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On the Morphology and Physiology of the Brain and Sense Organs of Limulus.

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With Plates 1, 2, 3, 4, 5.

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Introduction.

Some two years ago I published a short paper in this Journal calling attention to many striking resemblances between Arachnids and Vertebrates. I maintained that the Vertebrates are descended from a great group of Arthropods, in which I included the Arachnids, Trilobites, and Merostomata; and that the remarkable palæozoic fishes Pterichthys, Bothriolepis, and Cephalaspis resemble merostomatous Arthropods like Pterygotus, Eurypterus, &c. Now, the internal structure of the Merostomata and Trilobites cannot differ greatly from that of Limulus, judging from their resemblance in external characters; therefore, although Limulus itself is not in the main line of Vertebrate descent, a study of its structure will best enable us to understand that of the Merostomata and of the primitive Vertebrates.

In my preliminary paper the lines were indicated along which I had found evidence of relationship between Vertebrates and Arachnids. It was shown—and this first drew my attention to the subject—that the invaginations, which in insects give rise to the optic ganglia, in scorpions and Limulus

become so extensive as to enfold not only the optic ganglia, but the eyes and the fore-brain as well. A cerebral vesicle is thus formed, from the floor of which arise the fore-brain and the optic ganglia, and from the roof a tubular outgrowth, at the end of which lie the inverted retinas of the parietal eye. Such a condition is found only in Arachnids and Vertebrates; and in my judgment this fact, when all its bearings are considered, affords as trustworthy evidence of relationship as the presence of a notochord or of gill-slits.

It was further shown (1) that the lateral eyes of Limulus could be compared with the lateral eyes of Vertebrates; (2) that there is in Arachnids a cartilaginous endocranium similar in position, shape, and development to the primordial cranium of Vertebrates; (3) that there is in scorpions and in other Arthropods a subneural rod similar to the notochord of Vertebrates; (4) that in the Arachnids and in the Vertebrates the brain contains approximately the same number of neuromeres; it is divided into the same number of regions, i. e. fore-brain, mid-brain, hind brain, and accessory brain; and while in each region there is a different number of neuromeres, i. e. 3, 1, 5, 2 to 4, the number in the corresponding regions in Vertebrates and Arachnids is very nearly the same; (5) the nerves of each brain region in both Vertebrates and Arachnids show in a general way the same relation to sense organs, the same ganglia, and the same distribution and fusion with one another; (6) finally, there is a striking similarity between the cephalo-thoracic shields of Arthropods and the cephalic bucklers of the earliest fishes, such as Zenaspis, Bothriolepis, Pteraspis, Auchenaspis, &c. The shape and microscopic structure of the shields, and the arrangement of the eyes upon them, are practically the same in both groups. The three median and two lateral eyes of Cephalaspis Campbelltonensis as figured by Whiteaves ('Trans. Roy. Soc. Canada,' vol. vi, 1888, pl. x) have exactly the same arrangement as those of Limulus. I have carefully revised the palæontological aspect of the subject, and I hope in a separate paper to give it the careful consideration it deserves.

Attention was also called to resemblances of a more general character between Vertebrates and Arachnids, such as, for example, in the structure of the muscles, the nerves, and the liver; in the position and the net-like structure of the sexual organs; in the origin of the ova and the spermatozoa; in the origin of the germ-layers, and in the general formation of the embryos.

To this formidable array of evidence I can now add the following:—(1) It is possible to identify nearly all the important lobes and cavities characteristic of the Vertebrate fore-brain in the fore-brain of Limulus. (2) The coxal sense organs are shown by conclusive experiments to be gustatory, and to correspond to the supra-branchial sense organs of Vertebrates. (3) An extraordinary organ has been discovered in Limulus, having all the characteristic morphological features of the olfactory organ in Vertebrates. It is united with olfactory lobes that arise as outgrowths from the fore-brain. It consists of an upright layer of epithelium containing olfactory buds similar to those in the coxal (supra-branchial) sense organs. It is supplied by four nerves, the median ones resembling, in histological structure, those of Vertebrates. One pair of these nerves arises from the cerebral hemispheres, the other from the optic thalami. I long ago looked for something answering to the olfactory organ in Vertebrates, but finally gave up the search because that part of the cephalic lobes where, as I supposed, they ought to appear was invaginated, consequently any sense organ derived from that region must also be invaginated, and could not be homologous with the olfactory organ of Vertebrates.

Most of the physiological results were obtained during the summer of 1892 at the United States Fish Commission Laboratory at Wood's Holl, Mass., the facilities of which were generously placed at my disposal by Commissioner MacDonald.

The descriptive part of this paper deals mainly with Limulus, but incidental references are made to scorpions and other Arthropods. As I aim to point out resemblances between Vertebrates and Arachnids, I shall not enter into histological

details that might obscure the meaning of the broad facts I wish to present. However, I shall describe the morphology and physiology of the olfactory organ, and of the gustatory and temperature organs on the appendages and elsewhere in detail. In order to justify the comparisons instituted between these sense organs and those of Vertebrates I have given a general account of the structure and development of the brain and median eyes.

I do not hesitate to say that I believe the results herewith presented prove beyond any reasonable doubt that the Vertebrates are descended from the Arachnids.

Part I.—Sense Organs.

I. Gustatory Organs.

a. Experiments on the Gustatory Organs of the Mandibles.—If an adult horseshoe crab be placed on its back with its abdomen hanging over the edge of the table, it makes fruitless movements of the legs and abdomen to recover its natural position. The muscles, however, soon relax, and the animal usually becomes perfectly quiet, except that after long intervals the gills are raised and lowered a few times, and then held up motionless a few seconds till every part, expanded to its full extent, is thoroughly aërated; they then sink slowly back to their original position. If, while in the quiescent condition, the jaw-like spurs or mandibles (Pl. 1, fig. 3, *o. md.*) at the base of the legs are gently rubbed with some hard object, such as a piece of wood, glass, or iron; or if water or air, the temperature of the surrounding medium, be gently poured over them; or if the animal be vigorously fanned, or loud noises be made near it, only slight aimless movements of the legs or abdomen are produced, usually none at all. But if a very small piece of clam or other edible substance be rubbed ever so gently over the stout spines that arm the mandibles, very characteristic chewing movements are immediately produced. If all the mandibles are touched in this way, or even moistened with a few drops of water in which pieces of clam have been soaked,

the cheliceræ snap and work alternately back and forth, as though tucking something into the mouth; at the same time the metastoma are moved forward and backward. But the most constant feature is the following movement of the second to the fifth pair of appendages; the second and fourth pairs of mandibles move in unison inward toward the median plane, and downward toward the mouth; then back again in the reverse order. When they are farthest from the mouth the corresponding legs (except the second pair in both males and females) are quickly raised, flexed, and the tips carried toward the mouth, where they remain an instant, and then fall back on to the under side of the carapace; the corresponding jaw movement then begins again. The third and fifth pairs of appendages and the corresponding jaws work in unison in the same manner, but they alternate with those of the second and fourth. At intervals these movements cease, the abdomen is raised, and the stout crushing mandibles on the sixth pair of appendages, which have heretofore remained motionless, are slowly closed with great force, as though to crush some object too large to be swallowed whole, or to kill some struggling prey. These powerful jaws then slowly relax their convulsive grasp, and the chewing movements are resumed. All these movements go on with the greatest precision and regularity, so that any food placed on the jaws is forced into the mouth, and gradually disappears down the œsophagus. These chewing movements are produced when drops of soluble food, or almost any bit of animal matter, or wads of blotting-paper wet in nutritive animal fluids, come in contact with the mandibles. Drops of water from the interior of a clam will set the whole complicated mechanism to working in exactly the same manner as during the actual process of eating. Again, chewing movements of the mandibles are produced whenever ammonia vapour, ether, or chloroform is blown over them with a medicine-dropper, or when they are stimulated by a weak interrupted current; but in such cases these movements are rarely accompanied by the leg movement. If the irritation with ammonia or acids has been rather great the mandibles work apparently as

in eating, but the cheliceræ move rapidly back and forth, making frantic snapping movements toward the mouth, as though to pick away some disagreeable object. These movements usually last a long time. If wads of blotting-paper wet in ammonia or picric acid are used the chewing movements are reversed, and the offensive object is sometimes snapped up by the cheliceræ and rejected.

Holding strong-smelling food as close as possible to the mouth or to the jaws produces no effect, although chewing movements are instantly produced when the jaws are touched by it.

When a very small piece of clam or of pecten is touched to the surface—say of the third mandible on the left side—care being taken not to touch any other parts, that leg will be promptly raised and the tip bent toward the mouth; it soon falls back on to the cephalo-thorax again, and then its mandible moves back and forth, alternating with the leg movement, as in eating; but all the other mandibles and appendages remain quiet. One may start in this way one appendage after the other (except the cheliceræ, which have no mandibles), until all of them, first on one side and then on the other, are working in perfect rhythm.

If the mandibles of the post-oral appendages on one side are stimulated, the chelicera of that side, although not stimulated itself, soon has its tip extended straight backward as far as it can reach, and may remain some time in this rigid, unnatural position; or else it begins those characteristic back and forward movements, snapping its chela from time to time, as though to seize something and lift it up, or else thrusting the chela down the mouth as though forcing some piece of food into the œsophagus. If the jaws on the opposite side are now stimulated, the chelicera of that side begins to work also. It is a curious fact that the cheliceræ rarely move when the mandibles of the second or third appendages are stimulated; not till the last one or two pairs of mandibles are set in motion do they begin their characteristic movements. It is evident that the chewing movement produced in these various ways

is a reflex act caused by the stimulation of gustatory organs about the mouth.

The following experiments show that there are three kinds of these gustatory organs, and that they are situated in the inner and outer mandibles of the second to the fifth pair of appendages. The organs of the first kind are located in the mandibular spines, the second on the smooth concave margin of the inner mandible (fig. 3, *g. b.*), and the third are scattered over the surface of the mandibles between the spines.

(1) If the outer surface of one of the mandibles—say the second or third on the right side—be very gently touched with a piece of clam, as small even as the head of a pin, the characteristic chewing movements of that mandible and the corresponding leg are alone produced. With care all the appendages on one side, or any number of them, can be made in this way to go through the chewing movements, while the other legs and mandibles remain perfectly quiet.

(2) If any one mandible, or any combination of them, be amputated, then stimulation of the mouth region with food produces chewing movement of the remaining mandibles and of the corresponding legs, but those legs from which the mandibles have been removed remain perfectly quiet, even though food be rubbed on the mouth, the rostrum, the soft skin about the base of the legs, or on the proximal part of the leg itself. This proves that the gustatory organs are located in the mandibles alone, and not in or around the mouth, in the rostrum, or in the base of the leg.

(3) If the stiff spines be shaved off of one or more mandibles and a piece of clam rubbed over the outer anterior surface of the shaved mandibles, either no effect at all is produced, or else feeble movements of the mandibles only, without the leg movement. But the least contact of the clam against the unshaved mandibles produces at once the characteristic mandible and leg movements; it makes no difference whether the shaved mandibles are all on one side or not, or what combination may be selected. This proves that a large proportion of the gustatory organs are situated in the mandibular spines; it also

shows that the reflex in each leg and mandible is due solely to the stimulation of its own gustatory organs, and that it is entirely independent of the reflex in the adjacent appendages, either of the same or opposite side: this, however, does not apply to the cheliceræ.

(4) If the shaved mandibles be rubbed on their outer anterior surface, movements are rarely produced; but they are fairly well marked whenever a piece of clam is touched against the smooth concave surface of the inner mandible (fig. 3, *g. b.*). In performing this experiment, the shaved or unshaved mandible, it is immaterial which, is gently raised with a pair of forceps, care being taken not to touch the animal with the fingers, and a very small piece of clam rubbed on the smooth surface in question. Immediately the inner mandible is retracted by the muscle shown in fig. 3, *m. i. m.*, and then the whole mandible begins its rhythmic movements. This proves that, besides those organs in the spines, the smooth inner surface of the inner mandible contains a second set of gustatory organs, which when stimulated produce reflex chewing movements.

(5) The results of the following experiments may be stated in this way:—Destroying a certain number of gustatory organs in one or more mandibles suspends the reflex in the mutilated appendages. The reflex may be partially restored by destroying a corresponding number of sense organs in each of the remaining mandibles.

The following experiments were performed successively on the same individual:—(*a*) A healthy crab that was known to be very sensitive to gustatory stimulation was deprived of a part of its gustatory organs by shaving off all the gustatory spines on the mandibles of the right side. Ten days after, on equal stimulation of both sides, the shaved appendages remained perfectly motionless, while the unshaved ones began the normal chewing movements. But the shaved mandibles could be made to act when vigorously stimulated. (*b*) When the mandibles on the left side also were shaved, the reflexes were impaired for some time. However,

a week or ten days after, vigorous movements on both sides were produced by rubbing pieces of clam well over the mandibles. As might be supposed from experiment No. 4, the movements were especially well marked when the clam was rubbed over the under side of the inner mandibles, but they could be produced when only the outer part of the mandibles was touched. After the reflex had been once restored it required but little more stimulation to start the reflex in the shaved mandibles than it did before in the unshaved ones. (*c*) Now if we cut off the inner mandible of the right side the reflex on that side will again be suspended, but it can be once more partially restored by (*d*) cutting off the inner mandibles of the other side. The restored powers are each time feebler than before, but nothing short of amputating both inner and outer mandibles will completely and permanently destroy the reflex. The feeble movements caused by stimulation after the spines and the inner mandibles have been removed are produced by scattered gustatory buds distributed between the spines over the anterior face of the mandibles. The reflex chewing movements in one appendage, therefore, are not lost in proportion to the destruction of sense organs in it, but depend rather on the relation between the number of sense organs retained in it and those in the other appendages. Hence if the reflex in one appendage is suspended by destruction of a certain number of sense organs, it may be partially restored by reducing the number of sense organs in the other appendages in a like degree. In other words, the reflex impulse enters the widest door.

The reflex flows more readily along the most recently used lines, as shown by the following experiment :—If mandible A be slightly stimulated with food, whether enough to produce reflex movements or not, subsequent (say five minutes after) stimulation of all the mandibles to the same extent will produce reflex movement in mandible A first, and afterwards feebler movements in all the others.

B. Structure of the Gustatory Organs of the Man-

dibular Spines.—It is not difficult to find the three sets of gustatory organs, the existence of which is demonstrated above. Examination under a low magnifying power shows that the mandibular spines are thickly covered on their sides nearest the mouth with minute pores arranged in from eight to ten interrupted vertical lines (fig. 2). Each line consists of several subordinate groups composed of from two to twelve or more pores. Longitudinal sections of one of the spines (fig. 1) show that the cuticula is perforated by parallel canals, in each of which is a delicate chitinous tubule (*ch. t.*); the latter terminates at the outer opening of the canal flush with the surface; at the opposite end it bends nearly at right angles towards the base of the spine, where it soon expands into a clear, spindle-shaped body (*sp.*); beyond the spindle it is continued as a very long slender process that constitutes the body of the gustatory cell, the nucleated end of which unites with other cells to form great ganglion-like masses (*gsc.*).

The spindle, when more highly magnified (fig. 5), seems to be merely an inflation of the cell-wall, and contains, besides a watery fluid, a number of fibrillæ, arranged in a single layer along its inner wall, and continuous with fibrillæ in the stalk of the cell. The proximal half of the spindle is usually stained a little darker than the rest, and in it each fibril expands into a fusiform thickening that stains deeply in acetic acid carmine. The fibrillæ converge toward the distal half of the spindle to form an axial bundle that can be followed nearly to the free outer end of the chitinous tubule. From two to eight slender bipolar cells surround each spindle, and send their fibrous processes outward along the outer surface of the chitinous tubule (figs. 5, 6, *g. c.*).

It is difficult to tell just where the tubule begins. A short distance beyond the spindle (figs. 5 and 6) it appears to be continuous with the cell-wall, which there becomes rather distinct owing to its separation from the cell contents. It is slightly indented in some places, and is apparently enclosed by a second membrane, probably the outward prolongation of the nerve-like cells surrounding the spindle, for when

thoroughly macerated these cells fall off, and the outer membrane is then absent. The sharp inner wall, the investing membrane, and the axial nerve produce a picture very much like that of a Vertebrate medullated nerve.

In macerated preparations the cell usually breaks just beyond the spindle, as in figs. 5—7, but in some instances nearly the whole tubule is isolated. When the tubule breaks near the spindle, the axial nerve usually projects a long distance from its open end, either as a single fibre (figs. 5, 6) or as a delicate brush of fibrillæ (fig. 7). Beyond this region it breaks with a clean fracture, as though made of glass, and a protruding axial fibre is rarely seen. Towards its outermost end the axial nerve cannot be seen under any circumstances, but this is due to the fact, I believe, that the canal in the tubule is so small that it is completely filled by the nerve. The tubule is thickest where it enters the cuticular canal (fig. 1, *s. ch. t.*); but it becomes smaller and smaller, as well as the canal in which it lies, until, at the surface, the tubule just fills the canal. In surface views (fig. 2) the black dot is the pore of the canal, completely filled by the tubule; the clear halo surrounding it is cuticula more transparent than the rest.

The stalk of the gustatory cells, just below the spindle, is very finely striated, and when macerated and broken, as in fig. 5, the striation is seen to be due to the presence of excessively fine fibrillæ, much more numerous than those in the spindles. There are no nuclei to be seen on the long nerve-like stalk of the cell, between the spindle and the nucleus. On the distal side of the nucleated part of the cell are usually a few yellowish-brown pigment granules, that in some cases are large and very numerous, as in fig. 4.

The nucleated ends of the gustatory cells are arranged in long clusters along roughly parallel lines, each band of cells corresponding to a line of pores. Judging from the number of cells it contains, the cluster at *gs. c.*, fig. 1, is probably connected with a row of pores running the whole length of the spine, although this is difficult to determine with certainty.

There are a few very large ganglion-cells, with coarse, anastomosing, plasmodia-like processes, that run at pretty regular intervals one above the other around the inside of the spines (fig. 1). One may often isolate great masses of this finely fibrillate reticulum without finding a nucleus in it. It usually overlies and unites large bundles of the slender stalks of the gustatory cells, but the latter appear to pass through the plasmodium without change.

The body of the gustatory cells resembles that of the double retinal cells of Molluscs and Arthropods, described by me in Arca, Pecten, Acilius, Lycosa, and others, in that the large nucleus is nearly always excentric, and a spiral partition separates the cell into two more or less distinct portions (fig. 12). In one lies the main nucleus, n^1; in the other a small unstained body that I regard as the aborted nucleus of the second cell, n^2. The proximal end of the cell is sometimes forked, each branch being continued into a nerve-fibre. The interesting fact is thus established that double cells are not confined to the retina.

The cuticular canal of the gustatory organ can be readily distinguished from other canals by the presence of a peculiar swollen knee, or bend, near its outer third, the surrounding cuticula of which stains more deeply in borax carmine than elsewhere (fig. 1). Under favorable conditions one can see in the "knee" a kind of spiral thread, caused by what in some cases appears to be a spiral ridge on the wall of the canal, in others by a spiral nerve-like fibril that seems to surround more or less loosely the chitinous tubule (fig. 13). A similar spiral thread is sometimes seen on the isolated chitinous tubule (fig. 6, *a*); when treated with dilute potash the tubule swells a little, and a thin, finely granular layer is seen about it, together with what appears to be an extremely delicate fibril, wound spirally about the tubule and its sheath (fig. 14).

In some of the cuticular canals there are a few slender nerve-like cells loosely surrounding the chitinous tubule (fig. 1); in other cases these cells seem to be absent. That the tubules of the gustatory organs and of the others described

below are chitinous is shown by their resistance to caustic potash, and by the fact that they are shed during ecdysis. On examining cast-off shells one can see the perfectly preserved tubule projecting out of the inner ends of the cuticular canals.

Each spine on the jaws is crowded with the organs just described, and contains also a large blood-vessel. There can be no doubt that they are the gustatory organs, which, when stimulated, produce the reflex chewing movements described above.

c. Experiments on the Gustatory and the Temperature Organs of the Chelæ.—Two varieties of organs, having nearly the same histological structure and arrangement as those on the mandibular spines, are found on the last two joints, or chelæ, of the first to sixth appendage. One kind is a gustatory, the other, in all probability, a temperature organ.

The presence of the gustatory organs may be demonstrated by the following experiments:—Place a crab on its back and allow it to become quiet; then if the chelæ, which are usually lightly closed, are rubbed with a small piece of clam, they will open wide, and remaining so, move about rather vaguely, as though trying to grasp something. They are specially sensitive at the tips and along the cutting edge. Ammonia vapour will produce the same result, but it cannot be produced by any purely mechanical stimulus.

The temperature organs betray their presence by an entirely different action, for if one breathes very gently, or blows warm air on the chelæ, they suddenly close and open once, and will repeat the action without variation as often as they are stimulated in this way.

A very curious fact is the following:—If the chelæ are amputated at the penultimate joint, they remain perfectly quiet if left alone. But for five or ten minutes after the operation they will snap once, i.e. close and immediately open again, every time they are gently

breathed upon. But stimulation with food or ammonia produces no effect whatever. These facts seem to indicate that there is a reflex centre in the chelæ for the temperature impulses, but none for the gustatory ones. A rather hurried examination, however, failed to show the presence of any centre there, unless the few scattered tripolar ganglionic cells found everywhere in the subdermal nerve-plexus can be regarded as such.

Description of the Organs.—As one might expect after the above experiments, surface views of the chelæ show the presence of two kinds of organs. Those of one kind appear as small pores surrounded by a clear halo, and resembling, in their arrangement in lines and in every other particular, the gustatory organs on the mandibular spines (figs. 10 and 11, *g. o.*). See section C. The others are less numerous than the first, but larger, and over each canal there is a saucer-shaped depression, from which projects a short blunt spine. A chitinous tubule passes up the wide cuticular canal, and terminates in the spine. The same kind of organ is also found about the bases of the larger mandibular spines. As the first organs are just like those in the mandibular spines, and as no other organs are found near the tips of the chelæ, they are without doubt the gustatory organs. The second kind must, in all probability, be the temperature ones. Sections of the chelæ, blackened in osmic acid to show the distribution of the gustatory canals (Pl. 3, fig. 44), show that they are very abundant along the cutting edge of the chelæ, and even more numerous at the flattened apex of the fingers—in other words, just where they are most sensitive to taste, and where they would be most likely to come in contact with foreign bodies.

The pedal nerve in the propodite, or the next to the last joint, divides into four branches which run along the anterior and posterior margin respectively of each arm of the chelæ. Whether this division of the nerve is due to a separation of fibres into nerves, going some to temperature organs and others to gustatory ones, could not be determined.

II. Olfactory Organs.

A. Structure of the Olfactory Organ.—The olfactory organ is visible from the exterior as an irregular yellowish-brown, wart-like thickening of the cuticula, from 5 to 8 mm. broad, and situated about 25 or 30 mm. in front of the mouth. In specimens from 2 to 4 inches long it is usually raised into a beak-like projection directed backwards.

Directly beneath the ectoderm are a great many—at a rough estimate from 1500 to 2000—clear, flask-shaped sense buds, each of which is connected by a narrow neck with a cuticular canal. The distribution of the olfactory buds, as I shall call them, is fairly well shown by surface views of the olfactory region (Pl. 2, fig. 19). In this preparation, which is probably from an adult male, there are two unusually well-defined median elevations containing many more canals than elsewhere. The olfactory buds underlying this portion are supplied by a large median nerve (fig. 18, *m. ol. n.*); and this fact, together with the method of development, shows that it constitutes a distinct part of the olfactory organ. The lateral portions are clearer and smoother, and contain comparatively few canals; this is specially the case on the posterior lateral borders immediately over the bulb-like termination of the lateral olfactory nerve (fig. 19). In young individuals, 2—4 inches long, the cuticula of this part is more transparent than elsewhere, and looks like a small lens. This fact, together with the presence of pigment there in the early stages, was what led me, in my paper on the "Origin of Vertebrates," to regard this organ as a degenerate pair of eyes.

In the adult the cuticula over the olfactory organ often appears a dirty silvery white in reflected light, and black by transmitted light, owing to the inclusions of air in the extremely minute "pore canals." These "pore canals" are found equally abundant elsewhere, but they do not contain air. The cuticula in the median part of the olfactory organ often contains irregular cavities (fig. 19), as though some animal

had eaten into it, or it may contain a network of membranous canals, evidently the tubes of some Annelid; they are usually most abundant in the median portion of the olfactory organ, and either lie on the surface or are buried in the cuticula.

Scattered over the olfactory organ are many blunt backwardly curved spines. The olfactory cuticula can be easily peeled off in successive layers, but it adheres strongly around the pores leading to these spines. When it does come away large tufts of pigment-cells are seen projecting from the spine pores, and the outer surface of the inner layer of cuticle projects in a crater-like collar around the pore. There are similar spines on the cuticle surrounding the olfactory organ, but they do not act like the ones just described. For this reason I supposed at first that the olfactory spines were perhaps the true sense organs supplied by the olfactory nerves, but I can find but little evidence in support of this view. The large pores leading up into the spines over the olfactory organ are lined with thin cells and crowded with pigmented tissue, and in some cases contain a transparent, fragmented coagulum. A chitinous tubule, similar to that of the gustatory cells, usually runs the whole length of the canal, and becomes continuous with a minute canal extending from base to summit of the spine. The latter is pinnately striated in section, as though its central canal were connected with the exterior by innumerable radiating canals. Under favorable conditions a rather large nerve may be seen to enter the base of the spine canal. The spines are suspended in sockets, and are moveable. They are undoubtedly of a sensory nature, but they seem to play only a very subordinate part in the olfactory region, and, contrary to what I at first surmised, cannot be compared with the large gustatory spines on the mandible.

A section through the olfactory organ (fig. 21) shows the larger branches of the nerve-plexus arising, in the main, from the median olfactory nerve; also the densely pigmented layer of ectoderm confined to the olfactory region, the clear olfactory buds (*ol. b.*), the small clusters of dark cells looking like ganglia, and numerous branching blood-vessels. One layer of

cuticula has been peeled off, so that below where the tooth-like spines should be there are large pores with projecting crater-like summits (*s. sp.*), containing many pigmented cells.

The olfactory buds vary a good deal in size and form. They are usually spherical or pear-shaped, and composed of a varying number of large pyramidal cells, the apices of which sometimes surround a perfectly clear spherical lumen. The appearance of the buds varies greatly according to the method of preparation, and other causes not clearly understood. In some they appear perfectly empty, so that the organs look like so many blank spaces in the tissue, with only a few cell outlines visible; or, in organs isolated by maceration in Bela Haller's fluid, a few cells may contain a very delicate spongy reticulum (fig. 9), while others in the same organ may be densely crowded with refractive globules, so that they resemble certain gland-like cells that I have found associated with sensory cells on the tentacles and mantle edge of Molluscs, such as Arca, Pecten, and Lima ('Eyes of Molluscs and Arthropods,' p. 722). A dark multipolar cell with a large nucleus can usually be seen in the interior of the organ; it looks like a ganglion-cell with two or more fibrous ends, the course of which cannot be followed very far in sections. It is undoubtedly the same dark ganglion-like cell so conspicuous in the young stages of these organs (fig. 23).

The clear lumen seen in the younger specimens, the chitinous, duct-like tubule, and the whole appearance of these remarkable structures point toward their glandular nature. On the other hand, their extraordinarily rich nerve-supply, and the unquestionable derivation of the whole group of organs from a primitive segmental sense organ, seem to show equally clearly that they are sense buds.

Not till I was able to demonstrate experimentally that similar organs in the inner mandibles were gustatory organs did I feel satisfied that the "olfactory buds" were of a sensory nature. I then studied them anew by macerating fresh material, paying special attention to the central ganglion-cell and its relation to the chitinous tubule. I did not succeed in

getting such perfect isolation as with the gustatory cells, and there still remain some points of importance unanswered. But the cardinal point at issue, whether the buds are glandular or sensory, was settled beyond doubt, for I was able to demonstrate that there is rarely a lumen in the fully formed buds, and that when it does occur it does not communicate with the exterior. Moreover I proved that the chitinous tubule cannot be a duct to the gland, since it is in reality a direct prolongation of the central ganglion-cell, and may be compared with the tubule in the distal end of the gustatory cells. Thus every reason for regarding these sense buds as glands disappears.

The lumen of the buds varies greatly in its appearance. It is most commonly present in the newly formed buds, where it is spherical and sharply circumscribed (fig. 23). In the adults it seldom has this appearance, and may be entirely absent, or it may be reduced to a small irregular space between the cells. Although I have looked carefully for them I have never seen any of the clear globules of the gland-like cells in the lumen of the gustatory buds. The lumen appears to be something like a much-retarded invagination cavity, although, as nearly as I can make out, the organs arise by a solid ingrowth from the ectoderm.

The tubule can be followed in sections from near the centre of the bud, through the cuticular canals, to a point very near the outer surface; here it becomes very faint or disappears. It may be either straight, very much coiled, broken at intervals as though it were very brittle, or may have one or more spindle-shaped swellings. The tubule is undoubtedly composed of chitin, for, as with the gustatory tubules, they can still be seen in the cast-off shells of immature specimens and in the fresh shells cleaned with potash.

The cuticular canals of the olfactory buds are easily distinguished from the gustatory ones by their shape. Each canal in the adult has a nearly uniform diameter, except that near the outer surface it suddenly narrows and communicates with the exterior by a transverse slit (figs. 9 and 12, C and D).

In the younger specimens they are slightly expanded near the top, and a flange-like rim is formed by the projection of the cuticula into the outer end of the canal (fig. 23).

The canals contain, besides the chitinous tubule, a varying number of fibres, with here and there a few minute nuclei (fig. 9, *n. c.*). They can be traced a short distance only toward the outer end of the canal; in the opposite direction they seem to run either over the outer surface of the olfactory bud, or apparently between its cells toward the interior. I have never been able to trace them with certainty up to nerve-fibres, although they appear to have such connections.

The tubules isolated by maceration in Haller's fluid stain deeply in methyl green and in acetic acid carmine, resembling the chitinous tubules of the gustatory organs. They are usually collapsed, and appear to be perfectly empty. Although I have examined them in many ways, paying special attention to their broken ends for protruding fibres, I have never seen a trace of the axial fibres so conspicuous in the other gustatory tubules. Isolated tubules from the gustatory buds on the inner mandible sometimes show, when treated with potash, a protoplasmic-like envelope with spiral markings (fig. 14); others have two or more coarse refractive fibres, often thrown into numerous irregular folds, extending along their outer surfaces (fig. 16, *a*). The tubules in the olfactory organ are very rarely convoluted, and they never have the two refractive fibres just mentioned. When the olfactory buds are isolated and examined whole, spindle-shaped cells are often seen adhering to their outer surface, also a few scattered nuclei that appear to belong to the delicate membranous investment of each organ.

The nerves that supply the olfactory buds are small strands arising from an extensive anastomosing plexus found everywhere beneath and around the olfactory buds. The plexus itself arises from three large nerves, that I shall call the lateral and the median olfactory nerves (Pl. 2, fig. 18; see also Pl. 4, figs. 48 and 49).

The lateral olfactory nerves arise apparently from the anterior part of the brain, but in sections one can follow their roots on to the ventral surface into the optic ganglia (fig. 49). In the adult the proximal ends of the nerves consist of coarse, transparent nerve-tubes, while their distal extremities contain many large ganglion-cells, which form an elongated swelling at the tip of the nerve: the latter terminates abruptly just beneath the cuticle on the lateral edge of the olfactory organ (fig. 49). The lateral olfactory nerve is accompanied by a large blood-vessel that divides into numerous branches, supplying the tissues in front of the olfactory organ (fig. 18, *bl. v.*); small nerve-filaments accompany some of these blood-vessels, and probably supply the ectoderm of the same region.

Several larger nerve branches leave the median border of the lateral nerves a little distance back of the olfactory organ, and mingle with the plexus formed by the median nerve (fig. 18). Some of these branches terminate in small, rounded, ommatidia-like clusters of large cells, which contain irregular refractive plates like those seen on the borders of the young lateral eyes. The lateral olfactory ganglion contains a great many of these clusters of cells. A fair idea of their appearance may be had from fig. 20, which represents some of them at the root of the lateral nerve in an immature specimen. Some near the tip of the lateral nerve, in a still younger specimen, are shown in fig. 22 (*g. ol. n.*). The lateral ganglion and the isolated clusters of cells are the remnants of a primitive sense organ[1] derived originally from the margin of the brain, but which is now converted into these ganglion-like cells. They are retinal cells that have lost their pigment, and now have all the appearances of ganglion-cells, except that they still retain their rods. In other words, we have caught a sense organ in the act of being transformed into a ganglion—the only case of this nature on record, so far as I know.

It is an extraordinary fact that the lateral nerve terminates abruptly in this great mass of ganglion-cells, which are

[1] See pp. 29–30.

apparently neither connected with surface sense organs nor themselves in a position to receive stimuli from without.

The median olfactory nerve is of an entirely different nature from the one just described. If differs greatly in size and complexity in different individuals, and if the supposition shortly to be advanced is right, it is better developed in the males than in the females. It arises long after the lateral nerves (after the third larval moult) as an outgrowth from the anterior wall of the median eye tube (Pl. 3, fig. 43). In the adult it is a solid nerve composed of two portions, a distal and a proximal one. The latter is composed of a mixture of nerve-fibres and ganglion-cells. The nerve-fibres are not the apparently hollow nerve-tubes seen in the lateral olfactory nerves and elsewhere, but appear to be more solid and refractive, with a yellowish cast, and nuclei here and there. At intervals throughout the proximal portion of the nerve there are spindle-shaped ganglia composed of small densely crowded and deeply stained nuclei, exactly like those in the fore-brain. They vary in number and size, and may extend directly into the brain-tissue at one end of the nerve, or up to the olfactory organ at the other (fig. 18).

The distal end of the median nerve divides into many diverging branches, which can be followed by means of a hand lens to the posterior edge of the olfactory organ; they there begin to anastomose, and form a dense plexus underlying the olfactory region, but a considerable number of fibres extend beyond the olfactory region to the neighbouring ectoderm. Here and there the larger strands of the plexus contain small groups of the dark-coloured nuclei, similar to those in the brain; or the smaller strands may contain a single large tripolar ganglion-cell, with granular protoplasm and a large clear nucleus (fig. 9, *g. c.*).

A large blood-vessel accompanies the median nerve; under the olfactory organ it breaks up into numerous branches, some of which are crammed full of blood-corpuscles that stain dark red, and under a low power might be mistaken for ganglia (fig. 18, *b. v.*).

Termination of the Nerves.—On the terminal joints of the exopodites to the abdominal appendages sense buds like those in the olfactory organ are very numerous; and as there is little surrounding tissue, one can peel off the cuticle organs, together with the after maceration in nitric acid. When such a preparation is placed in dilute glycerine and examined from the inner surface, the nerve-plexus underlying the organs can readily be seen. Branches like those shown in fig. 9 are seen anastomosing with one another in a most intricate manner. In the olfactory organs one cannot obtain such instructive surface preparations, owing to the crowding together of the sense buds and the abundance of connective tissue and blood-vessels; but sections and isolated sense buds show clearly that the plexus is much the same as in the abdominal appendages, only a little denser. In the olfactory region the larger branches of the plexus usually lie a little below the organs, but they may lie directly on their inner surfaces, or may be squeezed in between adjacent buds. In some cases large branches seem to penetrate into the interior of the organ, but such appearances may be deceptive owing to the crowding of the organs.

The nerves actually connected with the organ are delicate, transparent, nucleated filaments, easily overlooked; they spring from the coarse strands and spread over the surface of the buds, as shown in fig. 9. I could discover no uniformity in number, or any of that drawing out of the cells often seen where nerves unite with sense organs. They can be followed a short distance as very faint, but rather wide fibrillated bands, some of which appear to dip down between the cells toward the central cavity. Occasionally one sees an irregular, poorly defined body containing several nuclei, from which arise a few nerve strands that spread out over the surface of the bud (fig. 9, *g. c.*). They sometimes contain a single large multipolar ganglion-cell, with dark granular protoplasm and a clear nucleus. The exact method in which the nerve strands terminate is very difficult to determine.

About the neck of the buds are numerous fibrous strands

which extend outwards over the surface of the buds into the large pore canals (fig. 9, *n. c.*).

There are some remarkable organs scattered about in the subdermal tissue of the olfactory region of the adult (fig. 21, *g.*). They are irregular, usually oblong, spindle-shaped, or branched masses of small cells, whose nuclei stain deeply. They are consequently very conspicuous, and I at first took them for ganglia connected with the nerve-plexus; but I have found them in other parts of the body where there seemed to be no plexus, so their nature is doubtful. When examined under a high power the nuclei appear to be surrounded by concentric layers of protoplasm, giving the whole mass a very characteristic appearance. In some places the cells are so loosely packed that they fail to touch one another; they then lose their concentric striations, and appear like masses of blood-corpuscles. Usually a blood-vessel passes through the centre or along the side of the bodies in question. In cross sections the central blood-channel is seen to be either empty, filled with a dark coagulum, or crammed with blood-corpuscles, which in some cases are difficult to distinguish from the cells composing the surrounding tissue. The nuclei of the cells usually arrange themselves concentrically about the blood-vessel. In other cases these problematical organs contain a central canal, or irregular space, through which passes one or more nerve-strands. The nerve-strands may run over or through these organs, dividing into several branches on the way, but emerging at the opposite end without any apparent diminution in size, and without any intimate connection with the organ.

B. Physiology of the Olfactory Organ.—The anatomical features of the olfactory organs are sufficiently remarkable to make any physiological experiments as to their function of great interest and importance. Their similarity to the gustatory organs on the inner mandibles might lead one to expect that stimulating them with food would produce reflex chewing movements. But although I have made repeated

attempts to stimulate the olfactory organ with various kinds of food, with acids and ammonia, I have never succeeded in producing any characteristic reflex movements in that way. Even drops of strong hydrochloric acid or ammonia seem to have no more effect than when applied to other parts of the body; they cause a slight start, nothing more.

