleasure in its tube; all these tubes that compose this Alcyonium are connected together by minute reticulated fibres; these enclose a stiff gelatinous substance which seems to be the flesh of this compound animal, and these fibres, with their enclosed contents, to be the muscles; for by the excretion of these it assists in opening or closing the stars on the surface, while the suckers or polype-like figures are pushing themselves out in search of food, or when they are retreating to secure themselves from danger."

In 1816 Savigny clearly pointed out that the species Alcyonium digitatum, A. exos, and A. arborea, in the possession of eight pinnate tentacles, must not be classed with the Ascidians, the anatomy of which he described in some detail. Alcyonium exos was probably the Alcyonium palmatum of the Mediterranean, and Alcyonium arborea the Paragorgia of Norway.

In Cuvier's 'Regne Animale' it is stated that in Alcyonium, as in the Pennatuleæ, we observe polyps with eight denticulated tentacles, and a stomach prolonged into the ovaries; but the first detailed description of an Alcyonid was that given by Milne Edwards in 1835 (9).

This distinguished naturalist obtained some specimens of Paralcyonium (?), to which he applied the generic name "Alcyonide," at Cape Matifou, in Algeria, and he gave to the Académie des Sciences a description of the anatomy, which, considering the simplicity of his methods, is remarkably accurate and detailed. He pointed out the individuality of the polyps, and that each of them is capable of independent action, although acting in response to stimuli with its neighbours. He cut off a single polyp, opened it longitudinally, and discovered the stomodæum, with its free opening into the cœlenteron, the eight mesenteries with their attachments, the ovaries, and the cavities in the tentacles. With regard to the structure of the colony, he pointed out that tubes (loges) running down into the colony are really the continuation of the polyps, "Il suffit d'un examen superficiel pour se

convaincre que ces loges ne sont autre chose que la continuation du corps des polypes eux-mêmes;" and asserted that the polyps do not retreat into their tubes like the Serpulæ, &c., do, but into themselves by a sort of invagination. He noticed, moreover, the mesenterial filaments, which he called "les organes intestinaux," and added with extraordinary perspicuity that they ought to be considered organs of secretion, "analogues aux canaux biliaires des insectes."

In more recent times the only account we have of the anatomy of Alcyonium digitatum is that given by Carl Vogt and Jung in their 'Lehrbuch' (37); but it contains so many blunders, and the illustrations are so exceedingly difficult of interpretation, that it is really of very little use to the student of zoology.

Von Koch's (21) account of the anatomy of Alcyonium palmatum contains some points of interest, but as it is very brief and but imperfectly illustrated it is not of much practical value.

It was the absence of any good account of this common British Alcyonarian which first induced me to make a careful study of some of the details of its anatomy; but I soon found that I must defer the investigation of many details until an opportunity presented itself of studying the living animal at the sea-side. I found, however, that many points presented greater difficulties than I anticipated, and I have been obliged to delay the publication of my results until I felt quite certain by repeated observation and experiment of their accuracy. Just before the close of last year I learned that the late Prof. Milnes Marshall was preparing for publication a description of the anatomy of Alcyonium palmatum, and the last letter I received from him, only a few weeks before the lamentable accident which caused his death, was on the subject of the formation of the buds in Alcyonium. With characteristic generosity he proposed to postpone the publication of his work until my paper was finished, and invited me to see his preparations before I went to press.

Since his death his brother has forwarded to me the few

rough notes and drawings on this subject that were found among his papers, for which kindness I am glad to have the opportunity of expressing my warmest thanks. The notes are only those made in the course of his work in the laboratory, and were not prepared in any kind of way for publication, but whatever help or suggestion I may have gained from them I have expressed in the text; and as the few drawings that were finished are those of another species of the genus to that I have myself investigated, I have not incorporated them at all into this memoir.

Section II.—General Form and Natural History of the Colony.

Alcyonium digitatum in its younger stages occurs in the form of small incrusting masses on shells, weeds, worm-tubes, &c.[1] As it grows older and larger it becomes dome-shaped, then spherical and cylindrical, and finally branches into five to eight blunt processes which become slightly flattened in one plane when the colony reaches its largest size. In the branched condition it is commonly called "cow's paps," "dead men's toes," or "dead men's fingers," all of which terms are more or less appropriate to its lifeless, sodden, pulpy appearance.

The largest colonies I have measured are eight inches long from the base to the tip of the longest branch, and six inches in breadth.

The colour of Alcyonium varies very considerably with the locality. The specimens caught in deep water off the Eddystone Lighthouse are almost invariably of a pale pinkish colour when alive, but they rapidly bleach just before death. Those living in shallower water are quite white. Johnston states that he occasionally met with it of a reddish-orange colour, and I have myself dredged up yellow varieties off the west coast of Scotland, and have seen similar specimens from the Shetlands, the Bristol Channel, and elsewhere.

[1] The youngest specimen I have found consists of a single polyp with one bud sprouting from its side.

The yellow variety owes its colour to a pale yellow tint in the spicules, which is retained after the colony is dead and preserved in spirit.

When first brought on deck from the dredge or trawl, the colonies are of a soft, flabby consistency, with the extensible portion of the polyps retracted, but still visible as transparent circles on the surface. If a colony be allowed to remain out of water for some little while the transparent circles become smaller and smaller, until at last only a star-shaped depression is left to indicate the position of the polyps. At the same time the colony itself contracts considerably until it reaches a size not much more than two thirds of its original bulk.

When the colonies are placed in a sea-water aquarium they soon regain their former size by the absorption of water, and in a few hours the polyps expand. The colonies, however, do not, if they remain in a healthy condition, continue expanded indefinitely, but are seen to contract and remain contracted for some time at intervals. These periodic contractions are quite independent of light, but as a rule seem to be synchronous with tide intervals,—that is to say, they occur twice in every twenty-four hours.

My experiments at Plymouth, two years ago, proved that for the first three or four days after capture they contracted regularly twice in every twenty-four hours, but after that their times of contraction became irregular.

A number of experiments were tried with some Alcyoniums placed in a tank in which an artificial tide, rising and falling only once in twenty-four hours, was introduced, and at the end of a fortnight I found that the two colonies which remained healthy were contracting regularly only once in twenty-four hours.

My observations were not sufficiently satisfactory to enable me to draw any general conclusions from them, but they seem to indicate that there is a normal rhythmic contraction of the polyps of Alcyonium corresponding with some state of the tides —probably low water, and that a new rhythm may be induced by an artificial tide of different duration from the normal one.

The matter seems to me to be worthy of further investigation, and it is to be hoped that some one who is able to remain at the Plymouth laboratory for some months will repeat the experiments.

The sexual reproduction of Alcyonium digitatum occurs on our southern coasts during the months of December and January. The colonies are invariably diœcious. Although I have opened several hundred colonies, I have never found a single instance of ova and spermatozoa occurring together.

Early in March the gonads may be seen as minute swellings on the ventral and lateral mesenteries. These gradually get larger and larger as the year progresses, but it is not until August or September that any difference can be seen between the ova and the sperm-sacs. The ova then acquire a pale yellow tinge, which becomes redder and redder as the winter approaches. The gonads do not reach their full size until December.

The spawning takes place in an aquarium very slowly, the ova being shot out of the mouth, one by one, at considerable intervals, and it may occur either by night or during the daytime. Whether this is also the case in the natural conditions I am, of course, unable to say; but colonies may be found at the end of December in all conditions between those densely packed with ova or sperm-sacs and those completely shotten.

Early in January the number of pregnant colonies becomes smaller and smaller, until in the last week of that month scarcely a single ovum can be found.

The extraordinary long period (nine months) that elapses from the first appearance of the gonads to the time they reach maturity is particularly noteworthy. I can offer no satisfactory explanation of it.

I may say that there are no external indications of the sex of a colony of Alcyonium. In shape, size, and colour the male and female are the same. Nor is there any probability that, as in some Hydrozoa, they are protandrous or protogynous, for small colonies not more than an inch in height may be either males or females.

The time of year at which the spawning takes place is also remarkable.

According to Wilson, the spawning of the Pennatulid Renilla and the Gorgonid Leptogorgia takes place in the summer months. Moreover, in the case of Renilla, "the eggs are always laid at very nearly the same hour of the day, viz. 6 a.m."

The following statistics as to the breeding times of certain Alcyonaria at Naples are given by Lo Biancho (28), the numbers referring to the months.

"Alcyonium palmatum.—Con uova mature ii—iii, deposizione ix—x.

"Gorgonia Cavolinii.—Con uova mature e deposizione di larve v—vi.

"Pennatula phosphorea.—Uova mature nei polipi xi.

"Pennatula rubra.—Esemplari con uova iii."

It would seem, then, from these facts that the breeding season of different Alcyonaria, and even species of Alcyonium, varies very considerably.

There is not sufficient evidence at present to enable us to give a very exact account of the distribution of Alcyonium digitatum. It extends from between tide-marks to a depth of forty or fifty fathoms, and does not, I believe, extend into deep water.

Professor Herdman informs me that Alcyonium digitatum is very common in the Firths of Forth and Clyde, and at various places in the Irish Sea. It is also common between tide-marks at Hilbre Island (Cheshire), Puffin Island, and at the south end of the Isle of Man.

Canon Norman informs me that he has dredged the species in the Bergen and Hardanger fjords in Norway, and believes that it occurs much further north.

As the determination of the species of Alcyonium is a matter that requires some care, all that can be said at present is that there is no evidence that it occurs outside the area of the North European coasts.

There are only two species of the genus Alcyonium found in

the British area, namely A. digitatum and A. glomeratum (Pl. 36, figs. 1 and 2). The yellow variety of A. digitatum referred to above cannot be separated as a distinct species from the white variety. There are no features in the mode of growth, character of the spicules, or general anatomy which distinguish the two varieties, the only difference being the yellowish tint in the spicules of the yellow one.

Alcyonium glomeratum was first discovered by Hassall in 1841, and he referred it to Müller's species Alcyonium rubrum; but on being told by a Mr. Macgillivray that it differed from A. rubrum, gave it the name Alcyonidium glomeratum (14). (Throughout his descriptions Hassall uses the word Alcyonidium in place of Alcyonium.) The species is defined as follows—"Polypidoms massive, of no very definite outline; colour a deep uniform red, the shade of which approaches to vermilion."

In the second edition of Johnston's 'Zoophytes,' vol. i, p. 178, there is a long quotation from Couch on A. glomeratum, who says, "The colour externally is of a deep blood-colour, and internally is but slightly lighter. The lobes are very numerous, and divide nearly as low down as the base. The spicula are numerous and irregularly arranged; they are linear, elongate, pointed at both extremities, with uneven or granular spaces between; sometimes they are simple, and at others united into K-shaped bodies, and occasionally wanting one or other of its members, forming an imperfect K.

When Gray examined the species in 1865 (12) he made it into a new genus, giving it the name Rhodophyton Couchii. The description of the genus is as follows:—"Coral flesh cellular, covered with a hard calcareous coat, contracted at the base, expanded above, and divided into several oblong lobes or branches, covered with short cylindrical tubes with a circular mouth. Polyps half retractile, forming when contracted a white tubular termination to the cells. The more developed cells of the polyps, especially those at the end of the lobes, are longitudinally grooved."

"This genus," he continues, "differs from the typical

Alcyonia or Lobulariæ, taking A. digitatum for the type, in the outer surface being covered with a continuous crustaceous coat, and in each of the polyps being enclosed in a distinct tubular sheath projecting from the general surface. It differs from all the Alcyonia in the polyps being only half retractile."

I have examined a couple of specimens sent to me from the Marine Biological Association's laboratory at Plymouth, and I am able to give a figure of the spicules (fig. 5). The descriptions given by both Couch and Gray are fairly accurate, but, as I shall point out presently, the condition of retraction of the polyps is not a character upon which too much reliance can be placed for purposes of classification, as it is one which depends so largely upon the means employed to kill and preserve the colony.

A. glomeratum (Hassall), then, differs from A. digitatum (Linn.), in that the lobes are more pointed and more deeply divided, the spicules are reddish in colour, and there are no dumb-bell-shaped spicules.

Section III.—General Anatomy.

The colony of Alcyonium digitatum is composed of a number of polyps which are fused together for the greater part of their length, but have each a free extensible and retractile portion which bears the mouth and tentacles. The mesoglœa of the larger portion of the body-wall of each polyp is considerably thickened, and in direct connection with that of its neighbours, so that no limits can be drawn in this region between one polyp and another.

In most of the text-books of zoology it is customary to restrict the term "polyp" to the free extensible portions of the individuals, and to give the name "cœnosarc" to the part of the colony which is composed of their fused basal portions. It cannot be too strongly insisted upon that this is an erroneous conception of the structure of Alcyonium. The dorsal mesenterial filaments of each primary polyp extend to the base of the colony, the ventral mesenteries are visible as ridge-like

projections for a long distance down the cœlentera of the fused portion of the polyps, and the endoderm shows no important modification in that region. Consequently the cœlenteron of the fused portion of each polyp is no more "colonial" in character than that of the free extensible portion. It is, in fact, simply a part of the individual polyp.

A comparison with the genus Tubipora may render this matter clear. In this genus the polyps remain separate from one another for the greater part of their course, being connected together only by the horizontal platforms and the basal stolon. The only part of the colony that is, strictly speaking, "colonial," or, in other words, common to more than one polyp, are the stolon at the base and the horizontal platforms. In these structures there runs a number of branching canals, lined by endoderm, which establish a communication between the polyps, and form a basis from which new polyps originate as buds. In the case of the colonial Hydrozoa it is often a matter of difficulty to determine where the cœlenteron of a polyp ends and the cœnosarcal canal begins, and consequently the distinction between polyp and cœnosarc is indefinite. But in the case of the Alcyonarian there is no such difficulty. The cœlenteron of a polyp of Tubipora, for example, can be distinguished from a canal in a horizontal platform at once by the fact that it contains, either complete or in a shrivelled condition, the dorsal mesenteries and mesenterial filaments.

In Tubipora, then, there can be no doubt that the cœnosarc is composed of the horizontal platforms and basal stolon, and by these structures only.

In the case of Alcyonium the distinction between the polyps and cœnosarc cannot be defined. In consequence of the enormous development of the mesoglœa in this genus, the parts corresponding to the polyp walls and horizontal platforms and basal stolon of Tubipora are all fused together, and consequently it is impossible to say where the polyp wall ends and cœnosarc begins.

The colony, then, is mainly composed of a number of polyps partially fused together. Some of these—the primary polyps

—extend from the surface right down to the base; but others —the secondary polyps, which are formed during the later stages of growth as buds from endodermic canals proceeding from the cœlentera of the primary polyps—terminate at various depths in the substance of the colony, according to the stage of growth at which they were formed.

My first impression on examining the anatomy of the colony was that the cœlentera of the secondary polyps fused directly with those of the primaries, but I am now convinced that this impression was an erroneous one. The secondary polyps terminate in the mesoglœa, but as the terminal end is usually connected with neighbouring cœlentera by short open canals, they cannot be said to terminate blindly (Pl. 37, fig. 8).

When a thoroughly contracted specimen of Alcyonium is examined the surface may be seen to be covered with a number of rounded prominences, each bearing in its centre a star-shaped depression. These prominences indicate the position of the polyps, and are on an average 1·5 mm. in diameter. In a few instances I have measured prominences as much as 2 mm. in diameter, and several young buds are very much smaller than the average dimensions given above.

On the upper free parts of the colony the polyps are very closely packed, so that in the contracted condition there is very little space between the protuberances; but on the sides near the base of the colony the protuberances are frequently separated by considerable intervals. In a specimen I obtained some years ago off the coast of North Wales the base of the colony is spread out like a membrane over a Serpula tube, and in the outer portions, where the base is thinnest, the polyp protuberances are as much as 1—3 mm. distant from one another.

In a fully expanded colony the base of the extensible portion of each polyp averages 2 mm. in diameter, but it is not so easy to give the exact length from the mouth to the surface of the colony, on account of the extreme sensibility of the polyps during life.

It is quite probable that in the fully expanded condition, in

their natural habitat, they stretch themselves out much further than they do under any circumstances in the tanks of an aquarium. All that can be said, then, about this measurement is that, of the polyps expanded in the tanks of an aquarium, the distance from the mouth to the surface of the colony does not exceed 5 mm.[1] Similarly it is impossible to give any reliable figures as to the exact length of the tentacles, but, from observations made upon numerous living polyps with a simple magnifying glass, my impression is that, when fully expanded, they are normally about three quarters the height of the extended portion of the polyp, that is 3 mm.

In living Alcyoniums, as they are observed in an aquarium, the tentacles are more often contracted than not, so that the crown has the appearance of an eight-pointed star, as it is figured in pl. xxxiv,[1] fig. 3, of the second edition of Johnson's 'Zoophytes,' and they will remain in this contracted condition for a very long time together. It is only on exceptional occasions that the tentacles expand themselves completely, so as to show clearly their characteristic pinnate Alcyonarian appearance.

When the colony is killed, expanded by sudden immersion in Lo Biancho's No. 2 chrom-acetic solution, and then transferred to methylated spirit for preservation, the zooids usually lose their transparency, and become opaque, excepting at their lower extremities, where the body-wall usually remains translucent. There is a good deal of difference, however, in the degree to which they lose their transparency when preserved. In some young colonies the body-walls remain perfectly transparent, only the tentacles and stomodæum being opaque; and occasionally the same transparency may be seen in the polyps of the larger and older colonies.

In Alcyonium palmatum of the Mediterranean the expanded zooids, when preserved, are as a rule transparent; this is due to the fact that the body-wall is much thinner than it is in the British species.

[1] In the MS. notes on the anatomy of A. palmatum, by the late Professor Marshall, I find 10 mm. given for this measurement.

The degree to which the extensible portions of the polyps contract on death is sometimes used as a character for the determination of specific characters, but although it may be used at times with advantage, it is one which should be used with great caution. The group of spicules, which may be seen in fig. 6 to extend from the surface of the colony a short distance into the base of the extended portion, may be much more considerable in some specimens than in others, and may afford a physical obstruction to the complete retraction of the polyps. I have observed, too, that when a colony has remained in imperfectly aërated water the polyps will die expanded, as sea-anemones do; and a colony thus moribund when placed in spirit will present a large number of expanded polyps. The condition of the retraction of the polyps of a spirit specimen of an Alcyonarian may depend, then, not only on anatomical characters, but also on the condition of vitality, or want of it, at the time the colony was preserved. These facts should not be ignored, as they too frequently have been, in the determination of species and the descriptions of new ones.

Colour.—All the extended polyps of Alcyonium digitatum I have examined are perfectly white and transparent when alive; but in the specimens obtained in Provence the spicules of the Halskranse (crown) are, according to Vogt and Jung, sometimes reddish in colour.

Tentacles.—The eight tentacles of Alcyonium digitatum are equal in size, and in the fully expanded condition stand at right angles to the long axis of the polyps. They are provided with a number of pinnæ on each side, which varies from twelve to twenty-two.

It is not possible to count with accuracy the number of pinnæ in contracted or preserved specimens. Some of the proximal pinnæ are capable of being so completely withdrawn that they are not visible except in the fully expanded condition. My numbers are given from the careful observation of a great many fully expanded polyps in the Plymouth laboratory.

In his description of the tentacles of A. palmatum, von

Koch (21) says, "The tentacles possess twelve to fourteen pairs of pinnæ (Fiederpaare)." In our English species it is quite impossible to recognise the pinnæ as pairs. Not only do we find an uneven number of pinnæ on the two sides of many of the tentacles, but a minute examination of the free end of the tentacle usually shows a small pinna on one side without any corresponding one on the other (see fig. 10).

Carl Vogt and Jung's description (37) of the tentacles is as follows:—"Bei mässiger Ausdehnung bieten sie die Form langen Blumenblätter mit sägeförmigen Rändern, aber in gewissen Fällen verlängern sich alle Theile dermaassen, dass die Fühler Hirschhörnern ähneln, welche auf beiden Seiten mit sehr langen Zacken besetzt sind."

I have never seen this "antler" appearance of the tentacles except in spirit specimens, and I am inclined to think it is entirely due to the action of the preservative fluid, for in life the tentacles appear to be perfectly regular (fig. 11).

The tentacles of fully expanded polyps when examined alive with a low power of the microscope vary very considerably in appearance. In Pl. 37, fig. 11, I have drawn the crown of a polyp I observed in a recently caught specimen which expanded in the bucket of sea water in which it was placed immediately after its removal from the sea. The tentacles were extremely extended, and at the base of each of them there was a bullate swelling, on the side of which there were no pinnæ. From time to time a sudden and simultaneous movement of contraction would occur, caused perhaps by a slight shaking of the table, and the tentacles would assume a more or less rigid appearance, with the bullate portion very much extended, but the terminal portions and the pinnæ very considerably shortened (fig. 6). These contractions lasted only a few seconds, and then the tentacles extended themselves again and resumed the condition represented in fig. 11.

It is but rarely, however, that it is possible to observe the polyps so fully extended as this. A condition such as that figured in fig. 10, in which there is apparently no bullate portion, is not unfrequently seen, and it is quite possible to kill a

certain number of polyps in this condition by the chrom-acetic acid method.

Under the ordinary, somewhat unfavorable, conditions of life in an aquarium the polyps do not usually expand more fully than those represented in pl. xxxiv of Johnston's 'Zoophytes,' the body-walls being fully extended, but the tentacle lightly contracted, forming an eight-pointed star on the summit of the polyp (Pl. 37, fig. 9).

The condition shown in fig. 10, in which there is no bullate basal swelling to the tentacles, may be called the first stage of contraction.

The condition just described may be called the second stage of contraction (fig. 9).

In the third stage (fig. 7, 2′) the tentacles become bent on themselves, and a ring-shaped collar folds over the crown, partially hiding it.

In this condition the polyp contracts down to the level of the surface of the colony. If they contract rapidly in response to strong stimuli the body-wall is often very considerably distended; but if they contract slowly, either naturally or in consequence of something distasteful in the water, they withdraw below the surface of the colony without any alteration in their diameter.

When the crown has withdrawn below the surface of the colony the basal portion of the body-wall of the extensible portion of the polyp closes over the hole, the stomodæum becomes folded like a concertina (fig. 7, 4′, *St.*), and the tentacles become tightly drawn down.

In the last stage of contraction (fig. 7, 5′) the transparent basal portion of the extensible portion of the polyp is drawn down below the surface, and the aperture is tightly closed by the opaque and densely spiculated crust of the colony, and at the same time the tentacles, disc, and stomodæum become so tightly pressed together that it is difficult in sections to make out their outlines.

Each tentacle is, when fully expanded, provided with a wide lumen, as in all Alcyonaria. This lumen, which is a prolonga-

tion of the intermesenterial space of the cœlenteron, is continued into each of the pinnæ. In Alcyonium palmatum the ectoderm of the pinnæ is thickened in regular patches, which contain batteries of nematocysts. I have seen similar patches on the pinnæ of our English species in favorable preparations.

The mouth varies in appearance very considerably. When fortunate enough to be able to focus the microscope quite vertically down on to a fully expanded polyp, the mouth is seen to be a slit-shaped opening on a shallow ridge in the centre of the flat oral disc. At one end it has a crescentic widening, which indicates the position of the siphonoglyphe lower down in the stomodæum. When the polyp is slightly retracted, as is usually the case, the true mouth cannot be seen, since the oral disc is depressed in a funnel-shaped manner, forming, as it were, a secondary mouth, which is oval in outline (fig. 6).

The stomodæum of Alcyonium digitatum consists of a short, somewhat flattened tube about 1 mm. in length, opening to the exterior by a slit-shaped mouth, and into the cœlenteron by an oval aperture.

On the ventral side there is a siphonoglyphe extending through two thirds of the aboral end (fig. 14). The epithelium lining the siphonoglyphe is considerably thicker than it is in other parts of the stomodæum (see Section IV), and provided with numerous long flagella.

The epithelium of the stomodæum other than that of the siphonoglyphe is thrown into a series of six longitudinal folds, which are rather more pronounced at the oral than at the aboral end (Pl. 38, fig. 12).

In this respect the stomodæum of the polyps of A. digitatum show a marked contrast with that of the polyps of A. palmatum. In all the sections of the polyps of this species that I have examined the ectoderm of the stomodæum is quite smooth, as is represented in pl. 1, fig. 2, of my former paper (16).

The mesenteries of Alcyonium digitatum present no features that require special comment. As in all Alcyonarians, they bear on their ventral faces the large retractor muscles. The details of their histology are given in the next section.

The mesenterial filaments may be seen through the transparent body-wall of a fully expanded living polyp. The single pair of dorsal filaments may be readily distinguished from the other six by proceeding in almost straight lines from the edge of the stomodæum down into the depths of the cœlenteron; and if they be traced further in sections of preserved specimens, they may be seen to continue right down to the base of the polyp,—that is to say, in the case of the primary polyps to the base of the colony, and in the case of the secondary polyps to that part of the endodermal canal system from which they, as buds, arose (Pl. 37, fig. 6).

The four lateral and two ventral mesenterial filaments are very short, and even in the most fully expanded polyps present a very sinuous outline. It is difficult to say what their exact length is, as I have found it impossible to measure them accurately in the living polyp; but judging from the length of the tentacles, and other measurements that I have obtained accurately, I should say that they are never more than 2—2·5 mm. long.

The gonads are spherical bodies, reaching in their mature condition a diametrical measurement of about 0·5 mm. When ripe the ova are of an orange-red colour, the sperm-sacs milky white. They are covered by a coat of endoderm continuous with the endoderm of the lateral and ventral mesenteries, to which they are attached by short stalks. They are invariably attached either to the side or edge of the free portion of the mesenteries below the termination of the mesenterial filaments, and never extend for a distance of more than 10 mm.

The gonads do not occur, so far as my observations go, on the dorsal pair of mesenteries.

As stated above, the cœlentera of the different polyps composing the Alcyonium colony do not communicate with one another directly, but running in the mesogloea between the cavities there are canals, lined by endoderm, with a wide lumen, by means of which the water can flow from the cœlenteron of one polyp into that of its neighbours. In the peripheral portions these canals are fairly numerous, but they are very scanty in the deeper parts of the colony, being almost

confined to the basal portions of the secondary polyps and the adjacent portions of their neighbours (fig. 8).

In addition to these canals, the mesoglœa contains solid rods or rows of cells, isolated cells and spicules (fig. 26, &c.).

The skeleton of Alcyonium is entirely composed of isolated spicules of calcium carbonate.

The spicules occur only in the mesoglœa, and are far more numerous in the periphery, where they are densely crowded, than in the deeper parts of the colony.

When an expanded polyp is examined with the microscope, a group of spicules may be seen to extend some little distance up the walls of the base of the extensible portion between the mesenteries (fig. 6). In some polyps a few scattered spicules may extend right up to the slight constriction just below the base of the crown of tentacles. Immediately above this constriction there is almost invariably found a ring of scattered spicules, which sends a radiating row a certain distance along the aboral side of the tentacles.

When the crown is examined from above a ring of spicules may be seen surrounding the mouth, and these send off lines of scattered spicules along the sides of the oral surface of each tentacle (fig. 10).

Section IV.—Minute Anatomy.

The ectoderm of the general surface of the colony of Alcyonium is very liable to become detached, either by friction in the bottle in which the animal is preserved or during the process of decalcification, and it is only in sections of specimens that have been very carefully treated that the ectoderm can be seen at all.

It consists of a number of columnar, spindle-shaped and flask-shaped cells, 0·02 mm. long, connected at their outer borders, but free from one another for the greater part of their course. At the base of the epithelium there are a few spherical interstitial cells of different sizes, and here and there may be seen a cell which, like a ganglion cell (fig. 17), is irregularly star-shaped.

I have examined the ectoderm very carefully, to determine whether it is ciliated or not; but neither in the living animal nor in my best preparations can I find any trace of cilia on any of the cells, either on the general surface of the body, the extensible portion of the body-wall, the tentacles, or the oral disc.

This is not inconsistent with the work of other observers, for in no other work on the minute anatomy of Alcyonarians can I find the external ectoderm[1] described as being ciliated. This is a noteworthy point, because in the Actiniæ, according to the Hertwigs and other observers, the ectoderm is ciliated. In the Hydrozoa the ectoderm is never ciliated, so that in this respect the Alcyonaria apparently agree with the Hydrozoa and differ from the sea-anemones.

It is difficult to determine with certainty the origin of the cells that give rise to the spicules; but, for many reasons, I am inclined to agree with von Koch's (23) results on Gorgonia and Clavularia, and attribute them entirely to the ectoderm. Among the interstitial cells of this tissue one frequently finds large spherical cells which lie beneath their neighbours, and cells very similar to these may be seen isolated in the subjacent mesoglœa. Moreover large cavities may be seen in isolated cells in the same region, which, in all probability, contained a spicule before decalcification (fig. 17, *sp. c.*).

Attention has already been directed to the fact that the spicules are far more numerous at the periphery than in the deeper parts of the colony. This suggests very forcibly that the spicules are only formed at the periphery, and that with the growth of the mesoglœa they become more and more separated from one another. It is not likely that during the growth of the colony they are dissolved.

[1] Von Koch (23) says that in the Alcyonaria the ectoderm-cells are provided with cilia, which often disappear in old colonies; but he gives no figure to illustrate this ciliation. Kölliker (24) says that the ectoderm of Pennatulids shows ciliation, but he was unable to determine its extent in the specimens he examined, and Prof. Marshall did not describe nor figure it.

The ectoderm is almost invariably covered by a coat of transparent mucus, in which grains of sand, minute algæ, and other foreign bodies occur. This mucous coat is probably formed from the secretions of some of the flask-shaped cells.

With the possible exception of an occasional star-shaped interstitial cell I can find no trace of a "Nervenschicht" in the general ectoderm of the colony, nor can I find true muscular processes to any of the cells, nor any cnidoblasts or nematocysts.

The ectoderm-cells of the tentacles are very similar in general appearance to those of the general surface of the colony, but they are not quite so long, i. e. about 0·015 mm. They are prolonged below, however, into well-marked muscular processes, and between them may be seen numerous cnidoblasts and nematocysts. A nerve-sheath is very distinctly present in the form of minute star-shaped and round ganglion-cells connected with a plexus of very delicate anastomosing fibrils (fig. 22).

The ectoderm of the oral disc and body-wall is not so easy to investigate, as it becomes detached or partly so with the slightest friction. It seems to vary very considerably in thickness according to the condition of expansion of the polyp, but in other respects it does not differ in any very marked characters from that of the general surface of the polyp.

The ectoderm lining the stomodæum is composed of closely packed columnar cells, 0·017 mm. in length, provided with short cilia (fig. 14), as in Pennatulids, Clavularia, and A. palmatum (Wilson). The epithelium of the siphonoglyphe differs from that of the other parts of the stomodæum in that the cells are narrower, somewhat clearer, considerably deeper (0·025 mm. in length), and bear long (0·025 mm.) whip-like flagella (fig. 29).

The nematocysts of Alcyonium are extremely small (0·0075 mm.), and all of one kind. They may easily be seen by snipping off a tentacle of an expanded polyp and examining it with a microscope, when, on the addition of a little acid, they may be seen to shoot out their threads, and occasionally get loose from the ectoderm and float off freely in the surrounding

water. They seem to swell considerably in dilute acetic acid, so that some of the free nematocysts measured as much as 0·015 mm. in length, but I do not think they are ever as large as that in their natural position in the ectoderm. The filament is long, easily broken, and is not provided, so far as I could discover, with any barbs.

In sections of preserved specimens the nematocysts are not very easily observed, as they are usually shot and lost during the process of killing the colony in an expanded condition; but they may be demonstrated by staining sections of colonies killed contracted in spirit, or any other preservative, in eosin and hæmatoxylin. By this method the nematocysts are stained blue and the ectoderm-cells pink.

The extremely small size of the nematocysts of Alcyonium is not by any means exceptional in the case of the Alcyonarians. Moseley stated that in Sarcophyton there are no nematocysts, and the late Professor Marshall and his brother (29) were unable to find them in Virgularia mirabilis. In Pennatulids generally, according to Kölliker (24), they are exceedingly minute, and their presence could not always be determined in spirit specimens.

Wilson (38) says that in the endodermic mesenterial filaments of Paralcyonium there are "scattered irregularly through the filaments minute nettle capsules. They are remarkable for their very small size, being smaller than the nuclei of the endoderm-cells. They have an oval form, and each contains a spirally coiled filament. In the minuteness and rarity of the nettle capsules the mesenterial filaments of the Alcyonaria differ from those of the Actinians, and it seems possible that in the former group they are to be regarded as rudimentary organs."

The figure given by this author of the nematocysts of the endodermic filaments on Paralcyonium is the only figure yet published of the nematocysts of Alcyonarians.

The Mesenterial filaments.—I have nothing new to add to Wilson's excellent account of the structure of the dorsal mesenterial filaments.

The dorsal mesenterial filament consists of columnar ciliated

cells, "arranged so as to form a long solid band on the edge of the septum (i. e. mesentery). As seen in sections this band has a bilobed form, or, in other words, a longitudinal groove runs along its middle" (fig. 18). I have never seen the Y-shaped appearance of this groove in section which Wilson describes in Funiculina, but in several sections I have seen the lobes folded over so as to form an almost complete canal (fig. 16).

The cells of the groove are in appearance and in detail similar to those of the stomodæum.

As regards the ventral mesenterial filaments my observations do not coincide in all respects with Wilson's figures and description of these filaments in Paralcyonium. Like Wilson, I recognise that there are essential and very important differences between these filaments and the dorsal ones. There is no median groove, there is no sharp line of demarcation between the cells of the filament and the neighbouring endoderm-cells of the mesenterial epithelium, and the cells are not all of the same kind, some staining very much more deeply than others (fig. 19). Two distinct kinds of cells may be made out in good preparations with a high power: first, large non-ciliated gland-cells, which stain deeply with hæmatoxylin; and second, elongated columnar cells filled with numerous minute granules.

The mesoglœa of Alcyonium is composed of a thick homogeneous substance containing (1) endodermic canals; (2) solid cords of endoderm; (3) minute isolated cells connected with one another and with the endoderm by fine anastomosing fibrils; (4) spicules of calcium carbonate.

(1) The endoderm canals—that is to say, cords of endoderm containing a lumen which communicates with the cœlenteron of one or more of the polyps—are chiefly found in the superficial parts of the colony. They consist of a single layer of endoderm-cells embracing a narrow lumen, which becomes obliterated in some of the branches (figs. 13, 17). The canals then are continuous with—

(2) The solid cords of endoderm which are found throughout the mesoglœa of the colony, and may be easily studied by osmic acid or methyl blue preparations of fresh sec-

tions. The cords are in some cases fairly compact, resembling a canal in all respects except the presence of a lumen; but in others the cells are only loosely connected with one another, become elongated or star-shaped (fig. 26), giving off fine fibrils at their angles. There may be only a single row of oval or cubical cells, or in some cases the row may be drawn out into a chain of elongated spindle-shaped cells (fig. 27).

(When studying the mesoglœa of Alcyonium last December I noticed a number of oral bodies lying in and among the cells [fig. 36, *gr.*] of the endodermic cords. They may be readily distinguished from the endoderm-cells by their dark but homogeneous appearance. In one or two instances I have succeeded in making out a somewhat irregular body in the centre, which may be a nucleus. It is difficult to say with any degree of certainty what these bodies are, but it is possible that they may be some form of parasitic sporozoon.)

(3) The isolated mesoglœa cells may be seen in osmic acid and other preparations of fresh sections. They are star-shaped or bipolar, and are connected with one another by fine fibrils, which branch and anastomose in the homogeneous mesoglœa (fig. 26).

These cells are connected not only with one another, but also with some of the cells of the endoderm cords, and fine fibrils from them may also be seen passing into the endoderm lining the cœlentera.

This account of the histology of the mesoglœa differs in many respects from that given by Carl Vogt and Jung. These authors describe numerous small canals on the growing branches of the colony which they call the "Nährcanäle." These end blindly at the periphery, and are continuous with the cœlentera. These "Nährcanäle" are probably the same as the "endoderm canals" of my description.

In addition to these, the authors describe in the substance of the cœnenchym (mesoglœa) a network of canals, "Sammelcanäle," and capillaries. It is noteworthy that in none of the figures to which reference is made is any lumen drawn in the "Sammelcanäle" or capillaries, nor is there any indication in the text that the authors ever saw any lumina in these so-called canals. They are probably the same, then, as the solid endoderm cords of my account, but quite erroneously described. The network of fine fibrils has not been previously described, and was overlooked by Vogt and Jung.

I have searched in vain for anything corresponding to the muscular fibres of Vogt and Yung's account, and I am quite convinced that neither in Alcyonium digitatum nor in A. palmatum are there any muscular fibres in the mesogloea. It is quite possible that some of the cells lying in the mesogloea may be to a certain extent contractile, but they are quite different in appearance from the muscular processes of the endoderm-cells lining the cœlentera, which will be described later on.

The chemical characters of the homogeneous substance which forms the bulk of the mesogloea are described in a paper by Brown, which appears in this number of this Journal.

Spicules.—The spicules of Alcyonium digitatum present so many varieties of form, that it is not possible, without writing a long treatise on this subject, to do more than give a description of a few examples.

The general impression obtained by examining a slide of spicules, made by boiling a small branch of Alcyonium digitatum in potash, is that no two spicules in the field of the microscope are alike.

The majority, however, are somewhat like a dumb-bell in shape (fig. 3, *a*, *b*, *c*), with numerous irregular and blunt projections at each end, and about 0·1 mm. in length. In addition to these there is a considerable number of larger (0·2—0·3) spicules, which are branched (*d*) or shaped like a capital K, or simply crosses with numerous blunt projections. A few spicules may be seen scattered about among the more characteristic forms which are quite irregular in shape, some with a central plate-like centre from which spring a few short branches (fig. 3, *f*), some with two or three long arms and one or two short ones, some very small ones like spheres covered with irregular tuberosities, and others of many different shapes and sizes.

In all preparations such as the one mentioned, in which the tentacles, the disc, &c., of the polyps are boiled down with the other part of the colony, there may be seen a certain number of long unbranched lancet-shaped spicules (fig. 42, *e*) covered with irregular tuberosities. They vary very much in shape, some being like a thick pin (without its head), others

lancet- or spindle-shaped, and others again slightly curved like a boomerang. These long unbranched spicules occur chiefly in the tentacles and disc of the polyps. They do occur in other parts of the colony, but I do not remember to have seen any dumb-bell-shaped spicules in the extensible portion of the polyps.

When one examines a similar preparation of the spicules of the yellow variety of Alcyonium digitatum, one notices precisely the same forms in very much the same proportions. In fact, if it were not for the faint yellow colour which they all possess, it would be impossible to distinguish them from the spicules of the white variety (fig. 4).

The figures were drawn by Mr. Wilson, from preparations in my collection of the spicules of the yellow (fig. 4) and white (fig. 3) variety respectively; the specimens were selected by him quite at random from a very large number on each slide.

A preparation of the spicules of the other English species, Alcyonium glomeratum, presents many features of marked difference from those of the yellow and white varieties of A. digitatum. In the first place, the colour is of various shades from pale pink to blood-red. The majority of the spicules are elongated needles and spindles, and there is an entire absence of the small dumb-bell-shaped forms, very few K's and crosses, and there are several club-shaped forms (fig. 5), which I have never seen in any preparations of A. digitatum.

While, then, I have found it impossible to distinguish the preparations of the spicules of the varieties of A. digitatum by any other characters than the colour, a preparation of the spicules of A. glomeratum can be easily and immediately recognised by the numerous characters given above. It is true that many of the elongated and spindle-shaped spicules of A. glomeratum are almost exactly the same shape as the spicules of the tentacles and disc of the polyps of A. digitatum, but the clubs are peculiar to it, the dumb-bell absent, and the K's and crosses very rare.

Alcyonium digitatum is not a favorable form to take

for the study of the development of the spicules, as it is a matter of very great difficulty to make a thin section of the surface of the colony before decalcification.

We have two very different accounts of the development of these structures in Alcyonaria. According to von Koch (**20**) the spicules of Clavularia prolifera are formed within and continue to grow within a cell which is given off from the ectoderm, the young spicules being always enclosed in a nucleated protoplasmic sheath. In my decalcified sections I have seen these sheaths (fig. 17, *sp. c.*), which, I imagine, contained young spicules, and I have also seen cells containing a small vacuole lying close under the ectoderm. I believe, therefore, that von Koch's account is substantially correct.

Schneider (**35**), on the other hand, if I understand his description and figures correctly, believes that in Alcyonium acaule one or more cells gradually assume the shape of the future spicule, and then become calcified. I have seen nothing in my preparations to support this view.

The Nervous System.—In longitudinal sections of the tentacles a number of minute cells may be seen lying between the epithelium and the layer of muscular fibres. These cells are spindle-shaped, triangular, or star-shaped, and are continuous with a fine plexus of fibrils, some of which have a beaded appearance (Pl. 38, fig. 22 *n. p.*).

Whether this system of cells and fibrils is really nervous in nature and function has not been determined experimentally, but it is undoubtedly homologous with the layer called "Nervenschicht" by the Hertwigs in the ectoderm of the Actiniæ.

A similar plexus of cells and fibrils may be seen in the endoderm, when suitably prepared sections are made which cut the cœlenteric tubes longitudinally and slightly obliquely.

I have represented in fig. 33 a portion of a tube that has been cut somewhat obliquely, so that in the upper part of the section the endoderm-cells may be seen, but in the lower part these have been shaved off, and only the circular muscular

fibrils remain. If the part marked with an asterisk be examined with a high power, the plexus represented in fig. 35 will be visible.[1]

We have evidence, then, of the existence of a nervous plexus in both the ectoderm and endoderm of Alcyonium. It has not been found possible at present to trace it in the ectoderm and endoderm of all parts of the body.

The occurrence of a fine plexus of cells and fibrils in the mesoglœa was noted in a previous publication (15); and although, owing to technical difficulties, this mesoglœal nervous plexus has not been proved to be connected with the nervous plexus of the ectoderm, it is extremely probable that such a connection does exist. There is no difficulty in tracing its connection with the endodermic nerve plexus.

The nerve plexus of the mesoglœa is represented in fig. 26. The figure was drawn from a section through a part of the colony about one inch from the surface. The difficulty of tracing it nearer to the ectoderm is due to the fact that the only satisfactory preparations showing the plexus are made by cutting sections of the living colony as thin as possible with the free hand, and treating them immediately with osmic or pyrolignic acid. The superficial regions of the colony are, however, so densely crowded with spicules that it is impossible to get them in very thin sections. Sections of decalcified specimens which have been made with a microtome do not show the nerve plexus very satisfactorily.

Muscular System.—The walls of the cœlenteron of Alcyonium are provided with a complete sheath of endodermic circular muscular fibres (fig. 33). This sheath is probably used during life for causing the expansion of the polyps. Any contraction of the circular fibres would cause an increased water-pressure in the cœlentera and force the polyps out; but when the colony is disturbed or artificially wounded, and the great mesenteric retractor muscles are contracted, it forces

[1] Herdman (17) has described a number of fusiform, polygonal, and triangular cells in the endoderm of the mesenteries of Sarcodictyon.

water out of the tubes, and causes a considerable contraction of the whole colony.

If one takes an expanded colony out of the water one notices that, in the first place, the crown and free portion of the polyps are withdrawn ; but when these are completely concealed the colony undergoes still further contraction in size, and water may be seen to be oozing from openings in the centre of the star-shaped tubercles that mark the position of the polyps. This contraction may continue until the colony is reduced in size by about one third of its original bulk, and it is then a great deal harder to the touch than it was when fully expanded. Specimens of Alcyonium in museums and laboratories that have been killed slowly by immersion in weak spirit are always very much contracted in this way, and they offer a marked contrast in consistency to the soft spongy specimens which have been killed suddenly by Lo Biancho's No. 2 chrom-acetic acid solution.

A similar contraction of the colony may be brought about by rolling the specimen about in a bucket of sea water.

This circular muscular sheath may act, then, either as a protractor of the polyps, or, in extreme cases, as a constrictor of the colony.

The great retractor muscles of the polyps are situated, as in all Alcyonarians, on the ventral faces of the mesenteries. In the regions where the muscles lie, the mesoglœa projects in the form of simple or branched lamelliform folds. The muscle-fibres are situated on these folds (fig. 21), and are covered by an epithelium of endoderm-cells.

In most of my sections it is not easy to distinguish between the muscle-fibres and the processes of mesoglœa, as both of them are perfectly homogeneous ; but a double stain obtained by hæmatoxylin and eosin differentiates the two tissues most beautifully, the mesoglœa staining blue and the muscle-fibres pink.

The muscles run longitudinally on the mesenteries, but approach nearer and nearer to the stomodæum in their passage from below upwards (fig. 14), but their eventual insertion is on the disc close to the mouth.

On the opposite face of the mesenteries the muscular processes of the endoderm-cells run transversely to the long axis of the polyps. These may be seen quite clearly in any good series of sections taken longitudinally through an expanded polyp. They constitute what has been called the protractor muscular system of the polyps (figs. 14 and 21, *P. msc.*).

In the disc there is a circular band of muscle-fibres situated in the ectoderm, and from this band there run fibres along the borders of the inner surface of each tentacle. These fibres undoubtedly act as the retractors of the tentacles (fig. 10).

The Endoderm.—The method adopted for examining the minute structure of the endoderm of Alcyonium was to place some thin strips of a colony into $\frac{1}{2}$ per cent. osmic acid for thirty minutes, then wash in distilled water and transfer to picro-carmine for thirty minutes. The walls of the cœlenteric canals were then scraped with a fine scalpel, and the endoderm thus removed teased up with needles in glycerine. A modification of this method, which yielded some good results, was to substitute a weak solution of gentian violet for the picro-carmine.

The cells lining the cœlenteric canals are by these methods seen to be very similar in their general characters to the myo-epithelial cells (fig. 28).

The protoplasmic portion contains a number of coarse granules, which stain brown with osmic, and have strong affinities for gentian violet. They are usually irregular in outline, but, owing to their very minute size, it is not possible to assert that they have crystalline shapes. The granules are probably of the same nature as the nutritive spheres which occur in some of the endoderm-cells of the Hydrozoa.

A few cells present one or more large clear vacuoles.

The nucleus is round, and stains faintly in picro-carmine. A careful and prolonged examination of these nuclei with the highest powers at my disposal failed to reveal anything beyond a clear homogeneous structure. I have never seen any stages in the division of the nucleus.

Most of the cells show a single very delicate flagellum on

their free ends. It is possible that those cells (such as fig. 28, *a*, *c*, *o*, *n*) which do not show a flagellum may have lost it during the preparation and maceration of the specimen.

The muscular portion of the fibre is homogeneous and transparent. It is usually branched at the extremities. Although most of the cells possess a muscular portion, there are undoubtedly some cells in the endoderm which do not. The cell represented in fig. 28 *f* is a type of cell very frequently met with in these maceration preparations. It possesses two long protoplasmic processes.

A few cnidoblasts may be seen in such preparations of the endoderm-cells, and are represented in fig. 28, *h* and *k*; but it is very probable that they have been swallowed with the prey, and are not of endodermic origin.

Although the study of macerated preparations reveals many points of great interest which cannot be seen satisfactorily in any other way, it has the disadvantage of giving no evidence of the true relationship of the cells to the organs of the body.

The endoderm is not precisely the same in all parts of the cœlenteron, and consequently it is necessary to supplement our study of macerated specimens by a study of carefully prepared sections through different parts of the body.

In the lower part of the cœlenteric tube the endoderm has a very vacuolated appearance. This is due, not to the occurrence of intra-cellular vacuoles, but to great gaps between the endoderm-cells.

The individual cells are thinner than they are in other parts of the body, and loosely connected together in groups which are separated from one another by considerable rounded spaces.

The character of the endoderm in this region is very similar to that described by Lankester as occurring in the proximal third of the gastric tube of Limnocodium (27).

If sections be taken through the upper parts of the tube in the region of the ventral mesenterial filaments, the endoderm-cells (fig. 23) are seen to be much more closely set, and to contain larger granules.

A few round interstitial cells may be seen in the endoderm in nearly all good sections through this region. These cells are of different sizes, and probably replace and supplement the other endoderm-cells during the animal's life.

The endoderm covering the mesenteries is composed of short cubical cells which become flattened and fusiform over the muscular ridges on the ventral faces of the mesenteries; but the general appearance of this part of the endoderm, as well as that just described in the last paragraph, varies very much with the condition of contraction or expansion of the polyps. When the polyps are fully expanded, it is a thin flat layer of cells; but when they are contracted the cells are long, thin, and so crowded together that, in sections which do not pass through it quite vertically, it has the appearance of consisting of two or three layers.

The sheath of endoderm which covers the gonads and the endoderm forming the six mesenterial filaments are not affected in this way by the condition of the polyps. In the former case the cells are cubical in shape, finely granular in consistency, without very distinct walls, and without muscular processes (fig. 25).

The sections of partially retracted tentacles that I have made show that the endoderm in that condition is a compact tissue composed of short cubical cells with round nuclei. I can find no muscular processes connected with any of the cells, and I am convinced, after a prolonged study of these cells, that they are not present. Each cell is very finely granular. There are no nematocysts. The lumen of the tentacles is wide, and it is continued for a short distance into each of the pinnæ (fig. 10). The endoderm of the pinnæ is similar to that lining the axial cavity of the tentacles.

The sexual cells first make their appearance in the thickened border of the ventral mesenterial filaments. In both sexes they occur as a mass of polygonal cells (fig. 37) covered by a single layered sheath of endoderm. There can be no doubt whatever of their endodermic origin.

Oögenesis.—If a series of sections be made through a

female colony collected in the month of April or May, several of the mesenteries may be seen to bear close to their free margins a group of small, clear, round or polygonal cells. In the smaller groups the cells are all of approximately the same size, but in some of the larger groups a few of the cells are very much larger than the others, and contain two instead of one nucleus (fig. 37). An examination of such a group with an immersion lens shows that the nuclei are of two different kinds, and take up the staining reagents differently. Some present a well-marked nuclear membrane, and a number of highly refracting chromatin granules giving the characteristic appearance of a resting nucleus; the others are perfectly homogeneous, staining deeply in hæmatoxylin, and presenting no granules, nucleoli, or other structure.

In the cases of the larger cells which present two nuclei one of them is invariably of the former description, and the other of the latter.

The explanation of these facts seems to be that some of the young ova—namely, those containing the homogeneous nuclei—cease to grow, and divide at an early stage in their development, and these become absorbed by the others which continue to grow.

At the end of May, and in the months of June and July, ovaries may be found on the mesenteries containing one or two ova very much larger in size than the others, and in some of these young ova may be seen partially disintegrated (fig. 38).

There can be no doubt, then, that some of the young ova formed in the ovary are swallowed up by the larger ones; others, however, simply degenerate, and their shrivelled remains may be seen between the more mature ova.

During the later months of the year the ova continue to grow, and assuming a light yellow colour in August, they reach their full size and deep red colour at the end of November or the beginning of December.

The ripe ova in the follicles of a female Alcyonium in December are about 0·5 mm. in diameter, but I have noticed a very considerable variation in the actual size of the ova

when spawned. The germinal vesicle of such an ovum is extremely large, its diameter being about a quarter of that of the ovum, and is situated quite at its edge. In some cases a large germinal spot may be found, but more frequently it is absent. The vesicle contains a dense network of fibrils and a considerable number of granules, which stain very deeply in carmine, hæmatoxylin, Löffler's blue, and other stains (fig 40).

The protoplasm of the ovum is filled with a larger number of spherical or irregular globules and granules of yolk, which turn perfectly black when treated with any reagent which contains osmic acid.

Spermatogenesis.—The very young testis of Alcyonium cannot be distinguished from the very young ovary. It consists simply of a number of small round cells on the mesenteries covered by a layer of endoderm-cells. It soon shows a difference in that the cell outlines completely disappear, and the testis has then the appearance of a dense crowd of nuclei (fig. 42).

The nuclei increase immensely in numbers during the summer months, but, although I have made many series of sections, I have not satisfied myself of their mode of division. At present I have not been able to discover any signs of karyokinesis. When the testis has reached a certain size, about 0·1 mm. in diameter, a space free from nuclei filled with an irregular coagulum makes its appearance in the centre (fig. 43). The coagulum shrinks somewhat in the later months of the year and disappears entirely in the ripe testis, which is simply filled with spermatozoa.

The spermatozoa when mature consist of head with a cone-shaped anterior end, followed by a spherical body and a long flexible tail (fig. 45 *a*).

On making a teased preparation of a ripe testis, a number of spermatozoa may be seen bearing just behind the head a clear vesicular body, similar to that described and figured by Pictet (33) in Strongylocentrotus and other Invertebrates.

The details of the formation of the ripe spermatozoon from the spermatids I have not at present been able to follow.

Gemmation.—The buds of Alcyonium digitatum arise in the canals near the surface of the colony, which run in the mesoglœa between the cœlentera of the polyps. There seems to be no definite law governing their appearance, as they appear quite irregularly between the older polyps in all stages of the growth of the colony. On referring to Marshall's manuscript notes on the gemmation of Alcyonium palmatum I find a similar remark with regard to that species. The first rudiment of the bud is a diverticulum from the canal in the direction of the surface of the colony. The cells lining this diverticulum proliferate rapidly, and it is usually found to be almost filled up with loose cells. The diverticulum increases in size and becomes pouched. In the older buds there are undoubtedly eight of these pouches, but I have not been able to follow the order of their formation (fig. 46). The intervals between the pouches become the mesenteries of the polyp. At the time of the formation (fig. 46) of the pouches an invagination of the superficial ectoderm immediately above the bud rudiment. This ectodermic invagination sinks down, and opens eventually into the centre of the endodermic diverticulum from the canal (fig. 47).

The later stages are very much more difficult to follow, as it is extremely difficult to obtain sections that run exactly parallel with the central axis of the young polyp. In very young colonies—and these are the most favorable for the study of gemmation—the axis of the bud rudiment is by no means parallel with the axis of the neighbouring polyps, and consequently it is impossible to orient the colony before embedding; and in older colonies the young buds occur at such rare intervals that hundreds of slides of sections may be hunted through without furnishing a bud in such a condition as will lead to any important results. There can be no doubt, however, that the epithelium of the stomodæum is formed from the cells of the ectodermic invagination.

As in Kenilla, so in Alcyonium, the two dorsal mesenterial filaments are the first to be developed, and for a long time apparently these filaments are the only ones to be found in the

buds. The epithelium of the central portion of these filaments is precisely the same as that of the stomodæum, the cells being smaller, and staining more deeply in borax carmine than the endoderm-cells, so that there can be little doubt that Wilson (38) is correct in saying that (in Funiculina) the dorsal mesenterial filaments are of ectodermic origin.

The tentacles do not appear until quite late in the development of the bud,—not, in fact, until after the formation of the ventral mesenterial filaments, which arise as thickenings of the endoderm at the edge of the mesenteries.

Section V.—Note on the Circulation of the Fluids in the Polyps.

In a communication I made to the Royal Society in 1882, I pointed out that the action of the cilia in the siphonoglyphe of Alcyonium was to create a current of water flowing from the mouth into the cœlenteron. Almost simultaneously, Wilson discovered that the cilia of the dorsal mesenterial filaments produce a current flowing in the opposite direction.

During the month of December I examined these currents again with great care, and I can now confirm not only my own results, but also those of Wilson.

When the polyps of Alcyonium digitatum are fully expanded, the long cilia of the siphonoglyphe are in active movement, causing a swift current of water to flow into the cœlenteron on to the six endodermic—that is to say, ventral—mesenteries. A reverse current from below upwards towards the mouth is produced by the cilia of the two long dorsal or ectodermic mesenterial filaments.

The current produced is a tolerably swift one, and must cause a rapid distribution of any soluble products of the digestion caused by secretion of the gland-cells of the mesenterial filaments, and there can consequently be little doubt that the filaments are not alone the absorbents of the food materials in solution. These must be carried down by the ciliary currents to the lower parts of the cœlenteric tube, and absorbed by the endoderm in any region of the tube.

This appears to be the case from the following consideration:—The dorsal mesenterial filaments of the primary, i. e. the oldest, polyps of the colony extend right down to its base; and, so far as I could observe, there is no diminution of the vigour of the current produced by them in the lower regions of the tube. If there is a current of water in one direction at the side of a tube which is closed at one end, it is obvious that there must be a current in the reverse direction on the opposite side. The endoderm of the lower parts of the tube, although provided with large intercellular spaces as described above, is nevertheless living and active. (In Tubipora and Clavularia viridis the endoderm dies in the lower ends of the tubes.) I have no doubt, then, that the endoderm of all parts of the cœlenteron can and does absorb nourishment in the fluid form.

There is no evidence that particles of food are taken up in the solid form by the endoderm of Alcyonium. I have not been able to observe any solid particles of food other than the nutritive spheres in any of the endoderm-cells I have observed, either in sections or in teased preparations. I have seen no diatoms or other unicellular algæ or fragments of animals embedded in the endoderm. Particles of carmine suspended in the water are, however, readily seized by these cells wherever they occur; and in some cases I have seen them embedded in the cells of the mesoglœa, indicating that they may be handed on from cell to cell throughout the system.

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EXPLANATION OF PLATES 36—39,

Illustrating Mr. Sydney J. Hickson's paper on "The Anatomy of Alcyonium digitatum."

Lettering used throughout.

D. Dorsal intermesenterial space. *Ect.* Ectoderm. *End.* Endoderm. *can.* Canal in mesoglœa. *c. g.* Coagulum in spermagem. *Dmf.* Dorsal mesenterial filament. *f. c.* Flagellate cells of ventral mesenterial filaments. *g. c.* Gland cells of ventral mesenterial filaments. *gon.* Gonad. *gon.* ♀. Ovary. *gon.* ♂. Testis or spermagem. *gr.* Parasitic sporozoon? *g. v.* Germinal

vesicle. *i. c.* Interstitial cells of the ectoderm. *m.* Muscles. *mes.* Mesoglœa. *mf.* Mesenterial filaments. *nem.* Nematocyst. *n. p.* Nerve plexus. *P. Msc.* Protractor muscles. *R. Msc.* Retractor muscles. *Si.* Siphonoglyphe. *sp.* Spicules. *sp. c.* Spicule forming cells. *St.* Stomodæum. *sp. g.* Spermagem. *t.* Tentacles. *V.* Ventral intermesenterial space. *v. m. f.* Ventral mesenterial filaments. *y. s.* Yolk spaces in the ovum.

PLATE 36.

Fig. 1.—A specimen of Alcyonium digitatum in the Cambridge Museum.

Fig. 2.—A specimen of Alcyonium glomeratum from the Marine Biological Association's laboratory at Plymouth. Both of these illustrations were drawn from spirit specimens, and are for the purpose of illustrating the differences of external form of the two species as may be seen in ordinary museum specimens.

Fig. 3.—Spicules of Alcyonium digitatum (white or pink varieties) × 240.

Fig. 4.—Spicules of A. digitatum (yellow variety) × 240.

Fig. 5.—Spicules of A. glomeratum × 240.

PLATE 37.

Fig. 6.—A fully expanded polyp drawn from a living specimen. The bases of the tentacles are extended in a bullate manner, and the pinnæ are partly withdrawn. The stomodæum (*St.*) and the six short and two long mesenterial filaments may be seen through the transparent body-wall. At the base of the crown of tentacles, and at the base of the extensible portion of the polyp, the body-wall is more opaque, and contains clusters of spicules.

Fig. 7.—Vertical section through a portion of a colony (semi-diagrammatic) to show the polyps in different stages of retraction, the different sizes of the polyps, the canal system, the distribution of the spicules, &c. The mesenterial filaments are represented as being in the same plane as the tentacles, which they are not, for the sake of illustrating certain points in the arrangement of the structure. The following points are illustrated in this figure:—1′ represents a fully expanded polyp. 2′ represents a partially retracted polyp in which the tentacles are contracted and folded over towards the centre of the disc; the body-wall forms a circular fold over the crown of tentacles. 3′ represents a polyp in which the tentacles and stomodæum have sunk to the level of the general surface of the colony. 4′ represents a polyp in which these organs have sunk below the level of the general surface of the colony. 5′ represents a polyp which is completely retracted, the thin body-wall of the extensible portion having sunk below the surface, and the densely spiculated

mesoglœa closed over it. It will be noticed that the long dorsal mesenterial filaments are all on one side, i.e. the axial (Marshall) of the cœlentera. The spicules in the colony are much more numerous at the periphery than in the deeper parts of the colony. The cœlentera of neighbouring polyps do not actually run into one another, but they are in communication with one another by means of canals.

Fig. 8.—Diagram of a section through a portion of a colony to show that the secondary polyp cavities (II) do not run into the primaries (I), but are in communication with them by means of canals. The peripheral canals, spicules, and other details are omitted.

Fig. 9.—The disc of a polyp in the second stage of retraction. The tentacles are contracted, but have not yet begun to bend over.

Fig. 10.—The disc of a polyp in the first stage of retraction. This drawing was made from a preserved and stained preparation of an expanded polyp, and shows the arrangement of the muscles (*m.*) on the disc, and the spicules of the peristome and of two of the tentacles. These muscles are all ectodermic.

Fig. 11.—A drawing of the disc of a living and fully expanded polyp, showing the bullate base of the tentacles and the shape of the mouth.

PLATE 38.

Fig. 12.—Transverse section through an expanded polyp (× 130) in the region of the stomodæum, showing the folds in the epithelium lining the stomodæum (not present in the expanded polyp of A. palmatum), the siphonoglyphe, the arrangement of the muscles on the mesenteries, &c.

Fig. 13.—Vertical section through a retracted polyp. On the left-hand side the section passes through the side of a tentacle, and through one of the long dorsal mesenterial filaments; on the right-hand side it passes through the centre of the tentacle and the siphonoglyphe. The stomodæum is seen to be folded on itself.

Fig. 14.—Vertical section through the extensible portion of a polyp (× 70), showing the mesentery bearing the transverse protractor muscles, the longitudinal retractor muscles, one of the ventral mesenterial filaments, and the siphonoglyphe.

Fig. 15.—Transverse section through one of the pinnæ of a tentacle, showing the ectoderm with its nematocysts and the lumen in the endoderm.

Fig. 16.—Transverse section through one of the dorsal mesenterial filaments.

Fig. 17.—Vertical section through the surface of a decalcified colony of Alcyonium, showing the ectoderm with its covering of slime, the mesoglœa containing the endodermal canals, the empty spaces which contained the

spicules before they were dissolved away in acid, and the spicule-forming cells (*sp. c.*).