In order to remove all doubt as to its glandular nature, I have excised the olfactory organ, wiped its outer surface dry, and then stimulated with electricity the attached nerves, but have never seen any traces of a secretion, such as might be formed provided it were a gland.

Although these negative results are a little surprising, they do not render the sensory nature of the olfactory organ any less probable; for we cannot expect every sense organ to give on stimulation such beautifully "diagrammatic reflexes" as those on the mandibles. However, I finally discovered that electrical stimulation of the olfactory region produced at once very remarkable leg movements, such as are never seen under any other circumstances.[1] The experiment is not always successful, but when it is, the moment the electrodes are applied to the olfactory organs of the male, rapid chewing movements of the mandibles are produced, accompanied by vigorous snapping of the cheliceræ, which may finally become rigid and stretched out backward at full length. At the same time the second pair of legs, which during all our preceding experiments on the gustatory organs have remained motionless, are now quickly and repeatedly flexed, as though trying to hug or grasp some object and force it toward the mouth; all the other legs remain motionless. Stimulation of the region about the olfactory organ, or along the median line between the olfactory organ and the brain, or above the brain, may produce the same effect. It is a remarkable fact, for which I can give no explanation, that one does not always get the same results on stimulating the

[1] I subsequently observed similar movements of the second pair of appendages when the olfactory organ of a male was excised.

olfactory organ, although there can be no doubt about the character of the response when it does occur. For example, some males would never respond, although repeatedly stimulated at different times. Others would respond immediately and at almost every stimulation. There was one male upon which stimulation had at first no effect, but which responded beautifully a short time after its mandibles had been stimulated into action by rubbing clam upon them. Another would not respond at first, but did after it had lain on its back in the air for two or three hours. Again, it might happen that repeated stimulation produced at first no effect, when suddenly the characteristic movements of the second pair of legs and the chelicerae would begin of themselves. Subsequent stimulation, however, failed to reproduce this response, although the animal would start whenever the electrodes were applied, showing that the current had passed through the cuticula into the underlying tissue.

It is also a curious fact that persistent stimulation of both sides will sometimes produce the characteristic leg movements on one side only; then suddenly both sides, or perhaps the opposite side alone, respond. When the mandibles were shaved or amputated on one side, the opposite side usually responds first on stimulating the olfactory organ.

In one female, in which all the mandibles of the right side were amputated, stimulation of the olfactory organs produced sudden raising of the second to the fifth legs on the left side, and forcing of their tips toward the mouth; the movements of the second leg were most marked. The legs on the right side remained motionless. This is the nearest approach I have seen in the female to the movements so characteristic of the male under the same circumstances. In every other case that came under my observation (at least twenty) stimulation of the olfactory organ of the female produced only slight starts of the legs and abdomen.

These experiments point toward a double function of the olfactory organ. The reflex chewing movements indicate its

association either as a tasting or a smelling organ with the process of eating. But it is very difficult to explain why these movements are not produced by direct stimulation with food. On the other hand, the extraordinary hugging and grasping movements of the second pair of legs in the males clearly show that they are in some way functionally associated with the olfactory organs. Now it is well known that these legs in the male are specially modified for grasping the female during copulation; and, as I have shown that they are the only legs not involved in the reflex chewing movements caused by stimulating the mandibles, I can conceive no other explanation for these facts than that the olfactory organ is used by the males in detecting the females. This is, moreover, a function for which its position is well suited. That an organ for this purpose must be present seems certain, for the males during the breeding season hunt out the females and attach themselves to them with great precision. It is hardly probable that this could be done by means of touch or vision. While I can find no important difference between the structure of the olfactory organ in the adult males and females, in the young I find that the median olfactory nerve has an enormous ganglionic enlargement in some individuals and a much smaller one in others, and I suspect that the former are males and the latter are females. Again, when sound males and females are put in the same aquaria, the males usually attach themselves to the abdomen of the females, but I have never seen a male whose olfactory organ has been cut out (and I have had, at different times, half a dozen such specimens) attached to a female. This experiment might be conclusive if it were performed on a larger scale. Unfortunately I did not have sufficient material to do this.

I have tried several times to arouse movements of the second pair of legs in the male by rubbing the olfactory organ with fresh ova, and with the secretions of the oviduct, but without success. Renewed experiments are necessary here, for I have not given this aspect of the question the attention it deserves.

c. The Development of the Olfactory Organs.—The olfactory organs first appear in surface views as a pair of oval ectodermic thickenings, the "primary olfactory organs" on the lateral margin of the brain, just in front of the optic ganglion (figs. 24, 25, 46, 47, 49, and 61). Each organ soon separates from the brain and grows forward, leaving behind long, thick strands of ganglion-cells, which constitute the lateral olfactory nerves (fig. 25). As soon as the primary olfactory thickenings are separated from the brain they become filled with white pigment; at the same time branched cells filled with white pigment leave the thickening, and, extending under the ectoderm in all directions, form a gradually widening pigmented plexus; that in the adult may be several square inches in extent. In the young larvæ the pigmented or choroid plexus is attached to the anterior edge of the primary olfactory thickening by a stout stalk (figs. 46 and 47, *p. st.*). Each plexus lies beneath the ectoderm and a little in front of the brain, which even at this stage it more than equals in size. At a little later stage the two plexi become completely united (fig. 45). In fig. 22, *w. p.*, a part of the plexus is shown in section under a higher power.

The primitive olfactory thickenings soon unite in the median line to form an apparently unpaired organ lying some distance in front of the brain. Before this takes place the cells constituting the thickening arrange themselves in irregular clusters, and their walls develop those peculiar cuticula-like thickenings that look so much like groups of visual rods in the ommatidia. Some of these cell-clusters soon leave the main thickening, and lie scattered about under the ectoderm, but connected with the distal portion of the lateral olfactory nerve by branching nerve-bundles (fig. 18, *x*); others remain in the distal end of the nerve to form the terminal ganglionic swelling. In specimens about two inches long there is nothing left of the primitive olfactory thickening but an irregular mass of large cells, mostly pear-shaped, in which the lateral olfactory nerves terminate. There is nothing in the final position, shape, or structure of these cells to indicate that they now

communicate with the exterior, or could function as sensory cells, although there is every reason to believe that they are derived from what were once sensory cells, like those in the eyes.

The definitive olfactory organ does not appear till the changes just described are nearly finished, i. e. in the larvæ from one half to one inch in length. During this period buds begin to appear in the ectoderm between what is left of the primitive olfactory thickenings, or between what now constitute the swollen ends of the lateral olfactory nerves. At about the same time the median olfactory nerve arises from the median eye-tube, near where it joins the brain as an outgrowth from its anterior wall. Now this part of the tube may be considered morphologically as a part of the anterior neural wall or roof of the brain, just as a corresponding part of the stalk of the pineal eye in Vertebrates or in scorpions might be regarded as a part of the brain roof (compare Pl. 3, figs. 41 and 42). Moreover, as in their earliest stages the median olfactory nerves contain numerous small ganglion-cells which soon develop into two large irregular botryoidal lobes, having the identical histological structure as the cerebral hemispheres; and as the nerve soon unites directly with the cerebral hemispheres, from which it then appears to be a direct outgrowth, I think we are justified in regarding the median olfactory nerves and olfactory lobes as outgrowths from the anterior wall or roof of the cerebral hemispheres.

Of course the fully developed lobes, as seen in fig. 17, do not grow out from the cerebral hemispheres, but as the few cells from which they arise do so, it amounts to the same thing. It is evident that if the growth of the lobes and their separation from the brain took place at the same time the olfactory lobes would, as in most Vertebrates, make their appearance as massive outgrowths of the cerebral hemispheres.

The olfactory lobes vary greatly in size in different individuals. They are relatively largest in immature forms from about 3 to 6 inches long. They may consist of two very

distinct botryoidal masses composed of irregular lobes of small, densely packed, and deeply stained nuclei, each surrounding a central mass of medullary substance (fig. 17). The whole appearance of the lobes is very much like that of the convoluted parts of the cerebral hemispheres (fig. 52). In some cases there is only one large lobe, evidently formed by the more or less complete fusion of two like those in fig. 17. The stalk of the lobes in this stage is a solid column of small nuclei, and passes without any perceptible change into the cerebral hemispheres. The fusion is so complete that immediately after reaching the brain all distinction of olfactory nerve and cerebral substance is lost, for there is no trace of any medullary strands or strings of cells that can be regarded as roots to the nerve. After the young crabs reach a length of from 5 to 8 inches the lobes are less conspicuous, breaking up into spindle-shaped masses scattered along the middle third of the nerve. The distal third breaks up into many fine strands, that form a plexus beneath the olfactory buds, from which the buds are supplied. The proximal third shows a more or less clearly marked division into two main strands, corresponding with the olfactory lobes, and each strand goes to its respective cerebral hemisphere. Thus, although I have called this nerve a median nerve, it is in reality a paired one.

D. Nature of Olfactory Organs.—The primary olfactory thickenings are undoubtedly segmental sense organs serially homologous with the eyes, as first stated in my paper "On the Origin of Vertebrates from Arachnids," p. 337. They correspond exactly in position with the lateral eyes of scorpions (Pl. 5, figs. 58 and 59), and in their histological structure they show traces of ommatidia and retinal rods, like those in the lateral and median eyes of Limulus. The degeneration of this pair of eyes in Limulus was due probably to their being retained on the under side of a broad shield-like carapace, where they could be of little or no use. Their gradual degeneration and conversion into ganglion-cells, and their subsequent incorporation with a new set of sense organs with an entirely

different function, can be followed in great detail, and furnishes a most remarkable instance of change of function and structure.

A fact, however, of great morphological significance is the striking resemblance between the structure and mode of development of the olfactory organ in Limulus and that of the so-called "frontal Sinnesorgan" of Phyllopods, as described by Leydig, Claus, and others. Claus's description of this organ in Branchipus will serve equally well for Limulus. The peculiar "kolbenformige" cells described by him as originating from the brain; their position beneath the ectoderm in ommatidia-like clusters, and containing the refractive "zinkage" needles; their position in relation to the median eye, as well as their relatively late appearance, are much the same as in Limulus. It seems to me there can be but little doubt that the frontal organ of Branchipus, with its ganglion-cell masses arising from the median part of the brain, corresponds to the median nerve and median olfactory region of Limulus; while the "Kolbenzellen" organ, with its lateral ganglionic nerves, corresponds to the lateral nerve and primitive sense organs of Limulus. Of course at first sight the appearance of the organ in Limulus is different from that in Branchipus, but its fundamental structure and relations to the brain are the same. These facts point conclusively to a much closer genetic relationship between Limulus and the Phyllopods than has been recently supposed to exist, and this supposition has further support in the similarity in the development of the trifold median eyes. If a more careful comparative study of the frontal organ in other Phyllopods—Apus, for example—should confirm the above comparison, it would settle once for all the vexed question of the relation of Limulus to the Crustacea, and would furnish very strong evidence of the common ancestry of Crustacea and the Arachnids in the trilobites.

III. The Gustatory Buds of the Mandibles.

It will be remembered that our physiological experiments

demonstrated the existence of special gustatory organs on the under side of the inner mandibles (**4**, p. 11). On examining this place (fig. 3, *g. b.*) one can readily detect just beneath the smooth cuticle a yellowish granular mass, 7 or 8 mm. long and 2 or 3 mm. deep. Sections show that the cuticle is perforated with an immense number of canals, something like those in the olfactory region. The canals contain chitinous tubules extending into the yellowish mass, which consists of innumerable gustatory buds, apparently exactly like those in the olfactory organ, only much more numerous, and densely packed together many rows deep. The chitinous tubules are coarser than in the olfactory organ, and many of them are thrown into complicated folds, as in fig. 16, *b.* I have wiped off the surface over these organs very carefully, and stimulated the organs and their nerves with electricity, but have never seen a trace of any secretion appear there.

When treated with chromo-acetic osmic acid, and stained in hæmatoxylin, most of the organs are darkened around the base of the chitinous tubule, assuming the colour and appearance of sensory tissues when treated with this reagent, while the periphery stains a bright blue. It is possible that the peripheral gland-like cells secrete a substance having special powers to absorb certain chemical substances in the surrounding media, and in this way the stimulation of the centrally placed ganglion-cell is increased. But how the stimulus can reach the organ through these long tubules, which in some cases are much coiled, is not easily understood. Some of the older and larger organs seem to be quite empty and dead; others stain a dense blue-black in hæmatoxylin; while still others, apparently young ones, show very little of this blue colour, but stain dark brown in osmic acid, like ordinary sense organs.

The same kind of buds, but isolated, are found thinly scattered over the surface of the outer jaws, between the bases of the spines. It is probably these organs which, after the spines and the inner jaws have been removed, produce the faint reflex chewing movements referred to in our description of the physiological experiments (*d*, p. 12).

IV. Temperature Sense.

The whole body of Limulus is very sensitive to changes of temperature. This may be easily demonstrated in the following manner:—If a crab be placed on its back and allowed to become perfectly quiet, one may grasp the appendages or mandibles with forceps and gently move them about without arousing the animal; or one may touch the upper or lower surface of the carapace, or the gills, with any object the same temperature as the air, but the instant one touches any of these parts with the fingers, or drops water on them warmer or colder than the surrounding air, the animal at once becomes more or less agitated, and moves the appendages and abdomen about in vain efforts to regain its normal position. There is no way in which we can make the quiescent animals start more quickly or violently than by very gently breathing on the gills and under surface of the body, although quite violent fanning may produce no effect at all.

If, holding the head within about a foot of a crab, one blows little puffs of warm air on the parts about the mouth, the cheliceræ will snap with every puff. If the puffs of warm air are made a little stronger the chelaria are brought forward, and the chelæ of the first and third pairs of appendages close at every puff; the chelæ of the second pair meantime, in both males and females, remain motionless.

Whenever the sides of the cephalo-thorax of a quiescent crab are touched with the fingers prompt movements of the appendages follow, the legs opposite to the point of contact, and on the same side that was touched, beginning first.

The gentle warmth of the hands held within two or three inches of the sides of the cephalo-thorax, or from the face or body when watching closely the experiments, usually produces uneasy movements in crabs that were before perfectly quiet.

The temperature sense is very acute on the lateral margins of the thorax and abdomen, and on the tips of the legs, and of the abdominal appendages, being apparently most acute in the

last-mentioned organs. The flat triangular area on the anterior margin of the under side of the cephalo-thorax is unusually blunt to temperature changes.

It is a remarkable fact that the regions so sensitive to slight temperature changes can be touched with small wires hot enough to singe the cuticula without producing any movement. But if a rather large iron, about 2 or 3 mm. in diameter, be held for a quarter of a minute on an abdominal appendage, movements are produced, but they are evidently due to irritation of organs situated more deeply than those stimulated by gentle breathing.

A. Course of Temperature Impulses.—The following experiments show the course of the temperature impulses to a temperature centre, located somewhere in the fore-brain region.

Experiment[1] A.—When a shallow longitudinal cut is made on the ventral side through the skin along the lateral margin of the right row of appendages, the temperature sense of the carapace lateral to this cut is destroyed. On applying the hand to the right side of the cephalo-thorax, either on its dorsal or ventral surface, no movements are produced, but when the same is done to the left side the legs on both sides are set in motion. The roots of the great tegumentary nerves (fig. 48, 2—6, *a. m. p.*) lie close to the ventral surface along the outer margin of the legs, and as they are the only ones severed by this proceeding, the experiment shows that the temperature impulses travel centripetally along the anterior and posterior hæmal nerves of the thorax, not in all directions through the subdermal plexus. It shows also that the temperature impulses not only pass up and down the crura on the side stimulated, but on to the opposite side as well. This is in marked contrast with the gustatory impulses which give rise to reflexes in those mandibles only that are stimulated.

[1] In my later experiments a ligature was drawn under the skin and tied tightly around the nerve, thus obviating the disadvantage of excessive bleeding.

Exp. B.—Cutting across the ventral cord just back of the chelaria causes regular raising and lowering of the abdominal appendages about twenty times a minute. They finally come to rest, and are then left in an unnatural position, with the right and left appendages of the same pair crossed over the median line. The mandibles are pressed firmly together in a sort of tetanus, the line of the meeting being irregular and unsymmetrical. Strangely enough, stimulation of the mandibles with food produced, in this instance, no regular movements of mastication. But a warm hand placed on either side of the cephalo-thorax produced immediate and simultaneous movements of the legs of both sides. The temperature reaction of the cephalo-thorax, then, is apparently not influenced in the least by section of the ventral cord, but the respiratory, and perhaps the gustatory reflexes are strangely affected.

Exp. C.—In this experiment the crab operated upon had already had the mandibles belonging to the second right appendage removed, but was otherwise in perfect condition. A deep median longitudinal cut was made, severing (as shown by post-mortem examination) the post-oral cross-commissures of the crura, and cutting through the junction of the crura in the vagus region (Pl. 4, fig. 48). The animal lived nearly two months in apparently good condition. It was then killed to make sure of the direction of the cut. During this period it ate with the normal movements of the mandibles, except that they worked perhaps a little more slowly than usual, and the cheliceræ were not brought into action. On placing the warm hand on one side of the cephalo-thorax of the quiescent animal, responsive movements of the legs on both sides were at once produced. The temperature reaction was unaffected.

Exp. D.—In this specimen the crus of the left side was sectioned just back of the second pair of legs. The results were very clearly marked. After about five hours, when the crab had become perfectly quiet, placing the hand anywhere on the left side of the cephalo-thorax produced no movements what-

ever of the appendages back of the section, and only feeble movements of those on the same side in front of it. On placing the hand on the right side, however, all the legs on that side were immediately set in motion, and continued to move as long as the hand was held there, but they ceased immediately the hand was removed. These results could be produced repeatedly without any perceptible variation. After eighteen hours, during which period the crab had lain on its back on a table without attention, the reaction was less vigorous, but still very prompt and decided. There was no movement of the mandibles after this period in response to stimulation of the gustatory spines. Unfortunately I did not try this at an earlier stage; neither did I try, as I should have done, heat stimulation of the appendages back of the section in the left crus. These experiments are sufficiently definite, however, as regards the course of the temperature impulses, for they show that, starting in the cephalo-thorax, they travel inward along the hæmal nerves to the corresponding crus, which they ascend to the fore-brain; from there they must descend along both crura to the pedal nerves.

If, as seems probable from this experiment, the temperature centre is somewhere in the fore-brain, we ought to be able to destroy all temperature reflexes by cutting both crura close to the brain. This is very nearly what takes place, as shown by—

Exp. E.—In this specimen both crura were cut completely across, just back of the second pair of appendages. There was much loss of blood, and all the reactions were feeble. The only spontaneous movements were those of the cheliceræ and second pair of appendages. Five hours after the operation no response could be produced by heat stimulation of the sides of the cephalo-thorax, but feeble movements of the legs could be produced by breathing on them directly. These experiments indicate the existence of subordinate temperature centres in each crus (see also experiment on amputated chelæ, p. 16), and prove that the main temperature centre is located somewhere in the fore-brain.

B. There is some doubt in regard to the position of the Gustatory Centre. I did not have this point so much in mind during these experiments following section of the crura, and I did not always test for gustatory reactions. The centre for the mandibular gustatory organs probably lies in the fore-brain. One would naturally suppose, however, from the way a given leg can be made to chew when its gustatory organs are stimulated, that there was in each leg an independent gustatory centre, which I at first supposed might be located in the ganglion of the pedal nerves. However, if this be so, it is hard to understand why sectioning either the ventral cord, or the crura back of the fore-brain, should stop the reflexes, while a median longitudinal section across their union in the vagus region should have no effect (see pp. 37–8). There is also a difficulty in the fact that I have amputated the whole leg, including a portion of the crura with the attached pedal nerve and its ganglion, and on stimulating the gustatory spines with food have failed to produce reflex contraction of the leg, although movements could be easily produced by applying the electrodes directly to the pedal ganglion or nerve.

C. Structure of Temperature Organs.—It is not so easy to identify the temperature organs as the gustatory ones. However, I have found close beneath the epidermis, in all the parts examined, including various regions on the upper and lower walls of the carapace, the gills, the legs, &c., a loose subdermal plexus of nerve-fibres and ganglion-cells. The cuticula of all these regions is perforated with canals under which are buds, in nerve-supply and structure like those in the mandibles and in the olfactory region. As I can find nothing else there that looks like sense organs, it is very probable that these buds are the organs that are specially susceptible to changes of temperature.

D. Function.—The temperature sense seems to be remarkably acute in Limulus. I know of no other Invertebrate that approaches it in this respect. As Limulus spends the fall and

winter months in deep water, where there is comparatively little variation in temperature, it is hard to imagine what this sense can be used for if not to aid the animal in migrating to warmer shallow water during the breeding season.

V. Tactile Sense.

There are some wart-like sense organs about 5 mm. in diameter on the endopodites of the abdominal appendages, to the structure and function of which I have not given special attention. They have already been briefly described by Gegenbaur. The underlying cuticula is richly perforated with peculiarly shaped canals, and chitinous tubules extend into them similar to those in the gustatory spines. They are sensitive to ammonia vapour, tactile impressions, and temperature changes, but stimulation of them in this way does not produce any definite reflex action. If a camel's-hair brush be drawn gently across the terminal joint of the expodites to the abdominal appendages, or over the region about the olfactory organs on the abdominal appendages, prompt movements of the legs and abdomen follow. This is not the case when the basal joints of the legs, or the soft flexible skin about the joints of the legs, or about the anus, are brushed.

VI. Summary.

If we review the results of our observations described in the preceding pages, we see that there are two distinct kinds of sense organs in Limulus. There are the single-celled sense organs, each with a long chitinous tubule, the best representations of which are found in the mandibular spines and in the chelæ of all the walking appendages; they are pre-eminently gustatory: a slightly modified kind in the chelæ probably serve as temperature organs.

The second kind are rounded, solid clusters of gland-like cells, containing a large multipolar ganglion-cell provided with a chitinous tubular prolongation, the distal end of which

terminates in canals near the outer surface of the cuticula. These sensory buds are distributed over the whole body, but they are much more abundant in some places than in others. They are as a rule innervated by delicate branches from an everywhere present subdermal nerve-plexus, which is itself connected with branches of the tegumentary nerves; but special aggregations of buds may be supplied by special nerves, such as the olfactory buds, and the gustatory cells and buds of the mandibles; both sets of the latter organs being supplied by the two branches of the mandibular nerve (coxal nerve of scorpions, supra-branchial nerve of Vertebrates).

All these sense buds or sense cells in Limulus multiply by division. This process is easily studied in the buds of the inner mandibles, and in the gustatory cells of the mandibular spines. The division begins at the summit of a cuticular canal, and gradually extends down into the organ, the process being the same in both sense cells and sense buds. The chitinous tubule itself does not divide; a new one is formed alongside of the old one; a longitudinal constriction then appears at the summit of the old cuticular canal, dividing it into two diverging arms, the separation of which gradually progresses toward either the sense buds or cells, as the case may be. I do not know how the latter divide.

There are at least five varieties of these two types that can be recognised by their termination at the surface. (1) The olfactory buds are connected with nearly straight canals, which contract near the top into a very narrow slit (fig. 12, *e* and *f*). (2) The gustatory buds of the inner mandibles are connected with straight canals, resembling the ones just described, except that they open out by excessively small pores (fig. 12, *a*, *b*, and *c*). Just before reaching the surface the tubule expands into an irregular, spindle-shaped body; beyond this it becomes extremely small, but still it can be followed with certainty to the outer surface, where it terminates in a very shallow depression (fig. 12, *a*). A dark coagulum of some kind usually fills the cuticular canal, and completely surrounds the chitinous tubule. (3) The buds scattered over the surface of the man-

dibles terminate in canals opening by a rather wide slit (fig. 12, *d*). (4) The gustatory cells always lie in bent canals that gradually taper to a very fine opening (fig. 1). (5) Finally, there is a set of canals like those of the gustatory buds, except that they are capped by short spines of varying shape and size, into which extends a chitinous tubule (fig. 10).

The sense buds of Limulus have a certain resemblance to the organs which in the Gephyreans have been described by recent authors either as glands or sense organs. Organs similar to those in Limulus, but generally described as glands, are widely distributed in Arthropods.

The only thing resembling the gustatory cells in the mandibular spines are certain organs in the extremities of the palps and in the first pair of legs of Galeodes, as recently described by P. Gaubert. They resemble the chitinous tubules seen in Limulus, but histological details concerning them are so meagre that it is impossible to identify them with certainty.

I will also call attention to the "olfactory cones" of Mutilla, as described by Franz Ruland. They consist of spindle-shaped clusters of cells from which a delicate hyaline tubule runs to the perforated summit of the overlying cone. At the base of the tube is a spindle-shaped swelling with longitudinal striations resembling those on the spindle of the gustatory cells of Limulus. The organ also contains a single large nucleus that may be compared with that in the sense buds of Limulus.

The young stages of the sensory buds are much alike, whatever the subsequent modification. They are probably derived from the multiplication of a few ectodermic cells; but however that may be, the cells soon arrange themselves to form a rounded body with a small central lumen (fig. 23). The buds in this early stage stain more deeply in osmic acid, and have a different appearance from that of the older buds (fig. 22). The large ganglion-cell and the halo around the central cavity are much more conspicuous at this time than afterwards. Moreover the ends of the cells that converge toward the central cavity are provided with refractive rod-like

thickenings, which are highly suggestive of the rods on the ommatidial cells of the lateral eye.

Very similar figures are seen in the inner mandibles of immature specimens, as in fig. 8. Here one can see a distinct fibrous process passing outward from the ganglion-cell, which in this instance lies well to one side of the central cavity.

These buds, which in the young are found in all parts of the body, and at first are everywhere alike, finally undergo various modifications. Some may degenerate, some may become olfactory buds, others gustatory, and still others temperature organs. The resemblance of these buds to ommatidia is so striking that we must include them both in the same category. We can therefore reduce the whole system of sense organs either to isolated sense cells or sense buds, or aggregations of the same. This agrees with my conclusion concerning the sense organs of insects (see 'Zool. Anz.,' 1890, Nos. 13, 14), where I maintained that the ommatidia of the compound eye were nothing more than specialised cell clusters, which in other parts of the body were supplied with various forms of spines or hairs, and served as tactile, auditory, gustatory, or olfactory organs. Moreover, in my earlier observations on the "Eyes of Molluscs and Arthropods," I showed that the eyes even of Molluscs were composed of circles of cells or ommatidia, which were also widely distributed over the surface of the body. In Vertebrates we have evidence of the same condition. Isolated sensory cells are there widely distributed, and, while essentially alike in structure and appearance, have very diverse functions. The olfactory and gustatory organs are but aggregates of sense buds like those widely distributed over the body. The presence of similar sense buds in the eye is shown by the circles of rod-cells surrounding the cones.

Part II.—On the Morphology of the Arthropod Brain.

The Brain of Insects and Myriapods.

Among the most remarkable features of the brain of Limulus

are its various cavities, its cerebral hemispheres, and its infundibulum. Although some of these parts can be identified with a fair degree of certainty in insects, Myriapods, and Arachnids, the whole appearance of the fore-brain region in the adult Limulus is totally unlike that of any other known Arthropod. On the other hand, it bears such a striking resemblance to a Vertebrate brain that I believe no competent person need hesitate a moment in picking out the corresponding parts.

Before one can understand the structure and development of the brain of Limulus one must first have a clear idea of its structure in those Arthropods in which the cephalic lobes are present in their simplest and most primitive condition. I shall therefore first call attention to certain features of the brain of Myriapods, Insects, and Arachnids, that are probably common to the brain of all Arthropods. I have not paid special attention to the Crustacea, and I do not, unless expressly stated, include them in my speculations on the Arthropod brain.

By a comparative study of the cephalic lobes of Acilius, Blatta, Vespa, Hydrophilus, Scorpions, several species of Spiders, and Limulus, specially prepared to bring out surface contours, I am able to demonstrate that the cephalic lobes of a typical Arthropod are composed of three distinct segments, each containing a segment of the brain, optic ganglion, and optic plate; between the two latter is an invagination by means of which the optic ganglia are more or less impeded. These results have been in part confirmed by Wheeler and Heider. Viallanes, who has made a careful study of the anatomy and physiology of the adult brain, has quite a different conception of its structure. Embryological studies, however, do not support his views; they show that each of his three segments, protocerebron, deutocerebron, and tritocerebron, comprise very heterogeneous centres, not at all arranged according to their morphological affinities. St. Remy, in his valuable work on the brain of Myriapods and Arachnids, has followed Viallanes. For lack of space I cannot review the works of these two

authors as I would like. But in what follows I shall try to show that their basis of classification of the brain-lobes is founded on vital misconceptions. They fail to recognise the difference between the organs derived from the cephalic lobes and those derived from the ventral cord, as well as the segmental nature of the primitive optic ganglia in such forms as Acilius and Scorpions, and their relation to the compound eye. Moreover, when Viallanes subsequently attempted to confirm his views by embryological study, he not only selected in Mantis a poor type, but mistook the well-known trachea-like invaginations for the ganglionic ones described by me, and consequently he is quite right in asserting that they do not give rise to any part of the optic ganglion.

Those who have heretofore touched on the development of the brain of Insects have failed to appreciate the far-reaching morphological importance of the ganglionic invaginations. Korschelt and Heider, in their text-book of embryology, did not understand their relations in Insects, Scorpions, and Limulus. As I consider these invaginations the key to the morphology of the Arthropod and Vertebrate fore-brain, I shall try to explain in more detail my interpretation of their significance.

In studying the development of the convex eyes great confusion and difficulty was at first occasioned by the failure to recognise that two distinct invaginations are sometimes present, one for the optic ganglion, the other, less commonly present, for the eye. Reichenbach and Kingsley made this mistake; both supposed that the purely ganglionic invagination described by them gave rise either to the whole (Kingsley) or a part (Reichenbach) of the ommateum.

I was the first to show in Vespa that the two invaginations are absolutely distinct, one giving rise to the three lobes of the optic ganglion, the other to the compound eye. The latter invagination, although very deep, probably does not close up, and its outer or middle wall produce the corneagen, as I at first supposed. Strangely enough, the invagination soon straightens out, and the corneagen, as I found out subse-

quently, is formed by the union, over the crystalline cones, of two cells derived from the sides of each ommatidium. The same process undoubtedly occurs in Crustacea.

I studied the subject again in Acilius, and fortunately this insect furnished the most primitive and least modified type of cephalic lobes yet described, and apparently the one from which all others have been derived. I do not by any means believe that the Coleoptera are the most primitive Arthropods. But since we find the Acilius type of fore-brain clearly repeated with more or less modification in other insects, in Spiders, Scorpions, Limulus, and probably with still greater modifications in the Crustaceans (although our knowledge of the last group is too imperfect as yet to speak definitely about them), it must be the nearest in structure to the ancestral type.

A. The Cephalic Lobes of Insects.—Basing our conclusions mainly on Acilius, we find that in insects the true fore-brain is derived from the cephalic lobes, which are composed of three segments. In each segment one can readily distinguish a pair of brain-lobes, a pair of optic ganglia, and two ocelli opposite each ganglion (Pl. 5, fig. 57). Between the ocelli and the ganglia are three pairs of invaginations, which decrease in depth and extent from the first to the third. The ocelli, after the closure of the invagination, still remain on the margin of the cephalic lobes in their original upright position.

The antennary neuromere is usually regarded as a part of the cephalic lobes. I believe this is a mistake, as can be very readily shown by comparison with Scorpions and Limulus. One finds there, as well as in Acilius, no evidence whatever that it forms part of the true cephalic lobes; but it is not possible to show this without the proper material properly prepared. I regard the antennary neuromere as morphologically post-oral —it is unquestionably derived from the ventral cord of the trunk. It moves gradually forward till it occupies a position beside, or in front of, the mouth, constituting what I shall call the mid-brain.

B. The cephalic, sympathetic, or the stomodæal nerves are very important landmarks in this region. The anterior stomodæal, or "frontal" ganglion and its nerve arise as a solid linear outgrowth from the median anterior wall of the stomodæum. Its outer or proximal end is united by two cross-arms with the lateral stomodæal ganglia situated either on the median border of the antennary neuromere, or on the œsophageal commissures (Pl. 5, fig. 57). This cross-commissure I shall call the anterior pons stomodæi (*a. p. st.*). In the later stages it is usually shaped like an elongated V, owing to the fact that the frontal ganglion, which is situated at the apex of the V, is carried a long distance inward by the growth of the œsophagus. From the lateral stomodæal ganglia the lateral stomodæal nerves extend inwards, one on either side of the œsophagus (fig. 57, *l. n.*). Their exact point of union with the brain I have not been able to determine, because in the forms that I have studied these nerves are either absent or imperfectly developed. However, from all I can gather about them from the works of others, they must arise very near this point; moreover that is their point of origin in Scorpions and Limulus.

The lateral and median stomodæal nerves expand at intervals into ganglia united with one another by commissural strands. These ganglia and strands are specially well developed in Iulus, as shown by Newport (fig. 62).

The labrum, which in Acilius I have shown to be unquestionably a paired organ, arising from the very anterior margin of the cephalic lobes, is innervated, strangely enough, from the roots of the anterior pons stomodæi, a condition which seems to prevail throughout the Myriapods, Insects, Arachnids, and probably the Crustacea. Moreover there appears to be present in a sufficient number of cases to make it typical for the whole group of Insects a small unpaired nerve directed outward and backward from the frontal ganglion toward the labrum (fig. 53). It is obvious that the innervation of the labrum is different from that of any other Arthropod appendage, and I believe it has special significance, as I shall presently indicate.

The lateral stomodæal ganglia are usually situated on the posterior median margin of the antennal neuromere, or sometimes on the œsophageal commissures. They are always united with one another by a large transverse commissure that contains, unlike the other post-oral commissures, many ganglion-cells. In the young stages of Acilius (see 'Eyes of Acilius,' fig. 44) I have figured a segment of the median sympathetic nerve which seems to be united with this commissure to form a rudimentary ganglion, and in the fourth larval stage of the lobster I have found a very distinct ganglion in the middle of this commissure. No doubt this commissure is characteristic of the whole group of Arthropods; it is well known in Myriapods, Insects, and Crustacea, and I have found it in Limulus. Its apparent absence in the Arachnids is probably due to the crowding together of the neuromeres in the mouth region. This commissure, I am convinced, also belongs to the cephalic sympathetic system; but it may perhaps contain fibres representing the cross-commissures of the antennal neuromere. I shall call it the posterior pons stomodæi.

Besides these nerves, most insects, as shown in the diagrammatic fig. 53, are provided with a system of trunk-sympathetics consisting of a chain of lateral and median sympathetic ganglia; the latter, as I have shown elsewhere, is derived from the "Mittelstrang" of Hatschek, and probably terminates anteriorly in the posterior pons stomodæi.

c. The Convex Eyes and their Ganglia.—The convex eyes form such an important part of the brain of Insects that it is of great importance to determine exactly where they belong. They are apparently so intimately associated with the fore-brain that there seems little reason to doubt their derivation from the cephalic lobes. But there are strong reasons for supposing that they belong, not to the cephalic lobes proper, but to the trunk, probably to the mid-brain neuromere.

That they did not belong to the cephalic lobes originally is indicated by the fact that in Acilius not a trace of them appears

till long after the cephalic lobes, as such, have disappeared (i. e. beginning of the pupal stage), but it is then impossible to determine exactly their relation to the cephalic lobes. In the Scorpion there is nothing comparable to the convex eye. In Limulus they certainly are not derived from the cephalic lobes, although as in insects the optic ganglion does have this derivation. My recent observations have shown that in Limulus the convex eye arises farther forward than I at first supposed. In its earliest stages it lies about opposite the cheliceral segment to which it in all probability belongs; its subsequent union with the optic ganglion of the cephalic lobes is therefore a secondary affair. The same thing apparently takes place in Acilius, for the convex eyes when they appear at the beginning of the pupal stage, instead of being provided with a special ganglion of their own, become united with the ganglia of the degenerative ocelli.

These facts suggest a very interesting comparison with Myriapods. There seems to be present in nearly all Myriapods a remarkable nerve arising from the optic ganglion and supplying a peculiar sense organ situated at the base of the antennæ. St. Remy calls them the nerve and sense organ of Tömösvary (fig. 62). Now I strongly suspect that this sense organ is the rudiment of the convex eye of the higher Arthropods, for it agrees in two important particulars with the convex eye of Limulus, and presumably with that of all other Arthropods, namely, in its situation at the base of the antennæ, and in the attachment of its nerve to the ganglion of the larval ocelli.

In Acilius, the compound eyes appear at the beginning of the pupal stage as a sickle-shaped band on the dorsal and median margin of the ocelli. They finally break away from the surface, and their degenerated remains become attached to the under side of the optic ganglia (fig. 60). The latter persist, and form the ganglion of the compound eyes. The conversion of the larval optic ganglion into that of the imago is brought about in the pupal stage by rapid growth along three very clearly marked regions (fig. 60, *o. g.* 1—3). In all three bands, or centres, which probably represent the three segments

of the larval ganglion, there is rapid multiplication of cells, but it is the middle lobe which increases most rapidly, and which forms the main part of the adult ganglion; traces of the other two bands may be seen even in the adult (see my paper on the "Eyes of Vespa"). Now it is a remarkable fact that we find these three identical bands of the pupæ of Acilius in the embryos of Vespa, but they are there formed by a direct invagination of the ectoderm of the cephalic lobes. Either the same kind of an invagination or a solid ingrowth is found in a variety of other forms, as in the Hymenoptera, Orthoptera, and Hemiptera—that is, in forms that do not pass through a free ocellate larval stage. This proves that the Acilius type of cephalic lobes is the most primitive, and that in such forms as the Hymenoptera and Orthoptera, &c., important embryological processes are omitted; it also shows that the optic ganglion of the convex eye of insects is formed by the fusion of the three larval ganglia.