Fig. 18.—Transverse section through a dorsal mesenterial filament and a portion of the mesentery. The groove in this case lies freely open instead of being nearly closed, as it is in fig. 16.

Fig. 19.—Transverse section through one of the ventral mesenterial filaments showing the gland-cells (*g. c.*) and flagellate cells (*f. c.*).

Fig. 20.—Transverse section of an expanded polyp (× 130) below the level of the stomodæum, to show in section the six endodermic mesenterial filaments (*Vmf.*) and the two ectodermic mesenterial filaments (*Vmf.*).

Fig. 21.—Transverse section through a portion of a mesentery to show the great retractor muscle-fibres (*R. msc.*) on their branched supports of mesoglœa (coloured darker in the figure), and the very delicate protractor muscles (*p. msc.*) on the opposite face of the mesentery.

Fig. 22.—Longitudinal section of a tentacle in a partially retracted condition such as that shown above in Fig. 9. The ectoderm (*ect.*) is shown containing several nematocysts, and the ectodermic nerve plexus (*n. p.*).

Fig. 23.—A small piece of the endoderm taken from the polyp tube a little way below the termination of the ventral mesenterial filaments to show the intercellular spaces.

Fig. 24.—The same taken considerably lower down, showing that the intercellular spaces are much larger and the endoderm-cells much more shrivelled in appearance.

Fig. 25.—A small portion of the endoderm covering a gonad, showing that in that region no intercellular spaces can be seen.

PLATE 39.

Fig. 26.—A section of a portion of the mesoglœa and endoderm of a polyp cavity taken about an inch below the surface of the colony, cut with the free hand from the living animal, stained in weak methyl blue, and subsequently treated with pyrolignic acid. In the mesoglœa there may be seen a number of very small stellate cells connected with one another by fine branching protoplasmic processes. In addition to these there are a number of spherical, spindle-shaped, or elongated cells collected together into strings or clumps, and connected at intervals with the plexus of fibres from the stellate cells. The plexus of fibrils may be seen to be connected with a plexus in the lower layer of the endoderm.

Fig. 27.—A string of cells in the mesoglœa from another preparation.

Fig. 28.—A number of cells obtained by scraping the walls of a polyp tube and treating with osmic acid and picro-carmine. *a.*, *k.*, *l.*, *o.*, and *n.* are all typical

myo-epithelial cells; *g.* probably represents a group of epithelial cells from the dorsal mesenterial filament; *h.* is a cnidoblast, but it is not possible to assert that it belongs to the endoderm, as they were not seen in situ in the endoderm in any of the sections.

Fig. 29.—A portion of the epithelium of the siphonoglyphe.

Fig. 30.—Two nematocysts after they have been exploded by the action of dilute acetic acid.

Fig. 31.—Two cnidoblasts found in a teased preparation of the ectoderm of a fresh tentacle.

Fig. 32.—Two unexploded nematocysts found in the same preparation.

Fig. 33.—A section cut very obliquely through a cœlenteron of a polyp some distance below the surface of the colony. In the upper portion the protoplasmic portion of the myo-epithelial cells are cut across; in the lower portion, marked by an asterisk, the circular arrangement of the muscular fibres of these cells may be seen. Outside the limits of the tube the mesoglœa may be seen with its strings and clumps of cells, stellate cells and fibres.

Fig. 34.—The appearance of the protoplasmic portion of the myo-epithelium as seen in such a section as that figured in 33, showing the large, irregular, intercellular spaces.

Fig. 35.—The circular muscle-fibres of the endoderm lining the cœlenteron, such as are represented at the point marked with an asterisk in Fig. 33, but more highly magnified, showing the endodermic nerve plexus.

Fig. 36.—A string of cells from the mesoglœa (osmic acid preparation) showing intra-cellular bodies (*gr.*) of doubtful meaning, but possibly parasitic sporozoa.

Fig. 37.—Transverse section through a mesentery in March, showing a young ovary (*gon.* ♀). Some of the young ova have a single nucleus containing a few chromatin granules in it; others have a single nucleus which stains deeply and is homogeneous; others again have two nuclei, one of each kind.

Fig. 38.—Transverse section taken through a mesentery later in the year, showing an older ovary. One of the ova (*ov'.*) has reached a considerable size; another (*ov''.*) contains two ova partly disentegrated.

Fig. 39.—Section through an ovum in the month of September, × 125 diameters, showing the nucleus (*g. v.*) containing a single large nucleolus situated at a short distance from the periphery.

Fig. 40.—Section through an ovum in the month of December, × 125 diameters. The nucleus (*g. v.*) contains a number of chromatin granules, but no large nucleolus. It is situated quite at the periphery.

Fig. 41.—Section through a portion of an ovum in December after treatment with alcohol, more highly magnified, showing the spaces occupied by the yolk.

Fig. 42.—Section of a mesentery in the early spring, showing a young spermagem (*sp. g.*).

Fig. 43.—Section through a spermagem in the autumn, showing a central coagulum and a crowd of densely packed nuclei in a matrix at the periphery.

Fig. 44.—Section of a portion of the above showing the nuclei more highly magnified.

Fig. 45.—A series of stages of the final development of the spermatozoa as seen in a preparation of a broken testis in December. *a.* A ripe spermatozoon. *b.*, *c.*, *d.* Stages in the formation of the tail. *f.* A cell containing four nuclei occasionally met with in these preparations.

Fig. 46.—A lobed diverticulum from an endodermal canal of Alcyonium meeting an ectodermic invagination to form a bud.

Fig. 47.—A later stage, when the endodermal diverticulum has joined the ectodermal invagination, but the mouth has not broken through. The dorsal mesenterial filaments have been formed.

Fig. 48.—A still later stage, less highly magnified, when the mouth has been formed. The connection with a neighbouring fully-formed polyp is shown. The tentacles have not yet been developed.

Note on the Chemical Constitution of the Mesoglœa of Alcyonium digitatum.

By

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The main organic constituent of the mesoglœa in Alcyonium digitatum would appear to belong to that class of bodies described by Kruckenberg[1] as hyalogens, so widely found among Invertebrate skeletal structures. Hyalogen is characterised by its insolubility, and its conversion by various reagents (e. g. 5 per cent. solution of caustic soda) into a soluble substance, hyalin. Hyalin is practically a mucin, yielding on decomposition a proteid-like body and a carbohydrate.

I. The following experiments show that mucin is readily obtainable from the mesoglœa; whether, in life, any of it is present as such, or whether it is derived from a hyalogen by the necessary preliminary treatment, it is, of course, difficult to say.

Fresh specimens, freed as far as possible from cellular layers, were washed well with distilled water and minced. They were then extracted with a 5 per cent. salt solution to remove any globulins, and left under lime water for seventy-two hours. The filtrate was then treated with acetic acid. A white precipitate was obtained, which, on standing for some hours, settled; it was then filtered off, and washed with water acidulated with acetic acid. It was again dissolved in lime water and re-precipitated. The collected precipitate was boiled with 2 per cent. sulphuric

[1] Kruckenberg, 'Zeit. f. Biol.,' xxii.

acid for about twenty minutes; on neutralisation a reducing sugar and a peptone-like body were obtained. A portion of the original colony, boiled direct with sulphuric acid, yielded a reducing sugar.

Throughout the investigation the endeavour was made to confirm and locate the results obtained from chemical analysis in bulk by the application of micro-chemical tests. For the micro-chemical detection of mucin, Waymouth Reid recommends thionin, as used by Hoyer,[1] which he considers to be thoroughly diagnostic. It appears probable, however, that mucin gives the ruddy purple reaction with this dye in common with other basophilic substances. Nevertheless this reaction serves to locate the position of the mucin or hyalin which had been extracted. Sections of specimens hardened in corrosive sublimate were cut and stained as recommended by Reid. The endoderm appeared blue, the mesoglœa a ruddy purple. This latter coloration was also seen in cover-slip films prepared from the precipitated substance extracted as above.

Hence we conclude that the mesoglœa yields a substance which resembles mucin—

(i) In its solubilities.
(ii) In its decomposition products.
(iii) In its micro-chemical reaction.

II. After treatment of the mesoglœa with dilute acids, however, lime water or baryta water is capable of dissolving a much larger proportion. Prolonged boiling or treatment with superheated water (120° C.) will effect the same change. Lime water or baryta water then leaves but a small granular residue, whereas the main mass of the mesoglœa is left by these reagents when repeatedly applied to the fresh material. Treatment for a few days with comparatively dilute (e. g. 50 per cent.) alcohol greatly impairs the solubility.

The filtrate from this lime water extract will on decomposition yield a reducing sugar and a proteid substance.

This recalls the condition of affairs met with in the edible

[1] Waymouth Reid, 'Journal of Physiology,' vol. xiv. Hoyer, 'Arch. f. Mikr. Anat.,' Bd. xxxvi.

bird's-nest which Kruckenberg (op. cit.) regards as composed of a hyalogen-neossidin, giving rise on solution to a hyalin-neossin. The mesoglœa further resembles the reactions of the edible bird's-nest as described by Green[1] in the absence of a cellulose reaction, and in the fact that on boiling with dilute sulphuric acid a pinkish coloration, which subsequently darkens, is seen.

Another feature frequently shown by hyalins, in which that under consideration agrees, is the absence of Millon's reaction. An application of this test micro-chemically to mesoglœa shows us that the cells alone stain red. This indicates that the proteid extracted by 5 per cent. salt solution (which presents all the characteristic features of a globulin) is located in the cells, and not in the mesoglœa; it need not then detain us.

The micro-chemistry of hyalogen presents a slight difference from that of mucin. Pieces of mesoglœa which yielded no further mucin to lime water were decalcified, and cut with the freezing microtome. Stained with thionin by Reid's method, the purple coloration of the mesoglœa only appears under artificial light, recalling the conditions of the basophilic reaction with methylene blue. The endoderm-cells appear blue both with natural and artificial light.

We shall not err greatly, then, if we describe the organic constituent of the mesoglœa as mainly a hyalogen, probably mixed with some amount of mucin. The hyalin obtained from the hyalogen is precipitated on saturation with ammonium sulphate as a gummy mass.

III. As stated above, after extraction of previously acidified mesoglœa with lime or baryta water a small amount of a somewhat granular residue is left. Examined under the microscope, it is seen to consist of clumps of globules which yield the xanthoproteic reaction. They recall in some measure the description given by Mall[2] of the appearances of elastin acted upon by acids or alkalies. The substance, however, is not elastin, as is seen by its having no affinity for Victoria blue or

[1] J. R. Green, 'Journal of Physiology,' vol. vi.

[2] Mall, 'Anat. Anzeig.,' iii Jahrg.

safranin, and in its resistance to prolonged tryptic action. It swells up, however, under the action of this enzyme. It is markedly insoluble, showing no diminution in bulk on addition of weak or strong acids and alkalies, cold or boiling. The only exception to this is concentrated sulphuric acid, which causes it to swell up, and after boiling for a short time to dissolve with an accompanying pink coloration, soon passing to a light brown. No leucin, peptone, or sugar could be demonstrated as a product of this action.

These reactions remind us somewhat of the insoluble residue described by Schäfer[1] as obtained from the entostemite of Limulus. The total amount, however, is but slight.

An artificial pancreatic digestion of mesoglœa yields the results which might be anticipated from the foregoing. The residue was composed of antialbumid (soluble in 1 per cent. caustic soda) calcareous matter (readily soluble in dilute acetic acid) and the granular substance just referred to. The filtrate yielded albumoses, peptones, a reducing sugar, crystals of leucin and of tyrosin—the last presumably from the cells, since in them alone is Millon's reaction successful.

IV. In investigating a material like the mesoglœa two questions naturally suggest themselves: does it contain gelatine? and does it contain nucleo-albumen? (*a*) Does the mesoglœa contain gelatine? In some experiments on this point, thin slices of mesoglœa were treated with distilled water under pressure at 120° C. for over two hours; in others, portions of it were minced and boiled with distilled water for 1½ hours. The subsequent procedure was that described by Young[2] for retiform tissue. The extract was filtered, while boiling, through a hot filter into alcohol. The precipitate which formed was collected after standing for some hours, dissolved in distilled water, boiled, and filtered. The filtrate was concentrated to a very small bulk. In all cases this did not "jelly" perceptibly. Hence we conclude—

[1] Schäfer, foot-note to Ray Lankester on "Skeleto-trophic Tissues," 'Q. J. M. S.,' vol. xxiv.

[2] R. A. Young, 'Journal of Physiology,' vol. xiii.

The mesoglœa does not contain gelatine. Hence also, it cannot contain chondrin-like bodies. The popular term "jelly" applied to this substance appears to have no basis in chemical fact.

(*b*) Does the mesoglœa contain nucleo-albumen? The recognition of this body in several non-cellular tissues suggests its presence here. To test this point a colony was stripped of its external layers and treated by Halliburton's method for extracting nucleo-albumen.[1] None could be detected.

Thanks to the method recently introduced by Lilienfeld and Monti,[2] the examination of this question micro-chemically is also feasible. Specimens hardened in osmic acid were cut with the freezing microtome, then washed thoroughly and placed in a solution of ammonium molybdate. After being washed for a few seconds in a mixture of ether (9 parts) and water (1 part), they were put into a 20 per cent. ethereal solution of pyrogallic acid. The cells in such specimens were seen under the microscope to be stained black, but the mesoglœa was not. Hence we conclude—

The mesoglœa does not contain nucleo-albumen.

V. Conclusions:

(i) The mesoglœa of Alcyonium digitatum is chiefly composed of a hyalogen.

(ii) Prior to the conversion of the hyalogen into hyalin the mesoglœa will yield a mucin.

(iii) It also contains a small amount of an insoluble albuminoid body, whose nature was not determined.

(iv) It does not contain gelatine or nucleo-albumen.

[1] Halliburton, 'Brit. Assoc. Reports,' 1888; 'Journal of Physiology,' vol. xiii.

[2] Lilienfeld and Monti, 'Zeit. f. physiol. Chem.,' Bd. xvii. See also Gourlay, 'Journal of Physiology,' vol. xvi.

A Study of Metamerism.

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With Plates 40—43.

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I. Typical Forms of Modification.

That there were occasionally to be found in the Annelids irregularities in the serial repetition of the rings seems to have been known to several of the earlier writers on descriptive and systematic zoology. The fact did not attract more than a passing attention, and such irregularities were relegated to that waste-heap of abnormalities from which subsequent investigation has often drawn valuable material.

Simultaneously in 1892 two articles appeared dealing with

these abnormal conditions, one by C. J. Cori (13), and the other by the present writer (33). Both writers pointed out the general interest attached to these modifications, and their importance for an interpretation of the general problem of metamerism. Subsequently a third paper appeared (10), recording the presence of similar abnormalities in many groups of Annelids, but without making any attempt to solve the problem itself.

My own paper was only a preliminary notice, and until the present time I have not had an opportunity of fully describing the material that I had at that time already accumulated, studied, and drawn. The present paper attempts to give a full consideration of the facts only touched on before, and to extend over a wider field the conclusions reached.

The modification of the rings, segments, or metameres of the Annelid fall into two general classes,—not, however, sharply separated from one another.

The first of these I shall speak of as the compound metamere, in contradistinction to the normal or simple metamere. In the earlier paper the shorter term "split metamere" was used, although it was there pointed out that the term was misleading.

The other type of modification may be spoken of as the spiral metamere or spiral modification, or briefly as the spiral.

The simplest case of the compound metamere is represented by the diagram on Pl. 40, fig. I, A, B, C. Fig. I, A, is a dorsal view of a compound segment. The segment is double on the right side, normal on the left. Fig. I, B, is the same, turned over so as to be seen from below, showing a similar doubling below on the same (right) side of the body. Fig. I, C, represents the compound segment as opened along the "split." It is conceived to be transparent, and the dotted line to represent the lower surface outlines. The general appearance is that a metamere has been split into two on one side of the body, but has retained on the other side its normal structure. The question at once arises, are we dealing here with a case of division of a metamere on one side of the body, or is it a case

of three half-metameres united together? It is one of the aims of the paper to answer this question.

To the other class of modifications belong the spirals, one of the simplest of which is shown in fig. VII, A, B, C. The spiral is produced by a combination of two half-compound metameres, i. e. one metamere is "split" below, but the same one is normal above. The next metamere is split above and on the same side of the body, but not below. The posterior arm of the anterior half-compound metamere is continuous at the side with the anterior arm of the posterior (upper) half-compound metamere. The same fact may be stated in a different and perhaps a clearer way. A half-metamere on the right side of the body unites in the mid-line below with a metamere in front of it, and above with a metamere behind it, fig. VII, C. We have formed as a result of this arrangement one more half-metamere on the right side of the body than on the left side, as is best shown by the construction in fig. VII, C.

We may proceed to study more in detail the many kinds of modification that group themselves around these two types. We shall find that nearly all of the geometrical combinations conceivable are to be found in the worms themselves.

Category I, fig. I, A, B, C.—This form has already been described as the type. It is equally common on the right and left sides of the body.

Category II, fig. II, A, B, C (above, below, and reconstructed, as seen from above).—In this form one (above) of the lines of the split turns to one side (posteriorly) to fuse with the septal line. This leaves, as shown in the reconstruction, a free end forming a modification of the compound metamere. This modification may appear either on the right or left side of the body, and the free end may occur above or below, anteriorly or posteriorly.

Category III, fig. III, A, B, C.—By a simple shifting of the lines in the last form we pass to the third category. Here both of the lines of the extra half-segment turn towards the same septal division, and we have formed an intercalated half-metamere.

The variations of this category are formed by the lines turning both anteriorly or both posteriorly, and on the right or left side of the body. In reality the half-segment is simply intercalated between two perfect segments; and when we speak of the lines turning either forward or backward we only imply that the intercalated piece has encroached more on the one or the other segment.

Category IV, fig. IV, A, B, C.—Here three half-segments of one side unite with one half-segment of the opposite side, so that the right side appears to be "split" by two incomplete divisions into three half-segments. This may be called a double compound metamere. Variations arise owing to the depth to which the incomplete lines (splits) pass towards the middle line. A combination of this form (really only a *half*-double compound) with others is shown in Pl. 40, fig. XIV. This same modification may be carried a step further, and in one case I found four half-metameres of one side united to one of the opposite. See Pl. 42, fig. 47.

Category V, fig. V, A, B, C.—Here on the upper side appear two compound segments—one on the right side, the other on the left. On the lower surface, B, two perfect metameres are found. By tracing the division lines between these segments from the lower to the upper side we see the result is brought about by the failure of these two septal lines to meet above; each turns somewhat to one side. The reconstruction shows that a middle connective is left to unite the two segments above. This is brought about by the fusion above of the middle of the right posterior segment with the left anterior.

The variations are few. The incomplete meeting may take place either above or below, and the lines turn anteriorly or posteriorly.

Category VI, fig. VI, A, B, C.—The arrangement found here may be produced from fig. V by extending the free ends of the segment lines till the one meets the segment in front, and the other the one behind. A reconstruction of such a figure gives the spiral shown in fig. VI, C. This spiral form terminates

both anteriorly and posteriorly in free ends. The two metameres involved take two complete turns around the body.

Variations of this (like those of category v) readily suggest themselves.

Category VII, fig. VII, A, B, C.—This spiral form has been already described as a type of the spiral.

Category VIII, fig. VIII, A, B, C.—On the upper side are two half-compound segments opening in opposite directions (A). On the lower side three normal segments are present (B). The reconstruction (C) shows that the posterior arm of the first half-compound segment takes a complete turn around the body, and then unites with the second half-compound segment as its anterior arm. The spiral involves three segments. The middle of the three, instead of forming its proper union above, unites above on the right side with the segment anterior to it, and on the left side and above with the segment posterior to it.

The variations of this form consist in having the union above or below, on the right and on the left, or on the left and on the right side.

Category IX, fig. IX, A, B, C.—This form is merely a longer spiral than that of category VII. The spiral turns one and a half times around the body, beginning and ending as in VII. Three segments on one side of the body correspond to four on the opposite.

This process may theoretically be continued through an indefinite number of segments, but generally a few turns suffice to bring the spiral to a close. The most extreme case that I have found involved twelve and a half turns to the spiral (Pl. 41, fig. 26).

Category X, fig. X, A, B, C.—This spiral is an extension of that of category VIII. It involves a greater number of metameres, and in consequence the spiral is longer. Two turns are taken around the body.

In both categories IX and X all the variations found possible for VIII and IX are here applicable.

Fig. XI, A, B, shows a further extension of VIII, with three turns.

There are several combinations of the preceding categories that are possible, and some of these I have found. It is not particularly important to discuss these possibilities, as they all reduce back to forms already described. As an example, however, one such is given in fig. XII, A, B, C; the reconstruction, C, sufficiently explains the conditions. It will be noted that five half-segments of one side correspond to three of the opposite.

Whenever a double half-compound metamere is introduced a more complicated form of spiral results (see fig. XIV, and Pl. 42, fig. 47, at x^1). This causes a double spiral, i.e. two spirals, to take a parallel course, as shown in fig. XIV. One or both of the spirals may end in a half-compound metamere—both so end in fig. XIII. This reconstruction will, I think, sufficiently explain the conditions, so that a further description would be superfluous.

The double spiral may be formed in other ways. Two successive compound metameres may be introduced in such a way that a new spiral is started along with the one already present. Such cases are shown in fig. XIII, and in fig. 47, Pl. 42, at x^2. Several variations of these combinations suggest themselves, and several have been found amongst the worms, but the discussion of these variations would not add materially to the former cases, and may be omitted. Even a triple spiral was found in one case, as shown in fig. 47 at x^2. It was of short duration, owing to the introduction of compound metameres in such a way that the spirals were quickly absorbed.

Lastly, the series of compound metameres, double and triple spirals shown in fig. 47, Pl. 42, were all drawn from the same worm. We find 134 half-metameres on the right side and 118 half-metameres on the left. In all there were fifty-two perfect rings, and the numbers in the plate between consecutive groups of compound metameres, spirals, &c., indicate the number of perfect rings in that locality. An examination of this reconstruction will show how far it is possible

for the variations—abnormalities—to be present, and the worm still be able to reach sexual maturity. In another worm containing many abnormalities there were 131 half-metameres on the right side of the body and 139 on the left side. In all there were seventy-nine normal rings. The number of half-rings in these worms is noticeably very large as compared with the normal number. It should also be noticed that the abnormalities are not confined to any one part of the body, but scattered throughout the whole length of the worm.

We may now study the relative proportion in which the different modifications, described above, are found. In a lot of 318 worms 218 were found to be normal externally with respect to the metameres, and 100 abnormal. This is in the proportion of 1 to 2·18, or in general one worm of every three was abnormal.

In the same worm there often occurred more than a single abnormality. Thus in the above 100—

With . .	1 abnormality . . .	65
„ . .	2 abnormalities . . .	16
„ . .	3 „ . . .	10
„ more than 3	„ . . .	9
		100

An examination of the material shows that there is practically no difference in the number of compound metameres on the right as compared with the left side of the body.

The examination shows, however, that a much larger number of "splits" are present on the dorsal surface of the body than on the ventral surface. In a compound metamere we have a split above for one below, but in such spirals as shown in figs. VIII, X, &c., we have two above and none below, and such spirals have their "splits" on the dorsal surface in the greater number of cases. I regard this as important, as it gives a clue to the solution of the problem.

An examination of the material shows that there are thirty-two cases of spirals beginning and ending above (category VIII), and only one case of a spiral beginning and ending below.

There were ten cases of failure of lines to meet above as in categories v and vi. No cases below.

There were twenty-five cases of spirals beginning above or below, and ending on the same side of body—below or above. Amongst these twenty-five cases there were fifteen that began above and ended below, and ten that began below and ended above. If we compare the data of the thirty-two cases with the twenty-five cases we find that we have ninety-nine cases where the split was on the dorsal surface, and twenty-five cases where the split appeared on the ventral surface.[1] That is to say, there were nearly four times as many cases of false unions above as below.

If we arrange the different forms of abnormalities found under the categories given in the preceding section, we find the relative proportion of the abnormalities in the 100 abnormal worms to be as follows. In this count no difference is made as to whether an abnormality occurred once or twice on the same worm. It is based on the results of all abnormalities found present.

Category I, II		42	
„ IV		1	
„ V, VI		10	
„ VII		20	26
„ IX		6	
„ VIII		16	32
„ X		8	
„ XI, &c.		8	
Mixed forms too irregular for classification		11	

[1] These numbers were derived from the data given above as follows:

32 (spirals beginning and ending above)	× 2	= 64
15 („ „ above and ending below)		= 15
10 („ „ below and ending above)		= 10
10 (cases where lines failed to meet above)		= 10
		99
15 (spirals beginning above and ending below)		= 15
10 („ „ below and ending above)		= 10
		25

II. Variations in the Position of the Reproductive Organs.

With the hope of throwing additional light on the modifications in the arrangement of the metameres, I was led to examine carefully those cases where the false arrangement occurred in the segments anterior to the fifteenth. Here there are definite landmarks, viz. on the outside are the openings of the vasa deferentia, oviducts, and seminal receptacle, and on the inside are the organs belonging respectively to these openings. The openings of the vasa deferentia on the fifteenth segment are the only ones of these openings that are readily and with certainty to be made out, so that I have confined my study largely to the modifications in the position of these openings. When we come to study the internal arrangement of the organs, then the position of ovaries and seminal receptacles will also be considered.

In utilising the fifteenth segment as a point of departure, it was first necessary to determine whether this was in reality a fixed point. In a lot of 799 worms, twenty were found with the vasa deferentia opening abnormally. In twelve cases of the twenty the openings were on the same segment, but this was not their normal segment (fifteenth). In six cases the openings (right and left) were not on the same segment. In two cases the openings were doubled on one side or the other. To summarise:

A. Openings on the same segment, but not on the 15th . 12
B. Right and left openings, not on the same segment . 6
C. Openings doubled on one side 2

A. The following table records the openings of the vasa deferentia in the first category.

No. of Worms.				
1	vasa deferentia opened on	10th	metamere.	
1	„	„	11th	„
2	„	„	12th	„
4	„	„	14th	„
4	„	„	16th	„
12				

In those cases in which the openings of the vasa deferentia occur on a segment anterior to the 15th metamere, we may be dealing with a case of incomplete regeneration of the anterior metameres. In fact, in the first case given there was evidence for believing that the anterior end had just regenerated, and even the second case gave slight evidence of the same thing. That all of the cases can be explained in this way is, I think, highly improbable. We find, as the table shows, the greatest variation on each side of the 15th metamere, pointing in favour of individual egg variation rather than regeneration. This view is also strongly supported by an examination of the internal conditions. Again, those cases recorded above in C and D show conclusively that the openings of the vasa deferentia may shift, and in these cases regeneration is largely out of the question.

In order, however, to satisfy myself completely, i.e. experimentally, on this point, I tried by (artificially) cutting off the anterior metameres of a large number of worms to determine what proportion of these regenerated the full number of anterior metameres. The results are given in a later section.

B. There were six cases out of the twenty in which the openings of the vasa deferentia were in different metameres of the same worm. These are figured in Pl. 42, figs. 48—58.

In the first case, fig. 48, the 15th metamere has on its right side (left of figure seen from below) an opening of the vas deferens. The left side of the 15th has no opening, but on the 16th metamere of that side occurs the opening of the left vas deferens. One such case is recorded.

In fig. 49 another variation is found. Here the 15th metamere has on the left side of the body its proper opening, but there is no opening on the right side of that segment. On the 16th metamere, however, and on the right side, the right vas deferens opens. Four such cases are recorded.

In fig. 50 we find that the right side of the 15th metamere has an opening, but not the left side. On the 14th metamere we find the opening of the left vas deferens. One such case is recorded.

There can be no doubt but that an examination of a larger number of worms would show other combinations, but these are sufficient to show that variations in the position of the vasa deferentia do occur.

c. Two worms in this lot showed a doubling of the openings of the vasa deferentia on one side.

In fig. 51 we find on the 15th metamere a pair of openings, right and left; but in addition to these we find on the left side of the 16th segment another opening of a vas deferens.

In fig. 52 we find the 15th metamere with its pair of openings, the right somewhat enlarged. On the left side of the 14th metamere another opening is present, as shown in the figure.

Dissections were made of nearly all of the worms recorded in categories A, B, C. There is always the possibility of mistakes when only surface views are examined, but fortunately in all cases recorded the evidence from internal structure has supported the conclusion from the external appearance. Moreover additional data of considerable interest has, I think, been gained.

We may examine first the results of dissections of worms in which the paired openings of the vasa deferentia are on some other segment than the 15th.

No. 1. Openings of the vasa deferentia on the 10th metamere, fig. 53. The ovaries were present two metameres in front of the vasa deferentia openings, i.e. the ovaries were in the 8th metamere. All of the other parts of the reproductive system were present and normally developed. The brain appeared in the 1st metamere.

No. 2. Openings of the vasa deferentia on the 11th metamere. Only one ovary was found in the 9th metamere (left side); the other probably lost in the process of dissection.

No. 3. Openings of the vasa deferentia on the 12th metamere. The ovaries were found in the 10th metamere, fig. 54. The first segment seemed incompletely regenerated.

No. 4. Openings of the vasa deferentia on the 14th metamere, fig. 55. The ovaries in the 12th metamere.

Seminal receptacles in the 8th and 9th metameres. In another worm similarly modified the ovaries were also present in the 12th metamere.

No. 5. Openings of the vasa deferentia on the 16th metamere. The ovaries were present in the 14th metamere.

We may next examine those cases where the openings of the vasa deferentia are, right and left, on consecutive metameres.

No. 6. Three worms were dissected in which the right vas deferens opened on the 15th metamere, and the left on the 16th, figs. 56 and 57 (projections from above). In two of the three worms the ovaries showed a similar variation, appearing on the 13th (right) and 14th (left) metameres,—that is, each two segments in front of its corresponding vas deferens. In the third worm both ovaries appeared in the same metamere, viz. the 13th.

No. 7. Two cases recorded with the left vas deferens opening on the 15th metamere, and the right on the 16th. The ovaries varied correspondingly, and were found in the 13th (left) and the 14th (right) metameres (see fig. 58). The seminal receptacles showed a similar displacement, and were found in the 9th and 10th (left) and 10th and 11th (right) metameres.

No. 8. The worm drawn in fig. 52 in which the left vas deferens is doubled (14th and 15th metameres) was dissected, and a corresponding doubling of the ovaries of the same side was found.

No. 9. In another case of doubling the vasa deferentia of the right side opened on the 16th and 17th metameres; that of the left side was single and appeared on the 16th metamere (fig. 59). The ovaries varied also, appearing in the 13th and 14th metameres of the right side, and in the 14th of the left side. The variation here is not the same in the ovaries and vasa deferentia on the right side, for if the ovaries had varied to correspond they would have appeared in the 14th and 15th metameres of the right side.

No. 10. The paired openings of the vasa deferentia are found on the 15th metamere. In addition there is another

opening on the left side of the 16th. The ovaries are found in the 13th metamere (fig. 60, A).

No. 11. The paired openings of the vasa deferentia are found on the 15th metamere. On the left side of the 14th there is an additional opening. One pair of ovaries are found in the 13th (fig. 60, B).

No. 12. One case of doubling is recorded for L. terrestris (the preceding cases were all for L. fœtidus) (fig. 74, A, 74, B). The opening of the right vas deferens is doubled, and opens both on the 15th and 16th metameres. The left only appears on the 15th. Dissection showed both ovaries present in the same segment and not doubled. The dissection shows also the course taken by the vasa deferentia (fig. 74, B); and we find a single vas deferens on the right side that supplies both openings of that side.

I regret that on account of the difficulty in dissecting out the vasa deferentia in L. fœtida I did not get further evidence as to the method of doubling of the external openings in those worms.

III. Abnormalities in Front of or including the 15th Somite.

In a lot of 799 worms (L. fœtidus), twenty, as recorded in the preceding section, showed abnormalities in the position of the openings of the vasa deferentia. In this same lot, after the removal of these twenty (leaving 779), there were thirty-two worms with abnormalities in front of or including the 15th metamere. This is in the proportion of 1 : 24.

In 1893 I again collected material in order to obtain a larger number of abnormalities in the anterior ends of the worms. From 957 worms eighteen were found showing modifications of the anterior metameres. This is in the proportion of 1 : 53.

The material from these two sources, with a few additions from other lots of worms, has been brought together and figured in Pls. 41 and 42.

The modifications may be classified under the following categories:

A. Part of a metamere compressed.

B. Failure of external septal lines to meet.

C. Spirals in front of the 15th metamere.

D. Spiral involving the 15th metamere.

E. Results of the introduction of compound metameres.

A. Plate 41, figs. 1—3.

In fig. 1, A B, the 4th metamere is not fully developed on the right side of the body. The ring is narrower, and has less pigment on the right side. The vasa deferentia open on the 16th metamere, as shown in B.

Fig. 2, A B, shows another worm with the 2nd metamere similarly modified. The vasa deferentia open on the 15th metamere.

Fig. 3, A B, shows the 5th metamere reduced on the left side. The vasa deferentia open on the 16th metamere.

Conclusion.—It is not a little surprising to find in two of the three cases where an anterior metamere is partially undeveloped, that the openings of the vasa deferentia have shifted one segment posteriorly. The data are too small to lead to any definite conclusion.

B. Plate 41, figs. 4—11.

In fig. 4 we see that the line of division between the 9th and 10th metameres failed to meet exactly on the upper surface. The vasa deferentia opened normally, if we count the 9th and 10th as true metameres.

In fig. 5 the line between the 5th and 6th metameres failed to meet above. Vasa deferentia normal.

In fig. 6 the line between the 4th and 5th metameres failed to meet. Vasa deferentia normal.

In fig. 7 the line between the 3rd and 4th metameres failed to meet. Vasa deferentia normal.

In fig. 8 the line between the 13th and 14th metameres failed to meet.

In fig. 9 the line between the 12th and 13th, and in fig. 10

the line between the 11th and 12th. In these last three cases all failures were above, and in all the vasa deferentia were normal.

In fig. 11 the line between the 1st and 2nd failed to meet below; in fig. 12 between 13th and 14th below. In fig. 13 the line between the 5th and 6th metameres failed to meet at the sides. Both in figs. 11 and 13 the vasa deferentia were normal, but in fig. 12 there were indications of an additional opening on the left side of the 16th metamere.

Conclusion.—In nine out of the ten cases the position of the openings of the vasa deferentia were not affected by the failure to meet of metameres anterior to the openings, nor was a shifting to be expected on a priori grounds, since the modification is clearly only a lack of perfect union between the right and left half-metameres (fig. 13 perhaps excepted).

Fig. 12 is, then, a case of doubling of the openings of one side, like those described in the last section, and can have nothing to do with the lack of perfect union of the more anterior segments.

It is noticeable that in seven out of these ten cases the imperfect union is on the dorsal surface. In only two cases is it found below, and in one at the side. We will return to a consideration of the point at another time.

C. Figs. 14—19, 20—26.

Two cases are to be considered under this head: 1st, those in which the spiral introduces no new half-metameres on either side; 2ndly, those cases where a new half-metamere is introduced in front of the 15th metamere, so that one half of 15 becomes united to 16 (15—16), and the other half that remains is united to half of 14 (15—14).

1st case.—In fig. 14 a spiral is found involving the 7th, 8th, and 9th metameres. It belongs to category VIII (Pl. 40, fig. VIII). No new half-metameres are introduced, and the vasa deferentia open on their normal (15th) metamere.

In fig. 15 a similar spiral involves the 12th, 13th, and 14th metameres. Vasa deferentia as before, normal.

In fig. 16 a similar spiral involves the 11th, 12th, and 13th metameres. Vasa deferentia normal.

In fig. 17 a spiral is present that involves the 12th, 13th, and 14th metameres. The lower end of the figure (posterior end of the spiral) turns to one side and ends in a point. The openings of the vasa deferentia were not apparent on the surface, but dissection showed the ovaries were in the 13th metamere, that is within the spiral. Presumably, then, the vasa deferentia were also in their proper metamere.

In fig. 18 a longer spiral involves the 6th, 7th, 8th, and 9th metameres. The spiral belongs to category x (Pl. 40, fig. x). This form does not introduce any extra half-metameres, and we find the vasa deferentia normal in position.

In fig. 19 a similar (reversed) spiral is found. It includes the 11th, 12th, 13th, and 14th metameres. The vasa deferentia open normally.

Conclusion.—In these six cases where a spiral is introduced in front of the 15th metamere the position of the openings of the vasa deferentia is not affected. No better demonstration is needed to show that the spiral is due simply to a rearrangement of the segments involving their union across the middle line. It will also be noticed that in all of these cases the false union is made on the dorsal side of the worm.

2nd case.—In fig. 20, A, B, C, are drawn the upper and lower surfaces of a worm, and (in C) a reconstruction of the spiral. The 2nd, 3rd, and 4th metameres are involved, and an extra half-metamere (3rd) is introduced upon the left side of the body. The modification belongs to category VII (Pl. 40, fig. VII). We find corresponding to this intercalation that the openings of the vasa deferentia are affected, and appear on different but consecutive segments. Each, however, opens on its own 15th half-metamere. In other words, the 15th half-metameres still develop their proper reproductive openings, and in consequence of an interpolated half-metamere in the spiral anteriorly the two openings do not fall into the same metamere.

In fig. 21, A, B, C, a somewhat similar spiral involves the 9th, 10th, and 11th metameres, and also introduces an extra half-metamere on the left side. The external openings of the vasa deferentia were not found, but dissection showed that the ovaries were present in consecutive segments, i.e. each in the 13th half-metamere of its side. The explanation is the same as in the last case.

In fig. 22, A, B, C, we find a spiral belonging to category IX (Pl. 40, fig. IX). An extra half-segment is introduced. Nevertheless the openings of the vasa deferentia seemed both present on the same ring (metamere). This metamere is made up of the 15th half-metamere of one side and the 16th half-metamere of the other. In fig. 23, A, B, C, D, are shown two modifications in the anterior end. The first of these is brought about by an incomplete lateral union (c) of the line between the 5th and 6th metameres. More posteriorly a spiral is introduced that involves the 13th and 14th metameres. An extra half-metamere, as shown in D, is introduced on the left side. The external openings of the vasa deferentia were not seen. Dissection showed that only one ovary was developed, and that on the right side (or at least only one was found). This was in its normal half-metamere. The seminal receptacles were in their normal segments (9th and 10th), which showed that the more anterior modification (failure of lines to meet) did not affect subsequent metameres.

In fig. 24, A, B, C, a short spiral involving the 5th and 6th metameres is present, and introduces an extra half-metamere on the left side. External openings of vasa deferentia were not found. Dissection showed that the ovaries were present in the same segment (13th of one side, 14th of other side). I could not fully decide whether this case was due to a true intercalation of a half-segment or to an overgrowth of the middle line by the right half 6th metamere, due to an incomplete dorsal growth of the left half 5th metamere. The latter interpretation would be more in accordance with the presence of the ovaries in the 13th—14th metamere, which would then be only the 13th metamere. Moreover, as this is the only case

recorded of such a modification, I think the latter interpretation is probably correct.

In fig. 25, A, B, C, a rather complicated spiral involves the 10th, 11th, 12th, and 13th metameres, as shown in C. An additional half-segment is introduced on the left side. Nevertheless both of the openings of the vasa deferentia appear on the same segment (15th—14th).

Conclusions.—The number of worms recorded is too small to give any conclusive data. In two cases (figs. 20, 21), where an extra half-segment is introduced, each opening of the vas deferens is on its normal half-segment, and therefore on consecutive rings.

In two other cases (figs. 22, 25) the openings of the vasa deferentia occur on the same ring (14th—15th in one case, 15th—16th in the other). In a third case only one ovary was found (fig. 23), and in a fourth the nature of the spiral was doubtful.

D. Figs. 26—32.

Two kinds of modification belong to this category: 1st, those where the spiral passes through the 15th metamere; and 2nd, those cases where the 15th metamere begins or ends a spiral.

1st Case.—In fig. 26 is shown a dorsal view of a worm with a spiral involving the 11th to the 24th metameres. The spiral winds around the body thirteen times. The number of half-segments is not increased by the spiral. Looking at the lower surface of the worm the vasa deferentia are found on the 15th metamere, and are in no way affected by the fact that the metamere is part of a long spiral, and not a closed ring.

In fig. 27, A, B, C, a spiral involves the 10th to the 16th metameres. Looked at from below, B, the vasa deferentia are seen on the 15th metamere. The reconstruction in C shows that the 15th metamere is a part of the spiral, and still carries the openings of the vasa deferentia.

In fig. 28 a spiral is found involving the 13th to the 24th

metameres. The openings of the vasa deferentia were not seen on the outside, but dissection showed the ovaries to be present in the 13th metamere.

Conclusion.—In these three cases where the spiral involves the 15th metamere the reproductive organs appear in their normal half-metameres. In all of these cases the false union of metameres that produces the spiral is above. The lower surfaces of the half-metameres join one another as under normal condition,—that is, the 14th below and on the right side joins the 14th, &c. On the dorsal side the reverse is true,—that is, the 14th joins the 15th half-metamere, &c. As the reproductive organs are in the lower part of the segment, we would expect to find them still in their normal segment (and not in any way modified), and this we do find in all cases recorded.

2nd Case.—In fig. 29, A, B, C, a spiral is drawn involving the 14th, 15th, and 16th metameres. The spiral begins in the 14th half compound metamere ($14—\frac{14}{15}$). The openings of the vasa deferentia are present in their normal half-metameres.

In fig. 30, A, B, C, a spiral involves the 15th, 16th, and 17th metameres. Here, as in the last case, the openings of the vasa deferentia are not affected.

In fig. 31, A, B, a similar spiral involves the 15th, 16th, 17th, and 18th metameres. Here a half compound metamere ($15—\frac{15}{16}$), beginning the spiral, carries the openings of the vasa deferentia on their normal 15th metamere.

In fig. 32, A, B, a spiral involves the 15th, 16th, and 17th metameres. The openings of the vasa deferentia occur on their normal metamere, which forms the anterior limb of the half compound metamere ($15—\frac{15}{16}$) that begins the spiral.

Conclusion.—In none of these last cases does the presence of a half compound metamere involving the 15th metamere affect the openings of the vasa deferentia.

General Conclusion from Category D.—Nearly every case points unmistakably to the conclusion that, however falsely the segments may unite in front of the 15th metamere, or so that the 15th metamere is involved in a spiral or half

compound metamere, still the openings of the vasa deferentia appear on their 15th half-metamere.

It is also very noticeable that in by far the larger majority of cases the false unions of the metameres are on the dorsal surface.

E. Figs. 33—46.

The results recorded in the preceding section are, as has been said, fully in accord with the statement that, however varied the union of the half-metameres across the median line may be, still the openings of the vasa deferentia occur in most cases on their normal (15th) half-metamere.

In the present category (E), where we should hope to find this same relation to hold, the matter stands otherwise. In only one case, viz. in that first given, does the anticipated result follow. Many of the cases are unintelligible, or nearly so.

I used in my earlier paper the first figure referred to above to illustrate the explanation offered as to the value of the compound metamere (there called the split metamere). I still believe that it does this, but I was not then aware how much in the minority, as far as numbers go, this modification really is. I fear that by picking out this one case as an illustration in my short preliminary communication I exaggerated the value of the modifications as furnishing evidence of my view. I was careful to state only that this was "one of the most instructive cases, ... and gives us a clue by which to interpret the split metamere." This statement I still think is true, but by far the weightier evidence for my conclusion is now furnished by the cases recorded in the preceding sections rather than in this one.

Pl. 41, fig. 33, A, B, shows the anterior end of a worm having the 10th metamere compound ($10—\frac{1}{1}\frac{0}{1}$); there is, therefore, one more metamere on one side of the body (the left) than on the other. Correspondingly the openings of the vasa deferentia are not on the same metamere, but on consecutive ones. Each opening is on the 15th half-metamere of its side. Each half-metamere has developed its normal structures, but

owing to the shifting of the metameres, due to the introduction of an extra half-metamere, the halves of the 15th metamere do not unite with one another.

In fig. 34 two modifications of the metameres are introduced in front of the 15th metamere. Anteriorly the line between the 4th and 5th metameres fails to meet above. The 7th metamere is compound, so that an extra half-metamere is introduced on one side (the left); nevertheless the two vasa deferentia open on the same segment (16—15).

In fig. 35, A, B, C, we find again two separate modifications. A small half of the 3rd metamere forms above a union with half of the same segment on the right side, and below it is wedged in between the 2nd and 4th metameres. Again, further back a half-segment is introduced on the left side between the 8th and 10th metameres (or, if the more anterior extra half be counted, between the 9th and 10th). The openings of the vasa deferentia were not made out, but the ovaries occurred in consecutive segments, i. e. on the 13th half-metamere of each side if the more anterior half-metamere (3rd) is not counted. If this was counted, then the ovaries would lie in the 14th half-metamere of the left side, and in the 13th half-metamere of the right side.

In fig. 36, A, B, C, a spiral[1] is introduced that involves the 11th, 12th, and 13th metameres, as shown in C. An extra half-metamere (12th) is introduced on the right side. The openings of the vasa deferentia were not seen, but dissection showed that the ovaries are in consecutive segments. In the left side the ovary occurred in the 14th half-metamere, and on the right side in the 16th half-metamere.

In fig. 37, A, B, the 12th metamere is compound, and we find that the vasa deferentia open on correspondingly shifted metameres. But, besides this, the openings of the right side of the body have doubled, so that the 15th—16th metamere has two openings, right and left.

In fig. 38, A, B, C (lateral), a half-metamere is introduced between metameres 3 and 4. The openings of the vasa

[1] This case belongs to a preceding category, D.

deferentia are on the same segment. (It seems to me very doubtful whether the surface line of the 4th metamere is due to a half-metamere introduced.)

In fig. 39, A, B, C, a compound metamere occurs on the 15th segment ($15—\frac{15}{16}$). The opening of the right vasa deferentia alone was visible on the exterior. The ovaries were found in the 13th metamere.

In fig. 40, A, B, we find a spiral involving the 8th, 9th, and 10th metameres, introducing an extra half-metamere on the right side.[1] The vasa deferentia open, nevertheless, on the same metamere (15th—16th). Another modification starts at the 15th metamere, and involves a few more posterior segments.

In fig. 41, A, B, C, we find four compound metameres (see c), followed by a spiral that involves segments immediately in front of the region of the opening of the vasa deferentia. Fifteen half-metameres of the right side correspond to twenty of the left side. The openings of the vasa deferentia appear on the same ring, and this ring is made up of the 16th half-metamere of one side and the 21st half-metamere of the other.

In fig. 42, A, B, C, we find a compound metamere ($3—\frac{3}{4}$), followed by a complicated spiral (c) that involves the 14th to the 21st metameres. The openings of the vasa deferentia were not seen on the outside. Unfortunately I have no records of the dissection of this worm.

In fig. 43, A, B, C, we find two spirals and a compound metamere in the anterior region. The openings of the vasa deferentia occur in the last spiral. The opening on the left side is in the 18th half-metamere, and on the right side in the 16th metamere.

In fig. 44, A, B, a compound metamere ($6—\frac{6}{7}$) is first found, followed by a spiral belonging to category vii, Pl. 40, fig. VII, and this followed by another compound metamere ($13—\frac{15}{16}$). Three extra half-metameres are introduced on the right side. The openings of the vasa deferentia are

[1] Belongs to another category, i. e. D.

doubled on both sides. On the left side the openings are found on the 15th and 16th half-metameres. On the right side of the body the openings appear on the 20th and 21st half-metameres.

In fig. 45, A, B, C, we find the 6th metamere somewhat reduced on the left side. We have an intercalated half-segment (15th) on the right side, followed by a spiral involving the 15th to the 19th metameres. The latter introduces an extra half-metamere on the right side. The openings of the vasa deferentia were not found. Dissection showed the ovaries in the 18th half-metamere of the left side, and in the 21st half-metamere of the right side (in the half-metamere that follows the 18th on the other side).

In fig. 46, A, B, C, an extremely modified anterior end of a worm is drawn. As the reconstruction shows, many extra metameres are introduced on the left side of the body. In the exterior of the 24th and 25th left half-metameres swollen areas seem to point to openings of the vasa deferentia, but none appear on the right side. In the dissection of this worm no ovaries could be found, and no data throwing any light on its "make-up" were obtained.

Conclusion.—It is very difficult to reach any conclusion in the face of so much conflicting evidence. It would seem as though an intercalated segment might or might not affect the position of the reproductive organs. If to get out of the dilemma we assume that some of these cases are not due to the intercalation of true half-metameres, but to unilateral division of metameres, then other difficulties are encountered as great or greater than those that appear on our first assumption. It was only after the preceding pages were written and the hopelessness of any explanation realised that a partial solution—or what seems at first to be such—suggested itself to me. Such cases as those drawn in figs. 41 and 46 might be due to a regeneration in the adult of anterior metameres. We see that this might offer an easy escape from the dilemma. It seemed also to explain why the segments in some of these worms that carry the openings of the vasa deferentia

and the ovaries are so far from the anterior end. Here more segments might have regenerated than were lost.

Fortunately we are dealing with an hypothesis that could be tested by experiment. I at once set to work to determine whether or not the same number of segments reappeared when the anterior ends were cut off. I also wished to find out whether a greater number of modifications than in the average worms were thus produced. A later section gives the result of these experiences.

IV. Study of Embryos.

Capsules containing embryos of L. fœtidus were collected, and the embryos killed immediately on their emergence from the cocoon. Out of 170 embryos there were twenty-five that showed abnormalities in the arrangement of the rings. This is in the proportion of 1 to 5·2. In other words, there are fewer cases of abnormalities in young worms than amongst adults. In the latter the proportion was one to two.

This apparent contradiction finds its explanation in the fact that the adults are often found regenerating lost metameres, and proportionately the number of abnormalities in these newly formed parts is greater than in the embryos. This will be taken up more fully in another section.

The number of metameres in the young worms that have just left the capsules depends to some extent, as the following table shows, upon the size of the young worms. The size seems to be in general connected with the number of embryos that develop in the capsule. Amongst the worms in the same capsule, however, there are variations in size, and we can only make some such general statement as this, that where many embryos are present in the same capsule they are each smaller when they leave the cocoon than when fewer embryos are present.

The following table indicates, in a rough way, the relation between the size of the embryos and the number of metameres:

		Size of Worm.
No. 1	111 metameres	Very large.
„ 2	102 „	Large.
„ 3	100 „	„
„ 4	85 „	Small.
„ 5	85 „	„
„ 6	78 „	„
„ 7	67 „	Very small.

Numbers 6 and 7 came from a capsule that contained 12 worms.

The following table gives the record of twelve young worms (including those of the preceding table), showing the number of metameres present:

No. 1	85
„ 2	100
„ 3	102
„ 4	85
„ 5	107
„ 6	106
„ 7	111
„ 8	78
„ 9	67
„ 10	87
„ 11	85
„ 12	97
	12) 1110
	92·5

I attempted to determine whether the young worms that had abnormalities came in proportionally larger numbers from individual capsules. Capsules were also examined to see whether the number of embryos in a capsule was a factor in the production of abnormalities. This examination ought also to show whether the eggs from certain worms had a tendency to produce abnormally joined metameres; if, for instance, in all the capsules that contained a large number of embryos many were abnormal, it would follow, with a great deal of probability, that the crowding (or smaller amount of food, &c.) brought about (directly or indirectly) the result. If, on the other hand, certain capsules, regardless of the number of

worms present, showed a large percentage of abnormalities, then the cause probably arose in the egg.

The following tables give the data that I have collected. The number of recorded cases is smaller than I could have wished, but the evidence is all in one direction and fairly convincing. The capsules were isolated and the worms collected as soon as they crawled out.

	Number of embryos in a capsule.	Number of abnormal worms.
No. 1	6	1
„ 2	3	0
„ 3	6	2
„ 4	6	0
„ 5	12	1
„ 6	5	1
„ 7	3	1
„ 8	4	0
„ 9	12	1
„ 10	5	1
„ 11	12	1
„ 12	8	1

Conclusion.—In none of the capsules is there a preponderance of abnormalities, nor does there seem to be any relation between the number of worms in a capsule and the presence or absence of wrongly united metameres.

We must conclude that neither of the possible causes suggested above is active in producing the abnormal embryos.

We get no clue from these data as to any outside forces producing the result; and although we have not by any means exhausted all the possibilities, still it seems not improbable that in each particular case local internal conditions determine the result.

All of the commoner forms of abnormalities recorded for the adult worms are also found in the embryo. This applies to abnormalities in the anterior part of the body as well as in other parts. There are more abnormalities in the tail end than in the head end.

A few typical abnormalities found in some of these embryos (young worms just out of the capsule) are shown in Pl. 42,

figs. 75—78. In fig. 75, A, B, is drawn a compound metamere; in fig. 76, A, B, a spiral of category VII; in fig. 77 a spiral of category VIII; and in fig. 78 a longer spiral of category X.

A number of adult worms was examined, and the following table shows the number of metameres in them. Mature worms were chosen as far as possible.

No. 1	101	
„ 2	102	
„ 3	92	
„ 4	85	(perhaps regenerating).
„ 5	97	
„ 6	97	
„ 7	105	
„ 8	95	
„ 9	101	
„ 10	88	(probably regenerating).
„ 11	92	
„ 12	85	
„ 13	97	
„ 14	84	
„ 15	95	
15)	1416	
	94·4	average.

It will be seen, if we may judge by the figures given, that few, if any, new segments are added to the worm after it leaves the capsule.

Another count of 25 worms gave the following figures:— 99, 89, 85, 100, 90, 96, 95, 95, 97, 101, 101, 105, 101, 106, 88, 84, 88, 95, 100, 92, 100, 91, 105, 92, 96. These numbers give an average of 99·6. If we combine this with the average of the 15 cases given above (94·4) we get 97 for the average number of segments of 40 worms.

V. Abnormalities at the Posterior End.

In one lot of 525 worms 40 were found showing newly formed posterior ends due to regeneration.

In these 40 worms we find—

A. Regenerated portion just beginning to show metameres . 4

B. Regenerated portion normal 2

a. Number of new metameres . . 6

b. ,, ,, . . 6

C. Regenerated portion having one abnormality . . 12

a. Number of new metameres . . 19

b. ,, ,, . . 6

c. ,, ,, . . 35

d. ,, ,, . . 16

e. ,, ,, . . 25

f. ,, ,, . . 3

g. ,, ,, . . 4

h. ,, ,, . . 11

i ,, ,, . . 4

j. ,, ,, . . (several)

k. ,, ,, . . 15

l. ,, ,, . . 6

D. Regenerated portion with two abnormalities . . 6
(Number of new metameres not recorded.)

E. Regenerated portion with three abnormalities . . 4

F. Regenerated end with more than three abnormalities . 12

In D, E, and F the number of new segments was not counted, but measurements were taken of the regenerated portions for comparison with C, and were as follows:

	C.	D.	E.	F.
1	18 mm.	26 mm.	27 mm.	25 mm.
2	13 ,,	21 ,,	26 ,,	25 ,,
3	10 ,,	21 ,,	9 ,,	25 ,,
4	7 ,,	16 ,,	8 ,,	22 ,,
5	6 ,,	8 ,,	4) 72 ,,	22 ,,
6	8 ,,	8 ,,	18 mm.	21 ,,
7	4 ,,	6) 98 ,,		19 ,,
8	4 ,,	$16\frac{1}{3}$ mm.		14 ,,
9	4 ,,			14 ,,
10	2 ,,			13 ,,
11	2 ,,			12 ,,
12	2 ,,			12 ,,
	12) 80 ,,			12) 224 ,,
	$6\frac{5}{6}$ mm. average length.			$18\frac{2}{3}$ mm.

These measurements show conclusively that the greater number of new abnormalities occur not more frequently at the beginning of the newly formed portion than throughout all the later period of growth.

Summary.—In the 40 cases mentioned above 4 were not well enough developed to furnish any data. In the 36 that remain there were only two that did not show any abnormality, and it is noticeable that each of these had only formed at the time a few new segments (six each). We must conclude from the data that about 18 worms out of every 19 would show abnormalities in the regenerated posterior end.

In these same worms the number of abnormalities in the region of the body anterior to the regenerated end was recorded. This gives us data to show whether the irregularities are due to inherited peculiarities of the tissue or to the conditions acting during regeneration. In the 12 cases recorded in c 9 were normal in front of the regenerated portion, and 3 showed abnormalities,—that is, as 1 to 3. In the 6 cases recorded in D, 5 were normal in front and 1 abnormal (1 to 5).

In the 4 cases in E there were no abnormalities in front. In the 12 cases recorded in F there were 2 cases unrecorded, and the remaining 10 had no abnormalities in front.

To sum up, the evidence points unmistakably to the conclusion that the abnormalities found so frequently in regenerated portions are due to the conditions acting during regeneration (from within or from without), and in no way connected with an hereditary tendency to be more abnormal in one case than in another. That is to say, the tissues of a worm that has developed normally from the egg are just as apt to develop irregularly in regenerating as are the tissues that have developed irregularities during embryonic growth. Heredity seems to have nothing to do with causing the abnormalities. In fact, one might say that the tissues inherit a strong tendency to regenerate normal metameres, but the means at command are so imperfect that abnormal results are frequent.

Will the large proportion of abnormalities present in regenerated worms, taken in connection with the number of worms found

regenerating naturally, account for the difference between adults and embryos? We have seen the number of worms found showing regenerated (and regenerating) posterior ends to be 1 in every 13 (1 to 12); therefore in a lot of 225 we should expect to find 19 worms having new posterior ends. In our lot of 225 worms we found 100 abnormal worms. If we deduct from this 100 the number of those that should have regenerated tails (19), we should have 81 cases remaining. The proportion would then be 81 to 225, or 1 to 2·8 (approximately 1 to 3). We found, however, that the proportion of abnormal to normal embryos in A. fœtida was only 1 to 5.

The difference that is still found is probably due to several causes, and these are not in the least of a hypothetical nature. Firstly, after a time a new regenerated end cannot be distinguished from the rest of the body. Secondly, a small percentage of regeneration must also take place in the anterior end. Thirdly, the difficulty of seeing the abnormalities in the embryos is much greater than in the adult, and defects may have occasionally escaped even a careful examination. Fourthly, the data is drawn from too small a number of cases to make an exact agreement very probable, even if it did exist.

In the light of these conditions I think the closeness of the result is as near as could be expected.

A number of worms were examined in which the posterior end of the body was regenerating, to see if any could be found in which the tip of the "tail" showed, in process of formation, modifications of the typical arrangement. Several were found, a few of which are drawn in Pl. 43, figs. 79—81.

The first of these (fig. 79, A, B, C) shows a compound metamere in process of formation from the terminal piece or telson. On the side that forms the "split" the telson is proportionately longer than on the other.

In fig. 80, A, B, C, the end of the body is very much modified, and a spiral is in process of formation. The reconstruction (C) shows sufficiently the conditions present.

In fig. 81, A, B, C, another modification is present. Above and on one side of the telson the outline of a metamere is seen, while

below this line only extends a third across. Whether this would ultimately form a complete ring, or whether we have here the beginning of a spiral starting with a half-compound metamere below, is uncertain.

Several vertical longitudinal sections were made through these "tails," but no additional information was gained from the sections. The irregularities in the positions of the rudimentary body cavities was very apparent.

VI. Modifications of Internal Structures.

Before any conclusion can be reached as to the value of the irregularities seen on the surface, we must examine the condition of the internal organs, particularly the arrangement of the septa.

We can formulate this general statement, that the arrangement of the septa generally conforms to the curves of the lines seen on the surface. This means that the phenomena are deep-seated, and that all the structures of the body are involved in the new arrangement, and not merely the external surface lines.

Exceptional cases are not uncommon, in which we find two principal departures from the rule. First, the arrangement of the septa does not always agree with the surface lines. Sometimes the arrangement of the septa is more perfect, and sometimes simply different. Secondly, the arrangement of the somatic attachment of the septa is sometimes different from the splanchnic attachment. Usually in this case the somatic portions of the septa follow the lines found on the surface, while the splanchnic attachment differs in its arrangement, and is usually more irregular in its form.

If a worm having a compound metamere be opened we find the septa arranged as shown in fig. 61, A, B. One septum (s) reaches only to the mid-dorsal line. If we remove the digestive tract we find the same septum ending freely below, just before reaching the middle line (s′). This half-septum is found to correspond to the "split" in the compound metamere, and lies between the two half-metameres of that side.

The septum is attached on the somatic side to the body-wall along the "split." Centrally it is attached to one half of the splanchnic wall of the digestive tract. We should find on sectioning such a worm that the half-septum had a double wall, and was like a true half of a septum in every respect. We should see also, as a result of this arrangement, that the body cavity of the single half-metamere is continuous in the mid-ventral and mid-dorsal lines with two half-metameres on the opposite side.

The number of the nephridia corresponds to the number of the half-metameres, as shown in fig. 62, so that there is one more nephridium on one side than on the other.

The nervous system is often modified, as shown in fig. 62. Here each of the half-metameres is seen to receive its full number of nerves from the ventral cord. This is not always the case, for, as shown in fig. 61, B, the double side gets only the supply normal for a single half-metamere. In figs. 63 and 64, B, we see other irregularities in the nerve-supply. This may be connected with the degree to which the half-septum approaches to the mid-ventral line. When it falls far short the nerve-supply is less than when it reaches the mid-line. The nephridia, however, that lie laterally in the body are invariably doubled. In fig. 64, A, the surface line between the half-metameres of the compound metameres is not very extensive. The figure represents the body-wall flattened out (the worm had been previously opened). Fig. 64, B, shows that the septum s is attached to the somatic wall over a correspondingly small area.

The condition of the septa in the spiral modification is very interesting. One of the simplest cases is shown in fig. 65, A, B. The first figure shows a short spiral beginning and ending above (category VIII). On opening the worm from below (fig. 65, B) the septa are seen to follow the same arrangement, both on the body-walls and on the intestine. There results a single spirally winding septum beginning with the half-septum of the compound metamere anteriorly, and ending with another compound metamere poste-

riorly. In other words, there is a continuous body cavity (cœlom) lying between the coils of the septum, and this cavity is continuous from the anterior to the posterior end of the spiral.

Fig. 66 shows a similar spiral, the only difference between this and the last being that in the former (fig. 65) the anterior end of the spiral abuts against the septum in front, while here it ends freely.