As to whether the three frontal ocelli found in many adult insects are derived from the larval ocelli, or are new formations, like the compound eye, is a question of great morphological importance, but we have as yet no evidence upon which to found an opinion concerning them. Their position and number suggest their identity with the three-lobed median eye of Limulus and Crustaceans. Careful investigation of some forms, the Sialidæ, for example, which pass through an ocellate larval stage and possess frontal ocelli in the adult, would probably settle this question. It is certain that early in the pupal stage of Acilius and Cecropia all the larval ocelli break away from the ectoderm and take up their position on the under side of the optic ganglion, where they seem to undergo complete degeneration.

II. The Brain of Arachnids.

A. The cephalic lobes of Arachnids have at first the same shape and appearance as in insects. They soon divide into three segments, which can be identified with even greater

ease than in Acilius, especially the three invaginations of the optic ganglion.

In the first segment of Scorpions[1] the ocelli, as well as the distinction between brain-lobe and optic ganglion, have disappeared. The invaginations, which in Acilius are separate, here unite to form a great transverse furrow with thick walls and small dark nuclei (fig. 58, *s. l.*); the whole lobe sinks below the surface, and moving backward, lies underneath the second segment, where it forms the semicircular lobes (l'organ stratifié, l'organ pédunculé), identical in almost every particular with the semicircular lobes of Limulus and Spiders. The second pair of invaginations are at first exactly like those in insects; but the fold on the lateral margin of the invagination soon advances rapidly inwards and backwards, and uniting in front with a similar fold in the first segment, and behind with one in the third, gradually extends backwards, in a broad amnion-like fold, over the whole of the cephalic lobes. During this process the eyes on the second segment are gradually infolded, so that they finally lie inverted on the middle wall of the fold. They then unite with one another over the median line, and growing forwards come to lie in a common sac at the distal end of a short tube (fig. 42). The eyes of the third segment are not involved in the ganglionic invagination, consequently they remain upright on the surface ectoderm, as in Acilius. Thus almost the whole of the cephalic lobes are invaginated, or, to speak more accurately, they are enclosed by amnion-like folds to form a primitive cerebral vesicle. The floor of the vesicle is formed by the fore-brain or the whole of the cephalic lobes, except that part containing the lateral eyes. The vesicle has a thin roof or pallium, from which arises a median tubular outgrowth, with its terminal pineal eye. There is nothing approaching this in any other animals, except in the Vertebrates, where this condition has long been regarded as typical of the group. I am convinced that this fact alone, when

[1] See also my figures of the cephalic lobes of the Scorpion "On the Origin of Vertebrates from Arachnids," vol. xxxi, part iii, of this Journal.

looked at without prejudice, is sufficient proof of the genetic relation between the Arachnids and Vertebrates. It certainly is not inferior to evidence based on the presence of a notochord or gill-slits. However, this fact only furnishes the key-note to the argument that is to follow.

In adult Scorpions and Spiders the brain shows more clearly its origin from three segments than is the case in insects. In fig. 61 I give a diagrammatic view of the brain of Arachnids based on my observations on Scorpions and Spiders. None of the tegumentary nerves are represented, and, excepting the position of the lateral ocelli, the diagram would do as well for one as for the other. I was the first one to point out the homology of the eyes of Spiders with those of Scorpions and Insects in my paper on the "Segmental Sense Organs of Arthropods." I stated, p. 601, "In Spiders the structure of the cephalic lobes is the same as that of Scorpions. The two anterior median eyes belong to the second segment, and are homologous with the median eyes of Scorpions, the development being the same in both cases. The three remaining pairs belong to the third segment, and are homologous with the lateral eyes of Scorpions. They are invaginated to form optic cups in the same way as those of Acilius." Korschelt and Heider have overlooked this statement, for they credit Kishinue and Purcell with having shown the similarity in the development of the median eyes of Scorpions and the anterior median eyes of Spiders, although their papers did not appear until two or three years after mine.

If further confirmation of this interesting fact is necessary, it is found in the structure of the adult brain. In Scorpions the optic ganglia of the second and third segments remain distinct through life. They are carried by the movements of the eyes on to the anterior face of the fore-brain, where they remain as two pairs of conical projections, the anterior pair united with the nerves to the median eyes (fig. 61). In the brain of some Spiders, notably Epeira, according to St. Remy, exactly the same condition prevails.[1] But the anterior ganglia

[1] It must be remembered that the terms superior, anterior, and posterior,

supply the median eyes, or "Hauptaugen;" while the others, irrespective of their position on the head of the adult, are supplied by the posterior ganglion, or that on the third segment of the cephalic lobes (fig. 61).

The brain-lobes of the second and third segments gradually become indistinguishable. In their place, in the adult, one sees a pair of deeply stained lobes (*c. h.*, fig. 61), which are probably homologous with the cerebral hemispheres of Limulus.

B. The Mid-brain.—There can be no doubt whatever that the whole of the cephalic lobes of the Scorpion are homologous with the whole of the cephalic lobes of Acilius. The next neuromere, that of the cheliceræ, must be homologous with the antennal neuromere of insects, because both bear identical relations to the stomodæal nerves.

In Scorpions and Limulus there are four nerves connected with the œsophagus. The most important are the lateral stomodæal nerves, extending from about the middle of the median margin of the cheliceral neuromere inwards along the sides of the œsophagus (figs. 59—61, *l. st. n.*). What I formerly regarded as the pre-oral cross-commissures of the cheliceral neuromere (*a. p. st.*) I am now convinced is merely the much shortened anterior pons stomodæi of insects. It is composed at first of numerous ganglion-cells surrounding a cortical "punct" substance, and in some Spiders, according to St. Remy, contains in the centre a ganglionic enlargement that I regard as the remnant of a frontal ganglion. The same commissure, called by St. Remy "pons stomatogastique," occurs in Myriapods (fig. 62, *a. p. st.*). It there strongly resembles the anterior pons stomodæi of Arachnids, and yet shows, by the presence of a distinct median ganglion, its undoubted homology with the cross-arms of the frontal ganglion of Insects (compare figs. 58—62).

when applied to the embryos, have entirely different meanings from those when applied to the adults. This is owing to the doubling of the cerebral lobes on to the back of the adult, so that the anterior border of the cephalic lobes becomes the posterior border.

An unpaired rostral nerve extends outward and backward along the anterior ventral wall of the œsophagus into the rostrum (figs. 59—61, *r. n.*). In Limulus, perhaps in Scorpions, there are also two lateral rostral nerves arising from the lateral stomodæal ganglion, and extending outwards to the rostrum (*l. r. n.*). They agree in origin and distribution with the lateral labral nerves of Insects, with which they are homologous.

In Limulus the first post-oral cross-commissure is much longer than the rest, and occupies a different position on the posterior under wall of the œsophagus (figs. 46, 47, c^2.). It is difficult to account for this commissure if we do not regard it as homologous with the posterior pons stomodæi of the œsophageal ring of Insects. I cannot identify it in Scorpions on account of the excessive crowding of the neuromeres about the mouth, but there is no reason to doubt that it is present.

c. Development of Lateral Stomodæal Nerves.—The lateral stomodæal nerves in Scorpions and Limulus arise in part from the walls of the œsophagus. Their development is best studied in scorpions. They first appear in surface views of Stage E ('Origin of Vertebrates from Arachnids,' fig. 2, *st. n.*) as a pair of invaginations on the median border of the cheliceral neuromere. The invagination gives rise to a string of cells which at its inner end is continuous with an evagination of the lateral wall of the œsophagus. By the inward growth of the œsophagus the nerves are gradually drawn out to their full extent; their inner ends are for a long time continuous with the proliferating thickening of the stomodæum, and their outer ends terminate in the lateral stomodæal ganglia derived from the thick-walled invaginations on the margin of the cheliceral neuromere.

Thus, if we combine our observations on scorpions and Limulus, we are able to identify in the Arachnids every characteristic nerve and ganglion of the stomodæal system of Insects except the unpaired stomodæal nerve. It is not impossible that further observation on other forms will show the

existence of this nerve in Arachnids. It is not easy to overestimate the importance of these facts. They prove beyond doubt that there has been no addition or suppression of neuromeres about the mouth either of Insects or of Arachnids.

It is also obvious that in Arachnids the fore-brain, the cheliceral neuromere, and the principal stomodæal nerves are respectively homologous with the fore-brain, the antennal neuromere, and stomodæal nerves of Insects and Myriapods. The "pons stomatogastric" of Myriapods is homologous with the frontal ganglion of Insects, and the cross-arms uniting it with the ganglion of the œsophageal commissure, or what I have called in Insects and Arachnids the anterior pons stomodæi. In spiders the same organ is called by St. Remy the "rostral ganglion," or "lobe rostral." The lateral rostral nerves and the labral nerves throughout Myriapods, Insects, and Arachnids, including Limulus, are the same, for in all these cases they arise either very near to or directly from the lateral stomodæal ganglion. It is hard to understand how St. Remy, after making his careful study of the brain of Myriapods and Arachnids, could overlook, as he seems to have done, these obvious homologies. It was probably due to the false a priori assumption that the antennæ are absent in Arachnids, and that their cheliceræ correspond to the mandibles of insects.

We are thus able to reduce the fore- and mid-brain of Myriapods, Insects, and Arachnids to the same ground plan. Such a comparison has been heretofore impossible, owing to the absence of the necessary embryological data. This, however, has been no serious obstacle to those who find it so easy to account for all discrepancies by assuming that one or more neuromeres have been omitted as the necessities of the comparison demanded. But there is apparently no biological law more constant than that head and anterior trunk segments, once formed, are never entirely omitted, or new ones intercalated between them ; and a careful study shows that the head of Arthropods offers no exception to this law. On the other

hand, to assume that there have been such changes raises an insurmountable barrier to any satisfactory comparison between the brain of Arachnids and other Arthropods. There is no reason to doubt that the brain of Crustacea will fall in line with the above comparisons.

D. Comparison with Annelids.—Still another advantage to be derived from my interpretation is that it enables us to compare the cephalic lobes and stomodæal nerves of Arthropods with those of Annelids. According to Lang, the paired stomodæal nerves of Annelids arise from the œsophageal collar, that is near the point of union of the ventral cord with the brain, just as they do in Arthropods. According to Kleinenberg, they originate in Lopadorhynchus from the lateral margin of the first post-oral neuromere. The origin of this nerve, therefore, in Annelids and in Arthropods marks the point of union of the neuromeres of the head and trunk. Moreover, since the stomodæum nerves arise from the first post-oral neuromere of Annelids and from the first neuromere of the ventral cord of Arthropods, i. e. from the antennal neuromere of Insects and Myriapods, the cheliceral of Arachnids, and the first antennal of Crustacea, these neuromeres must be homologous.

E. Nature of Stomodæal Nerves.—The remarkable mode of development of the stomodæal nerves from the walls of the stomodæum, their constant presence, and their voluminous size in the early embryonic stages emphasises their importance, and indicates that they belong to a system quite apart from that of the fore-brain and ventral cord. If so, what is their significance? The stomodæum undoubtedly represents the invaginated ectoderm formerly surrounding a primitive mouth leading directly into the mesenteron. That primitive mouth now lies at the junction of the mesenteron with the stomodæum. We have only to turn the stomodæum back to its primitive condition as ectoderm surrounding the mouth, in order to obtain a clearer idea of the original position

of the stomodæal nerves. I have made this change in the typical insect nervous system shown in figs. 54, 55, and 56, and the transformation is very suggestive. With only slight modifications of the nerve-rings back of the œsophagus, the pontes stomodæi, with the frontal and the lateral stomodæal ganglia, are seen to form a circumoral nerve-ring; and by continuing the nerves, uniting the lateral sympathetics, *p. p. n.*, till they meet on the dorsal side between the body and the head our ideal insect embryo appears like the larva of Lopadorhynchus, or of an advanced trochosphere; the stomodæal nerves forming on the sub-umbrella a system of circumoral nerve-rings, such as we might expect to find in a Cœlenterate. The labrum is carried forward by the evagination of the œsophagus to the umbrella, and its nerves now appear as umbrella nerves originating from a circumoral nerve-ring.

This transformation renders the homology of the labrum with the pre-oral antennæ of Annelids, as suggested by Korschelt and Heider, very plausible, and at the same time it explains their remarkable innervation from the stomodæal ganglia. The connection of the anterior ends of the sympathetic nerves of the trunk is not known. If they are connected with the system of stomodæal nerves, as indicated in the figures, they might be regarded as sub-umbrella nerves, drawn out to their present extent by the growth of the trunk. The median sympathetic nerve would then be antemeric with the unpaired stomodæal nerve, as in fig. 54. It is obvious, on inspection of the figures, that the invagination of the sub-umbrella must have been greater in front than elsewhere, for the lateral stomodæal ganglia are carried only to the edge of the permanent mouth, while the frontal ganglion is carried far into the œsophagus, bringing the labrum up to the anterior border of the mouth.

The advantages of this view are obvious. It affords a means of identifying, approximately, the "trochosphere" in the cephalic lobes of Arthropods; explains, among other things, the anomalous innervation of the labrum, and the limitation of the stomodæal nerves to the ectodermal portion of the alimentary

canal. I maintained, in my paper on "Acilius," that the segments of the cephalic lobes of Insects were originally post-oral, and comparable with those in the trunk. While I still recognise the great similarity of trunk and cephalic segments, I do not now believe that they are morphologically identical. The constant presence of the larval ocelli, and the absence of typical appendages, besides other considerations, indicate a sharp demarcation between the segments of the cephalic lobes and those of the body.

III. Development of the Brain of Limulus.

A. The cephalic lobes of Limulus, before they divide into segments, resemble more closely in shape and general appearance those of Insects and Scorpions than those of Crustaceans. We find there the same three pairs of invaginations seen in Acilius and Scorpions, but so strangely modified as to be at first sight hardly recognisable.

The first change that takes place is the formation of a great furrow along the whole of their anterior margin. As the furrow deepens, the lobes become a little shorter, and assume the shape shown in fig. 61; at the same time they come to be differentiated into four distinct swellings. The lateral ones constitute the optic ganglia (*op. g.*), or, as I shall sometimes call them, the thalamencephalon, owing to their double relation to the nerves of the lateral eyes and olfactory organ, and to the way they finally become incorporated into the adult brain. In front of the optic ganglia the furrow becomes deeper and broader, and constitutes the invaginations of the optic ganglia. Just in front of the latter, and united with them by thick nerves, is a pair of oval thickenings, the primitive olfactory organs (*p. ol. o.*). The compound eyes, with which this optic ganglion is subsequently united, have not yet appeared. They are first seen near the outer end, and a little back of, the ganglion, as though they belonged to the cheliceral segment.

The optic ganglia of Limulus evidently correspond to the optic ganglia of the third segment of Scorpions and Insects,

but the opening to the invagination is at right angles to the longitudinal axis of the body instead of parallel with it, as in the early stages of the Scorpion (fig. 59). But even in Scorpions it is at right angles with the long axis of the body at a later period. The sense organ on the anterior margin of this invagination in Limulus, the primitive olfactory organ, must be homologous with the lateral eyes of Scorpions. In the anterior median part of the cephalic lobes another enlargement of the furrow appears (*s. l.*); its walls are thicker and stain deeper than elsewhere, forming two oblong, slightly thickened lobes, very conspicuous in surface views, and having the same appearance as the semicircular lobes of Scorpions and Spiders, with which they are unquestionably homologous. This is shown not only by their position and appearance in the early stages, but by the fact that they develop into the same kind of organs in the adult. Back of the semicircular lobes are two poorly defined swellings (*br.*^{2} and *br.*^{3}), that I regard as the second and third segments of the fore-brain. From the anterior lobes arise the cerebral hemispheres; the posterior ones do not undergo any marked specialisation, they are concealed by the subsequent backward growth of the cerebral hemispheres, and seem to bear the same relation to the rest of the fore-brain that the "tween-brain" does to the cerebral hemisphere in Vertebrates.

About midway between the invagination of the optic ganglia and that of the semicircular lobes appears a small pore, which rapidly moves forward and inward till it lies in front of the invagination of the cephalic lobes. The position of this pore at first made me regard it as belonging to a segment in front of the semicircular lobes, as I stated in my paper on the "Origin of Vertebrates from Arachnids." Further study, however, convinced me that it originates between the invagination of the semicircular lobes and that of the optic ganglion, and consequently it belongs to the second segment of the cephalic lobes. The pore leads into a short tube formed by an invagination of the ectoderm. The tube, although easily seen in sections, is not very clear-cut, and the lumen extends

a short distance only into its interior. The distal end of the tube is not specialised, and shows no trace of the eye that is subsequently developed from it. These two tubes soon unite to form a common tube opening by a large pore, situated some distance in front of the brain (Pl. 3, fig. 24). Two bands of ectoderm, in which there is a shallow groove, lead on either side to the point where the original invaginations of the eye-tubes were situated (fig. 24, *c.m.e.t.*). The distal end of the tube is now slightly swollen, and constitutes the "anlage" of the median eye. The paired invaginations of the median eye-tubes in Limulus, therefore, are homologous with the invaginations on the second segment of Scorpions, with which they agree in their relation to the other invaginations. They are, however, much smaller than those of Scorpions, and instead of advancing backward and inward over the cephalic lobes, they advance forward and inward to a point in front of the brain, where they unite to form a single invagination extending right into the yolk, and apparently having no connection with the cephalic lobes. A comparison of figs. 24 and 25 and the sections shown in figs. 41 and 42 will show that the opening into the median eye-tube really has much the same position in Limulus as in the Scorpion, so that there can be no doubt that the median eyes in these two forms are homologous. The strange position of the median eye-tube in Limulus is caused partly by the forward migration of the invaginations, and partly by the shortening of the cephalic lobes, which brings all the invaginations into a nearly straight transverse line, instead of being distributed along a broad semicircular curve, as in Scorpions. Moreover the rapid invagination of the semicircular lobes and their backward growth under the rest of the brain have something to do with the apparent separation of the median eye-tube from the brain (figs. 41 and 42).

A narrow commissure, similar to that seen in Myriapods and in the early stages of Acilius, unites the right and left halves of the cephalic lobes (compare figs. 57, 59, 61, 62, *c'*.).

B. The Mid-brain.—The first post-oral neuromere, that of the cheliceræ, becomes intimately united with the cephalic lobes, as in Insects and Scorpions. Its stomodæal nerves, with one exception, are identical with those of Scorpions. There is a large "lateral stomodæal ganglion" on the median margin of the cheliceral neuromere, from which two large lateral stomodæal nerves extend inward the whole length of the stomodæum (figs. 43, 46, 49, 61, *st. n.*). There are also a small median and two lateral nerves extending outward and backward along the œsophagus to the rostrum, comparable with the paired and unpaired labral nerves of Insects and Myriapods (figs. 47 and 48). The first post-oral commissure differs from the others in that it is isolated from the rest, and forms a long loop around the posterior under side of the œsophagus. The other commissures extend straight across, and are bound together by firm connective tissue. I have not traced the ends of this commissure up to the lateral stomodæal ganglion, but nevertheless it seems to me very probable that it represents the "posterior pons stomodæi" of insects and Myriapods. The commissure just in front of the mouth unquestionably corresponds to the anterior pons stomodæi of insects and Myriapods.

I can find nothing in Limulus corresponding to the optic ganglion of the second segment in Scorpions. This seems to be due to the fact that the median eye-nerves in Limulus have shifted their points of attachment from the neural surface of the brain to the semicircular lobes on the opposite side.

C. Later Modifications of the Brain.—After the stage shown in fig. 59 the cephalic lobes rapidly change in aspect, and one finds less and less resemblance between them and the brain of other Arthropods; while, on the other hand, their resemblance to the fore-brain of Vertebrates becomes more apparent.

Among the important changes that take place in the disposition of the parts is the shortening of the optic ganglia, and the forward movement of their distal ends; at the same time they are drawn inward and backward till they lie in

about the middle of the hæmal surface of the brain, enclosed by a common envelope for the brain and optic ganglia (fig. 49). I know of no other Arthropod in which the optic ganglia have this position. They usually project from the sides, or, as in most Arachnids, from the neural surface of the brain. In their position on the hæmal surface the optic ganglia of Limulus resemble the thalamencephalon of Vertebrates, with which I regard them homologous. The other important changes that take place are in the cerebral hemispheres. They arise from disc-like thickenings of the cephalic lobes, and correspond in position with the brain-lobes of the second segment of Scorpions (compare figs. 24, 59, and 61, *c. h.*). They grow upward at first, and then their summits spread out mushroom-like, till they completely conceal the remainder of the cephalic lobes, including even the greater part of the segment of the mid-brain (compare figs. 24, 25, 43, 47—52).

By this method of growth a series of chambers are formed, some disappearing early, others persisting in the adult, that correspond to certain cavities of the Vertebrate brain, such as the fifth ventricle, the lateral ventricle, the primary cerebral vesicle, and the cavities leading from the third ventricle into the lateral eye-tubes.

We shall now describe these changes in more detail. I have carefully studied these stages, both in surface views and in sections, and have constructed a partial wax-plate model of an embryo in about this stage. In fig. 24 I have represented the cephalic lobes as they would appear in surface views. Some details have been omitted, and the clearness of some parts has been exaggerated, and in order to save repetition of figures strict attention was not paid to synchronism. It is therefore not so much an accurate picture of one stage, as a diagram of two or three closely joined stages. A series of sections of this period, drawn as accurately as possible, will serve to make the meaning of the drawing clearer, and will also show to what extent it is diagrammatic. Such a series of longitudinal sections is shown in figs. 35—40. They are taken

from a little earlier stage than that in fig. 24, and illustrate the continuity of the invagination cavities. There were fourteen sections in the series, of which the fourteenth is shown in fig. 35 ; the eleventh, ninth, fifth, and second, in the four succeeding figures. The other sections of the series showed nothing of interest, and so were not represented. The position of the sections is shown by the dotted lines in fig. 24. The invaginations do not persist very long, and the method of disappearing varies somewhat in the different parts. In fig. 39 the ectoderm has already broken away from the anterior wall of the invagination, and is now pushing its way backward over the optic ganglion, in order to unite with the thin ectoderm behind it. The anterior wall (*a. w.*), which assumes a little darker colour, finally fuses with and forms a part of the definitive optic ganglion. A section through this region in a little later stage (fig. 33) still shows traces of the anterior wall of the fold (*a. w.*). The figure also shows the characteristic way in which the thick layer of ectoderm, constituting the ganglion, becomes tilted over, so that its inner surface, where the medullary substance is just appearing, is turned outward. Another section of the same stage, across the distal end of the optic ganglion, and showing the first traces of the convex eye, is shown in fig. 44.

On the median side of the optic ganglion, to go back to the stage we started with, the invagination is already quite faint (figs. 37 and 38, *i. v.*), and in the next stage disappears, both walls forming brain tissue. The same is true of the semicircular lobes (fig. 36, *i. v. sl.*), although the invagination cavity and its walls can be distinguished as such for a considerably longer period than in the optic ganglion. In the median section (fig. 35) one sees the median eye-tube cut lengthwise; the relatively small commissure uniting the halves of the brain (*m. c.*), and, at the tip of the rostrum, a great mass of transitory tissue (*r. m. s.*), the nature of which could not be determined. In fig. 26 a cross-section of the anterior margin of the cephalic lobes of the same stage shows the extent of the two invaginations for the semicircular lobes.

Shortly after this stage the cerebral hemispheres become more conspicuous. An outline of a section showing their appearance at this time is seen in fig. 27. The whole brain here appears to be a very thick, proliferating layer of ectoderm, with no differentiation into an overlying hypodermis. The underlying semicircular lobes are seen with their cavities nearly obliterated (*c. sl.*).

Soon after this the ectoderm begins to advance in an obscure fold over the cerebral hemispheres, as in fig. 28, which represents a section just back of the posterior margin of the semicircular lobes. In some cases one can see a double-walled fold like that on the lower left side of the figure, but usually there is only a single layer, the edge of which creeps over the hemispheres, hugging closely to their outer surfaces. Where this layer has passed, one sees a layer of thin cells (*b. s.*), that probably develop into a part at least of the brain envelopes. Whether this layer comes from the brain itself, the mesoderm, or the middle wall of the ectodermic fold, could not be determined.

Similar but more distinct medullary folds are seen advancing over the margin of the mid-brain and ventral cords. Figs. 29—32 are selected from a series of cross-sections to illustrate these folds. In fig. 29 the section passes just back of the cerebral hemispheres in fig. 30, where the folds show best, through the mid-brain, just in front of the cheliceræ; in fig. 31 back of the second post-oral appendage; and in fig. 32 through about the middle of the third post-oral neuromere. These sections show that the nervous system is not separated from the surface by a process of delamination like that which occurs in nearly all other Arthropods, but by an infolding from the lateral margins, very similar to that which takes place in Vertebrates, especially of such forms as the sturgeon, as described by Salensky. The folds in the post-oral region never advance far enough to meet over the median line. What becomes of the middle layer of the fold when it does occur could not be determined. Over the middle of the ventral cords, mainly over the "Mittelstrang," there is formed by

delamination a thin layer of superficial ectoderm, which probably unites with the medullary folds to form the continuous layer that finally covers the ventral cords.

The margin of the ectodermic fold advances forward and medianly over the cerebral hemispheres, as shown by the line *m. c. f.*, fig. 24. At the same time the narrow bridge of ectoderm leading to the pineal eye-tube is drawn backward, diminishing the clear triangular area behind it. Its anterior lip then unites with the advancing fold on the cerebral hemispheres, so that the surface of the hemisphere, not yet covered by the ectoderm, has a contour something like that shown in fig. 25, *c. h.* The cross-bar of the ectoderm is finally converted, by the union of its anterior and posterior lips, into a tube opening by a small round pore immediately over the proximal end of the median eye-tube. This pore, which I shall call the anterior neuropore (*n. p.*), leads directly into the median eye-tube and through the cross-tube into all the cavities of the fore-brain. The lips of this pore also represent the last point of attachment of the fore-brain with the surface ectoderm.

Meantime the cerebral hemispheres have increased rapidly in size. From the very start each hemisphere shows a distinct separation into two lobes—a posterior one, drawn out into long sharp points, and an anterior lateral lobe. Both lobes are separated from each other by a deep fissure, in the angle of which lies the slender fibrous peduncle on which the hemisphere is supported (fig. 25, *p. ch.*). The shape of the hemispheres in the second larval stage is accurately shown in fig. 47. The fore-brain region is drawn from a wax-plate model, the rest from dissected specimens. The posterior lobes are not so slender and pointed as at an earlier stage, and both lobes show the first traces of the convolutions so characteristic of the brain at a later period. The hemispheres have united with each other along the median line, and the anterior lateral lobe is beginning to grow around on to the under side of the brain. Two cross-sections of the brain in this stage are shown, one through about the middle of the hemi-

spheres (fig. 51), another, farther back (fig. 50), showing the growth of the lobes over the rest of the brain. A third cerebral lobe, the median internal lobe, or "corpus striatum," is now visible on the anterior inner face of each hemisphere (fig. 51, *c. s.*). It is an oblong lobe, extending about half the length of the median face of the hemispheres. Even at a much later period its thick cortical layer of cells is never convoluted like the rest of the hemispheres, and it contains a great medullary core, terminating blindly in front, and behind in a great bundle of medullary substance that forms a part of the cerebral peduncles (fig. 43, *p. c. s.*). By comparing figs. 47—49 a fair idea of the enormous increase in size of the cerebral hemispheres may be obtained. These are all young specimens, however; the hemispheres are still larger in the adult, as may be seen in fig. 52, a camera drawing of a section through about the middle of the brain. The extraordinary convolution of the cortical substance, formed of very small, deeply stained nuclei, stands out in sharp contrast with the unstained medullary portions. One should observe the slender peduncles of the hemisphere, the large space or vesicle ($v.^5$), sometimes filled with a mass of loose connective tissue, and the now much-reduced semicircular lobes (*s. l.*).

The semicircular lobes undergo strange modifications. The two curved lobes seen in fig. 59 grow inward and backward beneath the brain till they cover its whole median hæmal surface. They are seen in cross-section in fig. 27, and their extent is dimly shown through the rest of the brain in figs. 24 and 25. They reach their greatest relative extent in the second larval stage, as shown in fig. 46 *s. l.* Some time before this there are split off from the median eye-tube two clear nerve-strands, which now terminate in conical swellings on the anterior ends of the semicircular lobes (fig. 46). The latter are now joined with each other at their posterior ends, and no longer show any trace of their dual origin. The lateral and posterior margins of the semicircular lobes contain small, densely crowded, and deeply stained nuclei, which are apparently multiplying rapidly, and when the brain is stained and

seen whole as a transparent object these cells appear as a very conspicuous narrow band, showing clearly the peripheral boundaries of the semicircular lobes. This narrow dark band is best seen in larvæ about four or five inches long, but may be distinguished even in the adults. After the stage shown in fig. 46 the semicircular lobes become narrower and relatively smaller (fig. 49). Its cortical layer is always smooth, and contains many large ganglion-cells. Within the lobes is a narrow stratified band of fine dense medullary substance, something like a horseshoe in shape. Its arms are directed forward and outward, and terminate blindly in the apex of the lobes: the curved posterior part constitutes the anterior commissure (fig. 43, *a. com.*).

The optic ganglia, soon after their first appearance, divide into four medullary cores or centres, each covered with a cortical layer of ganglion-cells. Three of these lobes are seen in fig. 49; the fourth, which differs in some respects from the rest, is situated at the base of the ganglion, concealed by the semicircular lobes.

D. Comparison with Vertebrata.—It cannot be justly denied that the brain of Limulus has a strong superficial resemblance to a Vertebrate brain, and it is equally certain that there is nothing else like its adult condition among other Arthropods. Is this resemblance real—that is, is it profound and varied, indicating genetic relationship, or merely a superficial resemblance, which on closer inspection is seen to be due to some deceptive quirk, but being in other respects totally and irreconcilably different? It must be either one or the other. Either Limulus is closely related to the Vertebrates, and will show this by a fundamental similarity of structure, or else it is a world apart, and will show this also by an equally profound difference in structure. The resemblances already pointed out can hardly be called superficial, involving as they do the similarity in the number of neuromeres, nerves, and sense organs. I shall not discuss these points now, but shall consider the various vesicles and

invagination cavities, which are so strikingly like the cavities of a Vertebrate brain that the resemblance cannot be due to a mere coincidence, or to similarity of function. Before studying the development carefully, it was a great puzzle to explain how the adult brain of Limulus could resemble so closely the brain of Vertebrates, and yet one be solid and the other hollow. How would it be possible to convert the solid brain of Limulus into the hollow one of Vertebrates, without at the same time destroying its resemblance to the Vertebrate type? A careful consideration indicates that we shall not have to deal with this difficulty, for the brain of Limulus already contains, either potentially or actually, all the important cavities of the Vertebrate brain. In order to show this, let us go back and consider the manner in which the brain and nerve-cord are folded off from the surface. The imperfect folds of ectoderm on the lateral margins of the ventral cord, and the single layer of cells advancing over the cerebral hemispheres, are, I believe, morphologically the same as typical double-layered folds, such as the medullary folds of Vertebrates. No one doubts, for instance, that the formation of the brain and spinal cord of Teleosts by solid ingrowth is anything more than a modification of the same process which in other Vertebrates is expressed in continuous folds. Either may be derived from the other. In order to prove the identity of these folds in Limulus with those in Vertebrates, it is necessary to show that they advance over the brain, not as they do now in a typical Vertebrate embryo, but as they did in the ancestral Vertebrate; or it will be sufficient if we can show that these imperfect folds in Limulus advance in such a manner that, if they were perfect duplicatures, the cavities they enclosed would have the same relation to one another and to the brain-lobes as those in Vertebrates. These requirements, that appear so formidable on any other theory, can be fairly met in Limulus. It is necessary to remember, however, that in Limulus the advancing layers of cells, representing folds of ectoderm, do not enclose spacious cavities between themselves and the brain; and that

neither these cavities nor the invagination cavities persist till the adult stage is reached.

Lateral Ventricles.—Referring now to figs. 24 and 25, it is evident the fold (*m. c. f.*), growing over the cerebral hemispheres, encloses a very broad but flat cavity, corresponding to the lateral ventricles of Vertebrates. The cavities are as extensive as the whole upper surface of the cerebral hemispheres, and they increase in extent as the latter increase in size. They communicate with each other in front (fig. 25), as in many fishes, and below with the cavity of the semicircular lobes or infundibulum. The roof consists of a thin, non-nervous membrane or pallium, and the floor of the thick posterior, anterior lateral, and the median internal, lobes of the cerebral hemispheres.

The Infundibulum.—On comparing figs. 24, 25, 41, and 43, it is seen that the anterior wall of the cerebral hemispheres passes downward and backward into the cavities of the semicircular lobes (*s. l.*). These two cavities, which are at first separate (fig. 59, *s. l.*), disappear about the time the lobes in which they lie unite. Had they persisted a little longer they would communicate with each other (as they do in Scorpions), and this broad, backwardly directed cavity thus formed would be similar to that in the infundibulum of Vertebrates. In my first paper on the "Origin of Vertebrates" I advocated the old view that the infundibulum represented a primitive œsophagus. I have now abandoned that position for what I consider a much stronger one, and we are thus left to account for the ancestral œsophagus in some other way. It is possible that the primitive Arthropod œsophagus broke through the narrow band of nerve-tissue in front of it, and moving forward, was converted into the one we now see in Vertebrates. However that may be, there are excellent reasons for regarding the semicircular lobes of Arachnids as homologous with the infundibulum of Vertebrates: (1) they agree in a general way in shape, and in their position on the hæmal surface of the brain; (2) they develop from invaginations in such a way as to bend the anterior end of the brain-tube downward and back-

wards on to the hæmal surface, and form cavities communicating directly with the third and the lateral ventricles; (3) their relations to the median or parietal eye are apparently the same in both Limulus and Vertebrates.

Concerning the last fact, it seemed at first impossible to satisfactorily account for what is apparently a great difference in the position and attachment to the brain of the parietal eye in Scorpions, Limulus, and Vertebrates. But these differences are very easily explained, and when thoroughly understood, help to strengthen the comparisons already made. For instance, in Vertebrates the parietal eye arises from the middle of the roof to the thalamencephalon, back of the cerebral hemispheres. In Limulus it seems to be about as far as possible from its apparent position in Vertebrates, for it lies in front of the cerebral hemispheres, and has its roots inserted into what, in Vertebrates, would be the floor of the infundibulum (figs. 43—49). But on looking at the spider's brain (fig. 61), which for the point under consideration will serve as well as that of the scorpion, we see that the parietal eye lies back of what corresponds to the cerebral hemispheres in Limulus (*c. h.*); and this position, as shown by the whole course of development, seems to be the primitive one for Arachnids. But by elongating its divergent nerve-roots the median eye can be moved forward in front of the cerebral hemispheres, so that its roots, instead of describing a half-circle around the posterior neural surface of the fore-brain, lie like an inverted Y on its anterior hæmal surface; the points of attachment, however, will be the same in either case. In Limulus this very change has taken place, owing to the movement of the eye farther and farther forward on to the hæmal surface. It is a very significant fact that in mammals there are two bands, the peduncles of the parietal eye, that extend around the "tween brain" and terminate in the posterior wall of the infundibulum, in the "corpora albicans." I have seen something similar to these bands in the brain of Petromyzon, but have not yet had time to work them out

carefully. I am not aware that their existence has been described in fishes, but it is hoped they will soon receive the careful attention they deserve. However, if we can regard the course and termination of these peduncles in mammals as typical for the Vertebrates, it is obvious that they correspond to the divergent roots of the parietal eye nerve of Limulus. On turning to fig. 43, it is evident that the parietal eye could be moved back to the position it occupies in mammals under the backwardly projecting lobes of the hemispheres, for the diverging roots (*l. n. m. e.*) could be slipped over the hemispheres, and when shortened would form nerve-bands or peduncles encircling the "tween brain" and terminating in the infundibulum. By turning to figs. 24, 25, and 59, it is seen that the olfactory organ would not stand in the way of this change provided it were made early enough, since the olfactory organs do not unite in the median line, and the median olfactory nerve is not formed, till long after the parietal eye has assumed its final position. The difference, then, between the position of the parietal eye in Limulus and Vertebrates is more apparent than real, and is probably due to the fact that Limulus moves about on its neural surface, and the parietal eye, to be of any use, must be on the opposite side; the extent to which it has wandered from its original position on the cephalic lobes is shown approximately by the great length of its nerve in the adult. In Vertebrates the neural surface is turned upward, hence the parietal eye can remain nearer its original position than in Limulus.

The, at first sight, apparently inexplicable position of the parietal eye in Limulus, and its connection with the infundibulum, are seen, therefore, to be in harmony with Vertebrate anatomy as soon as we recognise that the true roots of the nerve to the parietal eye in Vertebrates do not terminate in the roof of the "tween brain," but in the infundibulum.

The Third Ventricle.—It is evident that in Limulus the space between the lateral ventricles and the cavity of the infundibulum corresponds to the third ventricle of Vertebrates (compare figs. 25 and 43). The resemblance appears

greater when we recollect that the invagination cavity of the optic ganglion leads from the sides into this space at a level midway between the infundibulum and the cerebral hemispheres, just as the tubular lateral eye nerve of Vertebrates leads into the sides of the third ventricle. The principal difference between the optic cavities in the two cases is that in Vertebrates the canal extends the whole length of the nerve to the lateral eye, while in Limulus it only reaches to the root of the nerve. It must have extended further along the lateral eye nerve in forms more closely related to Vertebrates, otherwise the lateral eye could not have been inverted, as it is now in Vertebrates.

If this view is correct, then extending the ganglionic invagination to the lateral eyes in Limulus ought to give rise to conditions similar to those in the lateral eyes of Vertebrates. This is indeed the case. It is a well-known fact that the lateral eyes of Limulus, Trilobites, and the Merostomata are kidney-shaped, a configuration they must necessarily have in order to distribute the ommatidia economically over the convex surface of the cephalo-thorax. The concave edge of the eye is always directed hæmally, as I have shown, in a purely diagrammatic way, in fig. 24. Now if the invagination of the optic ganglion had progressed a little farther along the nerve the whole eye would have been invaginated, and the ommatæum would then form at the end of a long tube a kidney-shaped retina, but with its concave edge turned in the opposite direction from before. As a kidney-shaped retina would be no longer of any advantage it would tend to assume a circular outline; but owing to the peculiar distribution of the nerve-fibres and the predetermined method of growth, this could be most economically and advantageously accomplished by bringing the halves of the concave edge together, thus producing a choroid fissure, the position and direction of which would be like that in Vertebrates. In other words, the kidney-shaped retina of Vertebrates is due to the fact that the retinal cells multiply faster on the convex margin than elsewhere.