Fig. 67, A,B,C, shows a similar but longer spiral. Opened dorsally the septa are found as in B, and it will be seen that they follow the course of the outer spiral. In the middle line the septa over the intestine show a tendency to irregularity (even more so than the figure shows). In C the septa are drawn as seen from below. They run obliquely over the lower wall of the digestive tract. In both B and C the figures were drawn from preparations made by dissecting free the digestive tract and its attached septa from the body-walls.

These examples will suffice for the regular forms, where surface spirals and septal spirals agree; but this, as stated above, is not always the case.

In fig. 68, A B, we find on the surface a spiral shown in A; that belongs to category X (Pl. 40, fig. X). Opening the worm we find that the septa are also spirally arranged, but the spiral is shorter than the surface spiral by one turn, although both begin and end on the upper surface.

Fig. 69, A B, shows a parallel case, where the internal spiral is shorter than the external. In this case the septal spiral is shown only in its somatic attachment.

In fig. 70, A,B,C, we find a surface spiral like the last. We find the septa arranged dorsally as shown in B, and on the ventral as shown in C. It will be seen that the external and internal (septal) spirals belong to different forms (categories). The external belongs to category XI (Pl. 40, fig. XI), while the septal belongs to category IX (extended), Pl. 40, fig. IX.

Fig. 71 shows a form in which the external line between two metameres failed to meet in the mid-dorsal line. In dissection

the arrangement of the septa was found to be normal; that is to say, the septum has met normally across the mid-dorsal line, but the external lines have failed to do so.

In fig. 72, A B, a surface spiral is drawn, and in B the arrangement of the septa beneath is shown. The septa shows an abnormal arrangement, but this does not correspond to the surface lines: it is more of an approach to the normal.

Fig. 73 is from a compound metamere but in which the septa form a simple spiral making a little more than one turn of the body.

It is not always easy to picture to one's self the relation existing between septal and surface lines when the two do not correspond. In the simpler cases it is easily understood. When two spirals are formed of different lengths, it is due to the fact that the septa sometimes unite with one another beneath the surface before the surface spiral is brought to an end. That is, if both start together one may continue for a longer time than the other. We must conclude, then, that while the two usually vary together, yet they may vary independently. I have dissected a far greater number of worms than recorded above, and from that number have selected those given as the most interesting examples to illustrate the main points that concern us at present. Many other relations have suggested themselves, but I have only wished to go into the subject as far as concerns the matter in hand.

VII. Study of Polychætous Annelids and Leeches.

A large number of species of polychætous Annelids have been examined, and many modifications in the arrangement of the metameres have been found in them. From this number I have chosen a very few for description, since a large number of cases have been given in the papers by Cori and Buchanan.

The modifications that are figured here are all taken from specimens of Amphinome.

In fig. 82, A B, we find above B a compound metamere with the split on the left side. In the ventral side of the worm, A, we find the left anterior half of the compound metamere

wedged in ventrally between two segments, while the other left half completes the ring. We have here evidently a modification of the compound metamere similar to category II, Pl. 40, fig. II.

In Pl. 43, fig. 83, A, B, we see a metamere imperfectly developed on the right side. The dorsal parapodium and gill are absent on this side, but the ventral parapodium is present, as seen in side view in B. We have here probably a case of incomplete development of a metamere of one side.

Another somewhat similar modification is shown in fig. 84, A B. The first of these shows the ventral side, and the middle segment of the three drawn is seen to be imperfectly developed. The ventral parapodium of the right side (left of figure) is present, but the metamere does not reach as far dorsally as the line of dorsal parapodia (see B). On the left side of the body we find the metamere completely undeveloped. We seem to have here a half-segment of the right side that has not fully developed even on that side, but has overgrown the mid-ventral line on the left side.

Fig. 85 shows a spiral beginning and ending on the dorsal surface. Seven segments are involved, giving five turns to the spiral.

The large number of abnormal forms found in Amphinome is in part due no doubt to the frequent regeneration of portions of the body that takes place. The segments are broad, and for this reason it is surprising to find abnormal combination of the metameres so frequent. In other polychætous Annelids, where the metameres are very narrow, the false unions and imperfections of the metameres are very numerous.

Cori has figured several modifications, and I have found similar ones that are exceedingly difficult to explain as simply due to modifications of half-metameres. Such cases are particularly common in polychætous Annelids.

The majority of these very abnormal forms are, I think, due to regeneration rather than to egg-variation. Since the method of regeneration is more irregular than the growth of the

embryo from the egg, it becomes much more difficult to explain on the metamere assumption these variations due to regeneration.

A number of leeches were examined to see whether the annuli that make up the broad metameres ever showed false unions like those found in the earthworm's metameres. Such modifications are not rare.

In fig. 86, A B, the line between the second and third annuli of the ninth metamere failed to extend above over to the left side of the body. Again, in this same metamere we find that the line between the 5th and 6th annuli turns forward to join the line between the 4th and 5th, so that the last annulus of this metamere forms a compound annulus with the first annulus of the next metamere, producing a short spiral form.

A more complicated spiral is shown in fig. 87, A, B. The 16th and 17th metameres are involved. The spiral commencing in the 16th metamere runs over into the next metamere, as the reconstruction B shows. The annuli are also imperfectly joined at the sides, as shown in B. This last result is due to a failure of the annuli to unite perfectly with one another.

A third spiral-form, involving a double compound annulus, is shown in fig. 88, A, B. A half-compound annulus above and on the right side forms a spiral with a half-double compound annulus above and on the left side. The first half-compound ring is the 3rd.

We see that the annuli of leeches show many of the same sorts of false unions that are present in the metameres of the earthworm, but in the leech this represents only a superficial alteration.

The modifications in the leech would correspond to those modifications occasionally found in the earthworm, where the septa are normal but the surface markings abnormal in their arrangement.

It will be noticed that in the figures given no new annuli are introduced into the metameres, and only the method of union of the annuli varies. This result may only be due to the

relatively few leeches examined, although the number was sufficient to show that the modification involving additional half-annuli, if it occurs, must be rare.

VIII. Summary.

In the papers of Cori (13) and myself (32) the same explanation was offered as to the origin of the simpler cases of abnormal unions. Cori said, "Nun kann es sich ereigen, dass in der einen Körperhälfte während der Entwickelungsperiode ein Ursegment mehr gebildet wird, dem auf der Gegenseite kein Ursegment entspricht. Auf diese Weise wird die Bildung jener Fälle von Schaltsegmenten verständlich, wie sie im vorhergehenden anatomisch beschrieben worden. Betreffend das Verhältnis eines Schaltsegmentes zu den übrigen Metameren des Körpers sind zwei Möglichkeiten vorhanden. Es kann sich ein Schaltsegment vollständig ausbilden, und kann von den anderen Segmenten abgegrenzt bleiben oder es geht eine Verbindung mit dem vor oder nach folgenden Metamer ein."

My own statement was very similar:—"We know that in the embryo the metameres are laid down right and left of the middle line of the body in blocks of mesodermal tissue; that under normal conditions these hollow blocks come to lie exactly opposite (right and left of) one another, so that the opposite pairs unite across the median dorsal and ventral lines. If we conceive that the blocks are slightly displaced on one side, or that two consecutive blocks of one side are smaller than two of the opposite side, we may have, as a necessary mechanical result of the relative position of the block, a split metamere." To account for the spiral arrangement I said, and I am still of the opinion that this is the true explanation, "If we imagine one of the mesodermic blocks of one side to be larger either above or below (but not both above and below), so that above (let us say) it opens into two body-cavities of the opposite side, while below it opens into but one, then we have produced the conditions necessary to start the spiral. Each of the consecutive blocks on the same side as the supposed larger block will open below into its proper opposite block, but

above (on account of the first displacement) into the one lying in reality behind it. . . . The spirals when once started do not run on continuously, but end after passing around the body several times. The ending likewise finds its explanation in the inequality of the blocks of opposite sides." I did not in this preliminary paper consider a point with respect to the ending of these spirals that may be spoken of here.

If a spiral once started was dependent in order to end, on another accidental displacement appearing, so that the spiral is, as it were, satisfied, the chances are that most spirals would reach a prodigious length before terminating. As a matter of fact the spirals are generally short, the shorter the commoner. This shows, I think, that the conditions, after a spiral has been started, are of such a nature that the chances are the spiral will soon end itself. It is easy to offer a formal explanation of why this is so. The shifting of the blocks by the double union established at the anterior end[1] will tend to displace the block behind, so that two of one side will come to correspond to one of the other.

In the preceding section of the paper there are in reality two topics that have been discussed in close connection with each other. The question of the shifting of the reproductive openings was considered in connection with the origin of the compound metameres and spirals. The reason for doing so was based on the hope that the one relation might help in the interpretation of the other.

Moreover, in respect to the abnormal position of the openings of the reproductive organs, we have considered first those cases where, in worms with normal metameres, the openings have shifted or doubled; and secondly, those cases where there were both abnormal metameres and abnormal openings of the reproductive organs in the same worm. The former of these subjects has been already noticed by previous authors. Beddard (5) in 1886 published some most interesting observations on variations in the reproductive organs of Perionyx excavatus.

[1] It is assumed that the spiral starts at the anterior end. But it is also possible that the shorter spiral arrangement involves all the segments.

In a lot of 439 individuals there were found seventeen that showed such variations.

Benham (6) described in 1891 a variation in the openings of the reproductive organs of Lumbricus herculeus. The vasa deferentia appeared on the 14th and 15th metameres.[1]

With respect to the abnormal unions of metameres, and the relation borne to the position of the reproductive organs, there is little to be added to the conclusions reached in the sections dealing with abnormalities in front of or included in the 15th metamere. Two cases were there sharply separated,—those cases where there is a half-metamere more on one side than on the other (C, second case, E), and those cases where the spiral does not introduce an additional half-metamere on one side (B, C, first case, D).

The results from the latter cases are in full harmony with the interpretation of the spiral stated above. In those cases where an additional half-metamere is introduced there are certain examples that seem to verify the same statement; but there are other cases, and these form the majority, where the results will not bear this interpretation. We have, then, to assume, in some of the cases recorded, either that the half-metameres are not equivalent to the normal, or else that the reproductive organs do not give unfailing evidence of the change that has taken place. Anyone who has studied the facts will, I think, agree with me that it is far more probable that the reproductive organs have appeared on a wrong (?) half-metamere than that the half-blocks are not equivalent.

This leads us to another side of the problem, as yet not dealt with. It is natural to think of every half-block of one side as having a half-block that belongs to it on the other side; in other words, to look upon one of these blocks as predestined for the other, and we marvel to find them separated. I think there are no real grounds for such an

[1] Bateson's memoir, 'Materials for the Study of Variation,' that has just reached me, refers to numerous cases of abnormalities in the reproductive organs of earthworms recorded in a paper by Michaelsen, 'Jahrb. Hamb. wiss. Anat.,' viii, 1891. I have not had access to this paper.

expectation. If the facts recorded in the preceding pages indicate anything clearly, it is that the union of the blocks across the middle line is the natural result of their size and of their position. Under normal conditions the blocks are so placed that they unite pair for pair. If the order is disturbed they unite differently. This is brought out most strikingly in that case where there were 134 half-blocks on one side and 118 half-blocks on the other. Here throughout the body there are very few half-segments that are united with the one opposite bearing the same numerical relation from before backwards.

In the light of these statements I think we ought not to look to any one half-metamere as predestined to carry under all conditions the openings of this or that organ. Rather should we expect that those segments, which happen to get so placed in the body of the worm that they correspond to the normal position for a particular organ, will go ahead and develop that organ. We might say that the position in which an organ will develop is determined by a definite region of the body irrespective of how that region has gotten into the necessary position, provided this happened when the cells were still undifferentiated. The question is too large to discuss here, and the facts too meagre.

There is a liability to err if the statements made above in regard to the methods of union of the metameres be applied to all cases. All that I contend for is that the majority of cases will bear this interpretation. Sooner or later one is sure to come across individuals where it is impossible to see the application of the theory. Particularly have I found this true in the Polychæta. I have met such cases quite often in examining the regenerated anterior ends of worms where the head had been artificially cut off very obliquely. Sometimes pieces are left, where the new head joins the old body, that cannot be interpreted as half-metameres. Whether all of these unusual cases are the result of regeneration I am not prepared to say. Many of them seem to be; others, so far as I know, may have come in from the egg.

The results that have, on the whole, been the most puzzling to me, and which I cannot pretend to entirely explain, are some of those cases where the internal and the external spirals do not agree. It seems quite certain that in the far larger number of cases the false unions are at bottom, the result of imperfect joining of the mesodermic blocks, and that the ectodermic grooves mould themselves on the internal arrangement.

But in cases of disagreement it seems clear that the outer grooves have to a certain extent an independent individuality, and may behave differently from the spirals in the mesoderm. When we find similar spirals in antennæ, &c., it is not, after all, so surprising that the outer body-wall of the worm should show this independent variation.

IX. Modifications in Antennæ of Arthropods.

The antennæ of Arthropods are made up of a series of segments or rings. In some Arthropods the segments are long and relatively few in number. In others the segments are narrow rings and very numerous. The latter kind are more likely to show variations in the arrangement. The antennæ and antennules of the lobster (Homarus vulgaris) I found showed many variations. Perhaps the lobster's antennæ and antennules may owe many of these false arrangements to the fact that if they have been lost new ones will regenerate, but that all the abnormalities come from this source is extremely improbable.

I have found in the antennæ of the lobster nearly all the sorts of variation in the arrangement of the rings that are to be found in the earthworm. A few of these variations are shown in Pl. 43, figs. 90 to 95. Fig. 90, A, B, shows a compound ring. Fig. 91, A, B, shows a double compound ring, where three half-rings of one side are united to one of the other. Both of these modifications are common, particularly the former, at the broad base of the antennæ.

In nearly all cases the "splits" run in from the sides. Rarely a modification similar to that shown in fig. 92, A, B, is

found. Here the "split" is found over the broad surface of one side, and not over the other. This condition is never found in the falsely joined metameres of the Annelids, for even in those cases where a metamere appears below, and not above, it is also found to extend over and on one side (lateral) of the body.

Fig. 95, A, B, C, shows a spiral formed by a union of three compound rings above and one below, as shown in the reconstruction.

Fig. 93, A, B, C, shows a longer and more complicated spiral. In the middle of this spiral we find a "double spiral" developed.

Fig. 94, A, B, C, also shows a complicated spiral. These spirals in the antennæ are similar to the spirals found in the earthworm, and need no further description.

The antennules of the lobster also show the same modifications. Both the antennæ and the antennules are flattened from above downwards, and the splits extend as a rule on the lateral sides of the appendages. The basal joints of nearly all antennæ show imperfect rings. The greater number of abnormalities occur in the proximal portion of the antennæ, and are found in less and less frequency as far as the middle region. They are rarely found in the distal ends of the antennæ, and it is important to notice that distally the rings become much longer relatively to their breadth. Another interesting condition is found. By far the greater number of "splits" occur on the convex side of the antennæ and antennules, particularly in the latter. There results a larger number of half-rings on the convex side. In one case there were ten more half-rings on the convex side of one antennule than on the opposite side.

When we see that it is normal for the antennules to bend outward, i. e. to turn their convex side towards the middle line, it seems fair to draw the conclusion that the tendency of the antennule to turn to one side is the direct or indirect cause of the greater number of rings on the convex side. There is no reason for believing that the greater number of rings causes the turning, since the antenna is bent whether more rings are present on one side or not. Processes that go

on in the cells of the antennæ before each moult will determine where the new lines of division between the segments come in, and in some way the bent condition of the antenna seems to effect to a large extent the formation of the new lines.

I have also examined the development of the antennæ in the lobster. At the third (?) moult the terminal division of the endopodite of the antenna contains within it a rod of cells, and these show alternate constrictions to form the lines between consecutive rings. These lines of constriction are exceedingly near to one another, since the segment containing the new series is considerably shorter than the total number of segments that ultimately develop from it at the next moult. It is easy to see that under these cramped conditions the arrangement of the division lines might easily be disturbed by local causes.

An examination of the antennæ of certain insects where regeneration of the antennæ of the adult is not probable (and that in the larva hardly possible) shows that in those forms where there are a large number of rings, and each ring very short, variations exist in the arrangement, while in those forms with long rings such variations are absent (or not found commonly).

In the locust I have not seen any misformed rings in the antennæ (in as many as fifty individuals examined). In the cockroach (Blatta), where the rings are narrow, compound segments and short spirals are frequently found both in the larvæ and the adults.

X. Abnormal Metamerism of Locust.

It seemed to me highly improbable that such specialised metameric forms as the Crustacea and Insecta, in which the metameres are so definitely limited in function, number, and position, should show any variations comparable to those in the earthworm. However, I had brought to me a locust ("grasshopper") in which there was a half-segment more on one side of the abdomen than on the other.

In fig. 89, A, is drawn a dorsal view of this locust with the

wings removed. Corresponding to the 5th metamere of the abdomen we find a compound metamere, so that one half-metamere of the left side corresponds to two half-metameres of the right side. A figure of the abdomen from below is drawn in fig. 89, B, and the abdomen seen from the right side is seen in fig. 89, C. On account of the interpolated half-metamere the abdomen is bent towards the left. The interpolated segment reaches exactly to the median line above and below. The abdomen ends normally, having all of the accessory structures of the male perfect in all details.

Fig. 89, C, shows that each of the half-metameres on the right side of the compound metamere bears stigmata, so that there is one more of these on the right than on the left side of the body, i. e. eight on the right (one more than normal) and seven on the left.

If we assume each of the right halves of the compound metamere equivalent to a true half-metamere of the series, then each of the half-metameres following the compound metamere is joined to a half-metamere that is not its normal half. As a consequence, one new additional half-metamere will have to be formed at the end of the series on the right side to make a perfect ending to the abdomen. In so highly modified a form as the locust this view seems very improbable. An alternative view would be to suppose that a half-metamere has been intercalated between the 4th and 5th, or between the 5th and 6th, and that the intercalated half has united across the middle line to the 5th in common with the normal half of the 5th. We might think of this intercalation as due to a half of one metamere dividing into two, or we may suppose that when the ventral mesoblast broke up into a series of blocks it formed on one side one more block than on the other (one more than the normal). If the last view be true—and it seems more probable than any of the other suggestions—we see at once the futility of trying to explain the conditions on an assumption of predestined right and left half-metameres. When we recall that the whole ventral plate of the insect becomes segmented at the same

time, and when we recall the complicated modifications of the terminal segments, it seems highly probable that posterior to the compound metamere no shifting of the segments of one side can have taken place, and that no one segment has divided, but that the plate as a whole has broken up into a greater number of half-metameres on one side than on the other.

XI. Study of the Colour-bands of Echinoderms.

In the summer of 1891, while in Jamaica at the Marine Laboratory of the Johns Hopkins University, I began a study of the colour-bands on the arms of the Ophiurians in the hope of getting results that might help towards a solution of the problem of metamerism. The work was laid aside for two years, during which time the colour was so far removed from the alcoholic specimens as to render renewed study unprofitable. My earlier results, although not carried very far, point to certain conclusions that are not without interest in the present connection. In several species of Ophiurians ("brittle-stars") the arms are banded at regular intervals by pigment of a different colour from that of the rest of the arm. Each band of pigment is confined to a single segment—metamere—of the arm. In fig. 96 three such pigmented segments are shown. The colour of the band in this case is a rich orange-red, while the rest of the arm is an olive-green. Along the mid-ventral line there runs a longitudinal narrow line of orange-red pigment not shown in the figure.

We find that a definite number of uncoloured segments alternate with a coloured segment. Three uncoloured segments lie between the coloured segments, i. e. every fourth segment is coloured. Occasionally, however, the regularity of the arrangement is disturbed.

Fig. 97 shows a common variation. Instead of finding a fourth coloured segment between the upper and lower coloured segments of the figure, we find two consecutive segments coloured, each over half its extent. The middle line of the arm marks the extent to which each is pigmented, the first half-band lying to the right and the more distal to the left.

We see that the right and left sides of the arm vary independently, so that each forms its half-coloured band. In the present case something has disturbed the regularity of the process, so that the colour has appeared too soon on the right side, as the figure shows.

In fig. 98 we have a somewhat similar case. Here the half-bands are separated by an entire uncoloured segment. In other cases that I have seen the half-bands may be even farther separated by uncoloured segments.

From a study of these and similar variations we find that the following relations exist. When a segment is only half coloured on one side it is generally, though not invariably, followed, sooner or later, by a segment coloured only on the opposite side. We might say that the colour-ring had split, and its two halves had appeared on different segments, so that when we find a half-band on the right we would expect to find its other half on the left, and vice versâ.

When half-bands appear, one of the halves seems always to come in too soon proximally. The other half then appears on its normal segment or beyond it.

My material has been too limited to warrant any attempt at further explanation of the phenomenon, and no doubt a more extensive study would show exceptions to these statements that would render an explanation still more difficult.

The three following tabulations of the colour-bands show the main variations that come in. The × indicates a coloured ring. The figures between these indicate the number of uncoloured segments. The half-coloured bands are indicated by × L. ½ and × ½ R., depending whether the colour is on the left or right side of the arm as looked at from below.

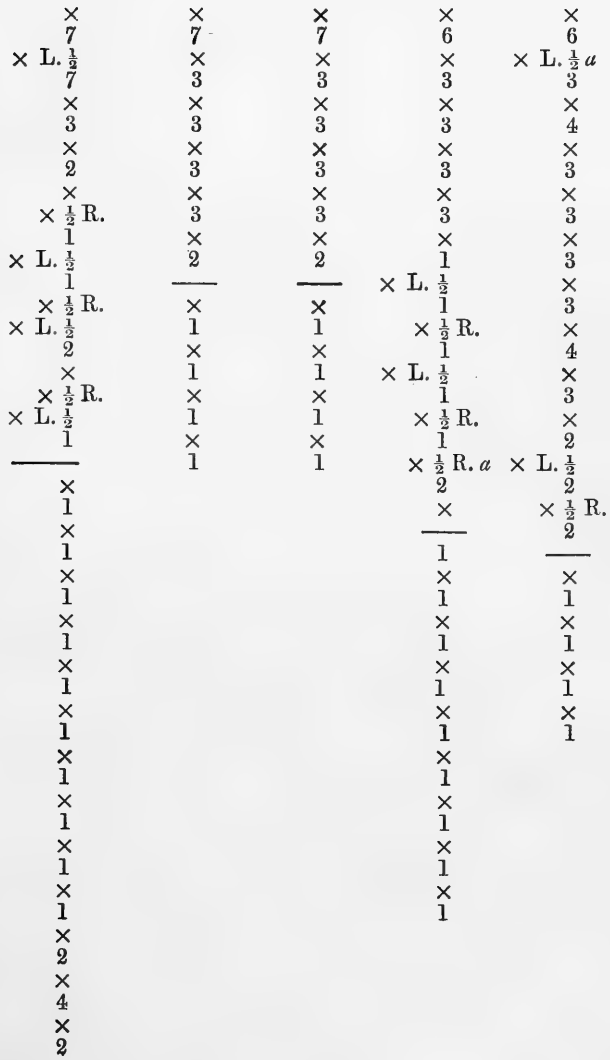
First Record.

×	×	×	×	×
3	3	3	×	2
×	×	×	3	×
3	3	3	×	3
×	×	×	3	×
3	3	3	×	3
×	×	×	3	×
3	3	3	×	3
×	×	×	3	×
3	3	3	×	3
×	×	×	3	×
3	3	3	×	3
×	×	×	3	×
2	3	3	×	3
× L. ½	×	×	3	×
2	2	2	×	3
× ½ R.	× L. ½	×	3	×
×	2	3	×	2
2	× ½ R.	×	3	× ½ R.
×	2	3	×	× L. ½
3	×	×	3	3
×	× L. ½	3	×	×
2	× ½ R.	×	3	3
×	2	3	×	×
3	×	×	3	3
Broken.	3	3	×	×
	×	×	3	3
	3	3	×	×
	×	×	3	3
	3	3	×	×
	×	×	3	3
	3	3	×	×
	×	×	3	3
	3	3	×	×
	Broken.	Broken.	3	3
			×	×
			3	3
			×	×
			3	3
			×	×
			3	3
				×
				3

SECOND RECORD.

×	×	×
3	3	3
×	×	×
3	3	3
×	×	1
1	1	×
× L. ½	× L. ½	× L. ½
2	2	1
× ½ R.	× ½ R.	× L. ½
× L. ½	× L. ½	2
2	2	× ½ R.
× ½ R.	× ½ R.	× L. ½
× L. ½	× L. ½	2
1	1	×
×	×	1
3	3	×
×	×	1
3	3	×
×		3
3		× L. ½
		× ½ R.
		1
		× L. ½
		× ½ R.
		2
		×
		3
		×
		3

THIRD RECORD.



A few of the more obvious relations that one finds in these tables may be pointed out. In some cases exceptions to the statements made above are found. At *a* in the third record, last (fifth) column, we see that a half-colour band is present on one side, but not on the other. And again this is seen at *a* in the third record, fourth column.

In the third record, first column, we find in one region many irregularities present, although taken altogether the same number of half-rings are present on the right and left sides.

In the third record each arm was found to have a new distal end that had regenerated. In this part every other segment was coloured. Whether this was a permanent or only a temporary coloration I cannot say.

It would be interesting to find out whether in the same individual similar variations showed a tendency to appear on the different arms. Even the limited data of the three tables give a slight amount of evidence in favour of such a view.

The regular arrangement of the colour-bands on every fourth segment finds an interesting parallel in certain Annelids, where the coloured rings bear a more or less definite relation to the metameres of the body.

Andrews (1) makes the following statement in regard to the arrangement of coloured bands in the polychætous Annelid Proceræa tardigrada, which belongs to the family Syllidæ:—"The female has a dark dorsal transverse band upon Somites 3, 6, 8, 9, 13, 17, 21, 25, 27, 29, 32, 35, 38, 42, 46, 49, 51, 53, 56, 57, 70, 71, 74, 77. . . . The non-sexual form has pigmented bands like those of the female, but arranged according to a definite law or general rule, to which the bands in the female conform also; bearing in mind that the female is formed as a cut-off part of the non-sexual stage, separating almost always just posterior to the thirteenth somite, and hence having thirteen less somites than that stage. In 110 individuals carefully studied, only three had the bud formed just posterior to the fourteenth somite; seventy-nine had an evident bud just posterior to thirteenth somite.

"Having tabulated the arrangement of the coloured bands in

these 110 individuals, there results the general rule that the bands occur upon the third and fourth somites, then upon every other or alternate one up to and including the twelfth, then (in the region of the bud) upon every fourth one up to and including the twenty-fifth, then upon every fifth one up to and including the forty-first, after which the exceptions become so numerous that no rule is evident. The examination of so many cases shows a definite tendency to limitation in the bands to certain somites in the anterior region, and a greater and greater irregularity in the posterior region." After illustrating some cases of failure in the normal arrangement of the bands, Andrews adds, "These facts seem sufficient to indicate that we have in this Syllid a marked tendency to the acquirement of a regular metameric marking, which, however, does not coincide with the metamerisation of the somites, but tends to follow a special law best expressed in the oldest part of the body in which certain alternating coloured and not coloured somites are distinguishable—a series of groups or combinations of somites thus following one another."

It is not without importance to find in the typically metameric Annelids regular serial markings following definite laws in each portion of the body. Groups of metameres seem here to act as a unit. This case is certainly paralleled by the colour-bands on the arms of the brittle-stars. The serial repetition of the appendages of the Crustacea furnish examples of somewhat similar regional variation. It is not improbable that if we find an explanation for one set of phenomena we will be able to explain them all.

XII. Regeneration in Earthworms.

In the winter of 1887–8 I made a small number of experiments to determine the extent of regeneration in the earthworm. Again, in the spring of 1892, another series of experiments were started, but an accident spoiled the results. In the past winter of 1893–4 I made a more elaborate and systematic attempt to work out the same problem. I am much

indebted to Miss Elizabeth Nichols, Fellow in Biology, Bryn Mawr College, who began this study of regeneration with me. Many of the early experiments were largely carried out by her; and later, when the work devolved on me, I profited much by the results of the previous work.

There were several main problems that I wished to work out. First, the extent to which the earthworm could regenerate; secondly, the number of new segments that would reappear in the anterior end after the removal of a definite number; thirdly, the presence or absence of abnormalities in the regenerated anterior segments.

Certain rough results were at first obtained, which showed that when many segments were cut off only a few segments replaced them. That is to say, there was no apparent connection between the number of segments that were cut off and the number that regenerated. Four and rarely five new segments came back.

I then set to work to determine what result would follow when only a few segments were cut off, for obviously if four came back when one, two, or three were cut off, the result would appear as though the reproductive organs had all shifted posteriorly. The tables below that are first given show the results of these latter experiments.

One of the most conspicuous results was the great decrease in size that the worms suffer during the period of regeneration. When only a few segments were cut off the regeneration was soon accomplished, and no great decrease in the size of the body of the worm was obvious; but where many segments were cut off, and regeneration only took place after several months, or not at all, the body dwindled until it got to be less than a half of its original size. I have not made a histological study of these worms to determine what organs have suffered most during the period.

The worms used were L. (or Allolobophora) fœtidus, which live in manure heaps. They were kept in ordinary flower-pots filled with the manure in which the worms were found living. The pots stood in about an inch of water, and each was covered

with a glass plate. The pots stood in a large glass case in one of the rooms of the laboratory. The temperature of the room varied from about 70° F. during the daytime to about 50° F. at night. Under the same conditions the normal worms kept perfectly healthy.

TABLE I.

Two segments cut off, 3/16, '94.
Killed, 4/28, '94.

2 segments regenerated	. . .	vas def. 15
2 „ „	. . .	„ 15
2 „ „	. . .	„ 15
2 „ „	. . .	„ 15

Two segments cut off, 3/4, '94.
Killed, 4/28, '94.

2 segments regenerated	. . .	vas def. 15

Two segments cut off, 3/16, '94.
Killed, 5/5, '94.

2 segments regenerated. Sem. recept. 9, 10, 11 (normal)

TABLE II.

Three segments cut off, 3/4, '94.
Killed, 4/28, '94.

3 segments regenerated	. . .	vas def. 15
3 „ „	(and piece of 4)	
3 „ „	. . .	„ 15
3 „ „	. . .	„ 15
3 „ „	. . .	„ 15
3 „ „	(and small piece of 4)	
2 „ „	(with indications of a third)	„ 14
2 „ „	„ „	

Three segments cut off, 3/16, '94.
Killed, 4/28, '94.

3 segments regenerated	. . .	vas def. 15
3 „ „	. . .	„ 15
2 „ „	(sem. recept. 8, 9, 10)	
3 „ „	(and little piece of 4)	„ 15

Three segments cut off, 3/16, '94.
Killed, 5/5, '94.

2 segments regenerated (sem. recept. 8, 9, 10)
2 „ „ („ „) vas def. 14

Table III.

Four segments cut off, 3/16, '94.
Killed, 5/5, '94.

4	segments regenerated	. . .	vas def.	15
3	,, ,,	. . .	,,	14
3	,, ,,	. . .	,,	14
3	,, ,,	. . .	,,	14
4	,, ,,	. . .	,,	15
3	,, ,,	. . .	,,	14
3	,, ,,	. . .	,,	14
3	,, ,,	. . .	,,	14
2	,, ,,	(+ 1/3 of 3) .	,,	13
3	,, ,,	(sem. recept. 8, 9, 10)		
4	,, ,,	. . .	,,	15

Four segments cut off, 3/4, '94.
Killed, 4/28, '94.