This method of growth forces the concave margins together and produces a choroid fissure. The shape and method of growth of the vertebrate retina are inexplicable, unless we assume it to be inherited from ancestral forms whose eyes must of necessity have had the shape and position that the eyes of Vertebrates would have if carried back to the exterior where they originally belonged. The only Invertebrates having such eyes are the marine Arachnids, Trilobites, Limulus, and Mirostomata, &c.

If we turn again to fig. 24 we see that the space over the mid-brain uncovered by the marginal folds (*m. f. cr.*) represents the imperfectly roofed-over cavity of the Sylvian aqueduct, and that it extends forward around the posterior median margins of the hemispheres into the uncovered lateral ventricles as a shallow groove (*F. M.*) which corresponds in position to the foramina of Monroe. Before a complete roof can be formed to the mid-brain (*Ne.*[1]), and to the third forebrain segment or the "tween brain" (*Ne.*[3]), by the union of their medullary folds, the cerebral hemispheres grow backward, as they do in Vertebrates, and, uniting in the median line, completely cover them (fig. 25). Therefore the cavity in Limulus corresponding to the "iter" must communicate freely above with the fifth ventricle, or the space between the inner median faces of the hemispheres (fig. 52, *v.*). But if the marginal folds of the embryo persisted they would occupy about the position indicated by the dotted line in fig. 52, and if they united completely two distinct chambers (*v.*[5] and *i.*) would be found corresponding exactly to the "iter" and to the fifth ventricle of Vertebrates.

If the marginal folds of the ventral cord united (fig. 32) they would produce a medullary canal, bounded at the bottom by the Mittelstrang, on the side by the ventral cords, and on the roof by the united medullary folds.

Owing to the divergence of the cords in the hind-brain region (fig. 48) a great rhomboidal cavity with a thin roof would be formed that would correspond to the fourth ventricle.

To sum up what has preceded, we find that in the brain of Limulus there is an actual or potential agreement throughout with the Vertebrate brain. There is (fig. 43) the folding of the anterior end of the cerebral vesicle downwards and backwards to form the infundibulum; the upward growth of the co-ordinating centres, or the cerebral hemispheres, and their expansion in all directions at the summit to cover the rest of the brain. There are the lateral ventricles communicating in front with each other, with the cavity in the epiphysis, or median eye tube, and with that of the olfactory lobes, below with the infundibulum, and backwards and downwards with the "iter" and the third ventricle. Its roof is a thin membrane or pallium; its floor the three great lobes of the cerebral hemisphere—the posterior, the anterior lateral, and the median internal or corpus striatum.

There is the great cavity in the centre of the brain corresponding to the imperfectly separated third and fifth ventricles and the "iter." It is bounded in front by a thin membrane, "lamina terminalis" (fig. 43, *l. t.*), above by the corpora striata (*c. s.*), and on the sides by the cerebral peduncles and optic thalami, and on the floor by the middle and inferior commissures. It communicates anteriorly and above with the lateral ventricles, behind with the unroofed "iter," and on the sides with a cavity leading to the roots of the lateral eye nerves.

There are three commissures to the brain—one the inferior commissure in the roof of the infundibulum (*a. com.*), the very large middle commissure in the floor of the third ventricle (*m. com.*), and the posterior commissure in what would be the roof of the mid-brain, just behind the posterior end of the cerebral hemispheres (*p. com.*).

There is the fourth ventricle, a large rhomboidal space imperfectly covered by the medullary folds (fig. 48). Its floor is composed of the united cross-commissures of the thoracic neuromeres in the position of the future "pons," and its sides are formed by the great diverging masses of nerve-substance, or crura, leading up to the fore-brain.

It is evident that the principal differences between the forebrain of Limulus and that of Vertebrates is a difference in degree and not in kind. In Limulus the infoldings are obscure, in not always showing the duplication of layers; individualized, in that in each important brain-lobe they progress independently of the others; and they are incomplete, in that they do not always unite so as to entirely enclose the infolded parts. Moreover some important cavities, such as the lateral ventricles and the cavities in the optic ganglia and infundibulum, disappear at an early stage, so that their relations to the permanent cavities are not very obvious. In Vertebrates, on the contrary, the infoldings are very simple because they have lost their individuality by fusing into one continuous fold, which appears very early, and is usually completed before the brain-lobes are specialized. In Limulus the phylogenetic processes from the very first stages dominate over the pure mechanics of ontogeny. In Vertebrates it is almost the reverse; purely embryological processes prevail at first, afterward phylogenetic ones are manifest.

There is no greater difficulty in identifying all the characteristic features of the Vertebrate brain in Limulus than there is in comparing a fish brain with that of mammals. No other Invertebrate will permit any approach to such comparison. On the other hand, it is impossible that the comparisons, such as I have instituted, could be carried so far without breaking down, unless they rested on a sure foundation of actual facts and correct premises.

It remains now to say a few words concerning the parietal eye and the olfactory organ. If the relation of the parietal eye to the primary cerebral vesicle and to the infundibulum are mere coincidences, having nothing to do with the genetic relationship of Limulus with Vertebrates, then in all probability the resemblance will cease there; it certainly cannot extend any farther, say to the structural details of its nerves and its terminal portions. But the resemblance does go a great deal farther, as we shall now see.

IV. The Parietal Eye.

After the median eye-tubes in fig. 59, *p. e.*, unite, a single tube is formed like that in figs. 24 and 25. The solid distal end (fig. 35, *m. e.*) soon divides into three lobes; the two outer ones just before hatching become filled with black pigment, and form the retinas to the ectoparietal eyes (*m. e.*); the inner, unpaired, one forms the endoparietal eye (*m. e'.*). There is no distinct cavity in these lobes, but as they are formed from the walls of an invaginated tube, and as the two outer ones are homologous with the median eyes of scorpions, where such cavities are present, we must regard them morphologically as sac-like diverticula from the end of a tube. Their similarity to the common vesicle of the median eyes of scorpions and their relation to the cerebral vesicle is easily seen in figs. 41 and 42. During the first larval stage the endoparietal eye is a solid cylindrical mass of cells with rod-like thickenings in their walls, like those in true retinal cells, and filled with dense white pigment (fig. 63). In sections the crumpled refractive plates on these degenerate retinal cells look like coiled or zigzag fibres something like those in fig. 20. As the animal grows older this mass of aborted retinal cells buries itself deeply in the underlying tissue, away from all connection with the exterior (fig. 74). In the adult it usually lies below a conical tubercle, situated a little behind the two lenses to the median eyes. In many old specimens this tubercle is replaced by a clear, transparent spot, which is no doubt the remnant of a lens. As the endoparietal eye must itself have been formed by the fusion of paired retinas, it is evident that the distal end of the parietal eye-tube contains the retinas of four originally distinct eyes. These facts are all the more interesting since Claus has shown that the median eye of crustacea, which in some cases is composed of three distinct eyes, is formed by invagination just as in scorpions and Limulus.

The distal portion of the primitive eye-tube is converted bodily into the median eye-nerve. Just before hatching its

distal end splits up into four branches, two of which plunge directly into the median diverticulum or endoparietal eye (fig. 63, *n. en. p. e.*), and the other two pass to the paired retinas of the ectoparietal eyes (figs. 63—65, *ec. p. e.*). Two delicate nerve-strands split off at a very early period from the proximal end of the primitive eye-tube and its diverging arms (figs. 35, *l. n. m. e.* and fig. 36, *r. m. e. n.*). Compare also surface view (figs. 46 and 47, *r. m. e. n.*). These two nerve-roots subsequently divide (fig. 49), so that there are at least four main roots to the nerve, not including the epiphysis. Two of these roots terminate in conspicuous medullary nuclei attached to the horseshoe-shaped medullary core in the interior of the semicircular lobes (*p. e. c.*). From each nucleus a delicate strand passes laterally to the peduncle of the lateral optic ganglia (*s. t. p.*).

The epiphysis, or what is left of the median eye-tube after splitting off the roots to the parietal eye-nerve, remains for some time unchanged, as in fig. 43, *m. e. t.*, also figs. 46—49 and 68—70. Finally its lumen disappears, and several swellings appear in it, composed of small nuclei like those in the cerebral hemispheres. In specimens four or five inches long these swellings may be seen embedded in the brain evelope, and extending downward from the root of the median olfactory nerve toward the parietal eye-nerve (fig. 49, *m. e. t.*). After this stage the epiphysis loses altogether its connection with the root of the parietal eye-nerve, and then disappears completely.

We have in the parietal eye seven distinct structures, which should be constantly borne in mind in making any comparison with the parietal eye of Vertebrates. (1) The primary brain diverticulum or primitive parietal eye-tube; (2) the paired, and (3) the unpaired diverticula at its distal end; (4) the solid nerve-stalk to these diverticula; (5) the epiphysis, or remnant of the primitive parietal eye-tube after splitting off the (6) roots of the median eye-nerve, and finally (7) the large blood-vessel accompanying the

nerve. The primary parietal eye-tube of Limulus corresponds to the epiphysial outgrowth from the brain-roof in Vertebrates. The ectoparietal eyes containing the two separate retinas, and the ventral diverticulum or endoparietal eye filled with white pigment, correspond to the two distinct terminal organs found in some Vertebrates. I venture to suggest that in Hatteria the "capsular-like structure," described by Spencer, at the base of the eye where the nerve enters, corresponds to the endoparietal eye of Limulus. Again, the extraordinary presence of dense pigment in the middle of the outer wall of the eye in Varanus giganteus, as described by the same author, may be regarded as the remnant of the pigmented epithelium originally separating the paired retinas of the ectoparietal eyes of Limulus and scorpions; and finally it is possible that the two separate pineal eyes that occur in certain reptiles, such as Anguis and Lacerta, may be due to shortening of the primary evagination, so that the ecto- and endo-parietal eyes arise as two independent outgrowths from the roof of the thalamencephalon.

The distal end of the primitive eye-tube in both Limulus and Vertebrates is converted bodily into the pineal eye-stalk. The proximal end in Limulus, after giving rise to the nerve-roots, persists as an inverted T-tube; the upright arm unquestionably corresponds to the epiphysis in Vertebrates, and the cross-bar, perhaps, to the ganglion habenulæ. The nerve-roots correspond to the "peduncles" of the pineal eye as seen in mammals. In both Limulus and Vertebrates a large blood-vessel accompanies the nerve to the pineal eye. I formerly regarded the epiphysis as one of the nerves to the parietal eyes, but it now seems to me to be nothing but the ectoderm along which the parietal nerves formerly extended. The nerves separate from it just as the peripheral nerves separate from the overlying surface ectoderm. It may be compared to the epithelium lining the interior of the hollow lateral eye-nerves.

A remarkable feature of the parietal eyes of Limulus and of Vertebrates is the presence in them of great quantities of

dense white pigment. In Petromyzon the solubility of this pigment in weak acids and its intensely white glistening appearance in reflected, and deep black appearance in transmitted light has been the cause of contradictory statements concerning it, some claiming it is black, others white, and others that it is absent, according to the method of preparation or the light (transmitted or reflected) by which it has been examined. I have examined the white pigment in the parietal eye of Limulus side by side with that in the parietal eye of Petromyzon, and have found them to be identical in appearance. In Limulus, as in Petromyzon, the white pigment is much more soluble in dilute nitric acid than the black. White pigment is comparatively rare in Invertebrates, and, so far as I know, is confined to the Arthropods. It is present about the base of the ommatidia in the compound eyes of the crustaceans Pinæus and Galatea (Patten), and in Limulus is found in the infolded margin of the young lateral eyes and in the choroid plexus lying in front of the brain. But nowhere in Arthropods is it more abundant than in the endoparietal eye of Limulus. This eye is apparently nothing but a solid mass of the pigment, and when ruptured with needles it flows out in a dense white chalky stream. Ahlborn claims that the white granules in Petromyzon are composed of calcium phosphate, like the "brain-sand" found in the parietal eye of the higher Vertebrates. It would be very interesting to learn its composition in Limulus.

Gaskell has compared the parietal eye of Vertebrates and Crustaceans, but he failed to see the real points of resemblance between them, for he knew nothing about their development in Arthropods. There is no more resemblance between the parietal eye of Vertebrates and the ocellus of Acilius, as he puts it, than there is between the lateral eyes of Vertebrates and those of a Cephalopod. If we fail to take into account the development of the parietal eye in Arthropods there can be no trustworthy grounds of comparison between it and the parietal eye of Vertebrates.

While there remains a good deal of doubt concerning the

significance of certain parts of the parietal eye in Limulus and Vertebrates, that they are homologous with each other as a whole is, I believe, beyond question.

V. The Olfactory Organs.

We have in the olfactory organ of Limulus a structure presenting the most striking and unusual features. It is as different from the other cerebral sense organs as the olfactory organ of Vertebrates is from the pineal eye. The features that in Limulus distinguish it from all other sense organs are the very ones characteristic of the olfactory organ in Vertebrates. The organ itself consists of an upright epithelium containing a large number of bud-like sense organs comparable with the sense organs described by Blaue in the olfactory organ of fishes. The whole organ, as it exists in the adult Limulus, may be compared to the unpaired olfactory organ found in the Cyclostomata. It is composed of three distinct parts—a pair of primitive sense organs growing forward from the brain, and a subsequently formed part lying between them.

It is probable that the dormant individuality of these parts has reasserted itself in the higher Vertebrates, and caused the separation of the olfactory organ into its constituent parts, or the olfactory organ proper, and the organ of Jacobson. The distribution of the nerves supports this view. In Limulus there are three distinct nerves: the paired lateral and hæmal ones, having their roots in one of the centres of the optic ganglia, or in the optic thalami; and an unpaired, median, neural nerve terminating in the cerebral hemispheres. The median nerve, which in its early stages shows evidence of its paired origin, arises at a late period as an outgrowth from the cerebral hemisphere. It is a new formation, in histological structure and origin utterly unlike any other Arthropod nerve. All four of these nerves are represented in Vertebrates, for it is now known that each olfactory nerve of the higher Vertebrates is represented in Amphibia by two distinct nerves, which have been likened to the dorsal and ventral roots of a spinal nerve.

But if this were so they would differ from all other spinal nerves in that both dorsal and ventral branches supply sense organs. Moreover, on any supposition they are entirely different from those belonging to the other sense organs of the fore-brain, such as the lateral and parietal eye, and the auditory organ. This condition is quite inexplicable on any theory founded on Vertebrate anatomy. But this very thing occurs in the olfactory organ of Limulus, although the meaning of it cannot be explained any more there than in Vertebrates.

Now in Pipa dorsigera and Epicrium glutinosum, according to Wiedersheim, there are four distinct olfactory nerves—a neural and hæmal pair. The Saracins in their Ceylon work, and more recently Burckhardt ('Z. f. w. Zool.,' 1891), have further shown that in Ichthyophis and Triton the hæmal pair innervate the organ of Jacobson, and the neural pair the rest of the olfactory organ. Exactly the same relations prevail in Limulus; the hæmal pair are also lateral, and they supply a distinct lateral depression in the olfactory organ. The neural pair in Limulus innervates the greater part, if not the whole, of the definitive olfactory organ; they are formed by an outgrowth from the cerebral hemispheres; they contain the olfactory bulbs or lobes, and they are composed of pale refractive nerve-fibres with a structureless nucleated sheath, differing strikingly from the thick-walled apparently empty nerve-tubes seen in the lateral olfactory and other nerves; moreover, as they supply by far the greater part of the olfactory buds with nerves, they may be regarded as the olfactory nerves proper. They therefore agree in these important morphological and histological features with the dorsal pair in Amphibians. In the higher Vertebrates all four nerves seem to have united, and the features of the median pair impressed on both.

I am not aware that the course of the roots to the olfactory nerves is accurately known in the lower Vertebrates; but in the Mammalia there are four large medullary nuclei in the thalamencephalon, in one of which terminate the lateral roots to the olfactory nerve. It certainly is not without significance that

the optic ganglion of Limulus, which for other reasons may be regarded as homologous with the thalamencephalon, also contains four nuclei, and from one of them (fig. 49) arises the lateral olfactory nerve.

But we have not yet reached the end of the similarity between the olfactory organs of Limulus and Vertebrates. In Vertebrates the olfactory organs are supposed to belong to a system of supra-branchial sense organs, from which arise the scattered sense organs of the head and lateral line. This supposition is based on the similarity between the method of development of the organs in both cases, and by the fact that the olfactory organ in many fishes is at first composed of a collection of sense buds similar to those in, or originating from the supra-branchial sense organs. Now, although I do not attribute much weight to these arguments, it is important to observe that we have in Limulus the same relation between the olfactory and other sense organs that we have in Vertebrates. We have, for instance, in the thorax of Limulus a series of sense organs and ganglia supplied by a purely sensory nerve, which in position and connection is much like the supra-branchial nerve of Vertebrates. Before I had paid much attention to these organs in Limulus I was led, from a knowledge of these relations in scorpions, to regard the epidermic thickening that gave rise to the "coxal ganglion" of Scorpions as homologous with a supra-branchial sense organ of Vertebrates. My recent observations on Limulus confirm this comparison, and broaden its significance in a most unexpected manner. In scorpions the great ganglionic thickening on the median edge of the coxal joint is a transitory structure, and there is no permanent or definite sense organ found there.[1] In Limulus, how-

[1] It seems to me now very probable that the mandibles on the walking appendages of Limulus and the coxal spur of Scorpions, with its rudimentary sense organ, represent much-shortened endopodites of the thoracic appendages. In Scorpions the sense organ comes in about the same position as the rudimentary appendages described by Jaworowski ('Zool. Anz.,' xiv Jr., 363) in Trochosa singoriensis, and regarded by him—rightly, I believe—as the endopodites of the thoracic appendages. But he has not, in my judgment, produced satisfactory evidence that the structure described by him as corresponding to the

ever, we find exactly the same nerves and the same ganglia, but they are connected with permanent organs of taste. Moreover some of these sense organs are single cells, strangely modified, and recalling the isolated gustatory, olfactory, and so-called "tactile" cells so widely distributed in Vertebrates; others are sensory buds, practically identical with those scattered so uniformly over the body, as well as with those in the olfactory organ.

There certainly can be no doubt that the great masses of sense organs in the jaws of Limulus have disappeared in Scorpions, leaving nothing but an enormous ganglion and nerve to indicate their former existence. This fact is of special interest in this connection, for it is exactly what Beard supposed had taken place in the supra-branchial sense organs of Vertebrates.

In the free swimming merostomata-like ancestors of the Vertebrates, we may assume that a change like that in the Scorpion has taken place. With the transformation of the appendages into gill arches the endopodites or the gustatory spurs of the walking appendages were probably reduced to mere sensory patches, appearing in the ontogeny of Vertebrates as transitory gangliogenic thickenings of the ectoderm, or supra-branchial sense organs. I have already pointed out that in Scorpions ("Origin of Vertebrates from Arachnids") the coxal sense organs, ganglia, and nerve, were like the supra-branchial sense organs of Vertebrates in their development and distribution and relations to the rest of the nervous system. Now we can show, in addition to this, that in Limulus the mandibles may be regarded as segmental gustatory organs. They are centres composed of aggregations of gustatory buds and cells, and from them, or near them, may arise diffusely distributed sense buds. The segmental aggregates and the diffuse organs in Limulus are largely gustatory, and the same is in all probability true of these organs in Vertebrates, various theories to the contrary notwithstanding.

antennæ of Insects is really an appendage; it may be either a sense organ, or the endopodite or exopodite of the cheliceral segment.

Moreover the gustatory cells in Limulus, and the various sense buds arising from the supra-branchial sense organs in Vertebrates, are arranged in straight lines, a condition, so far as I know, found in Arachnids and Vertebrates only. Now whether this arrangement in lines is due to the method of growth or to a physiological necessity is doubtful. Nevertheless this condition may be taken as evidence of genetic relationship between the forms in which it occurs. And finally, this being what I regard as of most importance, we see that in Limulus the olfactory organ, besides its other resemblances to the Vertebrate organ, contains the same kind of buds as those in the segmental gustatary organs. As is well known, the same thing occurs in Vertebrates. Now we might suppose, as has been done in Vertebrates, that the olfactory organ is serially homologous with the mandibular gustatory organs. But the whole development of the organ and its nerves shows that in Limulus this view cannot be entertained for a moment. The olfactory organ in Limulus is obviously a new growth on an old foundation. The latter, it is true, is a segmental sense organ, but it belongs to the series including the ocelli and compound eyes, not to that of the thoracic appendages.

The same interpretation applies, I believe, to the olfactory organ of Vertebrates, as Limulus appears to be less intelligent than animals like Lobsters, Scorpions, and Spiders, in which there are no cerebral hemispheres or any organ that may be regarded as a psychic centre. It is very surprising that it should possess gigantic cerebral hemispheres, and in shape, structure, and development like those of Vertebrates. This may be a coincidence. But can it be a coincidence that in both Limulus and Vertebrates these cerebral hemispheres are connected with only one pair of nerves? Is it a coincidence that these nerves arise from the same brain region, have the same lobes, and supply sense organs that show striking similarities in position, structure, development, and function? It cannot be. Not one of these characters obtains elsewhere, and, in my judgment, it is impossible that all of them should occur in

Limulus and Vertebrates unless they are genetically related.

VI. Comparison of other Brain Regions of Limulus with those of Vertebrates.

In any near relative of Limulus there might be from five to ten pairs of supra-branchial nerves, according as the cheliceræ and the vagus segments retained their sense organs. In Limulus there are five pairs, those belonging to the post-oral thoracic appendages (figs. 47, 48, *md. n.* 2—6). This number corresponds approximately to the number of supra-branchial sense organs in Vertebrates, and affords us a satisfactory explanation of their relation to the gill-arches and their absence from the trunk in the early stages of development.

I have already pointed out in my paper on the "Origin of Vertebrates" how the cephalo-thoracic neuromeres of Scorpions and Limulus are comparable with the entire brain of Vertebrates. This is clearly shown by the similarity in the grouping of the neuromeres, and by the modifications these groups have undergone.

The modifications and homologies of the three segments constituting the fore-brain of Limulus and Vertebrates have already been considered. The mid-brain of Vertebrates is generally conceded to consist of a single neuromere, distinct from those in front and behind, and characterised by the fact that it is provided with the only pair of cranial nerves arising from the neural surface of the brain. We find the same isolated neuromere in Arthropods, namely, the one belonging to the antennal segment of Insects and Myriapods, and the cheliceral segment of Arachnids. In the Arachnids it is easily distinguished from the true fore-brain on the one side and from the post-oral neuromeres on the other. Owing to the position of the cheliceræ, close together in front of the mouth, their nerves invariably arise from the neural surface of the neuromere, while all the others, except, perhaps, in the vagus region, arise from the sides.

The hind brain of Limulus (fig. 48, *H. B.*) is composed of five typical thoracic neuromeres, each having the following four pairs of nerves :—(1) The mandibular nerves (*md. n.*, 2—6) are purely sensory, and consist of two branches which terminate in the gustatory organs in the mandibles. They arise from the neural surface of the ganglion at the root of the pedal nerve, and usually possess well-defined swellings that contain a few small ganglion cells scattered through a mass of interwoven fibrous substance. Sections through the appendages of half-developed embryos (fig. 72) show two sensory thickenings, one corresponding to the inner mandible (*i. md.*) and the other to the outer (*o. md.*). In figs. 71 and 73 are seen ganglion cells separating from the sense organs to form the ganglia of the mandibular nerves in the adult (fig. 3, *md. n. g.*). The mandibular nerves correspond to the supra-branchial nerves and ganglia of Vertebrates. (2) The pedal nerve is a mixed nerve arising from a large neural ganglion common to it and the coxal nerve; it supplies the sense organs and muscles of the walking appendages. In Scorpions it is at an early stage probably connected with the lateral segmental sense organs, but the existence of such a connection could not be demonstrated in Limulus, owing probably to the transitory nature and imperfection of the segmental sense organs. This nerve corresponds to the ventral or branchial nerve of Vertebrates, with which it agrees in innervating the only important muscles of the head, namely, those of the visceral arches. All the other head muscles of Arachnids and Vertebrates, owing to the complete anchylosis of segments, have disappeared.

The neural and lateral ganglia of Beard probably correspond respectively to the pedal ganglion and the ganglia to the segmental sense organs of Limulus and Scorpions. The anterior and posterior hæmal nerves correspond to the pre- and post-trematic of Vertebrates.

All the nerves of the hind brain of neuromeres of Limulus agree with those of Vertebrates in being separate, while the same nerves in the trunk of both Vertebrates and Arachnids

are more or less united. This is especially true of the Scorpion, where I have shown that the abdominal nerves are built on the type of true spinal nerves, for their proximal ends remain separate to form two roots, the dorsal or neural one being ganglionated, the hæmal one being non-ganglionated.

The accessory brain forms the fourth and last brain region of Vertebrates and Arachnids. It is formed of from two to four neuromeres derived from the trunk and added at a comparatively late period to the head. In spite of their late origin, they form the most specialized and completely fused neuromeres of the head. In both cases almost every trace of the metameres to which they belong has disappeared, consequently their nerves have wandered backward to new territory. The nerves to these four neuromeres in Scorpions are very unlike all others in front or back of them, for the four ganglionated neural nerves are fused into one group, and the eight hæmal ones into another. They are, on the other hand, strikingly like the vagus nerves of Vertebrates in their direction, distribution, and general appearance (see "Origin of Vertebrates from Arachnids," fig. 1). In Limulus there are only one or at most two neuromeres in this region, and therefore it has not such striking vagus characters as in Scorpions.

The presence of these vagus segments is not confined to Scorpions and Limulus, but is of very wide occurrence in Arthropods. The last thoracic and first two or three abdominal segments in Insects, and the four abdominal appendages bearing segments in Spiders, probably belong here. Their presence in trilobites is shown by a well-marked "cervical suture" like that which in the adult Limulus marks the presence of these segments.

After we have torn off the deceptive Arthropod mask that disguises Limulus we discover that the nervous system, with all its complex and intricate modifications, shows as a whole a profound structural similarity to that of Vertebrates. This similarity extends to important aggregations of parts, as well as to many minute details, all of which could not on any reasonable assumption have occurred accidentally or be due to

similarity of function or condition. The mere enumeration of the resemblances which my as yet superficial study enables me to point out ought to convince the most sceptical that we are working in the right direction. Is there any group of Invertebrates besides the Arachnids which by any reasonable assumption can be made to resemble Vertebrates in the way that Limulus does? How far do the comparisons of either Ascidians, Balanoglossus, Nemertians, and Annelids with Vertebrates carry us? They explain nothing either in Vertebrate anatomy or in phylogeny, and only serve to render the whole problem of the ancestry of the Vertebrates hopelessly perplexing and obscure. If we accept the Arachnid theory all this is changed, for we can see, dimly perhaps, some way out of the intricate problems bound up in the morphology of the Vertebrate head. It is true many great problems will be left unsolved, but their discussion will be shifted to the vast field of Arthropod morphology, where there is hope of their ultimate solution. And the problems involved are great ones, notwithstanding a dawning tendency to subordinate phylogenetic questions to profound studies on the mechanics of cell division and kindred topics. If the Arachnid theory is true, the Ascidians, Amphioxus, and perhaps Balanoglossus and the Echinoderms become degenerate phyla, bearing somewhat the same relation to the Vertebrates that the parasitic Copepods do to the Arthropods. It will furnish us with a new basis for embryological interpretation, especially regarding the problems connected with the formation of germ layers. If the Arachnid theory is true, many current views on phylogeny, ontogeny, and important problems in comparative anatomy are based on false conceptions, and must be revised. That this is no exaggeration is obvious from the objections most frequently urged against the Arachnid theory, ones I had not anticipated would have any weight. Instead of considering the question on its own merits, it has been objected that "it was contrary to all our preconceived ideas," and "it is quite impossible to conceive highly specialized animals like Arachnids giving rise to such highly specialized animals as Vertebrates." I do not

repeat these objections to give them serious consideration, but only to emphasize the fact that the solution of the Vertebrate problem is not merely the filling in of a gap in our system of classification, but this solution will necessitate the reconstruction of a vast deal of that preconceived morphological philosophy which at present forms the most serious obstacle to the perception of the genetic relation between Vertebrates and Arachnids.

GRAND FORKS, N. D.; Nov. 29th, 1892.

EXPLANATION OF PLATES 1 to 5.

Illustrating Mr. William Patten's paper "On the Morphology and Physiology of the Brain and Sense Organs of Limulus."

EXPLANATION OF PLATES 1 and 2.

Reference Letters to Plates.

ax. n. Axial nerve. *bl. v.* Blood-vessels. *c. c.* Cuticular canal. *ch. t.* Chitinous tubule. *ch. t'.* Proximal end of chitinous tubule. *g. c.* Ganglion-cells. *g. o.* Gustatory organs. *g. p. n.* Ganglion to the pedal nerve. *gs. c.* Gustatory cells. *g.* and *t. or.* Gustatory and temperature organs. *i. md.* Inner mandible. *l. ol. n.* Lateral olfactory nerve. *m.* Muscle. *md. n.* Mandibular nerve. *m. i. m.* Flexor muscles to inner mandibles. *m. ol. n.* Median olfactory nerve. *n.* Nerve. *n. c.* Slender nerve-like cells surrounding the base of the chitinous tubule. *n. co.* Nerve collar. *n.* and *n'.* Primary and secondary nuclei of the gustatory cells. *n. fbl.* Cone of nerve-fibrillæ inside the spindle. *o. md.* Outer mandible. *p. g.* Yellowish-brown pigment granules. *p. n.* Pedal nerve. *s. c. c.* Swelling in cuticular canal. *s. ch. t.* Swelling on chitinous tubule. *sp.* Spindle on gustatory cells. *sp. f.* Spiral fibre. *s. sp.* Canal containing pigment and fibrous cells leading to spines. *t. o.* Temperature organs. *v.* Deeply stained varicosities on the nerve-fibrillæ within the spindle. *w. p.* White pigment-cells.

PLATE 1.

Fig. 1.—Longitudinal section through the anterior wall of one of the stout gustatory spines of the mandibles, showing the cuticular canals, each containing a chitinous tubule.

Fig. 2.—A gustatory spine from the mandibles, seen from its anterior surface, and showing the lines of pores into each of which runs a chitinous tubule. × 30.

Fig. 3.—Third appendage on right side of an adult female, seen from in front, with the anterior wall removed, and showing the nerve to the appendage with its mandibular branches. Natural size.

Fig. 4.—Nucleated end of one of the gustatory cells of the mandibular spines, showing the peculiar yellowish-brown granules that are sometimes found in these cells. Isolated by Bela Haller's fluid.

Fig. 5.—Distal end of a gustatory cell from the mandibular spines, showing fibrillar structure of the cell body and the spindle with its internal cone of fibrillæ, each fibrilla having an enlargement at the base of the spindle, and all converging toward the apex, where they unite to form the axial nerve-bundle that runs outwards through the chitinous tubule. × about 2000. Macerated in Haller's fluid and stained in acetic acid carmine.

Figs. 6 and 7.—Spindles from gustatory cells of mandibular spine, showing the partly detached nerve-cells, and the projecting axial nerve-bundle, which in Fig. 7 is broken up into its constituent fibrillæ. Haller's fluid and methyl green.

Fig. 8.—Very young gustatory bud from the inner mandible, showing the circle of refractive rod-like plates and the single ganglion-cell with its fibrous prolongation.

Fig. 9.—Two sense buds from the olfactory organ, showing the chitinous tubule, central ganglion-cell, and the nerve-plexus. Slightly diagrammatic.

Fig. 10.—Three cuticular canals from the base of a mandibular spine. A. Larger canals with short spine in a saucer-shaped depression at the summit. May be one of the temperature organs. B. Ordinary gustatory canal. C. Same, with a few bead-like globules in the chitinous tubule.

Fig. 11.—Surface view of the cuticle from the chelæ of the walking appendages of a young Limulus about 8 inches long, showing the two kinds of sensory pores, the gustatory pores at *g. o.*, and the temperature organs at *t. o.*

Fig. 12.—The nucleated portions of two gustatory cells from the mandibular spines, showing that they are formed of two imperfectly fused cells similar to the double ones described by the author in 'The Eyes of Molluscs and Arthropods.'

Fig. 12 A.—Outer end of a cuticular canal of the inner mandibular gustatory buds. *a*. In longitudinal section. *b*. Seen from above. *c*. Gustatory canal (outer end) from the chelæ.

Fig. 12 D.—Surface view of the outer end of a sensory bud of canal, from the thorax.

Fig. 12 E.—Same view of olfactory bud of canal from olfactory organ of female.

Fig. 12 F.—Same of male. All of Figs. 12 A—D drawn to same scale.

Fig. 13.—One of the node-like swellings in the cuticular canals seen in Fig. 1 much enlarged, and showing the chitinous tubule within and the spiral fibre.

PLATE 2.

Fig. 14.—Isolated chitinous tubule from the gustatory buds of the mandibles, showing the thin, granular, protoplasmic investment, with the spiral lines. Treated with caustic potash.

Fig. 15.—Isolated ganglion-cell from the centre of a gustatory bud. Hertwig's mac. fluid, 5 days.

Fig. 16.—Chitinous tubules from the mandibular gustatory buds. In A the tubule is stained dark by the acetic acid carmine, and is surrounded by two sinuous, refractive and colourless fibres. B shows the coiled tubules that are frequently found in the mandibles.

Fig. 17.—Longitudinal horizontal section of the median olfactory nerves and olfactory lobes of a young Limulus about 4 inches long. The lateral olfactory nerves, *l. ol. n.*, which bend outwards beneath the olfactory organ, are cut transversely. × 90.

Fig. 18.—Outline of the fore-brain, olfactory organ, and nerves, of an adult female Limulus. × 4.

Fig. 19.—Surface view of the olfactory organ of an adult female, showing the distribution of the cuticular canals leading to the olfactory buds. × 15.

Fig. 20.—Longitudinal section through the base of the lateral olfactory nerve, showing the ommatidia-like clusters of cells with their refractive, rod-like thickenings. × 200.

Fig. 21.—Cross-section of the adult olfactory organ. The outer layers of the cuticula have been removed. × 46.

Fig. 22.—Cross-section of the olfactory organ in a young Limulus about 7 inches long. Flemming's fluid (strong). × 90.

Fig. 23.—Portion of the preceding figure still further enlarged. The thickness of the cuticula is not increased in the same proportion. × 550.

EXPLANATION OF PLATES 3 and 4.

Reference Letters to Plates 3 *and* 4.

a. 2—6. Anterior hæmal nerves of the second to the sixth neuromere. *A. B.* Accessory brain. *a. e.* Anterior edge of dactylopodite. *a. w.* Anterior wall of the optic ganglion invagination. *a. w. c.* Anterior wall of cerebral vesicle. *b. e.* Last point of union of cerebral hemispheres with surface ectoderm. *c.* Posterior fore-brain commissure. c^2. First post-oral commissure. *c. h.* Cerebral hemispheres. *c. h'.* Uncovered part of cerebral hemispheres. *c. m.* Canal between the cerebral hemispheres and the thin median ectoderm. *c. m. e. t.* Canal leading to the median eye-tube. com^3. Posterior cerebral commissure. *c. r.* crura cerebri. *c. s.* Internal cerebral lobe = corpus striatum. *c. sl.* Invagination cavity of the semicircular lobes. *cu.* Cuticula. *c. v.* Cerebral vesicle. *D. S.* Dorsal surface. *F. B.* Fore-brain. *g. l. ol. n.* Ganglion to the lateral olfactory nerve. *g. n.* 1—5. Nerves to gills. *g. p. n.* Ganglion to pedal nerve = "neural ganglion" of a Vertebrate cranial nerve. *g. st. n.* Ganglion to stomodæal nerve. *H. B.* Hind brain. *h. n.* 2—9. Hæmal nerves or peripheral tegumentary nerves. *i.* Unroofed space corresponding to "iter." *i. v.* Continuation of the invagination of the optic ganglion with that of semicircular lobes. *i. v. op. g.* Invagination cavity in optic ganglion. *i. v. sl.* Cavity of semicircular lobes. *l. e.* Lateral eye. *l. e. n.* Lateral eye-nerve. *l. ol. n.* Lateral olfactory nerve. *l. t.* Lamina terminalis. *m.* 2—6. Median hæmal nerve of the 2—6 neuromeres. *M. B.* Mid-brain. *m. c.* Middle commissure of the brain. *m. c.* 1—2. Two cortical masses of ganglion-cells in front of cheliceral nerves. *m. cbr. l.* Median cerebral lobe. *m. c. f.* Margin of fold growing over cerebral hemispheres. *md. n.* 1—5. Mandibular nerve, or nerve to the endopodite. *m. e.* Median eye. *m. e'.* Diverticulum from median eye. *m. e. t.* Median eye-tube. *m. f.* Furrow leading from uncovered portion of cerebral hemispheres to the uncovered portion of the crura. *m. f. c. r.* Margin of the ectodermic fold growing over the crura. *m. ol. n.* Median olfactory nerve. *m. p. com.* Medulla of posterior commissure. *m. st.* Mittelstrang. *mt. n.* nerve to chelaria. *ne.* 1—4. Neuromeres. *n. p.* Anterior neuropore; leads into brain cavity and into median eye-tube. *ol. b.* Olfactory buds. *ol. l.* Olfactory lobes. *ol. o.* Olfactory organ. *op. g.* Optic ganglion. *o. g.* 1—3. Lobes to optic ganglion. *op. n'.* Nerve to operculum. *p.* 2—6. Posterior hæmal nerve of 2—6 neuromeres. *p. c.* Posterior commissure. *p. c. s.* Peduncle to internal cerebral lobe (corpus striatum). *p. e.* Posterior edge. *p. e. c.* Medullary nucleus at root of parietal eye-nerve. *p. g.* Pigment granules. *p. n.* 1—6. Pedal nerves. *p. op. g.* Peduncle to optic ganglion. *p. ol. o.* Primary olfactory organ. *p. st.* Stalk to the pigmented plexus arising from the primary olfactory organ. *r.* Rostrum. *r. m. e. n.* Roots

to median eye-nerve. *r. m. e. t.* Remnants to median eye-tube. *r. ms.* Rostral mesoderm. *s. l.* Semicircular lobes = infundibulum = l'organ stratifié. *st. g.* Stomodæal ganglion. *st. n.* Stomodæal nerves. *s. t. p.* Strand connecting the nucleus to parietal eye-roots with the peduncle of optic ganglion. *t. e. c.* Thin ectoderm between cerebral hemispheres. *th. r.* Thickened ring surrounding the cephalo-thorax. *v.* 5th ventricle. *va. n.* Vagus neuromeres and nerves. *v. s.* Ventral surface.