3	segments regenerated	. . .	vas def.	14
4	,, ,,	(+ ½ of 5th) (3rd segment imperfect)	,,	15
4	,, ,,	(a small piece of 4 had been left) .	,,	15

Table IV.

Five segments cut off, 3/16, '94.
Killed, 5/5, '94.

4	segments regenerated	. .	vas def.	14
3	,, ,,	(+ ½ of 4th) .	,,	13

Five segments cut off, 3/16, '94.
Killed, 4/28, '94.

4	segments regenerated.	
3	,, ,,	
4	,, ,,	

Five segments cut off, 3/4, '94.
Killed, 4/28, '94.

4	segments regenerated	(+ ½ of 5th) .	vas def.	15
2	,, ,,	(+ ½ of 3rd) .	,,	13

Table V.

Attempts made to cut off 1 and 2 segments, 3/4 and 3/16, '94.
Killed, 5/2, '94.

1 segment regenerated	. . .	vas def.	14
1 „ „	. . .	„	15
1 „ „	(indications of a division into 2) .	„	14
2 segments „	. . .	„	15

Conclusions from Tables I—V.—In all cases recorded (6 cases) where two segments were cut off two regenerated.

When three segments were cut off (14 cases) three grew back in nine worms and two grew back in five worms.

In those worms (14) where four segments were cut off, four segments regenerated in five worms, and three segments grew back in eight worms, and two segments and a third[1] in one worm.

When five segments were cut off in no case did five grow back. In some of these worms a little more than five must have been cut off, so that a small piece of the sixth was taken off (see Nos. 2 and 6 and 7 of Table IV) and regenerated. In four worms four segments grew back, in two worms three segments grew back, and in one worm only two segments grew back.

Taking these four tables together, we see that up to four segments lost, and including this, there is a tendency for the worm to reproduce the number lost. This was actually done in all the cases where two segments were lost, in nine cases out of fourteen where three were lost, and in five cases out of fourteen where four were lost. More than four segments the worm does not seem to be able to regenerate, as a rule.

The data given in Table V are not as accurate as in the preceding tables, because there was some confusion in the numbers of the pots containing these worms. So far as the figures go, they show that in three cases two segments must have been cut off, that one individual then regenerated two, and two

[1] The amputation was oblique, and cut off a part of the fifth segment.

individuals regenerated only one segment each. In one case one segment must have been cut off and one regenerated.

The results of these tables have a direct bearing on the conditions found in adult worms, and recorded in a previous section. Those worms in which both openings of the vasa deferentia were found on a segment anterior to the fifteenth may have been, in most cases, the result of the loss of anterior segments, and a subsequent incomplete regeneration. We cannot affirm that all cases were the result of the process, because, in the light of other facts, it is not improbable that such variations may have come from the embryo.

These records also show us that regeneration of the anterior end will not account for any of those cases where the vasa deferentia open on a metamere posterior to the fifteenth, for in no cases were more segments generated than amputated.

What the result would have been in the cases recorded below where a worm regenerated many segments, after amputation far posterior to the fifteenth segment, I cannot tell. The worms were not kept for a long enough time to determine whether or not reproductive organs would ever have appeared.

In the preceding and following tables it will be noticed that in many cases the position of the openings of the vasa deferentia is recorded, and when these were not found the segments containing the seminal receptacles (9—10—11 normally) are recorded. These landmarks were located after regeneration had taken place. This served as a check for those cases where the number of segments amputated had been previously recorded, and in the other cases gave fairly accurate evidence as to the number of segments that had been cut off.

In the next two tables,—the results of the first series of experiments,—a large number of recorded segments were cut off to find the limit of the power to regenerate anterior segments.

TABLE VI.

Segments cut off, Oct. 12, '93.

No. cut off.	No. of worms.	Nov. 14—	Jan. 1—
10	(4)	(1) 4 segments regenerating. (1) 4 „ „ (1) 4 „ „ (1) Imperfect.	Same as Nov. 14

		Jan. 30—	May 5—
12	(5)	(1) ... (1) ... (1) ... (1) ... (1) ? 5 segments regenerating.	(1) 6 segs. regen. ... (1) 5 segs. regen. (1) $3\frac{2}{3}$ „ „ ...

		Jan. 30—	Apr. 14—	May 18—
14	(5)	(1) 5 seg. regenerating.	...	...
			(1) Imperfect.	(1) (1) (1) (1) } Dead.

		Jan. 30—	Apr. 14—	May 18—
16	(4)	(2) Alive.	(1) Alive.	(0) Alive.

		Jan. 30—	April 14—
19	(2)	(2) Both regenerating.	(1) Had regenerated 4 or 5 segments. (1) ?

		Jan. 30—
19—24	(5)	(1) Regenerating imperfectly.

		Jan. 30—	
24	(5)	(3) Not regenerating.	(0)

		Dec. 2.	Jan. 3—	Jan. 30—	Apr. 13—
26	(5)	(3)	(2)	(2)	(0)
27	(3)	(2)	(2)	(1)	(0)

Table VII.

Segments cut off, Jan. 12, '94.

No. cut off.	No. of worms.	Apr. 14—	May 18—	
19	(1)	(0)		
21	(1)	(0)		
22	(3)	(2).	(1) Regenerated imperfectly.	(2) Very imperfectly regenerated.
23	(1)	(1)	„	(0)
24	(1)	(0)		
25	(1)	(1)		(1)
26	(1)	(1)		(0)
27	(1)			(0)

The results from Tables VI and VII show that one cannot say definitely, "here the power of regeneration ends." The figures show that some worms regenerate where others fail to do so. It may be that the possibilities are different for different worms, or that at the time of operation certain worms were in better condition than others, or the external conditions (bacteria, &c.) may have been different in different cases.

The tables show that posterior to the twelfth segment the power of regeneration rapidly decreases. Worms that have lost more segments than this number may live for some time, and heal up the wound, or even regenerate imperfectly. But sooner or later the majority of these die. Occasionally remarkable exceptions are found. In Table VI the fifth record shows that a worm that had lost nineteen segments regenerated four or five new ones, and in the next tables more remarkable cases still will be recorded.

It is a tedious operation cutting off a definite number of segments from a living worm. In the three following tables the number of segments cut off was not counted at the time, but could be calculated with approximate certainty after regeneration by the position of the vasa deferentia or segments containing the seminal receptacles, or, when the amputation was behind these, something like an approximation could be obtained by utilising the anterior end of the clitellum, or even the posterior end of the body.

TABLE VIII.

Anterior segments cut off, 1/22, '94.

Killed, 4/13, '94.

No. of segments regenerated.	Vasa def.	Calculated No. cut off.
3	13	5
3 ($\frac{1}{2}$)	13	5 ($\frac{1}{2}$)
3 ($\frac{1}{2}$)	14	4 ($\frac{1}{2}$)
3	12	6
3	12	6
3 (irregular)	14	4
3	14	4
4	12	7
4	12	7
4 (irregularly united to body)	13	6
4	12	7
4 ($\frac{1}{2}$)	13	6 ($\frac{1}{2}$)
4 ($\frac{5}{6}$ + $\frac{1}{2}$)	14	5 ($\frac{5}{6}$ + $\frac{1}{2}$)
5	15	5

Anterior segments cut off, 1/22, '94.

Killed, 5/5, '94.

No. of segments regenerated.	Vasa def.	Calculated No. cut off.
3	13	5
3 ($\frac{1}{2}$ + $\frac{1}{2}$ + $\frac{1}{2}$ + $\frac{1}{2}$)	15	3 (+)
3 (with compound 1—$\frac{1}{2}$)	14 (or 14—15)	4
3 ($\frac{3}{4}$ + $\frac{1}{4}$ irregular)	13	5 (+)
3 (irregular)	12	6
4	12	7
4	12	7
4	14	5
4	12	7
4 ($\frac{1}{2}$)	14	5 ($\frac{1}{2}$)
4 ($\frac{2}{3}$)	12	7 ($\frac{2}{3}$)
5 ($\frac{1}{2}$)	14	6 ($\frac{1}{2}$)

Table IX.

Anterior segments cut off, 3/4, '94.

Killed, 4/28, '94.

No. of segments regenerated.	Vasa def.	Calculated No. cut off.
1	14	2
1	14	2
1 ($\frac{1}{2}$)	(vas def. double $\frac{14}{15}$ — $\frac{14}{15}$)	1 or 2 (?)
3 ($\frac{2}{3}$)	15	3 ($\frac{2}{3}$)
3		
3 ($\frac{1}{2}$)		
4		
4		
4		
4	vas def. 13	6
4	14	5

The Tables VIII and IX add a large number of data to those of the preceding tables. It will be noticed that there is one definite case of five new segments coming in for five cut off. There are also a number of irregular methods of union of new and old parts, and several cases of imperfectly formed new rings. The next table covers much the same ground as the preceding, but is interesting because the worms had been kept for a much longer time than those in the preceding table, and because the last record shows a case where from thirty to forty segments were in all probability cut off, and yet three and a half segments had regenerated.

TABLE X.

Anterior segments cut off, 10/18, '93.
Killed, 2/11, '94.

No. of segments regenerated.	Sem. recept. (Normal, 9—10—11)	Vas. def.	Calculated No. cut off.
2 ($\frac{2}{3}$ + $\frac{1}{4}$ irregular)	5—6—7	11	6 (+)
3	7—8—9	13	5
3	4—5—6	—	8
3 ($\frac{1}{3}$) (worm very small)	—	10	8 ($\frac{1}{3}$)
4	5—6—7	—	8
4	8—9—10	—	5
4	—	12	7
5 (irregularly joined)	—6—7 (?)	—	9 (?)
5	—	11	9
5 (small piece)			
5 (very small piece)			
4 ($\frac{6}{7}$)	—	13	6 ($\frac{6}{7}$)
6 (+ $\frac{1}{2}$)	—7—8	—	10 ($\frac{1}{2}$)
3 ($\frac{1}{2}$) small worm, with only 65 old segments and old anus.			30—40

In addition two other worms, where union of new head and body was too irregular to count even approximately the segments.

TABLE XI.

Anterior segments cut off, 11/15, '93.
Killed, 4/14, '94.

No. of segments regenerated.	Sem. recept.	Calculated No. cut off.
3	9—10—11	3
5	6—7—8	8
4	—	8

Not regenerated	10	in front of	clitellum	15
,,	6	,,	,,	19
,, (mouth present)	9	,,	,,	16
Scarcely regenerated (small piece)				
3 or 4 very irregular	9	,,	,,	16
3 imperfect segments	8	,,	,,	17
4 (1st partially divided)	17	,,	,,	8
4 ,, ,, above)	15	,,	,,	10
5$\frac{1}{2}$ (1st imperfect)	15	,,	,,	10
15 or more regenerating	63 segments in front of anus			35—40

This table is interesting because it shows a large number of

worms regenerating imperfectly a few segments in front of the clitellum. Moreover the last case is particularly instructive, because here again 30—40 segments were probably cut off. The number of worms that were operated on at the beginning of this experiment was unfortunately not recorded; hence we do not know what the per cent. of mortality has been.

TABLE XII.

Anterior segments cut off, 11/18, '93.
Killed, 1/21, '94.

No. of segments regenerated.	Sem. recept.	Vas. def.	Calc. No. cut off.
2	—3—4	8	9
3	—	10	8
3 (united irregularly)	8—9—10	14	4
3 (+ $\frac{2}{3}$ + $\frac{1}{3}$)	6—7—8	12	6 (+)
4 (+ $\frac{1}{3}$)	6—7—8	12	7 (+)
4 (+ $\frac{1}{2}$ + $\frac{1}{2}$ irregularly joined)	—	11	8 (+)
2	3—4—5	9	8
3	—4—5	—	9
3 ($\frac{2}{3}$)	5—6—7	—	7 (+)
4	7—8—9	13	6
4 ($\frac{1}{2}$)	—6—7	—	8 ($\frac{1}{2}$)
3	13 in front of clitellum		12
3 ($\frac{1}{2}$)	11 „ „		14
3	12 „ „		13
3 (or four imperfect)	just „ „		23
4	17 „ „		8
4	14 „ „		11
5	16 „ „		9
5	14 „ „		11
2 (or three very imperfect)	4 „ „		21
2 (or three very imperfect)	14 „ „		11
Very imperfectly healed	7 „ „		18
Imperfectly healed	12 „ „		13
Healed—evidence of few rings	9 „ „		16
Beginning to regenerate	10 „ „		15
„ „	15 „ „		15
Healed, not regenerated	15 „ „		10
Healed—regenerating (?)	5 „ „		20
8 { or more segments. Rings faint. Small piece }	56 old segments in front of anus.		

In several instances in the preceding tables records are found of worms that, in addition to the formation of new segments, completed parts of segments accidentally cut off in the operation. The following records are of worms in which purposely very oblique amputation had been made.

TABLE XIII.

Anterior segments obliquely amputated, 3/4, '94.
Killed, 4/28, '94.

8	segments	completed		(9—10—11)	sem. recept.	vas. def.	—
9	„	„ (dorsal)		—		„	15
11	„	„ (lateral)		—		„	15
12	„	„		(9—10—11)	„		—
4	„	„ (lat. vent.)	+1 new seg.	(9—10—11)	„		—
4	„	„ „	+3 new segments	—		„	15
6	„	„ (lateral)	+3 „	—			—
7	(1/8) „	„ (lateral)		—			—
7	„	„ (lateral)	+2 „	(8—9—10)	„		—

The last table shows very clearly that the power to complete segments that have in part been removed is much greater in the earthworm than the power to regenerate whole segments. In the table we find as many as twelve segments completed, and in a comparatively short time. Such a number of segments is never or rarely regenerated when the anterior segments are cut squarely off.

The latter records of this same table show that when anterior segments are entirely cut off, in addition to those cut obliquely, that we have both a regeneration of those lost (within a limit) and a rebuilding of those injured. Moreover, although the results are too few to speak with entire confidence, it would seem that the presence of segments completing themselves does not interfere with the formation of the full complement of new regenerated segments.

These facts, it seems to me, throw an interesting light on the problem of regeneration. They are too few to warrant at present any speculation. It is my intention to make a fuller and more accurate study of these phenomena.

The obliquity of the cut was lateral in most cases, in a few

the slice was taken off the dorsal surface, and in others off the ventral.

In a few cases no record was made. Whether or not the injured rings complete themselves more readily at the side than dorsally or ventrally cannot be determined from the table, for the position of the parts cut off in the first instance was not recorded.

In connection with the preceding experiments another was carried on. The anterior ends that were cut off were in many cases kept alive to see what power of regeneration remained in them. It became evident very soon that the length of the life of the pieces was in a general way proportionate to their linear length. Those with a few segments died in the course of a week; those with more lived longer, &c. No cases of survival of as few segments as fifteen were ever found. The two following tables show to what extent longer pieces remained alive, but with one exception (twenty-four anterior segments) they all died after a time. It is surprising to find this to be the case, for such pieces contain the mouth (so that the piece may feed) and all of the important (?) organs of the body.

TABLE XIV.

Record of anterior segments.

Date—Oct. 12. No. of anterior segments.	No. of worms.	Nov. 14.	Dec. 2.	Jan. 4.	
12	(5)	(1)	(0)		
14	(5)	(4)	(2)	(0)	
16	(4)	(0)			
19	(2)	(2)	(1)	(0)	
19—24	(5)	—	(2)	(0)	
24	(5)	(5)	(4)	(3)	May 5 (0)
26	(5)	(5)	(3)	(1)	Apr. 26 (1)

Jan. 12.		Apr. 14.	May 5.	May 18.
19	(1)	(0)		
22	(3)	(3)	(3) (?)	(0)
23	(1)	(1)	(0)	
24	(1)	Regenerated a half-inch.		
26	(1)			(0)
27	(1)	(1)		(0)

The worms that had the anterior segments amputated, the history of which is recorded in Tables I—XIII, were examined from time to time during the period of regeneration. One striking result was often apparent. When the amputation of the anterior segments had taken place obliquely, the new segments grew out approximately at right angles to the cut surface. It was not uncommon to find a new regenerating anterior end with its axis inclined at as much as (or even more than) 45° to the long axis of the body. I have no records as to whether the same thing happens when the oblique surface is above or below. Those referred to were from lateral oblique sections.

This result is comparable to that of Barfurth on the regeneration of the tadpole's tail. Here the new part appeared at right angles to the cut surface, and subsequently swung round into line.

To get the same result from the tail of the tadpole and the head of an earthworm suggests that there is some fundamental law of growth underlying both phenomena that should be more extensively investigated.

It was my intention to examine the power of regeneration in young worms for comparison with the adult, but only a few experiments have been made, and there has not been sufficient time to allow the completion of this side of the work.

Miss Adelene M. Fielde (**14**) has published a few fragmentary notes on the power of regeneration in L. terrestris. Pieces containing twenty to thirty segments from the posterior end of the worm lived forty days, but did not regenerate at either end. In these pieces new half-segments had been inserted, the authoress affirms, because she could not find any such modifications (compound metameres) in other worms! In nine worms five anterior segments were amputated. These "wholly regenerated." In ten worms five anterior and twenty to thirty posterior. These were found regenerating.

XIII. General Conclusions.

The solution of the problem of metamerism has often been attempted by morphologists with varying success. It has become more and more evident that the problem is a difficult one, and I think the results have shown that the final solution can only come with a better knowledge of the fundamental relations of the parts of the body to one another, with an acknowledgment of the imperfection of the phylogenetic method, and with a better insight into ontogenetic laws.

Darwin said over thirty years ago, in the 'Origin of Species,' "We need not here consider how the bodies of some animals first became divided into a series of segments, or how they became divided into right and left sides with corresponding organs, for such questions are almost beyond investigation." The attempt of morphologists to solve even the simpler of these phenomena, viz. metameric repetition, shows how true Darwin's words remain even to-day.

It may, therefore, seem doubtful whether anything will be gained by a new analysis of the problem of metamerism, or by a critical consideration of the numerous theories already advanced. It would certainly be unwise to add any new speculation to that already afloat. In the following pages no new theory is offered, and I have attempted no more than a consideration of those methods which have proved sterile or erroneous as contrasted with the methods that have been fruitful and suggestive.

During the last fifteen years the methods of the phylogenists have been applied to the solution of metameric repetition. Comparative anatomy has been the point of departure; but speculation has leaped far beyond its legitimate boundaries. The results have shown that the method of phylogenetic interpretation is subjective rather than objective, and the conclusions have given, therefore, at most a probable course of evolution, and often only a conceivable process of transition.

It is only fair to say that in many cases the speculation has been advanced tentatively, as suggestion rather than conclu-

sion, and the admirable work on which the speculation has been based has been in no degree vitiated by the attempts of the authors to push their conclusions beyond the limits deducible from their immediate results.

The Cœlenterates have formed the basis of Sedgwick's theory (34) of the origin of metamerism. The radial actinian has been worked over into a bilateral metameric form, and all the details of structure of the higher forms (cœlom, nephridia, gill-slits, trachea, &c.) have been evolved from the hypothetical actinian ancestor. Wilson (37) has supported the same view, "but only as a suggestion for further investigation of the facts." The Turbellaria have been the starting-point of Lang (25) and Meyer (29); but each author has described an entirely different course of transition from the flat-worm to the Annelid.

The Nemertian claims have been pressed forward by Hubrecht (22) and Balfour (2), and hinted at by others.

The Echinoderms have proved refractory, yet Wagner[1] has made out a possible phylogeny from these to metameric forms, and Haeckel (15) reversing the process, made a star-fish out of five fused Annelids.

The Enteropneusta, declared unsegmented by Bateson (3) and segmented (32) by the present writer, have been believed, nevertheless, by both authors to throw light upon the problem of metamerism.

With this divergence of opinion it is not surprising to find as great a divergence of method. In one case a radial form has given the starting-point, in the others a bilateral form. But the ways in which bilateral forms have been transformed into the metameric form have been very different. The budding theory has played perhaps the most conspicuous part. Nearly all of the older writers looked upon segmented forms as animal colonies—Quatrefages, Cuvier, Owen, Duges, Geoffroy-St. Hilaire, Lacaze-Duthier, Herbert Spencer, and Perrier. The same idea is often found in later speculation as well. In

[1] Quoted on the authority of Meyer (29). I have not been able to find the original.

the budding theory a short bilateral form is supposed to have elongated by a repetition of itself to form a long chain of united individuals. Other authors—Hubrecht, Lang, Meyer —have supposed metameric repetition to have appeared in a long animal which split up secondarily. Hubrecht (22, 23) has supposed that a long animal, such as a Nemertian, is continually in danger of injury from without, and the animal has met this danger by acquiring a remarkable power of regeneration! Those animals that had the main organs of the body repeated were better able to regenerate any pieces that were broken off; for such pieces would contain, in all probability, all of the essential organs of the body.

Lang's (23) description of the origin of metameric repetition from the flat-worm, Gunda segmentata, is too well known to need rehearsal.

In this elongated form the repetition of the digestive diverticula have been the centres around which the metameres have been built up.

Meyer (29) has contended that the repetition of the metameres is an expression of the alternate bendings of the sides of the body of a free-swimming elongated worm. A pair of elongated gonad-pouches have been broken up into a series of metameric compartments, owing to the swimming movements of the body, and around these as centres the metameres have evolved.

The attempts to find the solution of metamerism within the metameric groups have been equally unsuccessful. The simpler Annelids, such as Polygordius and Protodrilus, have been interpreted as archaic forms by Hatschek. This explanation has been rejected by Kleinenberg, Eisig, and Meyer. The lower Vertebrates have been equally difficult to interpret. Lankester (28) found that the tail of Appendicularia showed muscle-plates with corresponding ganglia in the nerve-cord. The Ascidian larva has no similar structures. Brooks (8) has argued that Appendicularia is a very old archaic form; while Willey (39) has interpreted the tadpole larva of the Ascidians as a secondary larval form derived from a fixed ancestor. By inference, therefore, Appendicularia is a sexually mature larva,

and its segmented tail either an antefact or a secondary acquirement.

Bateson (3) has attempted to show that Balanoglossus is related to the Chordata, and is unsegmented. I (32) have defended the same supposed relationship, and described Balanoglossus as segmented. Spengel (36) believes Balanoglossus not to be related at all to the Chordata.

Amphioxus is a typically segmented form, but has given no clue as to the origin of its metamerism.

By many morphologists (Semper, Dohrn, Van Wijhe, Minot, &c.) the group Chordata is supposed to be derived directly from segmented Annelids. But the Annelid-vertebral connection has as many opponents as defenders, and is one of the most illustrious results of the phylogenetic method.

It must be admitted, of course, that morphologists are dealing with complex and difficult problems in attempting to unravel the past connections between the larger phyla of the animal kingdom, and that their speculations have been in many cases ingenious working hypotheses; but the results show, I think, that the method is easily carried too far, and that, after many trials, it has not led us to any definite conclusion.

Ontogeny has been also fruitful in speculation. The cry has been that Ontogeny tended to repeat Phylogeny, and larval forms without end have been set up as archaic remains.

The rise, culmination, and decline (?) of the Gastræa theory illustrates in a concrete case the history of the ontogenetic method; and the Nauplius theory teaches a lesson of caution that ought not to be forgotten. The cœlom theory, built up on the splendid results of Agassiz, Metschnikoff, Kowalewski, Lankester, Hatschek, and Hertwig, while a most suggestive working hypothesis, has led to no settled conclusion; for the well-ascertained fact that in many groups (Sagitta, Amphioxus, Brachiopods, Echinoderms, &c.) gut-pouches give rise to the cœlom has not led us to any decision as to whether we have here an ontogenetic performance or a phylogenetic repe-

tition. In the Annelids, for instance, where there is a typical cœlom developed, there are no traces of gut-pouches, and this has to be interpreted as a secondary loss. Hatschek has suggestively remarked (15), "The two mesodermal teloblasts may correspond to the cœlom-sacs from which they were derived by a reduction in the number of their cells. In fact, we find this method of formation only in those cases where the number of cells of the embryo is very small."

Whatever be the conclusion reached as to the formation of the cœlom, we shall still be far from a decision as to how and when the cœlom repeated itself in the metameric forms.

The trochosphere of the Annelids, Mollusca, and Molluscoidea (?) has been utilised by Hatschek to support the whole family tree of the higher Metazoa (the Vertebrates perhaps excepted).

Kleinenberg has interpreted the trochosphere as a recapitulation of a hydro-medusa. E. B. Wilson and others have rejected the trochosphere ancestry, and believe the larva to be cœnogenic. Whitman wrote in 1887, "In spite of volumes devoted to the discussion of the subject, the larva of Polygordius still remains a morphological puzzle."

The trochosphere theory got a strong support from Semper's discovery of Trochosphæra æquatorialis.[1] Even Korschelt and Heider, who, as a rule, are most circumspect in accepting embryological speculation, wrote in 1890, "Höchst wahrscheinlich liegt in der Trochophora der Anneliden die ontogenetische Recapitulation einer Stammform vor, welche den Anneliden Mollusken und Molluscoiden gemeinsam war und von der aus sich diese Thierstämme als selbstständige Gruppen abzweigten."

Recognising the relationship existing between the trochosphere larvæ of Mollusca and Annelida, embryologists have not been satisfied to postulate only the archaic nature of the larva,

[1] Semper's Rotifer was known long before Hatschek's theory, and suggested to me the name "trochosphere" for the larval form ('Devel. of Lynnæus,' 1875), which some years later Hatschek adopted from me with the change of the word to "trochophore."—E. Ray Lankester.

but have gone further, and postulated it as the ancestral adult. They have been led to believe that such a minute few-celled larva has evolved into the complicated segmented Annelid, and into the equally complicated but unsegmented Mollusc. The resemblances between the adult Mollusc and Annelid they have been content to call "parallel developments."

For all the evidence we have at present we might satisfy the facts just as well by assuming that a large many-celled unsegmented form stood as the bottom of these two groups, having a trochosphere as its larval form. The Rotifers and related forms would then be interpreted as arrested forms. The one conclusion would be, I think, as justifiable and as easily maintained as the other, and both impossible to demonstrate.

In the face of so much conflicting embryological indecision, it seems to me we have in reality arrived with certainty no nearer to the solution of metamerism.

There have been only a few attempts to explain the metameric repetition as the result of mechanical action. Hubrecht's (22) explanation for the Nemertian is scarcely a mechanical explanation, since it presupposes a repetition of parts and an ability to regenerate in the worm itself. Kennel's (24) ingenious attempt to interpret division of animals as the result of external stimuli is scarcely a mechanical explanation.

His (19) has attempted to explain the metamerism of the Vertebrate as an embryological phenomenon—as the result of series of breaks occurring in the two lateral mesodermic sheets of the embryo. Meyer's (29) view, referred to above, is distinctly a mechanical hypothesis. The repetition that he assumes to have come into a long Nemerto-Turbellarian ancestor was the result of the movements of the body in swimming. Many objections are easily raised against Meyer's romantic speculation. As Hatschek has pointed out, those Annelids that are adapted for swimming are heteronomously segmented, while Meyer's hypothesis seems to demand first a homonomously segmented form as the result of its own activity. In the second place Meyer's sketch starts off on Lamarckian

principles. Although many naturalists still admit the principle of use and disuse as a factor of organic evolution, perhaps an equally large number reject the explanation. Hence, until we get definite proof of the truth or falsity of any such theory, it ought not to be used as the starting-point on which to build up other or new theories.

Caldwell (11) has offered a brief and interesting attempt to explain metameric repetition. So brief, indeed, is the presentation, and so obscurely worded, that I am not certain that I have entirely understood the meaning of the author. It is a mechanical theory par excellence. The theory assumes that as early as the blastula[1] stage the endoderm, as well as the mesoderm, is represented in cells or groups of cells at the surface of the sphere. Now the endoderm may before invagination get separated into two parts, owing to an early elongation of the blastula, so that when gastrulation sets in it may take place at two regions of the surface. One of the regions may contain much more endoderm than another, giving an oral or an anal gastrulation as a result. Similarly the mesodermal "Anlage" may be pulled apart and turn in with the endoderm at one region or the other, or may even have been left along the line where the two endodermal masses separated.

In many cases the whole of the mesoderm may be turned into the gastrula cavity with the endoderm, and subsequently set itself free from the endoderm by one, two, or many gut-pouches. When many gut-pouches arise, they mark the beginning of metameric repetition. The reason for many pouches appearing in some forms is to be explained as due to an early elongation of the invaginated endoderm forming the archenteron, so that the mesodermal "Anlagen" get pulled out and broken apart.

If, as Caldwell supposed, there is pre-formation for the mesoderm, there must be for all the other organs of the body, and this the author admitted.

[1] The author says planula, which makes his explanation obscure; unless he means technically a blastula.

It is difficult to see how by simple elongation of a larva to meet a supposed larval advantage, in the first place the organs in the ectoderm should get separated into exactly the same number as the number of gut-pouches, and in the second place that all of these should correspond so as to give organs repeated symmetrically in both ectoderm and mesoderm. I cannot, I admit, resist the conviction that metameric repetition is far too difficult and fundamental a problem to be explained as the result of mechanically pulling out the supposed Anlagen of all the organs of the body.

The phenomena of metameric repetition and apical growth are closely associated together. Whether the latter stands in any causal relation to the first cannot be definitely asserted, or if it could we should have no means of determining whether the relation is an ontogenetic or a phylogenetic one; whether the apical elongation was established in the embryo or in the adult (if, indeed, we can draw any line of any value between the embryonic and adult appearance of any organs).

Nevertheless it is important to emphasise the fact of the connection, for we find both in Vertebrates and Annelids the two phenomena closely bound up together. Further, we have the interesting case of the star-fishes and brittle-stars, where at five radial points there is apical growth, and the five arms are segmented. In the higher plants also there is a repetition of similar parts (phytomeres) and apical growth.

Whether the repetition of the calcareous skeleton and tube-feet of the arm of a star-fish is a repetition comparable to the repetition in the Vertebrate and Annelid will depend largely upon definition of terms. As to the fact of a symmetrical repetition and the presence of apical growth there can be no question, and that is the main point. There are no grounds for assuming that the repetition of the parts of the star-fish arm was ever connected with any attempt of the animal, in the past, to reproduce itself at five equidistant points, nor would such a suggestion be believed by anybody for a moment. The method of growth in these arms, where there is a terminal piece carrying the eyes and a subterminal growing region,

is so similar to the method of elongation of the Annelid body that even the most casual observer must be impressed by the comparison. The greatest drawback to any attempt to refer the two cases to a common method of growth (not phylogenetic, of course) would probably be met by the statement that the repetition of a metamere is something entirely different from the repetition of the vertebral ridge in the starfish's arm. If we can succeed in breaking down this conventional and artificial definition of the value of metameric repetition as compared with other repetitions of the body we shall have made a step forward I am confident. This question will be more fully dealt with in the next section.

I have asked the opinion of eminent botanists on several occasions as to the meaning of the repetition of the parts of the higher plants, particularly the Phanerogams. So far as I can learn, the botanical phylogenists have not had a much better time of it than the zoological phylogenists. The former have had the immense advantage of much palæontological material, but, as I understand, even with this the great gaps come just where the evidence is most wanted. I have not, however, found any botanist who believed that in the past a single stem and leaf or two leaves (phytomeres) represented the ancestral plant which grew long by repeating itself, as the embryo plant does at present.