PLATE 3.

Fig. 24.—Slightly diagrammatic view of the brain of an embryo in a stage when the legs and from two to three pairs of gills are developed. The drawing is constructed from surface views and sections, and is intended to show the relations of the various invaginations and the lines along which the imperfect ectodermic folds (*m. c. f.*, *m. f. c. r.*) advance to enclose the brain.

Fig. 25.—Similar view of an older embryo, showing the greatly increased cerebral hemispheres and the areas still uncovered by the advancing folds, *m. c. f.* and *m. f. cr.*

Fig. 26.—Camera drawing of a cross-section through the brain in a stage like that shown in Fig. 19. The section passes through the end of the line, p. inf.

Fig. 27.—Cross-section of a brain in a later stage than that shown in Fig. 24, and in a plane that would pass through about the end of the line *c. m.* of Fig. 24.

Figs. 28—32.—Cross-section of the brain and nerve-cord in about the same stage as that shown in Fig 24.

In Fig. 28 the section plane passes through about the middle of the cerebral hemispheres (compare Fig. 24).

In Fig. 29, just back of the cerebral hemispheres.

In Fig. 30, just in front of the first pair of appendages.

In Fig. 31, through one of the lateral nerve-cords, just posterior to the second pair of appendages.

In Fig. 32, through the ventral cords, a little back of the middle of the third post-oral neuromere. × 200.

Fig. 33.—Longitudinal section of a later stage than that of the preceding series, passing through the base of the optic ganglion. It shows the anterior wall of the invagination, and first differentiation of the primary olfactory organ. × 200.

Fig. 34.—Part of a longitudinal section in a little older stage than that of the preceding series. The section shows the early stages of the lateral eye, while it is in its primitive position close to the optic ganglion.

Figs. 35—40.—A series of longitudinal sections through the brain in about the stage shown in Fig. 24. There were 28 sections in the series, section 14, Fig. 25, passing through the sagittal plane. The position of the sections is shown on Fig. 24 by the numbers 35—40. Only those sections are represented that show some variation in the invaginations.

Fig. 41.—Diagrammatic longitudinal section through an early stage of the brain of Limulus to show the relation of the median eye-tube to the anterior wall of the brain.

Fig. 42.—Same of the scorpion.

Fig. 43.—Right half of the brain of a young Limulus about 3 inches long, viewed from its cut surface. The outlines and proportions of all the parts are drawn from a wax plate model, same as that in Fig. 49, and is diagrammatic only in so far as the invagination cavities of the infundibulum, optic ganglion, median eye, and olfactory organ and neuropore are represented as persisting up to this period instead of disappearing soon after their formation.

Fig. 44.—Cross-section through the dactylopodite of a Limulus about 8 inches long, showing general distribution of the cuticular canals for the gustatory and temperature organs. The gustatory canals are most abundant along the anterior cutting edge of the joint. The cuticula about the canals is in some places stained black by the osmic acid used for that purpose.

Fig. 45.—Under surface of a young Limulus 61 mm. long, showing the position of the olfactory organs in reference to the mouth, also its radiating choroid plexus of white pigment-cells. × 2.

EXPLANATION OF PLATE 4.

Fig. 46.—Nervous system of Limulus in its second larval stage seen from the dorsal or hæmal surface. × 66.

Fig. 47.—Cephalo-thoracic nervous system of the same seen from the ventral or neural surface. × 66.

Fig. 48.—Nervous system of Limulus about 2½ inches long, seen from the neural surface. Constructed from sections and dissections. The sympathetic system is not represented in Figs 36—38. × 15.

Fig. 49.—Fore-brain region of a young Limulus about 3 inches long, seen from the hæmal surface. The drawing is made from a wax plate model, enlarged about 100 diameters. The drawing is reduced to about 30 diameters.

Figs. 50 and 51.—Cross-sections of the brain of Limulus in the second larval stage (see Figs. 46 and 47).

In Fig. 50 the section plane lies some distance back of the peduncles, and shows the overlapping of the cerebral hemispheres.

In Fig. 51 it passes through the middle commissure, the peduncles of the cerebral hemispheres, and the last connection of the hemispheres with the ectoderm. × 275.

Fig. 52.—Cross-sections of the brain of an adult Limulus, passing through the slender peduncles on which the enormous cerebral hemispheres are supported. The plane of section cuts the anterior border of the middle commissure. Its position is shown on a younger brain by the line 21, Fig. 43. × 15.

EXPLANATION OF PLATE 5.

Reference Letters to Plate 5.

a. p. st. Anterior pons stomodæi. *at. n.* Nerve to antennæ. *at. neu.* Antennary neuromere. *a. st. g.* Anterior stomodæal ganglion. *a. st. n.* Anterior stomodæal nerve. *br.* 1—3. Brain lobes. *c.* Brain commissure. *c. e.* Convex eye. *ch. g.* Ganglion to cheliceral nerves. *c. l.* Cerebral lobes. *ch. n.* Cheliceral nerve. *ec. p. e.* Ectoparietal eye. *en. p. e.* Endoparietal eye. *ep.* Epiphysis. *F. B.* Fore-brain. *g. hab.* Transverse tube of nervous substance. *g. v.* 1—3. Invagination of optic ganglia. *i. md.* Inner mandible. *i. md. n.* Inner mandibular nerve. *l.* Labrum. *l. e. n.* Lateral eye-nerve. *l. st. g.* Lateral stomodæal ganglion. *l. sy.* Lateral sympathetic nerve. *M. B.* Mid-brain = antennal or cheliceral neuromere. *m. sy.* Median sympathetic nerve. *n. ec. p. e.* Nerve to ectoparietal eye. *n. en. p. e.* Nerve to endoparietal eye. *n. l.* Labral nerve. *n. oc.* Nerve to ocelli. *n. r. p. e.* Nerve-roots to parietal eye. *o. To.* Nerve of Tömösvary. *oc.* Ocelli. *o. e.* Œsophagus. *o. g.* 1—3. Optic ganglia. *o. md. n.* Outer mandibular nerve. *o. md.* Outer mandible. *p. e.* Parietal eye. *p. g.* Pedal ganglion. *p. m.* Primitive mouth. *p. n.* Pedal nerve. *p. p. st.* Posterior pons stomodæi. *s. l.* Semicircular lobes. *v. c.* Ventral cords.

Fig. 53.—Diagram of nervous system of an insect, showing relations of stomodæal and trunk sympathetics to the cephalic lobes and ventral cords.

Fig. 54.—The same, with the stomodæum evaginated, to illustrate what was probably the original distribution of the stomodæal nerves.

Fig. 55.—Hypothetical embryo seen from the side, showing distribution of stomodæal nerves.

Fig. 56.—The same, with the stomodæum evaginated.

Fig. 57.—Diagram of the cephalic lobes and stomodæal nerves of an insect embryo (Holometabolic).

Fig. 58.—Same of a scorpion.

Fig. 59.—Same of Limulus.

Fig. 60.—Brain of Acilius at beginning of pupal stage, slightly diagrammatic.

Fig. 61.—Diagram of a spider's brain, showing relation of the ocelli to the brain-segments.

Fig. 62.—Brain of a Myriapod (Iulus).

Fig. 63.—Cross-section of parietal eye, just after hatching (early trilobite stage); depigmented. × 265.

Figs. 64—70.—Series of cross-sections of the parietal eye, after first larval moult (trilobite stage); not depigmented.

Fig. 64. Section through about the middle of the endo- and ecto-parietal eyes. × 265.

Fig. 65. Section through the root of the eye, showing the four nerves. × 570.

Fig. 66. Section of parietal eye-stalk just below the eye. × 570.

Fig. 67. Section shows the primitive roots to the parietal eye-nerve separating from the epiphysis. × 570.

Fig. 68. Section of the epiphysis just below the preceding section. × 570.

Fig. 69. Section through the base of same. × 570.

Fig. 70. Section through the base of the epiphysis, showing its union with the transverse tube, and the communication of the cavities in the same. × 570.

Fig. 71.—Cross-section through the base of one of the legs, showing the developing sense organs, nerves, and ganglia in the inner and outer mandibles. Trilobite stage. × 265.

Fig. 72.—Section of same in an embryo just after the shedding of the chorion. × 265.

Fig. 73.—Same just before hatching. × 87.

Fig. 74.—Parietal eyes of a Limulus about 3 inches long. The eyes have been dissected away from the cuticle and surrounding connective tissue, mounted in glycerine and viewed from above. × 38.

Fig. 75.—Three sensory hairs from the cephalo-thoracic shield of Limulus just after the trilobite stage. Their position and relation to the adult sensory hairs were not determined.

Fig. 76.—Sensory bud from thorax of same stage.

The Structure of the Pharyngeal Bars of Amphioxus.

By

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With Plates 6 and 7.

It may be thought that the structure of the pharyngeal bars of Amphioxus is sufficiently known, after the description by Lankester, and more recently by Spengel; but there still remains a certain amount of doubt as to some points in the structure of the tongue or secondary bar, although recent authors are in agreement as to the general structure of the primary bar. It is to the tongue bar, therefore, that I have more particularly directed my attention.

Spengel contradicted Professor Lankester on several points, both with regard to matters of observation, and more especially with regard to certain interpretations, in a very dogmatic and, indeed, discourteous manner. I was surprised to find that Professor Spengel himself is by no means correct in sundry matters of mere observation.

It will be remembered that Lankester, in addition to his account of the structure of the gill bars—which was, like his figures, in great advance of the work of previous writers on the subject—made certain statements with regard to the spaces within these bars; he attempted to distinguish, not only in the

bars, but in Amphioxus as a whole, cœlomic spaces from blood-vessels. It is chiefly with regard to the interpretation of the spaces within the tongue bars that Spengel joins issue with him, and it was to this part of the subject that I directed my attention last summer in the endeavour more especially to decide whether the cavity traversing the "chitinoid" rod of the tongue bar be cœlom (Lankester) or blood-vessel (Spengel).

I hoped to decide the question by the examination of specimens which had been fed with carmine; and for this purpose Professor Lankester requested Mr. A. Willey, who was then at Naples, to feed some animals and preserve them for me. My thanks are due to Mr. Willey for so doing. Unfortunately, however, these carmine-fed specimens have not been of much value for my purpose, and I was compelled to fall back upon carefully preserved specimens (in picro-sulphuric acid) stained in various media. This was the method followed both by Lankester and by Spengel—careful tracing of spaces from section to section in order to ascertain their communication with other cavities about whose nature there is no doubt.

Before I had completed my work, Boveri's most interesting paper on the nephridia was published. Boveri found, as I had, that Spengel, both in incomplete observation and in certain interpretations, had fallen into errors similar to those which with an accompaniment of gratuitous insolence he had attributed to Lankester.

This short note may conveniently be divided as follows:

A. Description of the tongue bar, according to my own observations, and comparison of it with the primary bar.

B. Interpretations of the parts of the tongue bar.

C. The observations of recent observers.

D. Certain abnormalities in the pharyngeal bars.

The authors who have described and figured the gill bars of Amphioxus, to whom I shall have occasion to refer, are—

1. Stieda.—"Studien üb. d. Amphioxus lanceolatus," 'Mém. Ac. Sci. Pétersbourg,' xix, 1873.

2. Langerhans.—"Zur Anat. d. Amphioxus lanceolatus," 'Arch. f. mikr. Anat.,' xii, 1876.
3. Schneider.—'Beiträge zur Vergleich. Anat. und Entwickel. der Wirbelthiere,' 1879.
4. Lankester.—"Contributions to the Knowledge of Amphioxus lanceolatus," 'Quart. Journ. Micr. Sci.,' xxix.
5. Spengel.—"Beit. z. Kenntniss d. Kiemen des Amphioxus," 'Zool. Jahrbuch' (Anat.), iv, 1891.
6. Boveri.—"Die Nierencanalchen des Amphioxus," 'Zool. Jahrbuch' (Anat.), v, 1892.

In addition to these, a bibliography relating to Amphioxus will be found in Lankester's paper.

A. The Tongue Bar.

The tongue (or secondary) bar is usually distinguished from the primary bar (*a*) in being supported by a tubular skeletal rod in place of the double rod of the primary bar, (*b*) and in being without any cœlom between the rod and the atrial epithelium. It is unnecessary to describe the relations of the bars to one another or to neighbouring parts of the animal, as these matters have been fully described and illustrated by recent writers on the subject.

The structure of the bar is most readily seen in its transverse sections, but such sections—accurately transverse to the bar—are not so easily obtained; in sections transverse to the long axis of Amphioxus, only one or two bars on each side of the pharynx will be cut transversely, though in the pre-hepatic region more bars are so cut, and still more are very nearly transversely cut, than is the case posteriorly. But by varying the obliquity of the plane of section to that of the long axis of the body, I was able, ultimately, to obtain sections which cut nearly the whole series of bars in any section almost accurately at right angles to their length.

It appears to me that this is most important, for the discrepancies in various descriptions and figures of the bar are doubtless due to the more or less obliquity of the sections.

Another matter which must be taken into serious account is the mode of preparation and the character of the stain; for I

have noted in my series—treated differently in both these respects—various differences due to shrinkage and such effects, which suggest the cause of certain omissions by some authors, and of wrong interpretations and other errors.

I have found that Kleinenberg's fluid (picro-sulphuric acid) is the preservative which produces less distortion than other reagents. Cochineal stains, especially Mayer's alcoholic cochineal, serves best for the demonstration of blood in the vessels and for the examination of the skeletal tissues.

Hæmatoxylin is, of course, useful for the nuclei, but cochineal gives better results in the case of cell-bodies.

Some of the animals were stained in bulk; in other cases the sections were stained on the slide.

I proceed now to a description of a transverse section of a tongue bar, as elucidated by the examination of sections treated in different ways, as well as of isolated portions of pharyngeal wall.

Such a section is a narrow bar (about three times as long as it is wide), presenting two ends and two sides: one end, the Outer end, projects into the atrium, and is covered by atrial epithelium; the opposite end, or Inner end, projects into the cavity of the pharynx, and is lined by hypoblast. The sides are directed (roughly) anteriorly and posteriorly.

The bar consists almost entirely of columnar epithelial cells, the inner ends of which abut upon a membrane, the cutis (I follow here Professor Lankester), throughout the greater part of the bar; whilst at the outer end the cells rest upon the skeletal rod, which I regard as merely a specialised part of this cutis.

The character of the epithelium, however, differs at the two ends and at the sides. At the Inner or pharyngeal end the nuclei are arranged in three groups, a central group and a lateral group on each side, as Lankester was the first to point out. The nuclei of the central group are arranged in two more or less curved rows (as in fig. 1), but this arrangement is due not to the existence of two layers of cells, but to the fact that alternately the nuclei are situated nearer to or

further from the base of the cell. The nuclei are elongated and oval; they are placed much closer to one another than my figure represents, and as they stain deeply are very conspicuous. In thick sections it is difficult to determine whether one has to do with a single row of very elongated cells (as Lankester believed), or with several rows of them; this difficulty is emphasised when the sections are not accurately transverse. But in thin sections, successfully cut, it is easily seen that the nuclei are arranged as I describe them.

The cells to which these nuclei belong are, therefore, as long as the epithelium is thick; they are very narrow peripherally and swollen at the nuclear level, producing the flask-shaped appearance of the whole group. These cells carry cilia which are considerably longer than those carried by the lateral groups of cells at this inner end; and it is curious that of previous observers, only Spengel and Boveri have noted this special bundle of cilia. The free ends of the cells are provided with a very finely striated border, which comes out well in cochineal preparations, but which is not differentiated by other stains used.

Each lateral group of nuclei consists of a single row curving downwards from the central group towards each side, somewhat in the way represented by Lankester; but I find that these nuclei, which are long and narrow, are not arranged quite in the fan-shaped manner represented by him.

The cells containing these nuclei carry quite short cilia—entirely overlooked by Spengel and Boveri,—and their free ends are not provided with a striated border. The shape of this inner end has been very variously represented, as the copies of previous figures on Pl. 6 will show.

The side of the bar presents some four or five rows of small nuclei forming a broad band, about two thirds the whole width of the epithelium. These nuclei are not circular, as most observers have represented them (owing to the obliquity of the section, as I know from experience), but are oval, with the long axis directed vertically to the plane of the surface. Langerhans appears to have noted their oval shape.

Here, again, these rows of nuclei do not represent as many layers of cells, for there is but a single layer of very long and very narrow cells, with the nuclei at different levels in neighbouring cells. In some of my preparations these cells are more or less macerated, so that I was able to confirm the description of them given by Langerhans (see fig. 19). Each cell, or at any rate most cells—for it is impossible to be absolutely certain that all the cells reach the surface, though I believe this to be the case—carries a single very long cilium, and presents a finely striated border. The nuclei of the lowermost row appear to be slightly longer than those of other rows, and are close to the septal membrane or cutis, along which they form a very well-marked series. Towards the extremity of the side, both outwards and inwards, i. e. towards the atrial end and the pharyngeal end, the lowest row of nuclei curve upwards towards the surface, so that the epithelial cells become shorter and shorter, and the number of rows less and less, till finally there is but a single nucleus contained in a cell not much longer than itself (see fig. 1). At the inner end of the bar the thin membrane-like cutis curves outwards towards the surface, and naturally the row of nuclei take on this curvature. At the opposite end of the bar the row of nuclei follow the curve of the chitinoid rod; but at this end, for a short space, the epithelium is overlapped, as it were, by a row of three or four cubical cells containing pigment, and not carrying cilia. These cells are part of the atrial epithelium, the invaginated epiblast of the pharyngeal slits. These pigmented cells differ from those hitherto mentioned in containing circular (spherical) nuclei, and herein agree with the cells constituting the epithelium of the Outer end of the bar.

This atrial epithelium is characterised by the larger size of its cells, the absence of cilia, and the presence of relatively small round nuclei, which are placed at different levels in the cells (fig. 1). These cells are highest in the middle of this end —i. e. at the end of the long axis of the section through the bar —and decrease in length at each side of this point till they graduate into the cubical pigmented cells which overlap the

cells of the sides of the bar. In the tongue bar these cells are all of one kind (fig. 18), whereas in the primary bar two kinds of cell (fig. 17, *a*, *b*) are present, one (*a*) being vacuolated, the other (*b*) more granular. Langerhans noted this fact, though he exaggerated the difference. So much for the epithelium of the bar. I shall point out later how far these statements of fact differ from those of my predecessors.[1]

Turning now to the cutis of the bar, i.e. the septal membrane and the chitinoid rod: the septal membrane forms a very thin sheet of tissue traversing the greater part of the long axis of the section, and separating the epithelium of the two sides. At the base of the epithelium of the Inner or pharyngeal end of the bar the septal membrane splits into two, and each of the two branches curves outwards towards the surface; this forking of the membrane leaves a V-shaped space, which is converted into a triangle by a membrane (cutis) at the base of the pharyngeal epithelium.

In this triangular space is a blood-vessel, as Spengel and Boveri have already described (figs. 7, 8). It may be called the internal or Visceral vessel. At the opposite end the septal membrane similarly divides into two, each half of which appears to be continuous with the corresponding side of the rod (fig. 1). This space, which differs somewhat in shape according to the shape of the rod, but which is, on the whole, triangular, also contains a blood-vessel—the external, or somatic vessel. This vessel was observed by Lankester (fig. 6), but overlooked by Spengel (fig. 7), although he represents the space here, whilst Boveri described it as existing only in the primary bar. From the fact that, at each extremity, this septal membrane forks, and from theoretical considerations, I believed this membrane to be in reality double, as indeed it is represented by Stieda's figure (Pl. 6, fig. 3). But I was for a long time unable to assure myself of this fact. I was unable to satisfy myself as to the presence of two membranes here, for, owing to the refrangibility of the structure, it is difficult to make certain whether

[1] I have not observed the "muscle-cells" in the bar described by Rohon and by Langerhans, J. Müller and Schneider.

one sees a double outline to the single membrane (such as Boveri represents, Pl. 6, fig. 8) or two closely apposed membranes.

Spengel vehemently animadverts on Lankester's interpretation of this membrane as a mesoblastic cutis, and insists on its being a "basement membrane," i.e. epiblastic. Lankester was, it seems to me, in error in referring the origin of this membrane to the deepest layer of nuclei in the sides of the bar; at the same time, if we consider the relations of this membrane to the rod and to the vessels at each end, we cannot doubt that the rod and the membrane have the same origin. The rod would scarcely, I presume, be referred by Spengel to the epiblast. I believe, however, that I have decided that the rod is mesoblastic by the discovery of the flattened nuclei pressed against its inner surface (Pl. 7, fig. 13); and we may conclude that the rods in both bars are produced by the flattened epithelium which, as Hatschek has pointed out, forms the "connective tissue" throughout the body of Amphioxus. If the rod is mesoblastic, then a priori we may believe the eptal membrane, which is absolutely continuous with it, to be also mesoblastic; but further, I have detected flattened nuclei in this axis of the bar, i. e. between the two halves of the closely apposed membranes. I searched long and carefully for any nuclei in relation to the septal membrane, and ultimately, in my series of accurately transverse sections, I was able, with the aid of Zeiss's apochromatic, to observe some structures which I believed to be nuclei. However, I was not absolutely certain of their existence, owing to the refrangibility of the membrane and the denseness with which the epithelial nuclei are packed; but in a series of sections intended to be horizontal, and stained in hæmatoxylin, some of the bars were cut longitudinally for a considerable distance—some six or seven times the length of an ordinary transverse section of a bar, —and here I saw distinctly elongated and much compressed nuclei (fig. 21) of fair size, lying between the two membranes. These, like the nuclei surrounding cœlomic spaces, are not rich in chromatin, and do not take the stain easily. We may,

therefore, conclude—as, from a priori reasoning, we should be led to believe—that this septal membrane is double, and is mesoblastic, and not a basement membrane.

The chitinoid rod of the tongue bar is distinguished, as is well known, from that in the primary bar by the presence of a canal—it is a perforated rod.

The shape of the section of the rod varies considerably, both in different bars and even in different parts of the same bar, but that represented in this figure may be regarded as the most general shape. Not only the general outline, but the shape and extent of the contained space are included in the above statement as to the variability of the rod. Professor Lankester has already figured several such variations (loc. cit., pl. xxxvi, B), and others will be found on the plate illustrating this note (Pl. 7, figs. 13—16). But most generally the rod presents a somewhat triangular section, with rounded angle at the base, and a notch—more or less profound—at the apex. This notch forms a part of the internal triangular cavity partially bounded by the septal membrane and occupied by the somatic blood-vessel.

The chief cavity—the canal—of the rod is similarly more or less triangular in section, rarely round, as Lankester represents it, though this shape does occur. The thickness of the outer wall presents very interesting variations: more usually it is nearly as thick as the sides, but it may be very much thinner (as in fig. 14); it may be represented merely by a thin membrane little thicker than the septal membrane, and much thinner than the cutis (basement membrane—Spengel) below the atrial epithelium of the primary bar. Further, this rod may be represented by a couple of curved pieces, which do not quite meet externally, so that the curtained cavity is without an outer wall (fig. 15). This is always the case at the points where a synapticulum joins the tongue bar (fig. 31), but it also occasionally occurs elsewhere.

This cavity frequently appears quite empty, invariably so in my hæmatoxylin preparations; but in sections stained with cochineal, granular matter is very frequently present in greater

or less abundance;[1] and I have sometimes noticed an apparent division of this cavity by a transverse partition, the granulations having a slightly different appearance in the two sides of this partition (fig. 14). Moreover in this same series of sections, as also in sections stained with Weigert's picro-carmine, the blood in undoubted blood-vessels, such as hepatic vessels, dorsal and subendostylar vessels, takes on a characteristic deep red colour—deeper than the tint taken by the skeletal tissues,—so that I am able most definitely to state that there are three blood-vessels traversing the tongue bar, not two, only as Spengel and Boveri believed (c.f. fig. 1 with figs. 7, 8).

Of these three vessels, two occur always at opposite extremities of the septal membrane, in the triangular spaces already mentioned; the third lies inside the rod, and may be called the skeletal vessel. Usually it has the position represented in fig. 1—in the chief canal of the rod, but it does not appear to fill this canal; I can generally detect a slight space around it. This may, of course, be due to shrinkage of the clotted blood; but the apparent existence in some cases of a partition (see fig. 14 and the explanation of it) favours my view, as also does the condition of things represented in fig. 13, where the vessel is passing out of the cavity, that this cavity of the rod is cœlom, which contains a blood-vessel. This view is further strengthened by the fact that, both in my hæmatoxylin preparations (where the blood-vessels are not evident) and in my cochineal sections, I have detected flattened nuclei pressed against the inner surface of the rod, as represented diagrammatically in fig. 1, and accurately drawn from the object in fig. 13. This, I may say, has not been an occasional occurrence, but can be seen in many accurately transverse sections of the bar.

In some of the variations from the normal the rod presents a small cavity about midway between the main canal and the apical notch (figs. 13, 14), and this cavity is usually connected

[1] Spengel mentions the presence of finely granular material in the canal of the hollow rod (loc. cit., p. 278).

with the former by a narrow channel; in this accessory cavity I have sometimes seen a vessel in addition to that in the chief canal, and sometimes I have not detected the latter. I take it that there may be occasional anastomosis between the "somatic" vessel in the notch and the skeletal vessel in the canal.

Comparison of the Tongue Bar and Primary Bar.

I wish now to compare such a transverse section of a tongue bar with that of a primary bar, so far as regards the Outer end. Compare my figures 1 and 2 and that copied from Boveri (fig. 12). In the primary bar Boveri describes, as I myself find, three vessels—(*a*) the inner, or visceral, and (*b*) outer, or somatic, as is the case in the tongue bar, and (*c*) the third or skeletal (first observed by Spengel) outside the cœlom of the primary bar, between the atrial epiblast and the "cutis" (basement membrane of Spengel). This last vessel may project through the cutis into the cœlom, and at the base of the bar, where it springs from the endostyle, and where the rod forks, this blood-vessel lies in the angle of the fork, i. e. in the cœlom itself (fig. 30, *a*), as Spengel has figured. In the tongue bar the first two of these vessels are identical with those of the primary, and one can scarcely resist the idea that the third, or skeletal vessel inside the rod, may correspond with the subepidermal vessel of the primary bar; it differs from it, however, in one very important point, namely, in the absence of any connection with the subendostylar vessel. But now turn to the rod itself. This is, in the primary bar, made up of two pieces, more or less fused according to the region of the bar (see fig. 30, *a*, *b*, *c*), so as to form a triangle, usually with a more or less pronounced notch, or linear channel, arising from near the base (see fig. 9); or again, as Schneider figured and as I have frequently observed, a triradiate split in its centre[1] (fig. 30, *c*). Outside the rod comes the cœlom—every one is agreed about that—lined by flattened cells; and outside them the cutis,

[1] This usually is due to the softer nature of the central part of this rod, and is not really a cavity: the rod presents irregularly concentric markings, as if shrunk, and is firmer externally.

which varies considerably in thickness, and can be traced, as Boveri's figure shows, into the rod at each extremity (Pl. 6, fig. 12). This cutis stains exactly like the rod in cochineal, and also like the rod is unstained in hæmatoxylin. Sometimes the cleft in the rod is more pronounced, and gives rise to a more definite channel (see Lankester, xxxvi B, fig. 3, *f*) open to the cœlom. Such a condition of things is represented in my Pl. 7, fig. 31, which passes through a primary rod (P. 1) at the level of a synapticulum,[1] and should be compared with certain variations in the rod of the tongue bar in the same figure and in fig. 15, and one is struck with the resemblance between the two.

I would suggest that the distinction between the two rods, viz. that of the primary bar and that of the tongue bar, is not so profound as one would be led to think from the use of the terms solid (or bifid) rod and hollow rod. We have seen that not infrequently the rod of the tongue bar is formed of two pieces (fig. 15), whilst, on the other hand, the rod of the primary bar may enclose a cavity. But I think the real distinction between them is that in the tongue rod the subepithelial portion is usually and typically as thick as the sides, and distinctly continuous therewith; whereas in the primary rod the extra-cœlomic piece (subepithelial) is thinner, and, owing to the greater development of the cœlom here, is more widely separated from the rest of the rod. This suggestion occurred to me forcibly in examining the connections of the synapticula with the two bars.

In a lucky series of sections, cutting the bars very accurately transversely, one often gets the whole synapticulum in sections, passing from one primary bar to the next, and showing the connection of the rod in the transverse bar with those of the main bars (fig. 31). Starting with the tongue bar (*T.*), the rod forks, so that its contained cavity is no longer bounded by a chitinoid wall; one branch of the fork passes towards each of the adjacent primary bars, and is continuous, not with the main part of the rod itself, but with

[1] Spengel gives a figure very similar to this one in pl. xvii, fig. 13, illustrating his paper.

the cutis (basement membrane of Spengel), or extra-cœlomic portion of the rod, as I would regard it. But this apparent forking of the tongue rod is due merely to the passage of the contained blood-vessel out of the rod to the vessels of the adjacent primary bars; so that in reality, as many sections show, the rod of the connecting bar, i. e. the synapticulum, is connected on the one hand with the extra-cœlomic portion of the tongue rod, and on the other with the extra-cœlomic "cutis" of the primary bar.

A second difference between the rods is that the skeletal vessel is inside the cœlom in the tongue bar, but outside it in the primary bar over the greater part of its extent, though, in the lower part of the latter bar, it is intra-cœlomic, as in the tongue bar (fig. 30, *a*). I have sometimes seen a small cavity outside the rod in the tongue bar, but I have not been able to satisfy myself as to its nature; it may be merely artifact.

B. Summary of my Observations and Interpretations.

1. The epithelium of the bar is everywhere only one cell in thickness.

2. The arrangement of the cells at the pharyngeal end of the bar, both in the primary and in the tongue, is much more definite than previous observers, except Lankester, have figured and described; the central group, contrary to Lankester's opinion, presents two rows of nuclei, and carries a bundle of very long cilia; the lateral groups carry quite short cilia.[1]

3. The nuclei at the sides of the bar are oval and not round, and the lowest row has nothing to do with the septal membrane.

4. There are three blood-vessels in the tongue, as in the

[1] This differentiation of the cilia round the bar may be compared with that occurring in the gill filaments of Lamellibranchs, and in the cirri of Brachiopods, where there are similarly bundles of long cilia, situated at the sides or angles, and shorter cilia elsewhere. The existence of a skeletal tissue in these cases is a further analogous resemblance.

primary, bar; (*a*) the visceral vessel at the pharyngeal end of the bar; (*b*) the somatic vessel in the apical notch of the rod; and (*c*) the skeletal vessel inside the rod: the last two anastomose here and there.

5. The cavity of the rod is cœlom, is lined by flat cells, and contains this skeletal blood-vessel.

6. The outer wall of this cœlom is homologous with the extra-cœlomic cutis (Spengel's basement membrane) of the primary bar.

7. The septal membrane and the rod are mesoblastic, and the nuclei of the cells forming them are here recorded for the first time.

c. The Observations of Recent Observers.

I will now pass on to a brief survey of the various descriptions and figures of the tongue bar of previous writers, copies of whose figures are represented on Pl. 6, and in the explanation of these, the references whence the figures are taken. Stieda, Langerhans, and Schneider saw no modifications of the epithelium at the pharyngeal end of the bar. Lankester was the first to observe this, and his figures represent more accurately than do those of his successors, Spengel and Boveri, the actual arrangement. As I have already pointed out, however, he missed the fact that the middle group presents two rows of nuclei. This is equally overlooked by Boveri, while Spengel's drawing is not quite clear on this point, and neither of these latter indicate the much greater length of the nuclei in this part of the bar. So far as the general shape of the pharyngeal end of the bar is concerned, Spengel's and Boveri's drawings show it fairly. Fig. 12 from Boveri is taken from quite the upper end of the bar, hence the peculiar shape of this end. Lankester is alone in regarding—as I believe wrongly—the epithelium of the sides of the bar as in several layers. Spengel points this out; but he, like all my predecessors, with the doubtful exception of Schneider and Langerhans, represents small round instead of oval nuclei here.

The authors, as will be seen from the reproductions of

their figures, represent the characters of the atrial epithelium at the upper end of the bar correctly, with the exception of Langerhans, who describes each cell here as bearing a flagellum.

Stieda and Langerhans did not recognise the differences in the length of the cilia round the bar. Schneider is the first to figure the long cilia at the sides, and the remaining authors follow him. Spengel is the first to note this tuft of long cilia at the pharyngeal end; but both he and Boveri[1] overlooked the short cilia carried by the lateral groups of cells at this end.

With regard to the contained vessels in the primary bar, Langerhans entirely missed the vessel at each end of the septal membrane. Schneider and Lankester saw only one vessel—that at the outer end of the bar at the apex of the rod—the somatic vessel. Spengel most unaccountably missed this vessel, although he states that he looked carefully for one here, and his figure of the tongue bar (Pl. 6, fig. 7) shows a very narrow cleft in its position, whilst he describes the inner or visceral vessel (*vn.*), which is usually less noticeable than the other. In this respect his figure shows no advance upon that of Stieda, who also saw, but did not describe, the space at the inner end of the bar.

Boveri, however, observed this vessel (and figures it) in the primary bar (fig. 12, *ve.* 1), in the position already given to it by Lankester, but overlooked it in the tongue bar (fig. 8). As a matter of fact, as I have stated above, there are three vessels in each bar. Schneider and Lankester found one, the "outer or somatic" vessel; Spengel found one, the "inner or visceral vessel," but denied the somatic vessel. Boveri found both these in the primary bar, but overlooked the "outer vessel" in the tongue bar.

As to the nature of the rod cavity, Stieda and Langerhans leave it aside.[2] Schneider represents (at *A*, fig. 5) "a blood-

[1] It should be borne in mind, however, that Boveri did not pretend to describe the bars except in so far as they are related to the nephridia.

[2] Stieda regards the granular substance in the centre of the rod (fig. 2, *a*) as an axial part of the rod itself.

vessel communicating with the branchial artery." Lankester, from its relation to the subendostylar cœlom, regarded this canal in the rod as "cœlom;" whereas Spengel and Boveri look upon it as a blood-vessel, and deny its cœlomic character. Lankester gives half a dozen figures (loc. cit., pl. xxxvi B, figs. 5—9) of as many consecutive sections representing the canal communicating with the subendostylar cœlom. Spengel denies this altogether, and states that the rod becomes solid before it reaches the endostyle, and there becomes continuous with the subendostylar skeleton. He further denies any communication of this rod cavity with the dorso-pharyngeal cœlom.

Now the tracing of these cavities is an extremely difficult matter, owing to the difficulty of making sections in the right plane, and I searched through section after section before I could satisfy myself as to the mode of termination of these rods. Time after time it seemed to me that Spengel was right, so far as the non-communication of the rod cavity was concerned; but in certain lucky sections I found that the plane was convenient for this purpose, and I represent five consecutive sections which show, as I believe, the continuation of the subendostylar cœlom into the cavity of the rod (Pl. 7, figs. 22—26). The series shows most certainly no continuity between the rod and the subendostylar skeleton on which Spengel insists.

I was not successful in tracing the rod cavity into the dorso-pharyngeal cœlom; but I attribute this to the difficulty of observation, and am by no means inclined to conclude that such a connection does not exist.

The "skeletal vessel" ceases some little way before the rod does, being connected with the vessels in the neighbouring primary bars at the lowest synapticulum.

If the hinder gill-slits be examined in a preparation of the pharynx, flattened out on a slide, and the mode of development of the "tongue" observed, it will be seen that the rod in the tongue is double at its upper end; the two pieces diverge and constitute the arcade connecting the series of rods. The tongue bar, as is known, is a downgrowth from the upper

boundary of a primary slit; the cœlom is here in the form of a canal giving off shoots to each bar, and fro them appearance presented in such a preparation, it is not an inconsistent interpretation to regard the cœlom as sharing in the downgrowth of its ventral wall. The appearance presented by the rod in the hinder gill-slits is thus: M.

d. Certain Abnormal Bars.

In one series of sections the pharynx presented a few bars containing sometimes three and sometimes two rods. In all cases these abnormal bars are "primary" bars, i. e. the rods contained within them are cleft rods, similar to those found in normal primary bars; and on each side of such abnormal bars there lies a normal tongue bar, separated by a pharyngeal slit from the abnormal one. On Plate 7, fig. 27, I represent one such bar cut at about the middle of its length, containing three rods, which are in all respects similar to one another. The bar itself is otherwise normal, and presents the usual characters of the epithelium in its various parts. Naturally the bar is much wider than an ordinary one, especially at its atrial end. The septal membrane (*s.m.*) is very short, but the two usual blood-vessels (*vix.v.* and *som.v.*) are present. There is not a vessel at the apex of each rod, as one might expect; the cœlom is very extensive, and dips in between the rods as the nuclei show. The usual subepidermic blood-vessel (*skel. v.*) is also present. But this bar, if traced upwards towards the epibranchial groove and downwards towards the endostyle, presents variations of this condition in different regions.

At its origin dorsally the three rods are fused together, but this occurs for only a very short space, and we soon find three rods. Towards its lower end two of these (Nos. 1 and 3) are fused, so that the bar has but two rods; and still further a junction between these two is effected (fig. 28), and there appear to be but one rod, rather larger than usual, but evidently consisting of two united rods. Soon, however, these separate again, and I take this union as representing a synapticulum.

But now the relative position of the two rods changes;

hitherto they have been side by side, or rather anterior and posterior; but now (fig. 29) one (No. 2) lies outside the other (Nos. 1 and 3), which occupies the normal position. I was unable to track the rods quite to their junction with the floor of the pharynx owing to the imperfection of some of the sections in this series.

Most of the other abnormal bars contained two rods, but I did not trace them all out from top to bottom, so that I am unable to state whether they always contain, at some part of their length, three rods. But I am inclined to answer this in the negative; so frequently are there only two rods that I think this is the more "usual" abnormality. There is another triple-rod bar on the opposite side of the pharynx, at about the same level as the one described, and usually, as far as I could observe, the abnormal bars are opposite.

A bar with a double rod might conceivably be produced by closure of a primary slit between two primary bars; but there is no evidence of any formation of a slit and subsequent closure and fusion of the bounding bars. One would expect if this had happened that the cœlom and subepidermic vessel would be doubled, and that the pharyngeal end of the bar would exhibit some peculiarity; this I did not find to be the case. A triple-rod bar might be explained by an extension of this suggestion, namely, that two slits had remained imperforate, whilst the rods had been formed nevertheless.

EXPLANATION OF PLATES 6 and 7,

Illustrating Dr. W. Blaxland Benham's paper on "The Structure of the Pharyngeal Bars of Amphioxus."

In most of the figures the blood-vessels are represented black, but in fig. 14 I have drawn the actual appearance presented by the sections.

Figs. 1 and 2.—Transverse sections of a tongue and a primary bar, so far diagrammatic in that each figure is a combination of several drawings of

different sections, which have been treated in different ways. The two figures are drawn to scale, and therefore represent the true relative sizes of the bars.

Fig. 1. A transverse section of a tongue bar of the pharynx of Amphioxus (Branchiostoma lubricum, Costa). It represents, accurately as I believe, a typical section of the bar. Most of the parts are fully named on the plate. The rod contains a cavity—the cœlom, lined by an epithelium, whose nuclei are shown (see also Fig. 13). Within this cœlom lies a blood-vessel—the skeletal vessel. Two other blood-vessels are present in the bar, the "visceral" (*Visc. Bl. vessel*) and the "somatic" blood-vessel; these lie at either end of the septal membrane, between the two layers of which are shown three nuclei (see also Figs. 20, 21). The atrial epithelium consists of only one kind of cell. The grouping of the epithelial nuclei at the pharyngeal end of the bar may be noted.

Fig. 2. A transverse section of a primary bar (see explanation of Fig. 1). Here the cœlom is more extensive, and the "cutis"—or outer wall of the cœlom—is thinner than in the tongue bar. The rod itself is made up of two pieces, meeting along the middle line of the bar, and a third piece wedged between. The skeletal blood-vessel is outside the cœlom, but is in reality surrounded by the rod, i. e. "cutis." The atrial epithelium consists of two kinds of cells (see Fig. 17), viz. *a*, the vacuolated, and *b*, the granular cells.

Figs. 3 to 12 are tracings of figures published by previous observers; the only alterations that have been made are (1) colouring the rod yellow, and (2) filling in with black the spaces regarded as blood-vessels by the authors.

Fig. 3. Copied from Stieda's fig. 6, pl. i. "Transverse section of a branchial plate. *a*. The rod. *b*. Deep layer of the epithelium. *c*. Superficial layer with cilia."

Fig. 4. Copied from Langerhans, fig. 24, pl. xiii. "Transverse section through a gill bar. *m*. Epithelium. *k*. Nuclei of branchial epithelium *p.e.* Pigmented epithelium. *h*. The atrial epithelium. *s*. Hollow gill rod."

Fig. 5. Copied from Schneider's fig. 4, pl. xiv. "Transverse section of a thin (that is, tongue) gill bar. *a*. Triangular space, a blood-vessel. *A*. A blood-vessel in communication with the branchial artery. k^1. The rod. *p.p.* Peritoneal (i. e. atrial) plate."

Fig. 6. Copied from Lankester, pl. xxxvi B, fig. 2. "Transverse section of a tongue bar. *al*. Left inner epithelial band. *ar*. Right inner epithelial band. *am*. Median inner epithelial band. *col*. Columnar lateral cells, with long cilia. *n*. Superficial nuclei. n^1. Deeper nuclei. *sept*. Clear septal tissue. *Bl. v*. Supposed blood-vessel, ending blindly at the ventral extremity. *pig*. Lateral groups of pigment in the atrial epithelium. *at. ep*. Atrial epithelium (epidermic)."

Fig. 7. Copied from Spengel, fig. 19, pl. xviii. "Transverse section of a tongue bar. *vz*. Chief vessel of the bar. *vn*. Accessory vessel."

Fig. 8. Copied from Boveri's fig. 14, pl. xxxiii. "Transverse section of tongue bar at the level of the nephridium. *neph.* Position of nephridium. *vi*[11]. Inner axial vessel. *ve*[11]. Outer axial vessel."

Fig. 9. Copied from Schneider, pl. xiv, fig. 3. "Transverse section of a thick (i.e. primary) gill bar. A^1, A^2. Blood-vessels which are in communication with the branchial artery. *a.* Triangular space, blood-vessel. *p. p.* Peritoneal (i.e. atrial) plate. *V.* Vein in communication with the subvertebral vein, and the venous space around the branchial artery. k_2. The thick rod."

Fig. 10. Copied from Lankester, pl. xxxvi B, fig. 1. "Transverse section of a primary bar. *B.v.* Blood-vessel connected with the lateral branches of the median endostylar vessel. *fiss.* Fissure due to the bilateral origin of the rod. *cœ. ep.* Cœlomic epithelium. *pg.* Pigmented atrial epithelium."

Fig. 11. Copied from Spengel's fig. 12, pl. xvii. "Outer part of a transverse section through a primary bar. *b. m.* Basal membrane. *cök.* Cœlomic canal of the bar. *sp.* Skeletal rod. *vk.* Chief vessel in the primary bar."

Fig. 12. Copied from Boveri, fig. 6, pl. xxxii. "Transverse section of a primary gill bar, *vi*[1]. Inner axial vessel. *ve*[1]. Outer axial vessel. *vc.* Cœlomic vessel. *cœ.* Cœlom."

Fig. 13.—A tongue rod in section, to show the skeletal blood-vessel (*sk. b. v.*) running in a special canal; it is probably about to anastomose with the somatic vessel (*som. b. v.*). Around the cœlom (*cœl.*) three nuclei (*n. c. ep.*) are present, embedded in granulations (? protoplasm). In this section the two blood-vessels, as is frequently the case in cochineal preparations, are stained deep red. The cœlom is quite clear. *cu.* cutis.

Fig. 14.—A rod from a tongue bar, exhibiting granulations in the cavity, which are of two kinds: a coarser, less deeply stained, in the outer part, which is probably the protoplasm of cœlomic epithelium—in that region marked *cœ.*—cœlom; and a finer and more deeply staining mass (*skel. b. v.*), the skeletal blood-vessel. The two are apparently separated by a partition. *n.* is the nucleus of the cœlomic epithelium. *som. b. v.* The outer or somatic blood-vessel. *cu.* The "cutis" or outer wall of cœlom. From a preparation stained with Mayer's alcoholic cochineal. Drawn under Zeiss's homogeneous immersion.

Fig. 15.—A modification in the shape of the section of the rod, which is not unusual, especially near the synapticula. The rod is here composed of two pieces. The outer wall of the cœlom is free of skeletal tissue (cf. Fig. 31), and the blood-vessel (*skel. b. v.*) is passing out of the cœlom (*cœl.*), and lies just below the atrial epithelium (*at. ep.*).

Fig. 16.—Another modification in the tongue bar, which occurs near the upper end of a bar.

Fig. 17.—Cells from the atrial epithelium of a primary bar, from a partially macerated section (cochineal). *a.* Vacuolated cells. *b.* More granular, narrower cells, the nucleus being near the free end. *c.* A portion of a cell, which is probably similar to *d*, with a narrow peripheral portion, and dilated nuclear region below.

Fig. 18.—Three cells of the atrial epithelium of a tongue bar, from a partially macerated section.

Fig. 19.—Cells from the lateral epithelium of a gill bar, from a partially macerated section. The nuclei of the cells are seen to be at different levels. Each cell carries a single long cilium. The small cell to the left may be a portion of a larger cell, or may be really a basal interstitial cell.

Fig. 20.—A small piece of the septal membrane, with a nucleus (*n.*) (from a transverse section of a bar). The membrane appears to be double, and the nucleus lies between the two sheets.

Fig. 21.—A portion of a longitudinal section of a bar, showing the septal membrane and three elongated, compressed nuclei (*n.*). Only a small portion of the epithelium of the bar is filled in, in order to show the relative size of the nuclei of the septal membrane and those of epithelium (*ep.*). (Hæmatoxylin. Under Zeiss's apochromatic 4 mm., aperture ·95, with compensating ocular 8.)

Figs. 22—26.—Five consecutive sections to show the passage of a tongue bar into the endostyle, and the communication of the rod cavity with the subendostylar cœlom.

Fig. 22. Transverse section of the endostyle and a neighbouring tongue bar. The details as to arrangement of nuclei are only approximately accurate. It is merely desired to exhibit the general topographical relations of the structures. *end.* Endostyle. *end. sk.* Subendostylar skeleton. *end. cœl.* Subendostylar cœlom. *cœ. ep.* Nuclei of the cœlomic epithelium. *at. ep.* Atrial epithelium. *art.* Subendostylar artery. *P.* The lowermost part of a primary bar fused with the endostyle. *x.* The peculiar tissue of elongated vacuolated cells (not reticular tissue). *T. T.* Tongue bars. *rod.* The contained hollow rod; on the left the bar has already reached and fused with the subendostylar tissue, on the right it is still free.

Fig. 23. The next section. The rod of the tongue bar on the left appears to have a narrow cleft in its wall, so that its cavity communicates with the subendostylar cœlom; but this is not so distinct as in the case of the right rod in Fig. 26. (The lithographer has exaggerated this cleft.)

Fig. 24. The right portion only of the next and following sections is drawn. Here the rod of the tongue bar (*T.*) is distinctly smaller, and approaches the corner of the subendostylar cœlom.

Fig. 25. The rod has passed further into the subendostylar tissue, and lies above the corner of the cœlom.

Fig. 26. The cavity of the rod communicates with the subendostylar cœlom most distinctly.

Figs. 27—29.—Transverse sections of an abnormal primary bar at different levels.

Fig. 27. Section about the middle of the length of the bar. The epithelium and general structure of the bar is normal, but contains three separate primary rods, 1, 2, 3, each partially surrounded by a layer of cœlomic epithelium. *visc. v.* The visceral blood-vessel. *som. v.* The somatic blood-vessel. *s.m.* The very short septal membrane. *cœ.* Cœlom. *skel. v.* Extra-cœlomic skeletal blood-vessel.

Fig. 28. A section of the same bar lower down. There are here only two rods, partially fused; the rod to the right is formed by the fusion of rods 1 and 3 (in Fig. 27).

Fig. 29. The same rod lower down, nearer the endostyle. The two rods have separated again, but have shifted their relative positions.

Fig. 30.—Three sections of a normal primary rod at different levels. (*a*) Close to the endostyle, where it consists of two independent pieces. (*b*) Slightly higher up, where a third piece (sometimes isolated) plugs the gap between the two pieces, with which it is more or less fused. (*c*) The general condition, in which the fusion of the three pieces is complete, except for a narrow axial cleft (? or less refractive substance). *R.* The rod, in the usual sense. *cu.* "Cutis," or outer wall of cœlom. *cœ.* Cœlom. *skel. b. v.* Skeletal blood-vessel. *som. v.* Somatic vessel. (Cf. Figs. 13—16.)

Fig. 31 *a*.—The connection of synapticulum with the primary and tongue bars. The section is accurately transverse, and includes three bars and the synapticulum. P^1, P^2. The two primary bars. *T.* The intervening tongue bar. *syn.* To the left, half a synapticulum cut along its whole length, and continuous, as is seen, with one half of the tongue rod and the extra-cœlomic "cutis" (*cu.*) of the primary rod. syn^1, syn^2. The two portions of the other half of the synapticulum (the intervening portion is in a section not figured). *b.* v^1. The subepidermic skeletal blood-vessels of the primary bar. *b.* v^2. The skeletal vessel of the tongue bar, which is seen to divide into a right and a left branch, which fall into *b.* v^1. of the primary bars. *cœ.* Cœlom of the primary bar.

Fig. 31 *b*.—The next section of the primary bar (P^2) showing the passage of syn^2. into the cutis (*cu.*). Other letters as in Fig. 31 *a*.

On the Perivisceral Cavity of Ciona.

By

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With Plate 8.

The genus Ciona differs from other simple Ascidians in having the alimentary canal (except the rectum) and other organs contained in a definite cavity, which is situated in the region of the body posterior to the pharynx—the so-called "body-cavity" of Ciona. From its position round the viscera this cavity may be called the perivisceral cavity, which implies nothing as to its nature, while the term body-cavity might be taken to assume its cœlomic nature as definitely known.

This investigation had for its object the determination of the exact nature of this cavity, and was carried on partly at the Zoological Station of Naples during the summer of 1891, and since then at Cambridge.

A. Previous Researches.

The existence of this cavity was first made known by Kupffer,[1] who described it as a cœlom or body-cavity. It is separated from the atrial cavity by a septum which is perforated by the œsophagus, rectum, and genital ducts. In this

[1] I take the account of Kupffer's work from the paper of van Beneden and Julin (2, p. 430). I regret that I have been unable to refer to Kupffer's original paper.

septum Kupffer described an orifice leading from the atrial cavity to the perivisceral cavity, by means of which water could penetrate the latter. He also described two orifices at the bottom of the pharynx, one on each side of the posterior groove which extends between the end of the endostyle and the œsophageal opening; these orifices he described as opening into the atrial cavity.

Roule (1), in his monograph on Ciona, gives a detailed account of the relations of this cavity, which he calls "la cavité générale du corps," but does not mention any openings into the atrial cavity or pharynx; on the contrary, he denies the existence of any possible communication between it and the atrial cavity: "vers chacune de ces ouvertures [i. e. the openings in the septum for the passage of the œsophagus, rectum, &c.], la lame péritonéale, insérée sur tous les organes qui la traversent, envoie entre eux de petits prolongements, de telle sorte qu'il ne peut exister aucune communication, si minime qu'elle soit, entre la cavité péribranchiale et la cavité générale" (p. 107). He considers the perivisceral cavity as the remains of the primary blastocœle of the larva, which has become reduced and, as it were, pushed back to the posterior end of the body by the development of the atrial cavity.

Van Beneden and Julin (2) discuss the question as to the nature of this cavity; they consider that it cannot be part of the original blastocœle, since, according to the description of the vascular system given both by Kupffer and Roule, it does not communicate at all with that system.

Two other possibilities remain:

(*a*) It may be derived from the atrial cavity; or

(*b*) it may be derived from the pharynx.

The latter view is considered by van Beneden and Julin to be the more probable one; they suppose the perivisceral cavity to be homologous with the epicardium described by them in Clavellina. In "l'opinion la plus probable, elle constitue une dilatation de l'épicarde, auquel cas elle devrait communiquer non pas avec les cavités péribranchiales, mais bien avec le sac branchial. . . . . Si l'espace périviscéral répond à la cavité épi-

cardique des autres Ascidiens, les communications avec le sac branchial doivent exister à droite et à gauche de ce sillon, à supposer toutefois que ces orifices persistent pendant toute la durée de la vie chez ces Ascidiens" (p. 432). Van Beneden and Julin state that they were able to confirm the existence of the two orifices at the posterior end of the pharynx mentioned by Kupffer, but not his statement that they opened into the atrial cavity: "Le seul point que nous n'ayons pas pu confirmer, c'est que ces orifices déboucheraient dans les cavités péribranchiales" (loc. cit., p. 432). They consider that these two orifiees most probably open into the perivisceral cavity and not into the atrial cavity, in which case water could enter into the perivisceral cavity, as described by Kupffer.

B. Perivisceral Cavity of the Adult.

A detailed account of the anatomy and relations of the perivisceral cavity of Ciona has been given by Roule (1, p. 105), so that it will be unnecessary to give a long description. The perivisceral cavity is separated from the atrial cavity by a septum or diaphragm attached all round to the body-wall externally, and internally to the posterior end of the pharynx on either side of the posterior groove which extends between the end of the endostyle and the mouth of the œsophagus; the atrial or outer aspect of this septum is lined by the atrial epithelium, and internally it is lined by the lining epithelium of the perivisceral cavity. This internal layer extends on to the œsophagus and rectum, which pierce the septum, and forms numerous folds or mesenteries passing to the various organs contained in the cavity; the chief of these mesenteries passes to the pericardium, completely surrounding it, and attaching it to the end of the pharynx underneath the posterior groove, the body-wall, and the stomach. The heart, V-shaped, lies in the pericardium, and the vessels going to and from it lie in the mesentery around the pericardium.

Two points require elucidation:

(i) Do the openings described by Kupffer at the end of the pharynx open into the atrial cavity, as he described, or do they

open into the perivisceral cavity, as supposed by van Beneden and Julin?

(ii) Is there any communication between the atrial cavity and perivisceral cavity, as described by Kupffer, and denied by Roule?

These points I hnve tried to work out by means of sections of small individuals, about 1—2 cm. in length; such individuals have completely undergone metamorphosis and acquired the adult form.

A transverse section of a small Ciona through the posterior end of the pharynx between the end of the endostyle and the œsophageal opening—i. e. through the posterior groove—is drawn in fig. 1, and a portion of the same section, more enlarged, in fig. 2.

The perivisceral cavity (*pv. c.*) is separated from the atrial cavity (*at.*) by the septum (*s.*), in which is seen a blood-vessel on one side. Inside the perivisceral cavity is seen the pericardium (*pc.*), attached by its mesentery (*m*, fig. 2) on the one hand to the base of the posterior groove (*p. g.*), and on the other hand to the stomach (*st.*); at its attachment to the posterior groove is a large vessel (*v.*, fig. 2) running beneath the groove. Inside the pericardium is the heart (*h.*), which is cut twice owing to its peculiar V-shape; each part is seen to be attached to the wall of the pericardium. On each side of the posterior groove of the pharynx is an opening (*o.*), by which the perivisceral cavity communicates with the pharynx. These two openings are situated, one on each side, between the posterior groove and the edge of the septum which elsewhere is attached to the base of the groove; they may be traced only through a few sections (12—15), and the left is distinctly larger than the right.

On working through a series of sections no communications between the perivisceral cavity and the atrial cavity can be seen.

The above section, shown in fig. 1, then, shows that definite communications between the pharynx and perivisceral cavity do exist; and, since these openings occur in the same position

as the orifices described by Kupffer, it is extremely probable that the supposition of van Beneden and Julin is correct, and that the orifices observed by Kupffer open into the perivisceral cavity, and not, as he described, into the atrial cavity.

Since no openings can be found in a whole series of sections between the atrial cavity and the perivisceral cavity, and since Roule expressly denies their possible existence, it appears certain that such communications between the two cavities do not exist.

c. Development in the Larva.

The early stages of development of Ciona are passed through very quickly; the tailed larvæ are formed in the first twenty-four hours of development, and fixation takes place on the second day, the changes at this time being passed through very rapidly, so that the development is very difficult to follow through all its stages.

I have unfortunately not been able to follow out completely the development of the perivisceral cavity in the early stages, but the comparison of the stages observed with the development of the epicardium of Clavellina, as observed by van Beneden and Julin (1), shows the process of development to be very similar in the two forms, and supports the hypothesis put forward by these authors that the perivisceral cavity of Ciona is homologous with the epicardiac tubes of Clavellina.

The process of development in Clavellina is shortly as follows:—Two tubes are first formed as outgrowths of the pharynx, called by van Beneden and Julin the "epicardiac tubes;" these later become fused with one another posteriorly, and the posterior fused portion then becomes separated from the epicardiac tubes to form the pericardium. The dorsal wall of the pericardium invaginates to form the heart, so that the heart is at first completely open along its whole length to the primitive blastocœle of the larva; later the pericardium becomes closed, while the heart remains attached to its dorsal wall by a kind of mesentery, and open only at its ends to the general blastocœlic space, which develops into the vascular system of

the adult. The epicardiac tubes become connected with the process of budding.

Van Beneden and Julin suggest that the perivisceral cavity of Ciona represents the epicardiac tubes of Clavellina, which have become enlarged, growing completely round the viscera and fused together.

The fact shown above that the perivisceral cavity communicates with the posterior end of the pharynx by a pair of openings affords a strong support to this view, which, however, can only be completely accepted if it be confirmed by a study of the embryological development of Ciona.

The first stage of development which I have been able to obtain is that of a larva which has recently become fixed. Figs. 3—5 are transverse sections of a larva at this stage. Fig. 3 is the most anterior, and passes through the lower end of the pharynx (*ph.*) below the opening of the œsophagus; below the pharynx are seen the stomach (*st.*) on one side, and the intestestine (*i.*) on the other, the whole being surrounded by the general blastocœle space (*b.*), containing numerous scattered cells.

A section a little further back (fig. 4) shows the endostyle (*end.*) completely separated from the more dorsal portion of the pharynx, which is now divided completely into two parts (*ep.*) by a septum; in a section still further back (fig. 5) we find these two portions fused together, and much less in size. From their mode of origin from the posterior end of the pharynx, their fusion at their posterior ends, and their similar relations, these two parts are evidently homologous with the epicardiac tubes of Clavellina, so that the early stages of development of Ciona are very similar to those observed in Clavellina.

The first formation of the pericardium from these epicardiac tubes I have not been able to follow; the next stage of which I have been able to get satisfactory preparations is one much later, when the pericardium is fully formed and separated from the epicardiac tubes which have grown round the viscera, and become completely fused dorsally, though still separated ventrally by the pericardium. This stage is one during the

middle of metamorphosis, while the larva is still fixed by a long stalk; it shows the formation of the heart by the invagination of the dorsal wall of the pericardium just as it has been shown to be formed in Clavellina.

Fig. 6 is a transverse section through a larva at this stage, passing through the heart (*h.*), which is seen to be formed by the invagination of the dorsal wall of the pericardium (*pc.*), and to be still open to the general blastocœle space (*b.*), which is now very much reduced, the chief portion of it being confined to a small space round the alimentary canal, into which the heart opens in the neighbourhood of the stomach (*st.*). The whole of the viscera are now surrounded by a large perivisceral space (*ep.*), which may be distinguished from the general blastocœle space by its containing no free cells. This space evidently corresponds to the epicardiac tubes of the preceding stage, now very much enlarged and fused together completely, except at the region of the pericardium between the pharynx and the stomach. A comparison of this stage with the preceding, and with the development of Clavellina as described by van Beneden and Julin, can leave no doubt that the pericardium in Ciona is separated off from the epicardiac tubes just as it is in Clavellina, and also that the perivisceral cavity of Ciona corresponds to the epicardium of Ciona.

The later stages of development, as regards the perivisceral cavity and associated organs, are very simple; the heart becomes completely closed except at each end, where it still opens to the original blastocœle space, which becomes reduced to the blood-vessels; the two halves of the perivisceral cavity approach each other between the pericardium and stomach till, on the closure of the heart and the reduction of the blood-space round the stomach, they only become separated by their thin walls, which unite to form the mesentery attaching the pericardium to the stomach, a similar process also taking place on the ventral side of the pericardium between it and the pharynx.

The derivation of the heart from the dorsal wall of the pericardium is still indicated in the adult, where the heart,

although it has become twisted, still remains attached to the wall of the pericardium along one edge, so that a study of the development of the perivisceral cavity shows it to be very different from the original blastocœle, and corroborates the hypothesis put forward by van Beneden and Julin that it is homologous with the epicardium of Clavellina.

One small point may here be noticed, which, though of little value by itself, yet when taken in conjunction with the other evidence affords another point of similarity between the perivisceral cavity of Ciona and the epicardiac tubes of Clavellina. In Clavellina van Beneden and Julin state that the left epicardiac tube is always larger than the right, and the same fact may be noticed in the openings from the perivisceral cavity into the pharynx in Ciona, the left opening in this case being similarly larger than the right.

D. Conclusions.

Two morphological conclusions may be drawn from the above facts.

(1) The primary condition of the epicardium is undoubtedly that found in Clavellina, where it has the function of a budding organ. The condition in Ciona is that of an organ which has become very much modified while losing its original function as an organ of budding. Since prolongations of the epicardium or perivisceral cavity extend into the stolons of Ciona, this supports the view which is adopted by Herdman (**3**, **4**, p. 139), that the stolons of Ciona are modified budding organs which have lost their original function; the opposite view, that the stolons of Ciona may be regarded as nascent organs, which have not yet acquired their function of budding, is negatived by the above facts, since the perivisceral cavity of Ciona, if it be a modified epicardium as shown above, cannot be regarded as a primitive condition.

(2) This leads us to the second conclusion to be drawn from the above facts. Roule (**1**) looks upon the perivisceral cavity of Ciona as a primitive condition, corresponding to the general blastocœle space which we find in the larvæ, as well as in

Appendicularia. He considers that the further development of the atrial cavity has reduced and obliterated this cavity in the other simple Ascidians. This view is not supported by the above facts, which lead us to look upon the perivisceral cavity of Ciona as a specially modified epicardium which has become greatly enlarged. The perivisceral cavity is certainly not homologous with the general blastocœle space of Appendicularia, and we have no reasons for believing that the other simple Ascidians pass in development through a stage in which the epicardium is modified and enlarged, as in Ciona, to be afterwards reduced; so that we cannot look upon the perivisceral cavity of Ciona as a primitive condition: in this respect Ciona is the most modified of the simple Ascidians.

In conclusion, it is my pleasant duty to express my sincere thanks to Mr. Harmer for his kind help and assistance, to the various members of the staff of the Zoological Station at Naples for the great kindness I received at their hands, and to the Master and Fellows of Christ's College for the grant of a scholarship during my residence at Naples.

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EXPLANATION OF PLATE 8,

Illustrating Mr. A. H. L. Newstead's paper "On the Perivisceral Cavity of Ciona."

Explanation of Figures.

at. Atrial cavity. *b.* Blastocœle. *end.* Endostyle. *ep.* Epicardiac tube. *g.* Generative organ. *h.* Heart. *i.* Intestine. *m.* Mesentery attaching the pericardium to the posterior end of the pharynx. *o.* Openings from the perivisceral cavity into the pharynx. *pc.* Pericardium. *p. g.* Posterior groove of pharynx. *ph.* Pharynx. *pv. c.* Perivisceral cavity. *s.* Septum between the perivisceral cavity and the atrial cavity. *st.* Stomach. *v.* Blood-vessels. *x.* Artificial space (in Fig. 6).

Fig. 1.—Transverse section of a small adult individual of Ciona through the posterior end of the pharynx (1 in. obj.).

Fig. 2.—Portion of the same section further magnified (¼ in. obj.).

Figs. 3—5.—Three transverse sections of a recently fixed larva, Fig. 3 being the most anterior (¼ in. obj.).

Fig. 6.—Transverse section of a larva at a more advanced stage of metamorphosis, showing the formation of the heart (¼ in. obj).

The Early Stages in the Development of Distichopora violacea, with a Short Essay on the Fragmentation of the Nucleus.

By

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With Plate 9.

The material upon which I have made my investigations was in part collected by me in N. Celebes, and in part by Professor A. C. Haddon in Torres Straits. Some of the specimens were treated with strong alcohol alone, others with corrosive sublimate followed by alcohol. For decalcification I have entirely used nitric acid.

I have tried a great many different stains and combinations of stains. Borax carmine, Biondi's fluid, methyl green, and hæmatoxylin all give fairly good results; but I find that the best treatment is to place the sections, when fastened to the slide, in a strong solution of eosin in 90 per cent. spirit for an hour, then to wash in 90 per cent. spirit and stain in weak hæmatoxylin for twenty minutes. This treatment gives a beautiful double stain which shows the nuclei and the chromatin granules better than I have seen them in any preparations treated with carmine.

My researches were entirely carried on in the morphological laboratory at Cambridge.

I. The Early Stages in the Development of Distichopora.

The ovum of Distichopora, like that of Allopora and other Stylasterids, is provided with a large amount of yolk, and lies in a cup-shaped trophodisc.

In young immature ova the germinal vesicle is situated in the middle of the egg, is spherical in shape, is provided with a well-defined membrana limitans, a germinal spot, and a fine network of protoplasmic fibrils with thickened nodes (Pl. 9, fig. 1).

When examined with a high power the germinal spot may be seen to contain a few clear vacuoles (fig. 2).

In some ova with a full complement of yolk-spheres the germinal vesicle is irregular in shape, and provided with processes resembling the pseudopodia of amœba. The outlines of these processes are usually difficult to observe, the membrana limitans being apparently wanting, and the intra-nuclear and extra-nuclear protoplasm perfectly continuous (fig. 3).

In these cases there may be seen a few large rod-shaped granules (the chromosomes), which stain deeply with carmine and other stains.

These amœboid germinal vesicles are without doubt passing from the centre of the ovum towards the periphery. In those that are near the periphery the chromosomes are more numerous and very much smaller than they are in those nearer the centre of the ovum. In one case I have observed these bodies arranged in a row parallel to the surface of the ovum, and dividing the nucleus into two unequal halves (fig. 5).

When and in what manner the polar bodies are formed I cannot say, but it is probable that in some cases the nuclei of the polar bodies are formed before the germinal vesicle reaches the periphery, and are absorbed in the substance of the ovum. The germinal vesicle finally reaches the periphery of the ovum, and when it is in that position the fertilisation most probably occurs.

It is clear that the germinal vesicle must remain at the

periphery for a very considerable time, for of the numerous unfertilised ova that I have examined a large majority have their germinal vesicles in that position.

In the next stage the membrana limitans of the inner half of the vesicle disappears, the network and the germinal spot break down into numerous very minute scattered granules (fig. 7).

Then the membrana limitans entirely disappears, and lastly, the substance of the vesicle, or, as it should now be called, the oosperm nucleus, becomes scattered through the substance of the ovum.

Fig. 8 is a careful drawing of a stage in which the membrana limitans has just disappeared, and I have three or four complete series of sections through ova in which no trace of nuclear structure can be found nor any area, such as that shown in this figure, which represents the vanished nucleus.

As these two stages are of the greatest importance in the consideration of what follows, it is necessary to say that notwithstanding very careful search with high powers, no trace of karyokinetic figures could be observed.

The ova of these stages are not sufficiently numerous, nor are the methods of preservation sufficiently perfect to enable one to assert that such figures do not occur. Corrosive sublimate followed by alcohol, although giving excellent general histological results, does not always bring out the full details of nuclear division; and it will be necessary to confirm these purely negative results as regards karyokinesis by observations made upon specimens preserved in Flemming's solution and other reagents before any general statements regarding fragmentation of the oosperm nucleus of Distichopora can be accepted.

Nevertheless it is my belief that we have here an instance of nuclear fragmentation, for reasons which I propose to discuss in the third section of this paper.

In the next stage that I have observed, a few small islands of protoplasm may be seen in the yolk (fig. 9), and the examination of broken sections, in which part of the yolk has

been washed away, shows that these islands are connected together by a very coarse mesh-work of fine protoplasmic strands.

In a later stage the islands are seen to be more numerous, and the protoplasmic mesh-work somewhat finer. A complete nucleus may be seen in some of these islands, but in others all that can be made out are a few deeply staining granules (figs. 10 and 12).

In a later stage the nuclei have increased in number in the midst of the yolk, and a few make their appearance in the protoplasmic sheath that surrounds the ovum.

In these last three stages I have described a process which can only be compared with the so-called free nuclear forma tion in early insect embryos. Nuclei make their appearance in places which were previously apparently devoid of any nucleus or nuclear structure. Moreover nuclei of various sizes and shapes may be seen in the embryo at the same time.

It is not reasonable, however, to assume on the insufficient evidence before us that "free nuclear formation" does actually occur. It seems to me to be much more probable that minute fragments of nuclear substance scattered through the protoplasmic mesh-work collect together in places, and form by their fusion true recognisable nuclei. In other words, the process we have under observation is rather one of "nuclear regeneration" than one of "free nuclear formation."

I have often noticed in ova of these stages an aggregation of the yolk into spherical, polygonal, or irregular lumps, suggesting that the egg has undergone some form of complete segmentation (fig. 13). This is not a true process of segmentation, however, since the distribution of the nuclei in the spaces between the aggregations and not in their centres shows that it affects the yolk only. It is remarkably similar in appearance to the so-called yolk segmentation of Arthropods, the appearance of the embryo at this stage being very much like that of such a form as Peripatus novæ-zealandiæ, as described by Miss Sheldon (53). This segmentation of the yolk seems to be only temporary, for in embryos in which the

ectoderm has commenced to be differentiated it cannot be observed. In later stages of the development the ectoderm is gradually formed. Nuclei appear in the peripheral sheath of protoplasm, and the protoplasm accumulates in the form of cellular blocks around each nucleus, as in Allopora.

I have carefully examined the endoderm in these stages in the hope of finding out the manner in which the nuclei divide, and although I have found a few dumb-bell-shaped forms, and no satisfactory evidence of karyokinesis, I do not feel justified in asserting that the nuclei always divide amitotically.

As far as the ectoderm is concerned, I can assert most positively that indirect nuclear division does occur.

Numerous dumb-bell-shaped nuclei and nuclei connected together in pairs may be seen in the developing ectoderm, and in these faint achromatic lines may be seen connecting the chromatin rodlets. The nuclei are too small to enable me to make out all the details of the process, but there can be no doubt that there is a true process of karyokinesis in the divisions of these nuclei (fig. 18, *a*, *b*, *c*, *d*, and *e*). I have not been able to decipher anything like the "spheres of attraction."

One very remarkable and important point in the development of all the Hydrocorallinæ, so far as they have at present been investigated, is the fact that there is no segmentation of the ovum, either complete or partial, nor is there any formation of cells with a definite outline until a very late stage.

At the time when (in Allopora and Distichopora) there are ten or fifteen nuclei, the young embryo is a simple multinucleated plasmodium, loaded with yolk. In the later stages the nuclei have increased in numbers, and a certain number of them are arranged in a row at the periphery of the embryo.

The yolk in the immediate neighbourhood of these peripheral nuclei disappears, probably by absorption, and thus they are situated in a clear peripheral sheath or envelope of protoplasm. In a later stage this peripheral sheath of nuclei breaks up into blocks, each block containing one nucleus, and thus the ectoderm is formed.

The ectoderm is, then, a differentiation of the periphery of a multinucleated plasmodium.

What becomes of the inner part of the plasmodium?

We have no answer to this question so far as the Hydrocorallines are concerned; but, judging from the other Cœlenterates, there can be little doubt, I think, that it becomes the endoderm.

In the development of Aglaophenia (Tichomiroff, 55) we find a stage that is almost precisely similar to the solid planula of Distichopora and Allopora, and in a later stage this central yolk-laden plasmodium breaks up into blocks, which become the endoderm-cells of the adult.

The difficulty that we have now to face is, how can these facts concerning the origin of the germ layers be brought into line with those of other Cœlenterates?

We find in the Stylasteridæ no segmentation, no process of invagination to form the endoderm, and no process that can be compared with ordinary primary delamination; but still it is probable that this method of the formation of the germ layers, if it is not itself the primitive one, has been derived from those of other Cœlenterates, and I shall endeavour to show in the next section how the transition has taken place.

II. On the Formation of the Germinal Layers in the Cœlenterata.

During the last ten years our knowledge of the early stages of the development of the Cœlenterata has very considerably widened, but still we seem to be no nearer to the solution of many interesting phylogenetic questions than we were before. The various theories that have been put forward, based upon the study of a few forms, have in no instance received the unqualified approval of the principal authorities on the group, and we find ourselves in a maze of conflicting theories, none of which seem to conform entirely to our knowledge of facts.

This unfortunate state of affairs is due to the fact that in the group of the Cœlenterata we find many very different types of

development, and no one of them seems to be particularly predominant.

The development of a gastrula by invagination probably occurs only in the group of Scyphomedusæ.

The formation of a planula by delamination (i. e. the primary delamination of Metschnikoff) occurs only in the group of the Geryonidæ.

The formation of a sterrula by secondary delamination occurs in most of the Anthozoa (McMurrich) and in many of the Hydroids.

The formation of a sterrula by hypotropic invagination occurs in many Sertularidæ and Campanularidæ.

The formation of a planula or sterrula by polypolar immigration of cells into a hollow blastula occurs in a few forms.

Lastly, the formation of a multinucleated plasmodium without segmentation, which is followed by the differentiation of epiblast-cells at the periphery of a solid plasmodium (the endoderm), occurs in the Hydrocorallinæ and in some Alcyonarians.

Between these various types of development many intermediate forms have been found, so that we have as it were a complete series of developmental histories, with the typical invaginate gastrula at one end and the multinucleated plasmodium at the other.

We may represent this series by the following plan :

A. Gastrula formed by invagination. Large segmentation cavity.

Examples: Cotylorhiza (Claus, **8**), Pelagia noctiluca, and Nausithoë (Metschnikoff, **42**).

a. Intermediate forms between type *A* and *B* are found in Aurelia flavidula (Smith, **52**), in which the clump of cells that are invaginated is at first solid, and in Cyanæa capillata (McMurrich, **41**), in which this clump of cells remains solid longer than in A. flavidula.

B. A solid planula (sterrula) formed by hypotropous immigration of cells into a large segmentation cavity.

Examples: Clytia, Tiara, Rathkea, Obelia, Tima, Æquorea (Metschnikoff, 42), and Cyanæa arctica (McMurrich, 41).

b. Intermediate forms, in which the migration takes place mainly at the hind end, occur in Mitrocoma (Metschnikoff, 42).