Haeckel, in his 'Generelle Morphologie,' 1866, used the term "promorphology" to include the fundamental relations of the parts of an animal to one another, in the same sense that the relations of the axes of a crystal are the expression of its form. The aim of promorphology, Haeckel said, is to determine the ideal fundamental form by a process of abstraction, and to discover the natural laws according to which organic matter develops its outer form. The relation of the parts, i.e. the form, results with absolute necessity from the architectural union of the constituent parts, in the same sense that an inorganic crystalline form results from the union of crystalline material, and from its relation to its environment.

Whether morphology will be ultimately driven to an inter-

pretation of the symmetry of organic form as the expression of physical laws of protoplasm, rather than due to slow adaptation of an amorphous or irregular substance to its surroundings, is too large a question to attempt to discuss. Whatever the explanation may be, there are certain well-established facts in this connection that have, it seems to me, an important bearing on metameric repetition.

The relation existing between bilateral and radial symmetry is one of the most suggestive fields of promorphology. We get from this source more suggestion as to a possible interpretation of the facts of metameric repetition than from any other source. It would lead too far to attempt anything like a full discussion, but I may cite two cases that will serve as simple illustrations of a large field of inquiry.

The radial symmetry of a sea-urchin is a very perfect type of five-rayed structure. The test, made up of a mosaic work of calcareous plates, is a marvellous piece of detailed fitting. Yet there are several cases on record of individuals that have a sixth ray (antimere) introduced. Each of the six antimeres may be a perfect copy of the others, as well as of the normal.[1] The same condition is not uncommon in the star-fish and other Echinoderms, but owing to the lack of a mosaic calcareous skeleton the result is not so impressive.

Again, in other individuals one of the five antimeres may be entirely or in part omitted, and yet the surface shows a perfect symmetry. The point here to be emphasised is that a whole section of the body (antimere) may be introduced or omitted, involving the introduction or loss of all the organs belonging to such a division.

More remarkable still are the triangular tapeworms described by Leuckart and others. A strongly marked bilateral animal repeats occasionally one of its halves, so that we may paradoxically speak of the worm as composed of three halves. Instead of a somewhat flattened bilateral animal, there results

[1] Bateson points out two cases which are to be distinguished. There may be a division into two of one antimere, or there may be a redistribution of the whole material into six parts. The text refers to the latter.

a radial (triradiate) form, having three sets of longitudinal organs where normally there are only two. Two other facts in connection with these variations ought to be emphasised. The scolices of the tapeworms arise on the large bladderworm by invagination of the surface wall. The relation of the invagination to the surface of the sphere is a radial rather than a bilateral relation, and it is interesting to find this occasionally expressed in the triangular scolices. In the second place, Leuckart records finding on the same bladder (Cysticercus) both radial and bilateral scolices. This seems to show that the radial type need not have come in from egg variation, but is the result of conditions acting at the time of the formation of the scolices.

Now both of these cases, the sea-urchin and the triangular tapeworms, show that a complete section of the body may be repeated and intercalated symmetrically amongst the other parts. The new portion has appeared at once and fully equipped, duplicating the structures of the body that lie in a similar axial position.

These and many other similar cases show us, I think, very positively that the variations appearing in a radial animal must have come simultaneously and all together into the antimeres.

Moreover I think no one will doubt that the relation existing between the repeated organs in a radiate animal is at bottom the same relation existing between the right and left sides of the body of a bilateral animal.

Mivart (**31**) and Brooks (**9**) have emphasised the further fact that the relation between the right and left sides of the body is the same relation that exists between the serially repeated parts of a metameric animal.

If this line of argument be admitted, it puts the problem of metamerism into a large category of well-established facts. That the final explanations of these facts is closely bound up with the solution of some of the most fundamental problems of biology is self-evident.

To hope, therefore, to solve the problem of metamerism in

the simple ways already tried by phylogenists and embryologists is, it seems to me, a vain hope. But a more vigorous study of the facts of metameric repetition from the standpoint reached above might lead us in the right direction. A study of a larger number of facts than we possess at present will at least tell us whether or not metamerism is to be referred to this category. If this should prove true, we shall know where to search for the key to the problem. Even if there is no immediate hope of reaching a definite conclusion, yet we shall have gained much if we can find in what direction the solution lies.

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DESCRIPTION OF PLATES 40—43,

Illustrating Mr. T. H. Morgan's paper, "A Study of Metamerism."

PLATE 40.

Type forms of modification of metameres of Annelids. See pages 395 to 403.

Fig. I, a, b, c.—Compound metamere (split metamere), as seen from dorsal (a), ventral (b) side, and reconstructed in c, as seen from above.

Fig. II, a b c.—Modification of last.

Fig. III, a b c.—Inserted half-metamere.

Fig. IV, a b c.—Double compound metamere.

Fig. V, a b c.—Failure of lines to meet dorsally.

Fig. VI, a, b, c.—Spiral of two metameres.

Fig. VII, a, b, c.—Spiral, with one more half-metamere on one side than on other. In all five half-metameres.

Fig. VIII, a, b, c.—Spiral, with same number of half-metameres on each side. In all six half-metameres.

Fig. IX, a, b, c.—Spiral of type VII, but longer.

Fig. X, a, b, c.—Spiral of type VIII, but longer.

Fig. XI, a, b.—Spiral of type VIII, but still longer.

Fig. XII, a, b, c.—Spiral formed by combination of half-compound metameres.

Fig. XIII.—Spiral, in part a double-spiral, formed by introduction of half-compound metameres.

Fig. XIV.—Spiral, in part a double-spiral, formed by the introduction of a half-double-compound metamere.

PLATE 41.

All figures are from the anterior end of L. (Allolobophora) fœtidus. See text pages 407 to 418.

Fig. 1, A B.—Fourth metamere, incompletely developed on right side.

Fig. 2, A B.—Second metamere, ditto.

Fig. 3, A B.—Fifth ditto, left side.

Fig. 4.—Failure of septal line between 9th and 10th metameres to meet above.

Fig. 5.—Ditto between 5th and 6th ditto.

Fig. 6.—Ditto 4th and 5th ditto.

Fig. 7.—Ditto 3rd and 4th ditto.

Fig. 8.—Ditto 13th and 14th ditto.

Fig. 9.—Ditto 12th and 13th ditto.

Fig. 10.—Ditto 11th and 12th ditto.

Fig. 11, A B.—Ditto 1st and 2nd below.

Fig. 12.—Ditto 13th and 14th below.

Fig. 13.—Ditto 5th and 6th at side.

Fig. 14.—Spiral of category VIII, involving 7th, 8th, and 9th metameres.

Fig. 15.—Ditto, involving 12th, 13th, and 14th.

Fig. 16.—Ditto, 11th, 12th, and 13th.

Fig. 17.—Ditto, 12th, 13th, and 14th.

Fig. 18.—Ditto category X, involving 6th, 7th, 8th, and 9th.

Fig. 19.—Ditto, 11th, 12th, 13th, and 14th.

Fig. 20, A, B, C.—Ditto category VII, involving 2nd, 3rd, and 4th.

Fig. 21, A, B, C.—Ditto, 9th, 10th, and 11th.

Fig. 22, A, B, C.—Ditto category IX, involving 6th, 7th, 8th, and 9th ditto.

Fig. 23, A B, C D.—Two modifications—categories II and V.

Fig. 24, A B C.—Short spiral; origin doubtful.

Fig. 25, A B C.—Spiral VIII and compound metamere I combined.

Fig. 26.—Spiral of category VIII, very long, involving 11th—24th metameres.

Fig. 27, A B C.—Ditto, long, involving 10th—16th.

Fig. 28.—Ditto, 13th—24th.

Fig. 29, A B C.—Spiral category VII, involving 14th, 15th, and 16th.

Fig. 30, A B C.—Ditto category VIII, involving 15th, 16th, and 17th.

Fig. 31, A B.—Ditto, 15th, 16th, 17th, and 18th.

FIG. 32, A B.—Ditto, 15th, 16th, and 17th.

FIG. 33, A B.—With compound metamere 10—$\frac{10}{11}$.

FIG. 34, A B.—Ditto 7—$\frac{7}{8}$. Also category V.

FIG. 35, A B C.—Ditto 9—$\frac{9}{10}$. Also category II.

FIG. 36, A B C.—Spiral VII, involving 11th, 12th, and 13th metameres.

FIG. 37, A B C.—Compound metamere 12—$\frac{12}{13}$.

FIG. 38, A B C.—Category II.

FIG. 39, A B C.—Compound metamere 15—$\frac{15}{16}$.

FIG. 40, A B.—Spiral VII, involving 8th, 9th, and 10th. Also another modification.

FIG. 41, A B C.—Four compound metameres I and spiral VII.

FIG. 42, A B C.—Compound metamere 3—$\frac{3}{4}$, followed by complicated spiral.

FIG. 43, A B C.—Two spirals VII, and compound metamere II.

FIG. 44, A B.—Compound metamere 6—$\frac{6}{7}$. Spiral VII. Compound metamere 13—$\frac{13}{14}$.

FIG. 45, A B C.—Intercalated half-metamere 15. III. Spiral involving 15th—19th metameres.

FIG. 46, A B C.—A much modified anterior end. See construction C.

PLATE 42.

All figures of L. fœtidus except Fig. 74 (L. terrestris).

FIG. 47.—All of the abnormalities drawn from one very abnormal worm. The numbers inserted between the spirals, compound metameres, &c., give the number of normal rings that have been omitted.

FIGS. 48—50.—Vasa deferentia opening on different metameres. The figures as seen from below.

FIGS. 51, 52.—Vasa deferentia doubled on one side. Seen from below.

FIGS. 53—55.—Both vasa deferentia on 10th, 12th, and 14th metameres respectively. Projections from above.

FIGS. 56—58.—Vasa deferentia on alternate segments. Diagram as seen from above, so that right and left of Figs. 53—60 are reversed as compared with Figs. 48—52 (below).

FIGS. 59, 60.—Vas deferens doubled on one side. Projections from above.

FIGS. 61—64.—Dissections of compound metameres to show arrangement of septa, &c.

FIGS. 65—70.—Dissection of spirals to show condition of septa.

FIG. 71.—Failure of surface line to meet above. Septa were normal.

Fig. 72, A B.—Disagreement between surface line (A) and septa (B).

Fig. 73.—Dissection of a compound metamere. Septa do not agree with surface lines.

Fig. 74, A B.—Vas deferens doubled on right side. L. terrestris.

Figs. 75—78.—Abnormal arrangement of metameres in young worms at time of emergence from coccoon. Fig. 75, category I; Fig. 76, category VII; Fig. 77, category VIII; Fig. 78, category X (Pl. 40).

PLATE 43.

Figs. 79—81.—Regenerating posterior ends of the body of L. fœtidus, See text for description.

Figs. 82—85.—Abnormal arrangement of the metameres in Amphinome.

Figs. 86—88.—Abnormal arrangements of the rings of leeches (Macrobdilla decora).

Fig. 89, A B C.—Compound metamere $5—\frac{5}{6}$ in abdomen of locust.

Figs. 90—95.—Abnormal arrangements of the rings of the lobster, Homarus americanus.

Fig. 96.—Nine segments of the arm of a brittle-star (Ophiuridea) from Jamaica, as seen from below. Every fourth segment is a pigmented colour-ring.

Fig. 97.—Nine segments of another individual, showing the middle pigmented ring broken.

Fig. 98.—To show a further separation of colour-rings.

On the Cœlom, Genital Ducts, and Nephridia.

By

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With Plates 44 and 45.

The chief object of this paper is to call attention to a theory of the homology of the cœlom which has been gradually gaining ground abroad, but has not, I venture to think, received in this country the notice which it deserves. The theory I refer to is, that the cavity which we know as the cœlom in the higher Cœlomata is represented by that of the genital follicles in the lower types of that grade. Ever since Hatschek wrote the often-quoted words: "Die secundäre Leibeshöhle verhält sich wie die Höhle der Geschlechtsdrüse der niedrigeren Formen," and pointed out that true mesoblastic metamerism is really due to the repetition of the gonads (**48**), so many favourable new facts have been brought to light, that from a suggestion the statement has become a well-established theory. In a most interesting and suggestive paper on the ancestry of the Annelids, Dr. E. Meyer has set forth the theory in some detail (**81**). After showing that the cœlomic cavities in the Polychætes are quite comparable in development and function with the genital follicles of the Planarians, he further maintains that the theory throws a flood of light on various otherwise obscure questions, such as the bilateral character of the cœlom, its invariable connection with the genital cells, the absence of a truly unpaired prosto-

mial cœlom, &c. He then treats of the nephridia and genital ducts, and it is with this part of the question that I wish more especially to deal in this paper. Meyer holds that the nephridium of the Platyhelminths is represented by the so-called head-kidney in the first segment, and by the tube of the nephridium in the trunk segments of the Annelids, while the genital duct of the Platyhelminths is represented by the wide funnel of the trunk nephridium which develops, independently of the tube from each genital or cœlomic follicle, and becomes grafted on to it afterwards. Unfortunately, the author restricts his remarks almost entirely to the Polychætes and those forms which appear directly to lead up to them. Although the theory has been at all events partially adopted by other writers (Lang, **71**; Korshelt and Heider, **67**), no one, as far as I am aware, has pushed it to its logical conclusion, and applied it to all the groups of Cœlomata. This is what I shall attempt to do in this paper. First of all, however, there is one thing to be noticed with regard to Meyer's general statement about the nephridial funnel, namely, that since the publication of his own researches on the Polychæta, and of those of Vejdovsky and others on the Oligochæta, there can be no doubt that the nephridial funnel in the latter forms part of the true nephridium (is, in fact, derived from the end-cell), and is not a grafted genital duct, as is the case in some at least of the Polychæta.

An unprejudiced review of the well-established and recently ascertained facts concerning the development of the excretory organs and genital ducts of the Cœlomata must, I think, inevitably lead us to the conclusion that we have been confusing two organs of totally different origin under the one name nephridium—the one organ the true nephridium, the other the morphological representative of the genital duct, which may be called the peritoneal funnel, to avoid confusion. Further, that while on the one hand in certain groups such as the Planaria, Nemertina, Hirudinea, Chætopoda, Rotifera, Entoprocta, besides the genital ducts or peritoneal funnels, we find true nephridia in the adult; on the other hand, in such

groups as the Mollusca, Arthropoda, Ectoprocta, Echinoderma, and Vertebrata, there are in the adult no certain traces of true nephridia. In these latter groups, as we shall see, the peritoneal funnels (primitive genital ducts) take on the excretory functions of the nephridia which they supersede.

In the following brief review of the various classes of Cœlomata, I shall endeavour to show that the two kinds of organs can always be distinguished; that the first, the nephridium, is primitively excretory in function, is developed centripetally as it were, and quite independently of the cœlom (indeed, is probably derived from the epiblast), possesses a lumen which is developed as the hollowing out of the nephridial cells, and is generally of an intracellular character, is closed within, and may secondarily acquire an internal opening either into a blood space or into the cœlom (true nephridial funnel as opposed to the peritoneal funnel); and that the second kind of organ, the peritoneal funnel, is primitively the outlet for the genital products, is invariably developed centrifugally as an outgrowth from the cœlomic epithelium or wall of the genital follicle, is therefore of undoubtedly mesoblastic origin, and possesses a lumen arising as an extension of the cœlom itself.

In the series of diagrams illustrating this paper, based on the most recent and accurate researches, it has been my constant endeavour to interpret the author's results correctly, and not to distort the facts in favour of the theory here advocated.

Planarians.

The nephridia of the Planarians, as is well known, are formed of a main duct, which branches out into fine tubules ending blindly internally in flame-cells (fig. 1); they do not develop beyond this "pronephridial" condition—protonephridium of Hatschek (55). The arrangement of this single pair of nephridia is extremely variable; the two organs may join and open by a median external pore near the mouth or behind, or they may open by a number of pores at the sides. Gunda segmentata, a most interesting form described by Professor Lang (69), possesses longitudinal main trunks into which

open the fine branches ending in flame cells, and from which pass segmental ducts to the exterior, corresponding to the segmentally arranged gonads. It is by the breaking up of such a system into separate organs that Lang would derive the nephridia of the higher Cœlomata.

Unfortunately, we know little about the origin of the nephridia in this group. Lang has described, in Discocelis, paired ingrowths of the epiblast, which he believes give rise to the nephridia (**70**). This observation strongly supports his theory as to the phylogenetic derivation of the nephridia from epidermal glands; and, indeed, it seems pretty certain that an incipient excretory organ to be efficient must have been derived from, or at all events situated close to, the surface layer in order to get rid of its excretory products.

It is in the Planarians, a group undoubtedly primitive[1] in some respects, that we should expect to discover the cœlom in its first stages of development, and, in fact, we do seem to be able to trace it from its first appearance. In some Acœla (Graff, **42**, **44**), and other simple forms, the gonads consist merely of the genital cells lying freely in the parenchyma. In others, these cells become surrounded by an epithelium formed by the adjacent cells; the epithelial sacs, one on either side of the body, may then become hollow, while the wall grows out to form two tubes, the genital ducts (peritoneal funnels). Another important stage is presented by these organs in Gunda segmentata (Lang, **69**). Here the genital follicles are repeated segmentally, the first pair being ovarian, the rest testicular sacs. If these

[1] One of the most useful lessons of modern research has been to teach us with what great care the word "primitive" should be applied to any group of existing animals. A few years ago naturalists readily derived one group of living animals directly from another, apparently more primitive; but their genealogical trees are now becoming reduced to bushes, in which the branches spring from a common base. Nevertheless, it is true that certain groups may retain, either in their general organisation or in some particular details, characteristics of the ancestors from which they have diverged. The Planarians, with their complicated nephridial and genital apparatus, their deeply-sunk nervous system, yet generally archaic plan of structure, are a striking case in point.

follicles were larger, Gunda segmentata could be called a truly segmented animal.[1] The inner ends of the genital ducts are formed as outgrowths from the genital follicles: "Der Oviduct ist bei Gunda segmentata, wie bei Planaria torva anfangs ein solider Zellenstrang. Zweifellos ensteht er durch Wucherung aus dem soliden ovarium selbst, ähnlich wie die Samenleiter Auswüchse der Hoden sind" (observations confirmed in his later work, **70**).[2] The oviduct becomes hollow and ciliated, and grows backwards to the genital pore; the vasa efferentia fuse to form the main sperm-duct.

To sum up, then. In the Planarians the excretory organs are a pair of pronephridia, probably derived from the epiblast; the gonads arise from a mass of cells in the mesoblast, which may become hollowed out into a genital follicle (cœlomic sac) from the wall of which arise the genital cells. The follicle grows out to form the genital duct (peritoneal funnel), which joins an epiblastic invagination at the genital pore (fig. 1).[3]

[1] E. Meyer believes (**81**) that the ancestor of the Annelids possessed a pair of long genital follicles, and that metamerism was brought about by their being broken up at intervals, chiefly to facilitate its serpentine motion; each portion would then have acquired its own duct to the exterior. It seems to me more probable that the metameric arrangement of the genital follicles is more directly due to that "tendency" to repetition by a sort of budding, which is seen in the case of the gonads, the penes (Anonymus), and even the pharynx (Phagocata; Woodworth, **112**) amongst the Planarians, and again, perhaps amongst the Mollusca (for a full discussion see Bateson, **3**).

[2] Jijima's account of the development of the sperm-ducts differs somewhat from that of Lang, but he derives both from the mesoderm (**60**).

[3] The spaces contained in the connective tissue or parenchyma have been sometimes compared with the cœlom; these spaces seem rather to represent the vascular system of the higher Cœlomata. I need not treat here of the homology of the vascular system, which is probably of quite separate origin from the cœlom. Professor Ray Lankester's view, that the blood-system is simply a liquefaction, as it were, of the mesoblast, seems to me to agree perfectly with the facts. Moreover, the theory held by many authors that it is directly derived from the blastocœl appears to be quite untenable. As Professor Lankester has pointed out to me, if this were the case we should expect to find the blood-spaces best developed amongst the Diploblastica; now it is just in the (adult) Cœlenterates that it is entirely absent. Indeed we may say that the blood-space, or hæmocœl, does not appear in phylogeny

About the Cestodes and Trematodes it need only be said that they are built (so far as concerns the question discussed in this paper) upon essentially the same plan as the Planarians;[1] while as to the Nematodes, of the development of which we know too little, it seems probable that here also the genital follicles represent the cœlom, while the body cavity is a blood-space corresponding in its relations to the parenchyma of the Planarians.

Rotifera.

The Rotifers, recently described in great detail by Platte (**87, 88**) and Zelinka (**115**), agree in the general structure of the nephridia, genital follicles, and genital ducts, so closely with the Platyhelminths that they may be dismissed with a very few words. The nephridia are a pair of branching tubes ending internally in flame cells, and opening behind into the cloaca.[2] The cœlom is represented by a pair of genital follicles, one of which only is generally developed; the wall of the follicle is produced backwards to form the genital duct, or peritoneal funnel opening into the cloaca.

The development of the nephridia has not yet been thoroughly worked out. Zelinka has traced them to a group of cells of doubtful origin. He adds: "das Exkretionssystem konnte ich in so fern mit Sicherheit auf das Ektoderm zurückführen, als es bestimmt nicht auf das Entoderm bezogen werden kann" (**115**).

until the mesoblast has been formed, and, generally speaking, that the greater the development of mesoblast the more definite is the vascular system. I should rather consider the connection of the blood-spaces with the blastocœl as arising for purely mechanical reasons, so to speak, and in no way of phylogenetic significance. The temporary continuity of the blastocœl with the blood-space during the ontogeny of certain of the higher forms would seem to be connected with that method of development by the folding of germ-layers or sheets of tissue, which no one would look upon as primitive. During this process, the cavities which will form the vascular system later on, are inevitably continuous with the space left between the germ-layers.

[1] Fraipont maintains that the flame end-cells of the nephridia of the Cestodes and Trematodes communicate internally by means of a small lateral aperture (**39**).

[2] As in the case of some Platyhelminths, the nephridia have been stated to open internally (Eckstein, **28**).

Entoprocta.

This group also, from our present point of view, differs little in its organisation from the Planarians.

A pair of nephridia—short tubes, generally with an intracellular lumen and ending in a flame-cell or a group of cells with cilia,—open by a median pore in front of the genital pore (fig. 4) (Hatschek, **47**; Harmer, **46a**; Ehlers, **29**; Davenport, **26**). Possibly in some forms they open internally (Joliet, **61**), and in Urnatella they may be connected with a system of branching tubules ending in flame-cells (Davonport, **26**). The origin of the nephridia is doubtful; Hatschek traced them in the embryo to cells which he believed to be mesoblastic (**47**).

Embedded in parenchymatous tissue are a pair of genital follicles (fig. 4). From each a typical peritoneal funnel leads to a median pore (Ehlers, **29**).

Mollusca.

We now have to deal with a group of animals in which, as I shall endeavour to show, the embryo is provided with a pair of true nephridia; later, when the two cœlomic sacs belonging to the unsegmented trunk have acquired a considerable size, the peritoneal funnels formed from their walls take on the excretory function, whilst the nephridia degenerate [1].

True nephridia have been described in all the groups of Mollusca except the Isopleura and the Cephalopoda, and have recently been the subject of a special study from Dr. R. von Erlanger (**34**, **35**). They consist of short tubules formed, as a rule, of one or of a small number of cells, pierced by a canal which communicates with the exterior by a pore behind the velum (fig. 20). The inner end of the nephridium is provided with a flame-like bunch of cilia, or with a flagellum. An internal opening into the blood-space or head-cavity has been observed in some forms, such as Lymnæus, Helix (de Meuron, **79**; Jourdain, **62**; Fol, **38**; Sarasin, **92**; Wolfson, **111**; v. Erlanger, **35**, &c.), Teredo (Hatschek, **50**), and perhaps in

[1] This view evidently obviates the difficulty some have felt as to the presence of two pairs of so-called nephridia in an animal composed of one segment.

Cyclas (Ziegler, **116**). In other cases, such as Paludina, and Bythinia (v. Erlanger, **32, 33**), the nephridium appears not to open internally, but to remain in the pronephridial stage.

The first stages in the development of the nephridium of the Mollusca have, unfortunately, not yet been satisfactorily worked out. Rabl (**88a**) derives the nephridium in Planorbis from a large cell which also gives rise to the mesoblast. Wolfson traced that of Lymnæus, which, he says, is derived from a large in-wandering epiblastic velar cell on either side (**111**). Hatschek (**50**), v. Erlanger (**32, 33**), and others trace it to cells which they consider to be of mesodermal character, but the exact origin of which is not clear.[1] In some cases a late epidermal invagination is said to take place at the nephridiopore which forms the peripheral end of the duct (v. Erlanger, **32, 34**).

We must now examine the development of the excretory organs of the adult Mollusca, which appear to be nothing but peritoneal funnels. An accurate description of the development of Paludina has recently been given by R. von Erlanger, and although the ontogeny is much modified owing to the asymmetry of the adult, I shall begin with it as it is the only detailed account we have. The genital follicles or cœlomic sacs (pericardium) arise as a cavity on either side, a hollowing out of the mesoblast (fig. 20). These cavities enlarge and fuse below the gut. On either side a thickening takes place on the ventral wall of the cœlom, which here grows out in the form of a typical peritoneal funnel (fig. 21). The right peritoneal funnel then enlarges and fuses with an epidermal invagination, which forms the outer end of the excretory organ. "Was die Niere anbelangt," says von Erlanger, "so bin ich der Ansicht, dass der secernirende Abschnitt derselben aus dem Mesoderm

[1] I think we may safely say that there is nothing which precludes the possibility of the nephridia being primitively derived from the epiblast, as they appear to be in the Platyhelminths, Rotifers, and Chætopods; although the forecasts may sink in at a very early stage and thus become included in that "Anlagecomplex" which we call mesoblast. In Helix, de Meuron derives them from the epiblast, but he is not positive about the internal extremity (**79**).

stammt und dass diejenigen Beobachter, welche ihr aus dem Ektoderm enstehen lassen, entweder nur den ausführenden Theil der Niere berücksichtigt haben oder, was noch häufiger geschicht, die beiden Abschnitte nicht in ihren Zusammenhaug erkannten: Der ausführende Theil wird nämlich von Allen, mit Ausnahme von Rabl, aus einem Theil der Mantelhöhle abgeleitet" (**32**). On the left side, to which the genital function is restricted, the gonad develops from the wall of the cœlom; then, together with the rudimentary left peritoneal funnel, it becomes constricted off from the main division of the cœlom (the pericardium), forming a small genital sac. From the wall of this sac the genital duct grows out, and joins an epidermal invagination, like the peritoneal funnel of the right side. Doubtless the genital duct is really the left peritoneal funnel, as v. Erlanger suggests: "Ich konnte . . . . feststellen, dass die Anlage der Genitaldrüse in der ursprüngliche linken Hälfte des Perikards ensteht, und zwar ungefähr da, wo sich die rudimentäre linke Niere zurückgebildet hat. Eben so ensteht auch die Anlage des Ausführganges an der Stelle, wo der rudimentäre Ausführgang der linken Niere sich befand, und scheint einfach aus diesem hervorzugehen." These observations have been fully confirmed in the case of Bythinia (**33**), and the conclusions, as we shall see shortly, are supported by the comparative anatomy of the Mollusca in general (fig. 22).

Ziegler has described the development of Cyclas cornea, which in some respects is less modified, the adult being symmetrical (**116**). Here also the cœlom (pericardium) arises as a right and left follicle. The two cavities enlarge, surround and fuse below the gut. On either side the organ of Bojanus develops as a peritoneal funnel, which meets an epidermal invagination (fig. 22).

In the Mollusca, then, we find at an early stage a pair of true nephridia (Urniere, head-kidneys), possibly of epiblastic origin. The cœlom develops as two cavities in the mesoblast, genital follicles, from the walls of which grow out two peritoneal funnels, the organs of excretion and carriers of the

genital cells of the adult. It can hardly be doubted that primitively the renal organs (organs of Bojanus, nephridia of authors) functioned as genital ducts. Such is, indeed, still the case in Chætoderma, the Neomenians, and the Zygobranchia. Also in the most primitive Lamellibranchs, such as Nucula and Solenomya, the peritoneal funnels still retain their original function. As Pelseneer has shown (**86**), gradually a separate genital duct has been split off, which in Anodon, Cardium, &c., opens independently. Intermediate stages are found in such forms as Pecten, and Spondylus, where the genital cells are shed into the kidney itself; and in Arca, Ostrea, &c., where the kidney and genital duct open into a common cloaca. Likewise in the Chitons a separation has taken place of the genital region of the cœlom from the renal; the gonad then acquires special ducts, which may not be homologous with peritoneal funnels. In the Cephalopoda, although the cœlom remains continuous, special apertures serve for the escape of the genital cells; whether these should be considered as a second pair of peritoneal funnels is also doubtful.[1]

(The aberrant form Rhodope veranii would appear to be amongst the Mollusca, if it be really of that class, the only one which retains true nephridia in the adult. It is provided with a pair of branching tubes, opening by a common pore, and ending internally in flame-cells. The cœlomic or genital follicles, ovarian and testicular, are small and numerous. Their ducts join to a common hermaphrodite duct, which again divides into two openings by male and female pores [**43, 12**].)

Dinophilus.

For our purpose it will be convenient to treat this genus separately, and not with the Archiannelida which will be included with the Polychæta. It is of great interest, since, in some species at least, the nephridia are metamerically repeated whilst the cœlom is represented by a single pair of genital follicles.

[1] Such a case may be compared to that of certain Nemertines, where numerous genital follicles and as many genital ducts are present in one segment, and to the similar arrangement in the Vertebrates.

In Dinophilus apatris, Korschelt has described flame-cells which he believed to be connected with a longitudinal canal opening posteriorly (**66**). E. Meyer, however, has figured in D. gyrociliatus five pairs of typical closed nephridia, or pronephridia, ending in flame-cells internally, and disposed metamerically according to the outer signs of segmentation (**80**). Harmer (**46**) describes five pairs of very similar nephridia in the female D. tæniatus. In the male there are four pairs of nephridia, perhaps with internal openings.[1]

The testes are a large right and left sac, which fuse across the median line; as Harmer himself says, it is "possible that in the connective-tissue lacunæ of the body of Dinophilus we have the representative of the so-called 'primary body-cavity,' whilst in the fully developed male the 'secondary body-cavity' is represented by the cavity of the testis, with which the funnels of the vesiculæ seminales are connected." It might be added that these funnels, which form the inner openings of the sperm-ducts, have all the appearance of true peritoneal funnels, comparable to the genital ducts of the Platyhelminths, and the other groups already spoken of.

The paired ovarian cavities appear to be provided with only very degenerate ducts, reduced to mere pores in D. vorticoides and D. apatris (compare the Archiannelida and the female ducts of certain Oligochæta such as the Enchytræids).

The structure of Dinophilus might be explained in one of two ways. Either it has acquired a number of nephridia, whilst retaining the primitive single pair of genital follicles; or it is a degenerate form which has lost its metamerically repeated genital follicles, whilst retaining a number of separate nephridia. Our present knowledge does not enable us to conclude for certain which of these explanations is the correct one.[2]

Histriodrilus Benedeni (Histriobdella homari) may

[1] The fifth pair is possibly, according to Harmer, represented by the distal portion of the genital ducts leading to the penis (compare with certain Polychæta where the nephridium fuses with the peritoneal funnel).