C. A sterrula is formed by polypolar immigration of cells into a large segmentation cavity, these cells being formed by the radial fission of the cells of the cœloblastula.

Example: Æginopsis (Metschnikoff, 42).

c. Intermediate form, in which the cells that immigrate are formed partly by radial and partly by tangential division.

Example: Hydra (Brauer, 5).

D. A planula is formed by primary delamination, the endoderm-cells formed by tangential division only. The segmentation cavity is large.

Example: Geryonia (Metschnikoff, 42).

d. Numerous intermediate forms in which the segmentation cavity is small.

Examples: Tubularia (Brauer, 5a), Bougainvillea (Gerd, 14).

E. A sterrula is formed by precocious delamination (secondary delamination of Metschnikoff). No segmentation cavity formed.

Examples: Aglaura (Metschnikoff, 42), Rhopalonema (Metschnikoff), Eudendrium and Sertularella (Tichomiroff, 55).

e. Intermediate forms in which the segmentation is at first incomplete.

Examples: Renilla (Wilson, 63), Gorgonia (von Koch, 36), and probably other Alcyonarians.

F. A multinucleated plasmodium is formed. There is no segmentation and no segmentation cavity.

Examples: Algaphenia (Tichomiroff, 55), Mille-

pora (Hickson, **17**), and the Stylasteridæ (Hickson, **18** and **19**).

It is not my purpose to discuss fully the various views that have been put forward concerning the origin of the Metazoa from the Protozoa. The gastrula theory, the planula theory, the plakula theory, and the phagocytella theory have each received in their turn the consideration of naturalists, and nothing would be gained were an attempt made in these pages to reopen the discussions that they gave rise to.

But I cannot pass on without expressing my opinion that the developmental history of the Hydrocorallinæ lends some support to the so-called "plasmodium" theory. Many years ago, Jehring (**27**) and Saville Kent (**32**) put forward the view that the Metazoa are derived from a multinucleated Protozoan like Opalina. Sedgwick (**57**) has supported this view, as a result of his important work on the development of Peripatus, and considers that the ancestral Metazoan was probably of "the nature of a multinucleated Infusorian, with a mouth leading into a central vacuolated mass of protoplasm."

In discussing Saville Kent's views Metschnikoff (**42**) says that there is no evidence of the formation of such a multinucleated cell in the lowest Metazoa.

Now I have already pointed out that in the earliest stages of the Stylasteridæ and of Millepora the embryo is nothing more nor less than a multinucleated cell; that is to say, it is a single undivided mass of protoplasm, containing numerous nuclei. It might be urged that it is a syncytium, a number of cells fused together; but there is no more evidence for such a view than for the view that it is a single multinucleated cell.

Similarly it may be urged that Tubularia (Brauer, **5 a**), Aglaophenia (Tichomiroff, **55**), Alcyonium (Kowalewsky, **37**), Gorgonia (von Koch, **36**), and Renilla (Wilson, **63**) all pass through a stage in their development in which the embryo is simply a multinucleated cell.

The fact that such a condition as this occurs in many different groups of the animal kingdom widely separated from

one other also lends support to the view that it may have some important phylogenetic significance.

Instances of the occurrence of an unsegmented multinucleated plasmodium are found not only in the Cœlenterata above mentioned, but in Peripatus, Myriapods, Spiders (Kishinouye, 34, and Morin, 44), Insects, Crustacea, Elasmobranchs, and probably many other forms with large eggs.

It might be urged as an argument against the plasmodium theory that the multinucleated plasmodium occurs principally in the development of those forms whose ova contain a large amount of food-yolk, that the segmentation is modified by the presence of this yolk, and that consequently the phylogeny is obscured.

But it does seem to me that in the ovum that is perfectly clear and homogeneous we have a cell that is any nearer to the ancestral Protozoan than the ovum that contains a moderate amount of yolk.

It is almost certain that the ancestral Protozoan normally contained some food-vacuoles, and it is quite as probable as not that it had some contractile or simple water-vacuoles for floatation purposes as well.

It is quite as reasonable to suppose that the Metazoa are derived from an Actinosphærium-like ancestor with vacuoles in the outer regions as well as in the inner mass, as it is to derive the Metazoa from a "multinucleated Infusorian with a mouth leading into a central vacuolated mass of protoplasm."

If this is the case, then we can no longer consider the yolk-bearing eggs to be secondarily modified, and the small transparent eggs to be the primitive types from which all the others are derived; but we may expect to find in the development of eggs with a moderate amount of yolk just as much or even more evidence of ancestral history as in eggs that are practically yolkless.

It must not be forgotten, moreover, that the occurrence of a multinucleated plasmodium is not confined to those cases in which the ovum contains a large amount of yolk.

In the ovum of Millepora there is no yolk, and yet the

oosperm nucleus fragments without any segmentation occurring, giving rise to a simple multinucleated plasmodium.

The eggs of Aphis (Will, 62) and some other insects contain very little yolk, and do not segment until a large number of nuclei are formed.

The segmentation of the ovum, then, and the subsequent formation of a morula mass of cells, are phenomena not entirely dependent upon the absence of yolk. Many, comparatively speaking, large eggs, such as that of Rana, segment, whilst others, such as that of Alcyonium, do not.

We cannot, consequently, assert that when an ovum segments it is simply repeating an ancestral phase, and that when it does not segment it is prevented from doing so by the physical obstruction of the yolk.

The reverse of this is more probably true. The recent brilliant researches of Driesch (9) prove that the segmentation of the ovum is due to physical or mechanical laws, and we cannot or should not derive any phylogenetic conclusion from the phenomena of segmentation.

We may even go further than this, and say that the developing ovum would not segment, but would naturally pass through the stage of a multinucleated plasmodium, were it not for the action of certain purely mechanical forces, with which we are not at present fully acquainted. When these forces cannot act upon the egg, or are in some way counteracted, the ovum does not segment, whether it is laden with yolk (Stylasteridæ, many Insects, Elasmobranchs, &c.) or not (Millepora).

III.—On the Fragmentation of the Oosperm Nucleus.

It is the belief of many eminent histologists that any process of division of the nucleus other than that by karyokinesis or mitotis is a sign of the degeneration of the nucleus, and the approaching end of the life of the cells.

Flemming says, "Fragmentation of the nucleus, with and without subsequent division of the cell, is universally a

process in the tissues of Vertebrates which does not lead to the physiological multiplication and reproduction of cells, but, on the contrary, represents where it occurs a degeneration or aberration, or perhaps, in many cases, is subservient to the metabolism of the cell by increasing the periphery of the nucleus."

Ziegler (**65**), who quotes the above passage from Flemming's work, discusses in detail some of the many instances of amitotic nuclear division, and comes to similar conclusions. He says that amitotic division of the nucleus always indicates the end of the series of divisions, and considers it hardly probable that nuclei which have arisen by amitotic division will ever again divide by mitosis.

If Flemming, Ziegler, and those who agree with them are right, then it is clear that the oosperm nucleus does not and cannot fragment. It must divide regularly by karyokinesis. But Ziegler's views are, it seems to me, altogether untenable. By simply denying, or passing over in silence, many instances of fragmentation of the nucleus, which do not support his views, he has given undue weight to mitosis, and leaves an unsatisfactory gap in the list of cases which support his theory.

Verson (**56**), Frenzel (**12**), and Löwit (**40**) have, since the publication of Ziegler's paper, called attention to cases of amitotic division of the nucleus which are most certainly not followed either by nuclear degeneration or by a cessation of cell multiplication.

A review of the recent literature of cell division shows that the cases given by these authors may be supplemented by many others, and, indeed, leads one to a conclusion quite different from that of Ziegler and Flemming, namely, that indirect nuclear division rarely occurs unless it is preceded by or accompanied by some partial or complete segmentation or division of the surrounding cell substance.

It is undoubtedly true that in many cases amitotic fragmentation of the nucleus is followed by its degeneration and the death of the cell. The numerous examples quoted by

Ziegler prove that this is the case. But I shall endeavour to show that we are by no means justified in assuming that amitotic fragmentation is a sign of degeneration.

In the first place, it can be shown that there is considerable evidence for believing that the oosperm nucleus of some ova does not divide by normal karyokinesis, but does split up amitotically into a large number of minute fragments.

I have already described (**17** and **18**) such a process of fragmentation in the case of Millepora, Allopora, and Distichopora, but the following considerations prove that the same is probably true of many other eggs.

Cœlenterata.—In the development of Alcyonium the germinal vesicle entirely disappears, and no traces of the karyokinetic division of the oosperm nucleus can be found. Kowalewsky[1] (**37**) gives a figure of the ovum without any nucleus, but my own observations show that at a stage corresponding to the one he figures the nucleus is in the form of a number of minute fragments scattered through the substance of the ovum.

The failure to find karyokinetic division of the oosperm nucleus cannot be attributed to imperfect methods of preservation or staining, because young embryos, preserved and stained in precisely the same way as the fertilised ova, exhibit beautiful and typical karyokinetic figures.

The early stages in the development of Gorgonia cævolini, described by G. von Koch (**36**), seem to be precisely similar to those of Alcyonium. In the unfertilised ovum there is a large germinal vesicle containing an excentrically placed germinal spot, but in the eggs that he believed to be fertilised there was no nucleus. "Ihre Structur weicht von der des unbefruchteten Eies wesentlich ab. Es fehlt nämlich vor allem der Kern, von dem ich keine Spur mehr auffinden konnte." The fact that von Koch, after carefully examining over a hundred series of sections through fertilised ova, could find neither traces of segmentation nor the division of the oosperm nucleus, suggests

[1] As Kowalewsky's paper is written in the Russian language I am unable to read it.

very forcibly that the ovum of Gorgonia does not segment at first, and that the oosperm nucleus fragments as it does in Alcyonium.

Arthropoda.—In the development of Peripatus capensis, Sedgwick (51) has described the division of the ovum into two blastomeres, and the large and easily seen karyokinetic figures which mark the first division of the oosperm nucleus. The fertilised ovum of Peripatus novæ-zealandiæ, however, does not segment, and Miss Sheldon (53) was unable to find any karyokinetic figures in the divisions of its nucleus.

It is a very striking fact in support of my views that in two species of the same genus we should find such a well-marked difference in this respect, the ovum that does segment showing clear and unmistakable nuclear mitosis, and the ovum that does not segment showing no signs of karyokinesis.

But this is not the only example of the relation between the segmentation and the division of the nucleus.

In a recent paper on the "Embryology of the Macroura" Herrick (6) states that it is a rule with the decapod Crustacea that the nuclei of the segmenting eggs divide with karyokinesis. There is an exception to this rule, however, in the case of Alpheus minus. "The fertile egg of A. minus is pervaded with a remarkably fine reticulum which encloses spherules of minute and uniform size. The nucleus is central or nearly so, and consists of an ill-defined mass of protoplasm, in which a fine chromatin network is suspended. In the next phase the nucleus is elongated and about to divide. Division appears to be direct and irregular. At a somewhat later stage the phenomena of the most interest occur. Each product of the first nucleus has developed a swarm of nuclear bodies which seem to arise by fragmentation. These bodies take the form of spherical nuclei in clear masses of protoplasm. . . . In the last stage obtained the whole egg is filled with several hundred very large elements, which are descended more or less directly from some of the nuclear

bodies just considered, but the intermediate stages have not been considered."

In the species Alpheus Saulcyi and Alpheus heterochelis (two varieties) the segmentation is normal and regular, of the centrolecithal type, and the division of the nuclei indirect. In Alpheus minus alone is the segmentation extremely irregular and the nuclear division direct.

Among Myriapoda we find that the ovum of Julus terrestris is very similar in many respects to that of the Stylasteridæ. There are no signs of segmentation, and there is no formation of cells until the time when the epiblast is formed. Heathcote (20), who carefully studied the early stages in the development of this species, could not find any signs of karyokinesis in the first divisions of the oosperm nucleus.

There is, according to Kingsley (33), a disappearance of the germinal vesicle of the American Limulus, and it is a suggestive fact that Kishinouye (35), in his careful paper on the development of Limulus longispina, does not refer to the first nuclear divisions.

It is possible that there may be a fragmentation of the oosperm nucleus in the ova of some other Arachnida.

In the development of many Insecta there are many facts that point to the conclusion that the oosperm nucleus fragments.

It is noteworthy in the first place that, notwithstanding the fact that several excellent embryologists have carefully studied the development of the common blow-fly, not one of them has been able to give a satisfactory account of the first division of the oosperm nucleus.

Blochman (3), who figures the spindles of the nuclear divisions in the formation of the polar bodies, and also the spindles of the nuclear divisions of the later stages of embryonic development, did not apparently observe the first division of the oosperm nucleus. He says, "Als erste Theilung des Eikernes kann man die Bilder wohl nicht auffassen, weil, wie ein Blick auf die späteren Figuren zeigt, bei Theilungen die

Tochter kernplatten stets so fort weit aus einander rücken." Henking (21), too, was unable to find the first division of the oosperm nucleus of Musca.

Now, in Musca, and in many other insects in which the early divisions of the oosperm nucleus have not been made out, the occurrence "of free nuclear formation" has been described in the young embryo. Whence come these free nuclei? It can hardly be believed that they are actually formed in the cell substance from something that is not directly derived from a pre-existing nucleus. All the evidence of modern histology tends to prove that nuclei are derived from nuclei, and nuclei only, and it is only reasonable to suppose that the so-called "free nuclei" of insect embryos are formed by the growth or fusion of fragments of the oosperm nucleus.

The evidence in support of this hypothesis is not the purely negative evidence of the absence of any direct proof of mitotic division of the first nuclei, but the fusion of minute chromatin bodies to form larger ones has actually been observed by Henking (23) in the embryos of Pieris, Pyrrochoris, and Lasius.

But it is extremely probable that fragmentation of the oosperm nucleus is of very frequent occurrence in the eggs of insects. In many cases, both in large yolk-laden eggs and in small yolk-free eggs, the fertilisation is followed by the appearance of numerous nuclei in the substance of the egg.

In Neophalax concinnus, one of the Phryganids, the division of the oosperm nucleus was not observed by Patten (46), and the following is his account of the early stages:— "Within ten or twelve hours after oviposition—the time varying with the temperature—a clear space makes its appearance at the surface of the egg, and gradually increases until it has attained the breadth of the future blastoderm. In this layer, which has been called the 'blastema,' the protoplasm has, under ordinary conditions, a very homogeneous appearance, with occasionally lighter, less refractive spots, which appear like vacuoles, but in which, when observed more closely and under slight pressure of a cover-glass, or especially when treated with a very little acetic acid, faintly marked

nuclei make their appearance in greater or less numbers according to the more or less advanced stage of the blastema." It is extremely improbable, if the minute nuclei in the blastema could be observed by the simple method of treatment with acetic acid, that the karyokinetic divisions of the large oosperm nucleus, if they really occur, would have been overlooked.

Many other instances could be given from the writings of naturalists during the last twenty years of the failure to trace the divisions of the oosperm nucleus in insect eggs, and of the occurrence of "free nuclear formation" in the eggs after fertilisation; but in many of these instances it might be urged that sufficient patience was not exercised, or that the methods of preservation and staining were imperfect.

An important paper has, however, been recently published by Henking (23) containing an extremely elaborate account of his investigations upon many different species of insects carried on with the aid of the best modern methods of research. It would take me far beyond the limits of this paper to give even an outline sketch of Henking's important results, but a brief reference to some of the points bearing upon the subject of this essay must be made.

In Pyrrochoris, one of the Hemiptera, Henking finds that in the formation of the polar bodies the nucleus divides by a process of karyokinesis, the chromatin bodies being of considerable size and definite in number.

After fertilisation a new spindle is formed with the chromosomes arranged in an equatorial plate, but before the division is completed the chromosomes disappear. Later on the chromosomes reappear in the form of extremely minute and numerous granules, which fuse together into threads, and arrange themselves in the equatorial plate of a new spindle.

Similarly, in Agelastica alni, a Coleopteran, the chromatin entirely disappears after the division of the segmentation nucleus.

In the Hymenopteran Lasius the chromatin of the first two segmentation nuclei completely disappears, and when the

nuclei are about to divide again reappears in the form of extremely minute granules, which fuse together to form the chromosomes of the next division.

A similar disappearance has been described in the unfertilised egg of Rhodites, and in this form there is no membrane surrounding the nuclei.

These researches prove, then, that in some insects there is a "disappearance" of the chromatin substance of the nucleus after its first division.

To what is this disappearance due? Henking thinks that it is due to some chemical change in the chromatin substance, as in some cases the outline of the chromosomes may be observed after the disappearance of the colouring matter. Nevertheless it is a fact that commonly the chromosomes lose their compact form during the colourless stage, and become very finely divided. We can attribute the disappearance, then, partly to the change in the chemical character of the chromatin, and partly to the very minute and scattered condition of its elements.

Further, in some cases (Rhodites) not only does the chromatin disappear, but also the membrane surrounding the nuclear area, so that we have (as in Distichopora, &c.) a condition in which the nucleus is practically indistinguishable from the surrounding protoplasm.

It is during this condition that some of the nuclear fragments may be distributed through the substance of the ovum, and give use to the nuclei of the so-called "free nuclear formation" by subsequent fusion.

It must be obvious to anyone who carefully studies Henking's figures that in many insects the spindle of the first division of the oosperm nucleus is very irregular, that the chromosomes are not always arranged with the same mathematical precision that they are in typical karyokinetic figures, and further, that in consequence of the disappearance and extremely fine division of the chromatin substances there are still some steps in the nuclear divisions at the commencement of development which have not been satisfactorily traced.

We may go further than this, though, and say that some of Henking's figures, such as figs. 335, 336, and 337 of Lasius, can only be interpreted on the supposition that the nucleus has fragmented. The little clusters of chromatin granules, of very irregular size and indefinite arrangement, that are here figured scattered through the substance of the ovum, cannot be considered to be the product of regular mitosis.

It seems to be extremely probable that in the group of insects we have a series of stages intermediate in condition between regular mitotic division of the oosperm nucleus or its immediate successors and irregular fragmentation.

In Aphis (Will, 62) we may have regular karyokinesis at all stages of the segmentation, the chromosomes being divided into two equal halves at each division of the nucleus; but in Musca, in Lasius, and perhaps in several others in which the earliest stages are passed through with great rapidity, the nuclei fragment with greater or less irregularity.

That the occurrence of karyokinesis is in some way dependent upon forces manifesting themselves in the cell substance of the ovum and acting upon the nuclei is rendered probable (1) by the fact that in Aphis, where the nuclei divide by karyokinesis in all stages, there is, as Will points out, a distinct aggregation of protoplasm round the nuclei, and (2) by the fact that in nearly all insects the karyokinetic figures of the nuclear divisions that take place in the formation of the polar bodies are much more regular and constant than they are in the early stages of development.

But I shall discuss this point and general significance of mitosis in greater detail later on.

That a similar process of fragmentation of the oosperm nucleus may also occur in some Vertebrata seems to be probable from the recent researches of Kastschenko (31) upon Elasmobranchs. It must be remembered that the early stages of the development of Elasmobranchs and birds have been carefully studied by numerous observers for the last twenty years, and although the karyokinetic spindles in the developing blastoderm and its surrounding yolk have been described by nearly

all of them, we have not received any account of the first division of the oosperm nucleus.

It is quite unreasonable to suppose that all these observers would have overlooked a nuclear division—which we might expect, if it exists at all, to be the largest and most conspicuous of the whole series. Nor can we suppose that the methods of preservation or staining was so consistently bad at the first stage as to prevent the observation of the figure, and so frequently good in the later stages as to show the whole process of karyokinesis clearly and distinctly.

Now Kastschenko (31) shows that in Elasmobranchs a number of nuclei appear in the blastoderm and the surrounding yolk before the formation of the segmentation furrows, which appear not in regular sequence, but simultaneously and irregularly. "Die bekannte regelmässige Reihenfolge des Erscheinens des Segmentationsfurchen existiert bei Selachiern fast gar nicht. Nur in seltenen Fällen bemerkt man das ursprüngliche Erscheinen einer Segmentationsfurche, welcher dann gleichzeitig mehrere andere unregelmässig sich kreuzende folgen. In den meisten Fällen aber erscheinen schon vom Anfang an mehrere Segmentationsfurchen gleichzeitig und somit zerfällt die Keimscheibe direct in mehrere verschieden grosse Segmentationskugeln, welche sich dann weiter aber nicht gleichzeitig theilen."

We have, then, at the commencement of the development of the Elasmobranch a multinucleated plasmodium, and Kastschenko is of opinion that all the nuclei of this plasmodium are formed by repeated divisions of the first segmentation nucleus. But, like all his predecessors, Kastschenko was apparently unable to observe these repeated divisions of the first nucleus, and it seems extremely probable that in Elasmobranchs, as in insects, Hydrocorallines, and others, we have at this stage a true process of nuclear fragmentation.

I have already called attention to the fact that in all of these cases in which the fragmentation of the oosperm nucleus probably occurs the ovum does not segment immediately after fertilisation; that there is, in fact, for a time in the early em-

bryonic development a multinucleated plasmodium without any definite cell walls or cell areas.

There can be little doubt, I think, that in all holoblastic eggs, such as those of Echinoderms, worms, Amphioxus, &c., the first segmentation is accompanied by typical karyokinetic division of the nucleus.

We may go further than this, and say that in many meroblastic eggs the first divison of the oosperm nucleus is also an indirect one. Vialleton (57) and Watase (59) have observed this division in the egg of Cephalopods, and Oppel (45) has observed it in the egg of the lizard, Anguis fragilis. But in both these cases the segmentation furrows occur regularly and in sequence from the commencement of development, and we have, consequently, evidence that the same forces are at work in the protoplasm as those which produce the more or less complete blastomeres of holoblastic eggs. Even in those eggs of insects in which the nuclei are known to divide by karyokinesis there is evidence of the drawing together of the protoplasm along certain lines of force in the "plasmatische Strahlungen" of Henking, which surround the nuclei.

But if there is any truth in the view that I have here put forward, that karyokinesis is primarily due to the forces which bring about cell division, and that in those cases in which cells or cell areas are not formed the nucleus may fragment or divide directly in some other way, then we should expect to find some further evidence of fragmentation of the nucleus in other tissues. There is ample evidence of this in other tissues.

In the formation of the spores in Protozoa the nucleus of the parent cell often divides long before there is any division of the cell protoplasm, and in nearly all such cases division of the nucleus is direct. In some cases the nucleus disappears, and it is probable, as in the case of the oosperm nucleus quoted above, that this may be due to the extremely fine division of the chromosomes and fragmentation.

I will give just a few examples to illustrate these points.

Wolters (64), in describing the conjugation of Monocystis magna and agilis, says, "Kurz nach erfolgter Encystirung

soll der Kern, respective die Kerne der beiden Copulanten sehr undeutlich werden. Sie entziehen sich zuletzt dem beobachtenden Auge ganz und sind im Inhalte der ausgequetschten Cyste nicht mehr zu finden." The author figures, it is true, an achromatic spindle in the encysted forms after the extrusion of the polar bodies, but the chromatin bodies are very minuteand irregularly scattered through the substance of the protoplasm. "Es gelangzwar nicht," he says, "eine zusammenhängende Reihe von Bildern für die Constatirung der mitotischen Theilung an den Sporogonien zusammen zustellen, doch liess sich mit Sicherheit constatiren, dass die Kernmembran an manchen Kernen der ungetheilten Sporogonie geschwunden war und die färbbare Substanz in zwei, durch einen grösseren Zwischenraum getrennte Reihen angeordnet war."

But the evidence in favour of a process of fragmentation of the nucleus seems to be much more conclusive in the case of Clepsidrina blattarum (Wolters, l. c.). In this form nuclei are found "in denen unzählige kleine chromatische Körner lagen, wie es schien regellos, ohne besondere Anordnung vertheilt. Allen bisheran geschilderten Kernformen war dagegen eine scharf contourirte Kermembran gemeinsam. Im Gegensatz da zu stehen Formen, die ebenfalls häufig beobachtet wurden, welche einer solchen Membran entbehrten. Der Kern breitet sich sternförmig mit seinen Fortsätzen in die Leibessubstanz des Thieres aus und steht mit dem protoplasmatischen Gefüge derselben in directen unterbrochenen Zusammenhange."

This account of the fragmentation of the nucleus of Clepsidrina blattarum is confirmed in all its essential details by the more recent work of Marshall (40 a), who was unable to find at any time any traces of karyokinesis. A very similar account is given by Schneider (49) of the division of the nucleus of Klossia.

It may be that in some forms, such as the Gregarina irregularis of Holothuria nigra (Minchin, 43), a regular form of division with mitosis does occur, but this does not detract from the importance of the fact that in many

Gregarines which form during encystment a vast number of spores, no karyokinetic figures can be observed.

Many years ago Hertwig (24) described a curious method of the fragmentation of the nucleus without karyokinesis in the spore formation of Thalassicola, and more recently Brandt (4) was unable to find karyokinesis in the divisions of the nucleus to form the nuclei of the spores of the Sphærozooids.

Gruber (16) has described several instances among the ciliate Infusoria in which the nucleus apparently fragments into extremely minute granules, which become scattered through the protoplasm of the body and collect again into lumps.

Jickeli (29) has described fragmentation of the nucleus of Stylonychia, Paramœcium, and other Ciliata.

Quite recently, too, Lister (39), in his researches upon Orbitolites, has not been able to discover any signs of karyokinesis in the division of the nuclei.

There is probably, too, a method of fragmentation in the spermatogenesis of many animals. I have myself carefully examined the earliest divisions of the nucleus of the sperm mother-cell of Millepora, Alloopora, Distichopora, and Alcyonium, and I can find no trace of karyokinesis. It is, in fact, only in a few exceptional cases, such as Ascaris (Hertwig, 25), where the cell outlines of the spermatocytes are very early delineated, that karyokinesis has been observed in the division of the nuclei of the sperm mother-cells.

Verson (56) shows that in Bombyx mori the primordial cells have at first a giant nucleus, which divides amitotically to form numerous secondary nuclei, and these divide mitotically to form the nuclei of the Spermatids.

Bolles Lee (38) found amitotic division of the nuclei of the spermatogonia of Chætognatha and Nemertines and regular karyokinesis in the division of the nuclei of the spermatocytes.

Dostojewski found the same thing in the spermatogenesis of Amphibia (see Waldeyer, 58, p. 39), and other examples could be quoted from the writings of La Valette St. George, Gilson, Sabatier, and others (see Waldeyer, 58, p. 39).

In some Annelid worms the nucleus of the spermatogonium disappears, and there is no evidence at present that the nuclei of the spermatocytes are derived by repeated mitotic divisions of this nucleus (Jensen, **28**, and others). In the recent work on spermatogenesis, by Pictet (**47**) no mention is made of the manner in which the nuclei of the spermatogonia divide in Polychætes.[1]

A study of the literature of spermatogenesis shows that when there is a distinct division of the protoplasm to form the spermatocytes or spermatogonia, distinct karyokinesis of the nuclei may generally be seen; but when, on the contrary, multinucleated cells are formed, which eventually give rise to the spermatocytes, the nucleus of the spermatogonia either disappears or divides amitotically.

It is not necessary for me to discuss in detail the numerous cases of indirect fragmentation of the nucleus that have been described by Arnold in his numerous papers in 'Virchow's Archiv' and the 'Archiv für mikroscopische Anatomie,' by Werner (**61**), Schottlaender (**50**), Hess (**26**), Geelmuyden (**13**), Beltzow (**2**), Ströbe (**54**), Göppert (**15**), and others. Many of these cases are those of the nuclear division of giant-cells, and I believe I am quite correct in saying that in all of them the fragmentation of the nucleus is not immediately followed by cell division.

The general conclusions to be drawn from the evidence before us are — 1. That fragmentation of the nucleus is a normal method of nuclear division, and is not always a sign of pathological change. 2. That in many of the instances in which the nucleus is supposed to disappear there is, as a matter of fact, minute fragmentation. 3. That fragmentation only occurs where there is no cell division; and 4. That karyokinetic division of the nuclei is caused by the forces in the cell protoplasm which bring about the division of the cytoplasm.

That there may be many forms of nuclear division inter-

[1] It is a noteworthy point that O. von Rath (**48**), who believes that the nuclei of the spermatogonia and spermatocytes always divide mitotically, does not refer at all in his paper to the spermatogenesis of Polychætes.

mediate in character between fragmentation and bipolar karyokinesis seems to be probable from the discovery of pluripolar mitosis in the inflamed cornea by Schottlaender (**50**), and other atypical nuclear divisions in the spleen of the white mouse by Arnold (**1**), &c.

We have, then, a series of phenomena in the division of nuclei, with typical karyokinesis at one end and direct fragmentation at the other. The occurrence of any one kind or the other is, in my opinion, determined by the forces which act simultaneously upon nucleus and cell plasm. If these forces are of such a kind as to drag the cell plasm into two equal halves, the nucleus is also dragged into two equal halves with mitosis; if, on the other hand, the forces are irregular and act from many centres at the same time, the nucleus fragments irregularly.

These views seem to me to be supported by the statement of Flemming (**11**) quoted by Sedgwick, that "the first change observable in a cell whose nucleus is about to divide is in the extra-nuclear protoplasm," and by Bürger's (**7**) recent conclusions concerning the meaning of the spheres of attraction.

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DESCRIPTION OF PLATE 9,

Illustrating Dr. Sydney J. Hickson's paper on the "Development of Distichopora."

Fig. 1.—Young ovum of Distichopora, situated in the cup-shaped trophodisc (*tr.*). The germinal vesicle, *G. v.*, is situated near the centre of the ovum.

Fig. 2.—Germinal vesicle of the same stage, showing the vacuoles in the nucleolus.

Fig. 3.—Germinal vesicle of the ovum of Distichopora, migrating from the centre to the periphery. The membrana limitans becomes obscure over the pseudopodial processes.

Fig. 4.—A peculiar condition of the germinal vesicle, observed in only one preparation.

Fig. 5.—A germinal vesicle, with chromatin granules arranged in a row.

Fig. 6.—Germinal vesicle, containing numerous minute chromatin granules situated at the periphery of the ovum.

Fig. 7.—A stage showing the disappearance of the inner part of the membrana limitans.

Fig. 8.—A stage showing the complete disappearance of the membrana limitans.

Fig. 9.—Section of a young embryo which shows only two large nodes of protoplasm, each of them containing a few deeply staining granules. The yolk is omitted from the lower part of the section in order to show the loose protoplasmic mesh-work which pervades the embryo.

Fig. 10.—A later stage in the development, showing several nodes of various sizes, some with nuclei, some without.

Fig. 11.—A stage in the development corresponding to that of Fig. 10, to show the relation of the nuclei to the yolk. Each nucleus is situated in a small protoplasmic area or node, and the yolk granules close to it are extremely small.

Fig. 12.—The same stage as Fig. 10, showing the different phases in the formation of the nuclei. The details of the yolk are omitted.

Fig. 13.—A section of a young embryo, which shows yolk segmentation.

Fig. 14.—A section through an older embryo, the yolk being omitted, showing the first stages in the formation of the ectoderm (*ect.*). *ec.* Ectoderm. *en.* Endoderm of the gonophore.

Fig. 15.—A section through a still older embryo, showing a later stage in the formation of the ectoderm and its connection with the central endoderm plasmodium.

Fig. 16.—A solid planula of Distichopora, just before it escapes from the gonophores.

Fig. 17.—A small portion of the endodermic plasmodium more highly magnified. Some of the smaller yolk granules are omitted.

Fig. 18.—*a*, *b*, *c*, *d*, *e*. Six stages in the division of the nuclei of the ectoderm.

Studies on the Comparative Anatomy of Sponges.

V. Observations on the Structure and Classification of the Calcarea Heterocœla.

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With Plates 10—14.

I. Preface.

The object of the present paper is to give a general account of the anatomy, histology, and classification of the Calcarea Heterocœla, from the point of view of one who has for some time past been engaged in an independent study of the group. The exceptionally fine collection of Calcarea Heterocœla which I am fortunate enough to have at my disposal must be my justification for making this attempt.

Concerning the anatomy and histology of the group, I have perhaps not much to say that is new, but I hope that by bringing together our information on the subject in a collected form I may be of some use to students of spongology. Serious errors have from time to time crept into our information as to the anatomy of the Heterocœla, and I can only hope that I have not reproduced any of them in this paper. As, however, by far the greater part of my information has been derived from personal observation, I feel tolerably safe in this respect.

With regard to classification, I have been obliged to depart

widely from the lines laid down by previous writers. The necessity for doing so was forced upon me when preparing my "Synopsis of the Australian Calcarea Heterocœla" (4). In that paper I proposed a classification of the group based upon a personal examination of forty-seven species, and a careful consideration of the published accounts of species which I had not seen. In the Synopsis, however, I had not space to justify the classification proposed, merely giving it as a skeleton upon which to arrange my descriptions of species. The task of justification I reserved for the present occasion, and I have endeavoured to fulfil it rather by a critical re-examination of the anatomy of the group than by a detailed criticism of the systems of other spongologists. A great deal depends upon whether one regards the canal system or the skeleton as affording the most reliable guide to the systematist, for the characters of the two certainly appear to contradict one another. Here, as in similar cases, I believe that a compromise is the only satisfactory way out of the difficulty, as neither set of characters is solely reliable. The skeleton evidently follows the canal system up to a certain stage of organisation, and then begins to vary independently. Up to this stage I believe the canal system to be most important; after it I think the skeleton has prior claims, while the canal system becomes of secondary value. According to this idea, which will be found elaborated later on, I regard the Leuconoid type of canal system as of polyphyletic origin, as also the "Sylleibid" type, and I abandon the old families Syconidæ (Sycones) and Leuconidæ (Leucones) of Haeckel, as well as the more recent Sylleibidæ of von Lendenfeld. I have previously shown (9) that the very generally accepted family Teichonidæ must be abandoned, and I am glad to see that von Lendenfeld follows my lead in this (10), although he declines to acknowledge any indebtedness to my writings.

As I have naturally adopted my own classification as a basis for the arrangement of the subject-matter of this paper, it may be convenient to the reader if I give an outline thereof at once, reserving further discussion as well as the

necessary diagnoses till later on. The following, then, is the classification proposed:

Families.	Genera.
1. Leucascidæ	1. Leucascus.
2. Sycettidæ	2. Sycetta.
	3. Sycon.
	4. Sycantha.
3. Grantidæ	5. Grantia.
	Sub-genus Grantiopsis.
	6. Ute.
	Sub-genus Synute.
	7. Utella.
	8. Anamixilla.
	9. Sycyssa.
	10. Leucandra.
	11. Lelapia.
	12. Leucyssa.
4. Heteropidæ	13. Grantessa.
	14. Heteropia.
	15. Vosmaeropsis.
5. Amphoriscidæ	16. Heteropegma.
	17. Amphoriscus.
	18. Syculmis.
	19. Leucilla.

Before concluding these introductory remarks it is my pleasant duty to express my sincere thanks to various friends, without whose assistance this paper could not have been prepared. To Mr. J. Bracebridge Wilson, M.A., I owe, as usual, the greater portion of my material, and I am also indebted to Professor W. Baldwin Spencer, Mr. T. Whitelegge, and the authorities of the Adelaide Museum for a number of very valuable Australian sponges; while numerous fragments of type specimens from the British Museum, most generously forwarded to me by Dr. Günther, have been of the greatest service. Lastly, I must again thank Professor G. B. Howes for most kindly undertaking the correction of the proof in my absence from England.

The numbers in parentheses, in the text, refer to the list of literature at the end. The technical descriptions of a large

number of the species referred to, or references to places where they are to be found, are given in my "Synopsis of the Australian Calcarea Heterocœla" (4).

MELBOURNE;
January, 1893.

II. The Canal System of the Calcarea Heterocœla.

The simplest type of canal system in the group is found in the genus Sycetta. This is, unfortunately, a genus which I have never had the opportunity of personally examining, and I am indebted for my information concerning it to Haeckel's great work on the Calcarea (5).

A Sycetta individual consists, in the first place, of a central tube, which bears at its summit a single osculum leading from the cavity of the tube (gastral cavity) to the exterior. This central tube gives off all around and throughout its length numerous short, hollow, conical diverticula (the radial flagellated chambers). Each radial chamber communicates by a single exhalant opening at its proximal end with the central gastral cavity, and each has its wall perforated by numerous much smaller apertures (the prosopyles), through which the water passes from the exterior into the cavity of the chamber. It is important to notice that the radial chambers, of which there may be a large number, all stand perfectly free from one another and do not touch at any point, so that the water is free to circulate between them without obstruction of any kind, and to penetrate right in to the outer surface of the wall of the central gastral cavity. Hence there is no true inhalant canal system, further than the small prosopyles by which the water gains access to the interior of each chamber. The wall of the central gastral cavity is very thin, so that the chambers open almost directly into the latter, the wide "exhalant canals" which conduct the water through the wall being comparatively short and inconspicuous.

The collared cells are, of course, confined to the interior of the radial chambers, while the central gastral cavity and the

outer surfaces of the radial chambers and of the central tube are doubtless caused by a layer of pavement epithelium, as in other Heterocœla.

Such, then, is the simplest type of canal system met with amongst the Heterocœla; and, as it appears to me, all the higher types met with in the group may be derived from some such simple radiate one by modification in one or more of the following ways:

(1) By the outer surfaces of adjacent radial chambers coming in contact with one another and fusing together. This fusion at first takes place irregularly and partially, and then more completely and throughout the length of the chambers, so as to divide the water-containing space which surrounds the chambers into a series of more or less well-defined inhalant canals, sometimes called "intercanals" (compare figs. 2—5).

(2) By the closing in of the inhalant canals at their distal ends by the outgrowth of a thin, pore-bearing, dermal membrane from the walls of the radial chambers at or near their distal extremities. In this way true "dermal pores" are formed, through which the water gains access to the inhalant canals (compare figs. 3, 6—8).