[2] In a paper which has just appeared Schimkewitsch describes a ladder-like nervous system, and traces of segmentation in the developing mesoblast ('Zeit. f. w. Zool.,' Bd. lix, 1895).

be closely related to Dinophilus. It possesses five pairs of pronephridia (four in the female), and a distinct cœlomic or genital cavity also opening by one pair only of peritoneal funnels to the exterior (Foettinger, **37**). Here it seems to be pretty certain that there was originally a segmented cœlom ; there is still a ventral chain of ganglia.

Nemertina.

The nephridia of the Nemertines consist essentially of a longitudinal canal on either side of the anterior region of the alimentary canal, opening to the exterior by one or by several pores situated laterally at more or less regular intervals (von Kennel, **63**; Oudemans, **85**; Hubrecht, **59**; Burger, **15, 17**). Internally they have been stated to open into the blood-vascular system ; the latest researches, however, do not support this view (**15**), and Bürger has shown that in several species the nephridial canals give off fine branches ending in bunches of flame-cells (**17**) (figs. 2, 3, and 24; in these diagrams the nephridia are represented in the same region as the gonads).

Although the development of the nephridia has not been followed out in detail, Hubrecht (**58**) and Bürger (**18**) have traced their origin from direct invaginations of the epiblast.

In this group of elongated worms the genital follicles are numerous, and generally arranged in pairs, alternating with the intestinal cæca. Each follicle communicates with the exterior by a duct or peritoneal funnel, formed as an outgrowth from its wall at a comparatively late period (figs. 2, 3, and 24). It is hardly necessary to emphasise the striking similarity between this metameric arrangement of follicles with their corresponding ducts and the almost identical metameric cœlomic follicles and genital ducts of the Chætopods.[1]

Oligochæta.

Thanks to the numerous researches of Profs. Hatschek,

[1] R. S. Bergh, in 1885, in a paper which I have not seen, pointed out the resemblance between the genital follicles of the Nemertines and the cœlomic cavities of the Chætopods; but he considered the ducts of the follicles to be homologous with the nephridia of the latter group (**6**).

Bergh, Wilson, Vejdovsky, and others, we have now a very detailed history of the development of the nephridia in the Oligochætes.

The observations of Hatschek, Wilson, and Bergh do not coincide in many particulars; but all these discrepancies have been so admirably reconciled by Vejdovsky in his careful work on Rhynchelmis and other forms (**101**), that we can, I think, be now quite confident that we have a satisfactory account of the embryology of these organs; more especially since these results have been in many respects amply confirmed by the researches of Whitman, Bergh, and Bürger on the Hirudinea.

The development of the nephridium in Rhynchelmis has been most carefully described by Vejdovsky (**101**). In the first stage he figures it consists of a large cell (trichterzelle or funnel-cell) within or on the posterior surface of the septum. This "funnel-cell" divides and gives off a string of small cells behind, from which is developed the canal of the nephridium. A large vacuole appears between the two cells formed from the funnel-cell itself, in which a flame-like flagellum is developed. Vacuoles now arise in the posterior string of cells, fuse together, and form the lumen of the canal which communicates with the end-chamber containing the "flame" (figs. 6 and 25). Finally this chamber opens into the cœlom, and the posterior loop joins the skin; a communication is established with the exterior by means of a secondary invagination of the epidermis—the end-vesicle. Quite similar is the development of the nephridium in Stylaster and Tubifex (Vejdovsky, **100**).

Bergh (**9** and **10**) traced back the nephridia in Criodrilus and Lumbricus to a large cell, the funnel-cell, lying close to the epiblast and between each successive pair of solid mesoblastic somites. When these become hollowed out, the funnel-cell buds off posteriorly a chain of cells, the future canal of the nephridium; vacuoles appear in these cells, as in Rhynchelmis, to form the lumen. Meanwhile the funnel-cell itself, which has retained its large size, pushes through the mesoblast to reach the cœlomic cavity in the segment in front; here it divides, acquires cilia, and becomes the funnel of the adult nephridium.

The posterior canal grows to the surface, where it opens through the epidermis; in some cases there is here an invagination of the epidermis to form the end-vesicle (Vejdovsky).

As to the origin of the funnel-cell—the forecast of the whole true nephridium: it arises from the primitive cell row, or nephric cord, formed by the repeated division of one of the teloblasts on either side. In the earlier stages this teloblast and the nephric cord to which it gives rise lie on the surface of the embryo; thus the funnel-cells are epiblastic in origin. From the nephric row one cell enlarges and enters into connection with each successive segment, as described above (fig. 5). To judge from the figures of Bergh, Wilson (**108** and **109**), and Vejdovsky, in some forms, such as Dendrobæna and Lumbricus, the funnel-cells give off the chain of posterior cells, whilst separating from the nephric row, thus remaining for some time in connection with it. In other cases, such as Criodrilus, the funnel-cells appear to separate first.[1]

In the embryo of most Oligochætes the nephridia of the first segment are developed precociously to perform the excretory functions at an early stage (fig. 25). Vejdovsky (**100** and **101**) has described these organs in Rhynchelmis, Chætogaster, Æolosoma, Nais, Allolobophora, Lumbricus, Dendrobæna, &c., and Bergh described those of Criodrilus (**9**). They consist of fine canals with an intracellular lumen, or sometimes of wider tubes; they are often ciliated, and occasionally end internally in a flame-cell; they appear to be always blind within. Externally they open either by a median dorsal pore or by two lateral pores on the first segment. In fact they closely resemble the closed pronephridia of the Platyhelminths, Entoprocta, and other groups we have already examined, or the pronephridial stage of the trunk nephridia.

The origin of the cells which form the (larval) nephridia of the first segment has not been traced; but since they arise (in some cases at least) before the division of the promesoblast cell,

[1] Bergh denies the derivation of the funnel-cell from the nephric row. Wilson rightly traced the development of the main body or canal of the nephridium from the nephric row, but failed to discover the origin of the funnel-cell itself.

Vejdovsky considers it probable that they are derived from the epiblast, a conclusion which agrees with the known development of the posterior nephridia.

The mesoblast in the Oligochætes is formed, as in all Annelids, as two germ bands, which become broken up into separate somites. The hollowing out of these gives rise to the cœlomic follicles, which increase in size, surround the gut (a stage resembling what we find in the Nemertines), and fuse below it (figs. 6 and 25). The transverse septa, between adjacent follicles, become pierced, allowing a communication from one to the other (Kowalevsky, **68**; Hatschek, **48**; Wilson, **109**; Vejdovsky, **101**). From the wall of certain of these follicles the gonads are developed, whilst others remain sterile. The number and position of the fertile follicles varies considerably according to the family of the worm in question, and even amongst different individuals of the same species (Woodward observed an earthworm with seven pairs of ovaries; **113, 114**).

The genital ducts (peritoneal funnels) develop as a thickening of the cœlomic epithelium in the fertile segments, which grows outwards towards the epidermis, with which it fuses (figs. 6, 7, and 25). Vejdovsky, who has followed the development of these organs in several forms, such as Stylaria, Chætogaster, the Enchytræids, and Tubificids, says: "Die Anlage des Samenleiters wiederholt sich nach dem oben Dargestellten in übereinstimmender weise bei allen bisher beoachtete Familien. Die zuerst zum Vorschein kommende Anlage des Samentrichters besteht aus einer Zellvermehrung des Peritoneums an den Dissepimenten der betreffenden Segemente" (**100**). His observations have been confirmed by Bergh (**8**) and Lehmann (**74**) in the Lumbricids.[1] In the case of the male ducts, these organs may be further complicated by the fusion of two con-

[1] Beddard (**4**) tried to show that the genital ducts were derived from the nephridia in Acanthodrilus. The few facts he brings forward from the very scanty material at his disposal do not, I think, prove his case. The theory of Claparède that the genital ducts of the Oligochætes were the modified nephridia of the genital segments was founded on an erroneous notion, since thoroughly disproved by Vejdovsky's observations.

secutive peritoneal funnels, and by the invagination of the epidermis at the genital pore to form an atrium and penis.

We may now sum up the main characteristics of the nephridia and genital ducts in this group, which has been treated at length owing to its great importance (figs. 5, 6, 7, and 25).

The nephridia of the Oligochætes are probably of epiblastic origin. They develop from large cells ("funnel-cells"), arranged metamerically outside and between each pair of somites. They pass through a more or less disguised pronephridial stage (comparable to that permanently retained in flatworms, &c.); in the first (most forms), and sometimes in the trunk segments (Chætogaster) they never develop beyond that stage. In the other segments the nephridia grow towards, and open into, the cœlom by means of a funnel formed from the original "funnel-cell."[1]

The genital ducts, on the other hand, are peritoneal funnels of undoubted mesoblastic origin, which grow outwards from the metameric genital follicles to open to the exterior. They thus have no connection with the nephridia, and differ from them entirely in their development.

Hirudinea.

So closely do the cœlom, genital ducts, and nephridia of the Leeches agree in their development with those of the Oligochætes, that their history need only be rapidly sketched.

Bürger (**16** and **19**) has carefully traced, in several forms, the origin of the whole nephridium proper (funnel and canal) from a large "funnel-cell," which comes to lie in the hinder wall of each cœlomic follicle. Just as in the previous group of worms, this cell buds off a row of cells behind which constitute the canal; the "funnel-cell" then divides up into a ring of small cells, which form the funnel of the adult nephridium (figs. 8 and 9). This organ remains closed in some forms, such as Hirudo, but opens into the cœlom in others, such as Nephelis.

[1] The branched so-called plectonephric condition of the nephridia in certain earthworms has recently been shown to arise by the secondary subdivision of originally paired nephridia (Vejdovsky, **102**; A. G. Bourne, **13**).

Meanwhile the lumen of the canal in the posterior chain of cells becomes hollowed out. The peripheral end of the canal fuses with an invagination of the epidermis, the vesicle, by means of which it opens to the exterior. The first origin of the "funnel-cells," from which the nephridia are formed, has not been traced in detail; it seems quite probable that they are derived from the nephric rows described by Whitman (**107**) in Clepsine. (They are possibly the large cells mistaken by Whitman [**106**] for the forecasts of the testes, as suggested by Bergh.)

As in the Oligochæta and Polychæta, so in the Hirudinea the nephridia of the anterior segments develop precociously in the larva. Bergh (**7**) has traced their origin as outgrowths from the epiblastic cell-rows. In Aulastoma there are four pairs, which never develop beyond the pronephridial stage, i.e. do not open internally. Bergh was also unable to find external openings (compare the nephridia of Capitella, **30** and **31**).

The cœlom develops in a normal manner as a hollowing out of the paired metameric blocks of mesoblast. Most of these cœlomic or genital follicles are fertile: an anterior pair develop the ovaries on the peritoneal wall; several posterior pairs develop the testes. That part of the cœlom which surrounds the gonads generally becomes partially separated off as a perigonadial cœlom (Bourne, **12a**; Bürger, **16**). That the genital ducts are peritoneal funnels is shown by their development, although it seems to be somewhat modified. The oviducts arise from the cœlomic epithelium surrounding the ovary, and fuse with the two ends of a forked, but median invagination of the epidermis (fig. 9) (Bürger, **19**). The vasa efferentia are similarly formed from the testes (fig. 28); they grow outwards and forwards, fusing with those in front. The most anterior join the ends of a median forked invagination of the epidermis (Nussbaum, **84**; Bürger, **19**). The complete genital ducts thus closely resemble those of some Planarians (Gunda), and differ essentially from those of the earthworm only in the number of peritoneal funnels which contribute to their formation (compare also the Vertebrates).

Archiannelida and Polychæta.

The first origin of the nephridia in these worms is not so well known as in the case of the Oligochæta. It is, however, to be remarked that in the only case where the forecast of the nephridium appears to have been traced from the beginning, it has been found to arise from the epiblast (the head-kidney in Nereis; Wilson, **110**).[1] This would agree with what we have seen occurs in most, if not all, of the groups we have already examined.

E. Meyer (**80**) has given us a most excellent description of the development of the nephridia in Polymnia and Psygmobranchus. They arise on either side from large cells, situated close to the epiblast between each pair of mesoblastic somites, with which they have no connection at this early stage. These large cells, which seem to me obviously homologous with the "funnel-cells" of the Oligochæta and Hirudinea, divide, forming a short chain of cells within which an intracellular lumen becomes hollowed out (figs. 10, 11, and 26). The mesoblastic somites become hollowed out to form the genital or cœlomic follicles, from the posterior wall of which the cœlomic epithelium becomes pushed out, forming a typical ciliated peritoneal funnel, which fuses with the internal blind end of the nephridium (figs. 11 and 26). This specialised portion of the cœlomic epithelium forms the wide-mouthed funnel of the adult nephridium (fig. 12). The lumen of the nephridial duct becomes intercellular by the multiplication of the cells which constitute its wall, and breaks through at its point of junction with the funnel on the one hand (opens, in fact, here into the cœlom), and establishes a communication with the exterior through the epidermis on the other. Such is the history of the wide-mouthed segmental organs of compound origin of the trunk. The nephridia of the first segment (or sometimes of several

[1] Hatschek (**48**, **51**, **54**), Salensky (**91**), and von Drasche (**27**) all consider the cells from which the nephridia are developed to be of mesoblastic origin, but the evidence on this point is not convincing. Possibly, however, as suggested for the Mollusca, they have secondarily come to be derived from the mesoblast.

anterior segments) develop in the same way as the posterior, but never pass beyond the pronephridial stage (head-kidneys); they end blindly internally with a typical flame-cell (fig. 26). Meyer figures as many as five pairs of such pronephridia in Nereis cultrifera (**80**)[1]. Although the head-kidneys of the Mollusca undoubtedly occasionally open internally, v. Drasche and Hatschek seem to have been mistaken in describing an internal opening in these organs in the Chætopods (see Fraipont, **40**; Meyer, **80**).

Fraipont appears to attribute a very similar history to the wide-mouthed trunk "nephridia" of Polygordius as Meyer has described for the trunk "nephridia" of the Tubicolous Polychætes, though his statements are less precise: "Le mésoblaste est representé de plus au niveau des muscles obliques par une masse de cellules assez confuse. C'est dans ce groupe de cellules situées au dessus des muscles obliques contre les champs musculaires longitudinaux que ce différencient les entonnoirs des organes ségmentaires et plus tard encore les organes sexuels. C'est un simple épaississement du péritoine" (**40**).

We see, then, that in the Polychæta nephridia are developed from large cells, which may be compared to the "funnel cells," giving rise to the nephridia in the Oligochæta. Whilst, however, in the latter the pronephridium acquires an opening into the cœlomic follicle independently of the peritoneal funnel, which acts as a genital duct; in the former, the Polychæta, the pronephridium may acquire an opening into the cœlomic follicle in the region where the peritoneal funnel is formed, fuse with it, and become an organ of double function—excretory and genital. In many cases division of labour leads to the restriction of the genital function to one set of cœlomic follicles and their funnels, and of the excretory function to another set

[1] There can now, I think, be no doubt that the head-kidneys are simply the precociously developed nephridia of the first segment; they do not open into the cœlom for the very good reason that at this stage there is, as a rule, no cœlom for them to open into. They preserve the same relations as the Platyhelminth nephridia (Hatschek, **48**, **51**, **54**; Meyer, **80**).

of cœlomic follicles and their funnels. This may lead to a corresponding differentiation of structure; in the first set the peritoneal funnel becomes the most important part, in the second the nephridial portion (such specialisation has been well described in many forms by Eisig, Meyer [**80**], Trautzsch [**98**], Cunningham [**25**], Marion and Bobratzky [**78**], &c.).

The fusion between the nephridium and peritoneal funnel does not occur in all Polychætes; fortunately, we appear still to have all the intermediate stages between this condition and that of the Oligochætes.

Meyer (**80**) has shown that in Nereis the nephridia of the first five segments have the typical pronephridial structure with a flame end-cell, and that in the posterior segments this end-cell (judging from his figures) opens into the cœlom (true nephrostome; compare Rhynchelmis).[1] I have also described in the Lycoridea (**41**) a ciliated region of the cœlomic epithelium which I believed to be the peritoneal funnel. It is, however, to Eisig that we owe the description of what appear to be intermediate stages. In Dasybranchus and Tremomastus we have conditions in which the peritoneal funnels (Genitalschlauche) are separate from the nephridia and open independently (fig. 14), and in which the two organs are connected but still open separately (fig. 13). Perhaps in certain segments of these forms and of Capitella we have the more usual Polychæte arrangement, in which the peritoneal funnel no longer acquires an independent opening (**31**).

Arthropoda.

It will be best to begin our review of this group with a brief recapitulation of the development of Peripatus, which has been so excellently described by Mr. Sedgwick (**94**), and Dr. von Kennel (**64**). Soon after the metameric somites have been hollowed out to form the cœlomic follicles, the upper half of each cœlomic cavity becomes nipped off from the lower half. From the wall of each of these lower cœlomic sacs a peritoneal

[1] Such would appear to be the condition in Protodrilus, where the nephridial funnels figured by Hatschek (**52**) are small, and provided with a flagellum.

funnel is formed as an outgrowth, which fuses with the epidermis (figs. 15 and 16). V. Kennel maintains that in Peripatus Edwardsii the mesoblastic funnel is met by an epiblastic invagination, and the question arises as to whether this invagination represents a true nephridium, in which case the segmental organ of Peripatus would be a compound organ similar to that of Psygmobranchus (see above), or whether it is merely a secondary invagination of the epidermis, such as occurs more or less pronounced in almost every case where a tube opens on to its surface (vesicle of the nephridia in the Hirudinea, peripheral end of the genital ducts of the Oligochæta, &c.). I am inclined to take the latter view, and consider the segmental organs of Peripatus as purely peritoneal funnels which have assumed the excretory functions. Whilst these organs have developed in this way, the dorsal or genital halves of the somites in the posterior segments have become fused, forming two genital tubes communicating posteriorly with the undivided cœlomic follicles of the last segment. The peritoneal funnels of this segment retain their primitive function, and develop into the genital ducts (fig. 17). The peripheral ends and median portion of the ducts are probably derived from the epidermis.

The history of the genital and excretory organs of the other groups of Arthropoda can easily be brought into agreement with the development of these parts in Peripatus. However, whereas in the latter all the cœlomic follicles give off peritoneal funnels, in the Crustacea, Arachnida, Myriapoda, and Hexapoda the peritoneal funnels are only fully developed in a very few segments. The shell glands and green glands of the Crustacea have been shown to bear the relations of peritoneal funnels (Grobben, **45**; Weldon, **104**; Marchall, **77**; Allen, **1**) and develop from the cœlomic follicles. "Bei Daphnia," says Lebedinsky (**73**), "entwickelt sich . . . die Schalendrüse als die Ausstülpung der Somatopleura, welche sich zur Max.^{2} richtet und hier sich mit dem Ectoderm vereinigt." The same author describes the development of the coxal gland of Phalangium as a typical peritoneal funnel derived from a cœlomic follicle (**73**),

which description agrees with that of Laurie (**72**) of the origin of the coxal gland in Scorpio, and of Kingsley (**65**) in Limulus.

The genital cells are derived from the walls of the cœlomic follicles [1] of many segments (Heymons, **57**; Wheeler, **105**). The dorsal portion of these follicles generally fuse to form continuous tubes, or a median genital sac (as in the Crustacea). The ducts are the peritoneal funnels of one segment. The particular segment selected, so to speak, for this purpose varies much in position in different groups, and also according to sex. Wheeler, in his admirable account of the development of the Orthoptera (**105**), shows that the cœlomic follicles of all the abdominal segments at an early stage begin to develop peritoneal funnels, but that those of one segment only reach the exterior and form the genital ducts (fig. 30).

As far as we can see, therefore, there are no certain traces of true nephridia in the Arthropoda. The segmental organs, the green glands, the shell glands, and the genital ducts are all developed as peritoneal funnels.[2]

Sipunculus.

The development of the excretory organ of Sipunculus nudus has been described by Hatschek (**53**). The nephridium arises from a large cell (? "funnel-cell") which comes to lie in the wall of the cœlomic follicle. This cell divides, forming a chain of cells in which a lumen is developed. The outer end joins and opens on to the epidermis; the inner end grows towards and opens into the cœlom. The ciliated funnel is formed from the cœlomic epithelium. From this it would appear that the excretory organ of Sipunculus is of a double origin, formed by the junction of the nephridium with the peritoneal funnel, as in most Polychætes. It functions as the carrier of both genital and excretory products.

[1] Sedgwick holds that they are, in Peripatus capensis, derived from the hypoblast. If this be the case, it must be considered as due to some secondary modification.

[2] The possibility of the segmental tracheæ of the Arthropods being derived from the true nephridia should not be lost sight of. The tracheæ arise comparatively late, as a rule, as invaginations of the epidermis, and it seems not

Phoronis.

In the larva of Phoronis, Caldwell describes a pair of nephridia, slender ciliated canals blind internally (**21**). Their origin is still doubtful. The cœlom is developed as two pairs of follicles, of which the larger "posterior" pair is alone fertile. The excretory organs of the adult consist in Phoronis psammophila and Ph. Kowalevskii of a pair of peritoneal funnels leading to the exterior from the "posterior" cœlomic follicles (Cori, **23**). According to Caldwell (**22**), they are developed in connection with the nephridia of the larva, and would appear to be of a compound nature like those of Polychætes. In Phoronis australis both the anterior and posterior pairs of cœlomic follicles are provided with their peritoneal funnels, which open by a common duct (Benham, **5**). These funnels in Phoronis serve both as renal and genital ducts.

Ectoprocta.

No true nephridia appear to have been found in these Polyzoa. On the other hand there are two peritoneal funnels, an excellent account of which has been given by Cori in Cristatella (**24**). They differ from those of Phoronis only in that they open by a common median pore.

Brachiopoda.

Here the cœlomic follicles, formed as paired archenteric pouches, are provided with a pair of wide-mouthed peritoneal funnels opening to the exterior. They function as the carriers both of excretory and of genital products, and possibly the distal end of the organ represents the true nephridium (also in the Ectoprocta), which has fused with the peritoneal funnel (Morse, **83**; Blochmann, **11**).

impossible that they may be formed not from the nephridia, but from those late epidermal invaginations which, as we have seen, so generally occur in connection with the external opening of the peritoneal funnels. In the Arthropods above mentioned these funnels disappear in those segments which possess tracheæ.

Sagitta.

Archenteric diverticula give rise to the three pairs of cœlomic follicles present in the adult Sagitta. The genital cells are precociously developed, and come to lie in the two posterior pairs of follicles (Hertwig, **56**). Whether the genital ducts—which in the male segment, at all events, open into the cœlom by ciliated funnels—are peritoneal funnels, or are partly formed from true nephridia, cannot be decided with our present incomplete knowledge of their development.

Echinoderma.[1]

Only a very brief reference can be made to this highly modified group. In the larva we find a right and left cœlomic follicle, the enterocœls (derived from unpaired or paired archenteric diverticula), which may give rise to a second pair of cœlomic follicles by constriction. The anterior follicles then develop peritoneal ciliated funnels (fig. 18), which open to the exterior, fusing with paired epiblastic invaginations. As a rule only the left peritoneal funnel becomes developed (Field, **36**; Bury, **20**). The genital cells are developed from the wall of the posterior cœlomic follicles (MacBride, **75**, **76**); but how far the genital ducts can be likened to peritoneal funnels is quite uncertain.

There appears to be no trace of true nephridia.

Vertebrata.[1]

As is well known, the cœlom in the Vertebrates arises by the hollowing out of a series of metameric blocks of mesoblast. In the lower forms (Balanoglossus and Amphioxus) several of the anterior cœlomic follicles are formed directly as pouches from the wall of the archenteron. From these follicles, produced by either method, peritoneal funnels are developed

[1] If the treatment of these last two groups (Echinoderma and Vertebrata) seems to be somewhat too brief and dogmatic, it is that space will not allow me to discuss the subject *in extenso*. Moreover, we are here treading on such uncertain ground that I do not feel competent to treat of the structure of these animals in full detail, and offer these remarks merely as a suggestion.

which communicate with the exterior. The gonads develop from the wall of the follicles; in the lower forms a large number are fertile, but in the higher forms the genital products become restricted to a very limited region.

No true nephridia have been discovered in this group; for, until the development of the interesting segmental tubules described by Weiss (**103**) and Boveri (**14**) in Amphioxus is known, it is not possible to decide for certain on their nature.

Hemichorda or Enteropneusta.—In the anterior or proboscis region is a cœlomic cavity which appears to represent a fused pair of follicles (fig. 23), as is evidenced by the fact that it communicates with the exterior by paired peritoneal funnels (ciliated proboscis pores) in Balanoglossus Kupfferi and B. canadensis, and occasionally in Ptychodera minuta and B. Kowalevskii (Spengel, **96** and **97**), and by its development as a bilobed sac in B. Kowalevskii (Bateson, **2**).[1] The collar region contains a second pair of cœlomic follicles, also provided each with a peritoneal funnel or collar pore (Bateson, **2**; Morgan, **82**; Spengel, **97**). Behind these is a third pair of follicles which do not develop funnels, but become of large size and encroach on neighbouring segments, sending back blind posterior prolongations. The posterior region contains a series of fertile cœlomic follicles, the gonads (fig. 23), each provided with a peritoneal funnel leading to the exterior.[2] Although the metamerism of this region is not very definitely pronounced, possibly having become obscured through degeneration, the genital follicles bear a remarkable resemblance, both in their structural relations and in their arrangement, to those of the Nemertines, as has already been noticed by Schimkewitsch (**93** and **93a**). As in the Nemertines, so also in the Enteropneusta, several genital follicles may occur in one segment.

[1] Compare the development of the corresponding anterior pair of cavities in Amphioxus (Hatschek, **49**).

[2] If this explanation be correct, the gill-slits of Balanoglossus are metameric and intersegmental as in other Vertebrates. If not correct, we have an almost incredible state of things in which the genital cells are shed into a cavity which is not the cœlom.

Craniata.—Owing mainly to the recent researches of van Wijhe (**99**), Rückert (**89** and **90**), Semon (**95**), and others, it is now generally concluded that the Craniates originally possessed a metameric series of pronephric tubules, or peritoneal funnels, opening independently to the exterior. In existing forms the funnels, which grow out from the cœlomic follicles, sometimes reach the epiblast as solid rods (fig. 19) ; they then fuse with each other to a common duct which opens into the cloaca (fig. 31), constituting what is known as the pronephros and its pronephric duct. In his excellent paper on the development of the renal system of Ichthyophis, speaking with regard to E. Meyer's theory, Semon says : "Die segmentalen Ausführgänge der segmentalen Genitalfollikel oder Ursegmente übernehmen neben ihrer ursprünglichen auch noch exkretorische Funktion, sie werden zu Vornierkanälchen" (**95**).

To conclude our general survey it may be said : that the cœlom can be traced from its smallest beginning as a cavity or cavities in which are developed the gonad-cells : it grows gradually in size and importance until it becomes the body cavity in which the viscera rest ; that the genital ducts (with a few possible exceptions due to secondary modifications) are homologous throughout the Cœlomata ; that the nephridia, which have often been confused with these ducts, can always, when they occur, be distinguished from them ; and finally, that the cœlom may secondarily acquire a renal function, in consequence of which the peritoneal funnels supersede the nephridia proper as excretory ducts.

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EXPLANATION OF PLATES 44 & 45,

Illustrating Mr. Edwin S. Goodrich's paper on "The Cœlom, Genital Ducts, and Nephridia."

Reference Letters.

c. Cœlomic or genital follicle. *ep. i.* Epidermal invagination. *neph.* True nephridium. *p.f.* Peritoneal funnel developed from the cœlomic epithelium.

In all the diagrams the outline of the cœlom, the peritoneal funnels, and the genital cells, are drawn in red. The epiblast and hypoblast are darkly shaded. The mesoblast is lightly shaded. The true nephridia are drawn in black.

Fig. 1.—Diagrammatic plan of the cœlom, genital ducts, and nephridia in the Planaria.

Fig. 2.—Similar plan of a young Nemertine.

Fig. 3.—Plan of a mature Nemertine.

Fig. 4.—Plan of the cœlom, &c., in the Entoprocta.

Fig. 5.—Plan of the cœlom and nephridium in an embryo Oligochæte.

Fig. 6.—Later stage of the same, showing the nephridia in the pronephridial condition.

Fig. 7.—Plan of the cœlom, &c., in an adult Oligochæte (Lumbricus).

Fig. 8.—Plan of the cœlom and nephridia in an embryo leech.

Fig. 9.—Plan of the cœlom, &c., in the Hirudinea.

Fig. 10.—Plan of the cœlom and nephridia in an embryo Polychæte.

Fig. 11.—Later stage of the same, showing the pronephridium about to fuse with the peritoneal funnel.

Fig. 12.—Plan of the cœlom, &c., in the Polychæta possessing wide-mouthed "compound nephridia," the nephridium having fused with the peritoneal funnel.

Fig. 13.—Plan of the cœlom, &c., in certain segments of Tremomastus and Dasybranchus in which the nephridial funnel is continuous with the genital or peritoneal funnel.

Fig. 14.—Plan of the cœlom, &c., in certain segments of Dasybranchus caducus, in which the nephridial funnels are independent of the peritoneal funnels.

Fig. 15.—Plan of the cœlom and developing peritoneal funnels in an embryo Peripatus.

Fig. 16.—Plan of the cœlom and peritoneal funnels in a trunk segment of Peripatus. The cœlomic follicles on either side have become separated into a ventral and a dorsal portion, forming the genital tube.

Fig. 17.—Plan of the cœlom and peritoneal funnels in the posterior segment of Peripatus.

Fig. 18.—Plan of the cœlomic follicles (enterocœls) and developing peritoneal funnels in an embryo Echinoderm (Asterias).

Fig. 19.—Plan of a stage in the development of the cœlom and peritoneal funnels (pronephric tubules) of an Elasmobranch.

Fig. 20.—Plan of the cœlom and nephridia (pronephridial stage) in an embryo Mollusc.

Fig. 21.—Plan of the cœlom and developing peritoneal funnels (excretory organs of the adult) of a Mollusc.

Fig. 22.—Farther stage of the same, after the peritoneal funnel has fused with the epidermal invagination.

Fig. 23.—Plan of a longitudinal section of Balanoglossus, showing the cœlomic follicles and peritoneal funnels.

Fig. 24.—Plan of a longitudinal section of a Nemertine, showing the cœlomic follicles and peritoneal funnels before the latter have reached the epidermis (the nephridia are represented in the same region as the genital follicles).

Fig. 25.—Plan of a longitudinal section of the nephridia, cœlom, and developing peritoneal funnels in a larval Oligochæte.

Fig. 26.—Similar plan of a larval Polychæte.

Fig. 27.—Plan of a longitudinal section of the cœlom and peritoneal funnels in an embryo Vertebrate.

Fig. 28.—Plan of a longitudinal section of the cœlom, peritoneal funnels, and nephridia of a Leech.

Fig. 29.—Plan of a longitudinal section of the cœlom, &c., of an Oligochæte.

Fig. 30.—Plan of a longitudinal section of the cœlom and developing peritoneal funnels of an embryo Insect.

Fig. 31.—Plan of cœlom and peritoneal funnels (pronephric tubules) of a Vertebrate.

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