(3) By the increase in thickness of the dermal membrane and the development in it of a special skeleton, so as to form a thick cortex, which not only stretches between but also covers over the ends of the radial chambers. The formation of such a thick cortex necessitates the development of a more or less highly specialised "cortical inhalant canal system," which places the dermal pores in communication with the deeper parts of the inhalant canal system (compare figs. 9—12, 23).

(4) By the branching of the radial chambers, and consequently of the inhalant canals which lie between them (compare figs. 7, 9, 10, 18, 20).

(5) By increase in thickness of the wall of the central tube (gastral cortex), and consequently also in the length of the exhalant canals (compare figs. 4, 9, 10).

(6) By retreat of the collared cells towards the distal

extremities of the chambers, their place being taken by pavement epithelium. This results in elongation and branching of the exhalant canals, and in corresponding shortening of the chambers, which may be thus converted from the elongated radial chambers of the Syconoid type to the short, rounded, and irregularly arranged chambers of the Leuconoid type. Branching of the primitively straight chambers is of course also necessary in order to effect this change (compare figs. 10, 19, 21, 17, 16).

(7) By evagination or folding of the wall of the central gastral cavity, which also results in elongation of the exhalant canals, and possibly also in branching of the same (compare fig. 7). As pointed out by Sollas (**6**), it is often extremely difficult, if not impossible, to decide which of the two sets of causes indicated in this and the preceding paragraph respectively have operated in the production of a particular type of canal system, though in some cases the arrangement of the skeleton (fig. 7) may furnish a clue to the problem. Though I do not doubt that in some cases a certain amount of folding of the wall of the gastral cavity has taken place, I do not believe that this cause has operated to any great extent, in the production of the Leuconoid from the Syconoid type of canal system.

(8) By the fusion of different Syconoid or Leuconoid individuals[1] of a branching colony to form a compact whole, in which the individuality of the different members is more or less completely obliterated (compare fig. 15).

Other minor causes have also doubtless aided in the production of various modifications in the canal system; such are the enormous dilatation of the gastral cavity and osculum in Grantia labyrinthica (**9**), and the strong development of the mesoderm in the walls of the flagellated chambers which takes place in some forms. The causes (or sets of causes)

[1] By the terms Syconoid and Leuconoid individuals I mean simply individuals or "persons" consisting each of a single, central osculum-bearing tube enclosing the gastral cavity, and surrounded by flagellated chambers and canals arranged according to the "Sycon" or "Leucon" type, as the case may be.

given above are, I believe, the principal ones to which we must look for an explanation of the peculiarities in the canal system about to be described in different genera of Heterocœla.

The various genera (with the possible exception of Leucascus) might, as regards the canal system, be arranged in a gradually ascending series, commencing with Sycetta and ending with those genera, such as Leucandra, in which the Leuconoid type reaches its maximum development. Such a series would not, however, as I believe, represent a natural arrangement of the group, for there is, as I hope to show later on, strong reason for concluding that the most highly modified Leuconoid type of canal system has been independently arrived at in several distinct genera. At present, however, we need not concern ourselves with this question, but pass on to consider to what extent the various causes indicated in the above scheme have operated in modifying the canal system in each separate genus. I propose to deal with the genera in the order in which they occur in my system of classification. In this manner, having first of all disposed of Leucascus, we shall be able to start with Sycetta, and trace the gradual evolution of the canal system from its simplest Syconoid form in that genus, to its most complex Leuconoid one as exhibited in Leucandra. We shall then, in each of the two remaining families (Heteropidæ and Amphoriscidæ) which are distinguished by skeletal peculiarities, be able to start fresh with a Syconoid type of canal system, and work up again to the Leuconoid.

Leucascus (fig. 1).

In this genus the sponge is more or less massive or lobate, and it is not possible to distinguish a single central gastral cavity. There may be several oscula, which perhaps indicates that the whole sponge is then to be regarded as a colony composed of several fused individuals. The arrangement of the canal system in L. simplex, as seen in a vertical section through the osculum, is shown in fig. 1. The flagellated chambers are very long, and copiously branched. Their blind distal extremities lie beneath the dermal surface, towards

which they are directed more or less at right angles, so that the chambers certainly exhibit a more or less radial arrangement. At their proximal ends the chambers open into wide and long exhalant canals, which converge towards the osculum. The distal ends of the chambers are covered over by a thin membrane, strengthened by spicules and perforated by numerous inhalant dermal pores. These pores lead into a series of quite irregular spaces lying between the branching chambers. These spaces represent the inhalant canals, and convey the water to the prosopyles in the thin walls of the chambers. In all specimens which I have seen of L. simplex and in one of L. clavatus (4) the mesoderm is feebly developed, so that the dermal membrane, the walls of the chambers, and the walls of the exhalant canals are all very thin, and the entire sponge has consequently a soft and delicate texture. In one of the specimens which I have referred to, L. clavatus, the mesoderm is very strongly developed; the sponge thereby acquires a dense and solid texture, and the canal system is correspondingly reduced in dimensions. This strong development of the mesoderm is perhaps to be associated with the fact that the specimen contains very numerous embryos; for the normal condition of the genus appears to be one with all the canals and chambers thin-walled.

This genus, as already indicated, does not appear to come into what may be called the typical series of Heterocœla; and its relationships and systematic position will be discussed later on. Though I do not suppose that it has ever passed through a Sycetta stage in its history, it is easy to see how its canal system may have been derived from a radiate ancestral type, by modification along the lines suggested above.

Sycetta.

The simple form of canal system met with in this genus has already been described. If we exclude from the genus Haeckel's S. strobulus, S. cupula, and S. stauridia, which are all corticate species, and include, as von Lendenfeld (10) has rightly done, Sycetta (Sycaltis—H.) conifera, we are

left with three species, all of which exhibit the same type of canal system, in which the radial chambers are short, straight, and unbranched, and project quite separately and independently from the wall of the central gastral cavity.

According to Haeckel (5) there is in Sycetta primitiva, and also in certain more highly organised species of Syconoid Heterocœla, a single larger aperture at the distal end of each radial chamber, which he terms a "dermal ostium." Poléjaeff, however, has (8) thrown considerable doubt on the existence of "dermal ostia" in any case, and I cannot help agreeing with him in attributing the supposed presence of these structures to an error of observation on the part of their describer.

Sycon (figs. 2—7).

In this genus we meet with a considerable amount of variation in the canal system. The simplest form is found in such species as the European S. ciliatum (Bauerbank's Grantia ciliata) and S. raphanus, and in the Australian S. Carteri and S. minutum (4). For a detailed account of S. raphanus I may refer the student to Schulze's well-known and admirable memoir (7).

These species mark but a slight step upwards from the Sycetta type. In Sycon Carteri, for example (fig. 2), the radial chambers are rather short, and more or less thimble-shaped; they touch one another in some places, and there fuse together by their outer surfaces. Their distal ends, however, project freely, and thus form well-marked "distal cones" on the surface of the sponge. By the fusion of adjacent chambers at the points of contact, the originally continuous water-containing space which surrounds all the chambers becomes broken up into more or less definite inhalant canals ("inter-canals"), whose exact form depends, of course, upon the shape of the chambers and the extent to which they fuse together, and is of no great importance. In the simplest cases, such as Sycon Carteri (fig. 2) these canals appear, in sections taken along the length of the chambers, as straight narrow gaps

between adjacent chambers. In the case of Sycon raphanus, Schulze (7) has shown that the different inhalant canals often inter-communicate through gaps left by the incomplete fusion of adjacent chambers, and this condition probably occurs to a greater or less extent in a great many cases.

Probably in most species of the genus Sycon, and certainly in the four with which we are now more immediately concerned, the distal ends of the inhalant canals ("inter-canals") are in no way closed in; in other words, the spaces between the distal cones of the radial chambers remain widely open. Thus the water still has direct access to the prosopyles, without having to pass through the pores of a dermal membrane. In Sycon ensiferum (4), a species closely related to S. raphanus, the basal rays of many of the most distally situated tuber triradiates are very strongly bent outwards from the walls of the chambers, so as to curve over and protect the entrances to the inhalant canals.

In the common Australian S. gelatinosum (figs. 3—6) we meet with a slight but very interesting advance in organisation. The radial chambers, though of considerable length, are still straight, and usually unbranched, subcylindrical tubes. The mesoderm of the chamber walls is strongly developed, and the inhalant canals between the chambers are very well defined and squarish in transverse section (fig. 8). The gastral cortex is rather thick, and consequently the exhalant canals take the form of distinct, though short and wide, tubes, sharply marked off from their respective chambers by well-developed chamber diaphragms, which appear to be almost universally present in calcareous sponges (fig. 4). A tangential section of the dermal surface (fig. 6) shows that the distal ends of the chambers, each crowned with its tuft of oxeote spicules, do not touch one another, but are separated by rather narrow gaps or spaces, across which stretches a delicate pore-bearing membrane (seen in section in fig. 3). This membrane is doubtless formed as an outgrowth of the ectoderm and mesoderm of the walls of the chambers. In Sycon gelatinosum it contains no spicules. The fusion

of adjacent chambers does not commence until a short distance within this pore-bearing membrane, so that the dermal pores lead at first into a continuous subdermal space, from which the true "inter-canals" penetrate between the chambers.

In another Australian species, to which I have given the name Sycon boomerang (4), we meet with yet further complication. The principal features in the anatomy of this species are shown in figs. 7 and 8. The radial chambers are very long, thin-walled, and very much branched, especially towards their distal ends.[1] The irregularity in the branching causes the tufts of oxea at the distal ends of the chambers to form an irregular series of prominences on the surface of the sponge (fig. 8). Owing to the branching of the chambers the inhalant canal system also becomes very irregular. The wall of the gastral cavity is rather thick, and the length of the exhalant canals appears to be further increased by an irregular and not very extensive folding of the same (fig. 7). As in S. gelatinosum, a thin pore-bearing membrane extends between the ends of the radial chambers, but in S. boomerang a few spicules are found in this membrane (fig. 8).

In Sycon giganteum (4), a very large species from the Gulf of St. Vincent, which closely resembles S. gelatinosum in structure, the radial chambers are narrow and greatly elongated; they branch repeatedly, and the branches run parallel with one another to the dermal surface. They communicate with the gastral cavity through rather long exhalant canals, which commence at some distance beneath the gastral cortex. These canals appear to have been formed by modification of the proximal portions of the radial chambers, from which they are separated as usual by diaphragms. They may unite together before opening on the gastral surface. The inhalant canals are irregular and very narrow, opening on the dermal surface through narrow, irregular chinks between the tufts of oxeote spicules which cram the distal ends of the chambers. I have not been able to detect any pore-bearing

[1] It appears from Schulze's researches that branching of the chambers may take place even in such a simple form as S. raphanus (7).

dermal membrane stretched across the openings, which are so narrow that such a structure could hardly be required.

In Sycon Ramsayi, a species common in Port Jackson and described by von Lendenfeld (**11**), the exhalant canals, though confined within the limits of the thick gastral cortex, are extraordinarily well-defined cylindrical tubes, and it is interesting to notice that although the long radial chambers are themselves unbranched, and there is no indication of the exhalant canals having been formed by modification of their proximal ends, yet these canals may unite together before opening on to the gastral surface, and thus present a branching structure. In other respects Sycon Ramsayi agrees in canal system with the simpler species of the genus.

Sycantha.

We owe our information concerning this sponge entirely to von Lendenfeld, who has recently described it from the Gulf of Trieste, and from his work (**10**) the following details as to the canal system are taken.

Sycantha tenella (the only species) is a large, tubular sponge, with a single terminal osculum. The gastral cortex is thin, and forms a cylindrical tube. From the outer surface of the gastral cortex project tufts, which are partially united together by membranes, and attain a length of 2—4 mm. Each tuft runs out into a number of flexible points. These points are the free distal ends of the flagellated chambers of which the tufts are composed. Ten to twenty chambers, united at their bases, form a tuft, each of which is thus a group of flagellated chambers. The chambers themselves are long and narrow. At the base they are irregularly prismatic, generally quadrangular in cross-section. The free, always unbranched, terminal portion is circular in cross-section, and runs out into a conical point. In their basal portions the chambers touch one another, and here there are no inhalant canals (inter-canals) between them, so that collared cells are found on both sides of all membranes which occur in the interior of the basal portions of the tufts. In these membranes,

which separate the different chambers from one another, roundish openings occur, which place the cavities of all the chambers of a group in direct communication with one another. One or several of the chambers of each group are placed in connection with the central gastral cavity through openings in the gastral cortex.[1] Small circular inhalant pores (prosopyles) occur in large numbers in the free distal portions of the chamber walls. Sycantha tenella is said to be distinguished from all other Sycons by the fact that the chambers do not all communicate directly with the central gastral cavity, and also by that of their non-communication with one another in groups, the stream of water passing partly from chamber to chamber before it reaches the gastral cavity.

I am inclined to think that the apparent intercommunication of the chambers may be due to an error of observation. It is by no means the first time that such a condition has been described. Poléjaeff observes (8) that "there is no doubt that what Haeckel declares to be 'dermal ostia' and 'dermal pores' in the individuals of his 'Syconusa type' were merely the pores of the intercanals; and what he calls 'conjunctive pores,' these latter uniting, according to him, the cavities of the radial tubes, were nothing but the common pores on the side walls of the radial tubes connecting these latter with the intercanals. To anyone who will notice Professor Haeckel's remark that these 'conjunctive pores' are best to be observed in sections of dry Sycones, the error into which he fell will be easily comprehended." It is a noteworthy fact in this connection that von Lendenfeld himself observes that his specimens of Sycantha tenella had been preserved for some time in not very strong spirit, so that he was unable to make any observations on the finer histological characters. He also refers to the extraordinary delicacy of the mesoderm in all parts.

I may also remind the reader that Carter (12) bases an

[1] Later on in the work referred to (p. 192) we are told that "jede Kammergruppe ist durch eine einzige grössere Oeffnung in der Gastralmembran mit dem centralen Oscularrohr in Verbindung."

entire genus (Hypograntia) on the supposed presence of such "large holes of inter-communication between the chambers."

On the whole it appears to me that Sycantha tenella probably presents a slight modification of the simpler Sycon type of canal system, having the radial chambers united in groups.

Grantia (figs. 9, 10).

The canal system of this genus may be regarded as derived from the Sycon type by the conversion of the thin, pore-bearing, dermal membrane which we first met with in Sycon gelatinosum (figs. 3 and 6) and then, with the addition of a few spicules, in S. boomerang (figs. 7 and 8), into a more or less strongly developed spicule-bearing cortex, which not only extends between the ends of the radial chambers, but also covers them over, so that we no longer find in this genus distal cones projecting from the dermal surface.

A thoroughly typical example of the genus is afforded by the Australian Grantia extusarticulata (4), the anatomy of which is represented in fig. 9. The radial chambers are almost straight, cylindrical, and only slightly branched; between them lies the irregular and more or less lacunar inhalant canal system. The dermal or inhalant pores are irregularly scattered through the dermal cortex, which is well developed and about 0·07 mm. thick. The gastral cortex is of about the same thickness, and is perforated by the short, wide exhalant canals; one coming from each chamber, and separated from it by a constricting diaphragm.

Grantia Vosmaeri (4), another Australian species, whose anatomy is represented in fig. 10, illustrates in an extremely interesting manner the gradual shortening of the radial chambers and the corresponding elongation of the exhalant canals, accompanied by a strong development of the mesoderm which surrounds the latter. Here, again, the junction between exhalant canals and flagellated chambers is clearly marked by well-developed diaphragms. The dermal cortex is very thick, and the dermal pores communicate with the deeper parts of

the inhalant canal system through distinct, though not very regular, cortical canals.

I have already, in an earlier paper (9), given a detailed account of the anatomy of the somewhat aberrant and very remarkable Grantia labyrinthica. In its essential features the canal system agrees with that of G. extusarticulata, and there are only two points to which I need refer again in this connection. The first concerns the inhalant pores, which, in this species, tend to be collected together in groups or pore areas, where the cortex forms only a thin dermal membrane, and overlies wide lacunar spaces perhaps comparable to the subdermal cavities of some higher sponges, though not at all sharply separated from the deeper parts of the inhalant canal system. The second concerns the central gastral cavity and osculum, which, though in most calcareous sponges so uniform in structure as to require no special notice, are here enormously enlarged, so that the entire sponge takes the form of a funnel whose thin wall is thrown into deep folds, and the margin of the osculum is no longer evenly curved, but extremely sinuous. Since my memoir on the species was written Mr. Bracebridge Wilson has dredged some very large specimens, one of which measures no less than five inches across the top, so that the actual circumference of the osculum is something enormous.

Grantia labyrinthica also illustrates very clearly the branching of the radial chambers, which is of very general occurrence in Syconoid sponges higher than the Sycetta type.

Grantiopsis (fig. 11).

The only species of this sub-genus, Grantiopsis cylindrica (4), though evidently derived from the ordinary Grantia type by no very great amount of modification, is in many ways a very remarkable sponge. The entire sponge forms long cylindrical tubes, which may branch and which are provided with single terminal oscula. The largest tube which I have seen is unbranched and slightly crooked, 57 mm. long, and with a nearly uniform diameter of 5 mm. The wall of the

tube surrounding the central gastral cavity is about 1 mm. thick, and is divided into two sharply defined concentric layers of about equal thickness. The outer of these layers forms a firm cortex, with a very strongly developed skeleton. The inner layer is soft and spongy, consisting almost entirely of the thin-walled radial chambers.

Fig. 11 represents a portion of a transverse section of the sponge. It will be seen from this that the radial chambers are arranged side by side with great regularity. Each is a straight, wide, unbranched (or very slightly branched), thin-walled tube, extending completely through the chamber layer. In cross-section the chambers vary from nearly square to nearly circular. Each opens directly and separately into the gastral cavity, the gastral cortex being so thin that no special exhalant canals are required. Each is provided at its proximal end with a membranous diaphragm, which, in spirit specimens, almost closes the exhalant opening. There is, of necessity, a well-developed cortical inhalant canal system. The inhalant pores, scattered over the dermal surface, lead into sharply defined cortical canals, which unite into larger trunks, which conduct the water to the ordinary "intercanals" between the radial chambers. Hence it appears that, as regards the canal system, the points to be specially noticed in Grantiopsis are the strong development of the cortical canal system and the great elongation of the gastral cavity.

Ute (figs. 12—14).

The canal system of a typical Ute agrees essentially with that of a typical Grantia. In Ute syconoides (4) the canal system is unusually regular, and the radial chambers have a remarkably definite and constant form. The anatomy of this species, which in general form closely resembles the European U. glabra, is represented in figs. 12—14. The radial chambers are straight, and circular in transverse section for the greater part of their length (fig. 13). Towards their distal extremities, however, they widen out in a direction at right angles to the long axis of the gastral cavity of the

sponge, and at the same time become hour-glass shaped in transverse section (fig. 14), while each has a slight depression at its extreme end (fig. 12). The extraordinary regularity with which these chambers are arranged, and the very definite relation which they bear to the lines of huge oxeote spicules in the dermal cortex, will be sufficiently evident from the figures referred to. The inhalant pores are thickly scattered over the dermal surface between the parallel lines of oxeote spicules. They lead into spaces which are at first somewhat lacunar, but soon give place to sharply defined "inter-canals" as they pass in between the radial chambers (vide figs.). The gastral cortex is very thin, and the exhalant canals are consequently very short.

The canal system of Ute argentea, another Australian species, has been figured by Polejaeff (8). It appears to differ from that of U. syconoides principally in the relatively greater thickness of the dermal cortex, and consequent shortness of the radial chambers, and in the less regular arrangement of the inhalant canal system.

In a colonial species from Port Jackson, which I have named Ute spiculosa (4), we find the canal system much more irregular, in accordance with an unusually strong development of the mesoderm and its contained skeleton. The gastral cavity is narrow and cylindrical, occupying about one third of the total diameter of the sponge. The flagellated chambers are long and narrow, and more or less radially arranged. They do not extend nearly through the entire thickness of the sponge wall, and they communicate with the gastral cavity through long, sometimes branched exhalant canals. The inhalant canal system consists of scattered pores on the dermal surface, leading into elongated canals which lead down between the chambers, but the typical radial arrangement of the canal system is greatly obscured by the strong development of the mesoderm, and the dense, irregular skeleton. There is a very thick dense cortex on both dermal and gastral surfaces.

In Ute Spenceri (4), from the same locality, we find another species with a very strongly developed mesoderm and

skeleton. This sponge is solitary, and is remarkable for its globular or subspherical shape, with correspondingly situated gastral cavity and narrow osculum. The dermal cortex is very strongly developed, occupying more than one third of the entire thickness of the sponge wall. The inhalant pores, scattered over the surface of the sponge, lead into wide, irregular subdermal cavities lying in the cortex, from which inhalant canals lead down between the radial chambers. The chambers themselves are arranged parallel to one another with considerable regularity. They are long and narrow, and at their distal ends they branch in a curiously irregular manner, the branches sometimes penetrating for some little distance into the dermal cortex. The proximal ends of the chambers are all situate at about the same level, which is some little distance from the gastral cavity, and even from the gastral cortex, which latter is very much thinner than the dermal cortex. Hence we find a number of rather short, cylindrical, radially arranged exhalant canals, which look exactly like continuations of the radial chambers without the collared cells, and which may unite together in groups before opening on the gastral surface. The points of junction of these exhalant canals with the radial chambers are marked as usual by diaphragms. The "inter-canals" between the chambers are narrow and irregular.

Ute spiculosa and U. Spenceri occupy a position in the genus Ute very similar to that occupied by Grantia Vosmaeri (fig. 10) in the genus Grantia. In both cases the typical radial arrangement of the canal system is more or less disturbed in accordance with the strong development of the mesoderm and skeleton.

Synute (fig. 15).

In this interesting sub-genus we meet with a very unusual, if not unparalleled condition, in the complete fusion of a large number of Syconoid individuals to form a compact, solid sponge invested in a common cortex. The anatomy of Synute pulchella, as seen in horizontal section, is represented in fig. 15. As I have already pointed out (13), the canal system,

apart from the fusion of the individuals, closely resembles that of Ute argentea, as figured by Poléjaeff (8). A horizontal section of the colony shows a number of circular spaces scattered at intervals, generally, but not always, in a single row. These are the gastral cavities of the Syconoid individuals cut across. Each cavity is surrounded by its own radial chambers, arranged in a perfectly normal manner, except for the fact that on the adjacent sides of any two neighbouring gastral cavities the chambers are much shortened, and sometimes appear bent outwards as though to avoid one another. The inhalant pores are scattered over the dermal surface, and lead into irregular canals, which pierce the thick cortex to reach the radial chambers. At their lower ends the gastral cavities of the fused Syconoid individuals all communicate with one another, indicating that this peculiar form of colony has arisen from fusion of adjoining individuals of a branching colony. The radial chambers are approximately octagonal in transverse section, while the much smaller "intercanals" between them are square. The exhalant openings of the chambers are protected by very well-developed diaphragms, and each gastral cavity has also a single large, well-developed diaphragm situate just within the osculum.

Probably Synute pulchella is the only known example of a calcisponge with a Syconoid canal system, which has become at all strikingly modified in accordance with the principle set forth in section 8 of the scheme given above.

Utella.

This genus, founded entirely on skeletal characters for the reception of Haeckel's Sycandra hystrix, and perhaps also Schmidt's Ute utriculus (5), presents no features of special interest in its canal system, which appears to conform to the ordinary corticate Syconoid type, like that of Grantia.

Anamixilla.

This genus is also founded entirely on skeletal characters, and the only known species, A. torresi, Poléjaeff (8), appears

to possess the typical corticate Syconoid canal system, well shown in Poléjaeff's figure of the anatomy of the sponge.

Sycyssa.

Here, again, the same remarks apply as in the case of the last two genera. The anatomy of Sycyssa Huxleyi is illustrated by Haeckel in his great monograph (5).

Leucandra (figs. 16, 17, 25).

In this genus we meet for the first time with the well-known Leuconoid type of canal system, the essential features of which are shown in fig. 16, representing a portion of a transverse section through the wall of Leucandra phillipensis (4). It will be seen from this figure that the flagellated chambers are small and more or less rounded, and scattered quite irregularly but abundantly through the thickness of the sponge wall. Each communicates by several small prosopyles with the irregular inhalant canal system, and by a single larger opening with the exhalant canal system. The gelatinous ground substance of the mesoderm is sparingly developed, except in the region of the dermal and gastral cortex, and the inhalant and exhalant canals are very wide and irregular. Numerous small inhalant pores, scattered over the dermal surface, lead first into small canals in the dermal cortex; these unite into much larger ones, which lead inward, and break up in the thickness of the sponge wall. The very wide exhalant canals open into a perfectly well-defined gastral cavity from which the water passes out through a single terminal osculum.

Such a sponge as Leucandra phillipensis forms a thoroughly typical Leuconoid individual, and we find the type of canal system above described repeated in a large number of species with singularly little variation; the flagellated chambers being irregularly scattered, ovoid or subspherical in shape (fig. 25), and about 0·1 mm. in diameter.

In a few species of Leucandra, however, we find the flagellated chambers much larger, more or less elongated in form,

and arranged radially around the exhalant canals, features which characterise von Lendenfeld's family Sylleibidæ (**10**).

The only instance of this Syllectoid condition which I have myself observed in the genus Leucandra is in the case of L. australiensis (**4**), the anatomy of which is represented in fig. 17. This is a rather large, solitary species, of the typical sac-shaped form. The flagellated chambers are irregularly sac-shaped, and average about 0·3 by 0·1 mm. in size, though very variable. Their radial arrangement around the exhalant canals is not nearly so well marked as in some other species to be mentioned directly; but it is obvious that the chambers of any sponge must be arranged more or less radially around these canals if, as in Leucandra, a large number open directly into them.

In Leucandra aspera, according to Vosmaer (**14**), we find much the same condition, except that the radial arrangement of the somewhat elongated, sac-shaped chambers around the exhalant canals is more regular, a condition which is very closely paralleled by Leucilla uter (vide infra and fig. 21).

Von Lendenfeld's Polejna telum and Vosmaeria corticata (**10**), both of which belong to the genus Leucandra as here understood, likewise afford, according to this author's figures, good illustrations of the more regular Sylleibid type of canal system, with the flagellated chambers somewhat elongated, and arranged radially around the exhalant canals.

This Sylleibid condition, first described by Poléjaeff (**8**), appears to be intermediate between the typical Syconoid and the typical Leuconoid conditions, but approaches more nearly to the latter than to the former. Indeed, it would be extremely difficult to say where the Sylleibid condition ends and the Leuconoid begins, for even in such a typically Leuconoid form as L. phillipensis (fig. 16) some of the chambers, especially towards the outside of the sponge, may be markedly elongated, and thus approach the Syconoid type; and the same variation in the form of the chambers is found to an even greater extent in some species belonging to other genera, as will be seen later on. This fact, and the fact that the Sylleibid type of canal

system is met with in species with very distinct types of skeletal arrangement (as will be seen subsequently), cause me to believe that it is of no value for systematic purposes, although it is of great interest, as probably representing a late stage in the evolution of the Leuconoid from the Syconoid type of canal system by branching of the radial chambers and restriction of the collared cells to the branches. (It is also conceivable that the Sylleibid type may have originated from the Syconoid by folding of the wall of the gastral cavity, but there is very little evidence in favour of this view; while, in some species of Sycon and Grantia, we have a certain amount of evidence, in the arrangement of the skeleton, for believing in the direct conversion of flagellated chambers into exhalant canals. That both processes may have taken place together I do not for a moment deny.)

In some species of Leucandra we find, as in the case of Synute pulchella, a very strong tendency towards the formation of massive, irregular colonies, by the more or less complete fusion of a number of Leuconoid individuals. Hence we find species which, instead of having a single well-defined central gastral cavity, with a single terminal osculum and a correspondingly definite sac-shaped external form, as in Leucandra phillipensis, exhibit an irregular massive form with a larger or smaller number of oscula scattered over the surface, each osculum being the outlet of a wide exhalant canal, which probably corresponds to the gastral cavity of a single Leuconoid individual. Synute pulchella is of great interest as showing us how such forms have probably arisen. As far as my experience goes, all such massive colonial species have in other respects a typical Leuconoid canal system with small rounded chambers, as in the Australian L. gladiator (4).

Lelapia.

The canal system of this remarkable genus is unfortunately unknown, but from the fact that Mr. Carter (12), who has personally studied it, places it amongst the "Leucones," it seems probable that it conforms to the Leuconoid type.

Leucyssa.

This genus also appears, from the very scanty information which we possess with regard to the canal system (5), to conform to the ordinary Leuconoid type.

Grantessa (fig. 18).

In this genus we find ourselves returning to a canal system which is emphatically Syconoid, and agrees closely with that which we have already described in the genus Grantia. This will be rendered obvious by a comparison of fig. 18, representing a portion of a transverse section of Grantessa intusarticulata with fig 9, representing a similar section of Grantia extusarticulata. In Grantessa sacca, one of the most beautiful of our Australian sponges, the chambers are very long and copiously branched (4). All the known species are solitary Syconoid individuals or branching colonies, never completely fused into solid, massive forms. In Grantessa erinaceus we meet with a very striking peculiarity, in the presence of ingrowths of mesoderm from the gastral cortex, covered by a single layer of flattened epithelium, into the gastral cavity. These ingrowths form a series of irregular "endogastric septa" without any spicules. They are present in both the specimens in my possession, and, as Mr. Carter also mentions them (12), they would seem to be constant in the species. In G. erinaceus also, the flagellated chambers are very irregular and much branched, and they communicate with the gastral cavity by unusually long exhalant canals, which unite together in groups.

The anatomy of Grantessa (Amphoriscus) poculum has been figured by Poléjaeff (8) and re-investigated by myself. The canal system of this species agrees closely with that of G. intusarticulata (fig. 18).

Heteropia.

The only species which I propose to retain in this genus is Carter's Aphroceras ramosa (15), which appears to have a typical radiate Syconoid canal system, complicated only by the development of a strong dermal cortex resembling that of the genus Ute.

Vosmaeropsis (fig. 19).

In this genus we find (4) at present only three species—V. macera, V. depressa, and V. Wilsoni,—all of which are interesting with regard to their canal system. The canal system is never truly radial and Syconoid, but the shape and size of the flagellated chambers, and in V. macera (fig. 19) the arrangement also, clearly indicate a condition intermediate between the typical Syconoid and the typical Leuconoid plan; in other words, a Sylleibid condition. In V. macera, the anatomy of which is represented in fig. 19, the chambers are thimble-shaped, and mostly widely separated from the central gastral cavity; they communicate with this by wide exhalant canals, into each of which a number of chambers discharge their contents. Each chamber has, as usual, whether it be large or small, a number of small inhalant prosopyles, and a single much larger exhalant aperture, guarded by a well-developed diaphragm (fig. 31). Those chambers which lie next to the dermal surface still exhibit a more or less radial arrangement in relation to the central gastral cavity.

Vosmaeropsis depressa is, unfortunately, known only from a single specimen, so that it is impossible to tell how far the peculiarities of its canal system are constant. There is no single wide gastral cavity, but several large branching exhalant canals converge to a single small osculum situate near the middle of the upper surface of the cushion-shaped sponge. The inhalant canal system is quite irregular, commencing in wide lacunar spaces beneath the thin pore-bearing dermal cortex. The flagellated chambers are irregularly but thickly scattered through the thickness of the sponge, with no trace of radial arrangement around a central gastral cavity. They are, however, more or less sac-shaped or thimble-shaped, measuring about 0·2 by 0·09 mm. This sponge, from the fact that it possesses but a single osculum, probably corresponds to a single individual, but it is interesting to note how the central gastral cavity has become indistinguishably merged into the branching system of wide exhalant canals.

Vosmaeropsis Wilsoni is a large species found abundantly

in the neighbourhood of Port Phillip. The sponge is colonial, and consists of short, thick, subcylindrical or truncatedly conical individuals, united together basally in larger or smaller agglomerations. Each individual has a single well-defined wide gastral cavity, with a single terminal osculum, protected by a remarkably distinct membranous diaphragm situated a short distance within its margin. The wall of the sponge surrounding the gastral cavity is thick, and there is a dense thick cortex on both gastral and dermal surfaces. Between the dermal and the gastral cortex lie the flagellated chambers, thickly but irregularly scattered. These chambers vary to a remarkable extent both in shape and size, from approximately spherical ones of about 0·072 mm. in diameter, to elongatedly sac-shaped ones of as much as 0·37 by 0·13 mm. It is right to state that these measurements were taken from different specimens, but the species is so well characterised that it would be difficult to make a mistake in identification, and we also find considerable variation in the chambers, even in the same section. Most remarkable, however, is the inhalant cortical canal system, a portion of which, from an osmic acid preparation, is represented in fig. 23. The inhalant pores, thickly scattered over the surface of the sponge, lead each into a separate narrow canal lined by a flattened epithelium. These canals unite together into larger and larger canals as they penetrate the dermal cortex, and also form frequent anastomoses by cross-branches. This canal system conducts the water to the chamber-bearing layer of the sponge wall and distributes it to the chambers; from these it is collected again by the exhalant canals, which, uniting into tolerably large trunks, penetrate the gastral cortex and open into the central gastral cavity. I have never seen the inhalant cortical canal system so well illustrated as in this sponge, doubtless because I took the precaution to treat a piece of a living specimen with osmic acid. It finds a close parallel in the corresponding cortical canal system of the Syconoid Grantiopsis cylindrica, and may probably be taken as representing the typical condition for Heterocœla having a very strongly developed dermal cortex.

Heteropegma (fig. 20).

In this genus we again go back to a Syconoid arrangement of the canal system, though not a very typical one. In H. nodus-gordii, the anatomy of which has been admirably illustrated by Poléjaeff (8), and of which I also venture to give a drawing (fig. 20) based upon personal examination, the sponge forms irregular agglomerations of small Syconoid individuals, each with a single osculum and a central gastral cavity. The flagellated chambers have extremely thin and delicate walls, and branch in an extraordinarily copious and irregular manner (fig. 20). The gastral cortex is also extremely thin, but the dermal cortex is very strongly developed, and penetrated by irregular canals which lead from the inhalant pores on the surface of the sponge into the quite irregular lacunar system of spaces ("inter-canals") between the chambers.

In Heteropegma latitubulata, which is found off the south coast of the continent of Australia (H. nodus-gordii being found off the north), we meet with an identical canal system and external form (4). The peculiarities of the canal system in these, the only known species of the genus, are brought about by the copious and irregular branching of the chambers and the extremely slight development of the mesoderm everywhere except in the dermal cortex.

Amphoriscus.

In this genus the canal system is more typically Syconoid, such as we find in any of the Syconoid genera, like Grantia, with a dermal cortex. As I have never myself met with the genus, I may refer the reader to the account of Amphoriscus (Sycilla) cyathiscus and A. (Sycilla) cylindrus given by Haeckel (5).

Syculmis.

Here, again, the canal system appears to differ in no particular from the typical corticate Syconoid plan, like that of Grantia. I must again refer the reader to Haeckel's great work (5) for an account of the only known species (Syculmis synapta).

Leucilla (figs. 21, 22).

This genus includes species which never possess a truly radiate Syconoid type of canal system, but either a thoroughly typical Leuconoid arrangement with small, more or less rounded chambers, as in Leucilla australiensis (fig. 22), L. saccharata, and L. prolifera (4), or an arrangement like that of Leucilla uter (fig. 21), which indicates by the elongated form of the chambers and their radial arrangement around the exhalant canals a Sylleiboid condition, intermediate between the Syconoid and Leuconoid types.

The genus Leucilla, in short, occupies a position in the family Amphoriscidæ exactly analogous to that occupied by Leucandra amongst the Grantidæ, and we find in both genera the same variations in canal system. Thus the canal system of Leucilla uter[1] appears to be almost exactly paralleled by that of Leucandra aspera (14), while that of Leucilla australiensis and the other species with small, rounded, and irregularly arranged chambers, is paralleled by the similar arrangement found in numerous species of Leucandra, as will be evident on comparing figures 16 and 22.

Most remarkable is the canal system of Poléjaeff's Leucetta vera (8), which appears to belong to the genus Leucilla as now constituted. In this species, according to Poléjaeff (8), the chambers of the inner half of the sponge wall are rounded and irregularly scattered, while those of the outer half are elongated and radially arranged, and it thus affords a noteworthy commentary on the value of the canal system for purposes of classification.

A similar variation in the form of the chambers, only on a much smaller scale, is to be found in Leucilla australiensis (fig. 22).

Another interesting variation in the canal system is afforded by Leucilla cucumis (Haeckel's Leucandra cucumis, 5).

[1] For further information as to this species vide Poléjaeff (8). My drawing is made from my own preparations of a portion of the type specimen from the British Museum.

In this species we find a series of very distinct subdermal cavities supported by a special skeleton, for further information with regard to which the reader is referred to the sections of the present paper dealing with the skeleton and classification of the Heterocœla.

We find in some species of this genus also the same tendency to form colonies, by complete fusion of Leuconoid individuals, as we met with in Leucandra. An admirable example of this is afforded by Leucilla (Teichonella) prolifera, of which I gave an illustration in a previous memoir (9), showing the oscula arranged side by side in rows.

Summary.

Thus we find that the canal system varies considerably in what appear to be closely related genera of Heterocœla Calcarea; and that, if my view of the relationships of the genera be correct, the Leuconoid type has been independently evolved from the Syconoid type, along the lines indicated above, no less than three times. The Leuconoid type cannot, however, be produced until the corticate Syconoid type has been arrived at; and when this condition has been reached the conversion of the originally long and radially disposed chambers into short, rounded, and irregularly arranged ones seems such a simple matter that it may well have taken place again and again. The variation in shape and size of the chambers, even in the same species, may, as I have already shown, be very great. The branching of the radial chambers in Syconoid forms is of such common occurrence in the most diverse genera as to excite no surprise wherever we meet with it; and the shortening of the chambers and corresponding elongation of the exhalant canals, due simply to shifting of the limits of the lining of collared cells, has been repeatedly observed in various genera.

We may sum up our observations on the canal system of the Heterocœla by indicating again the various stages which appear to have been passed through in the gradual evolution of the most complex Leuconoid from the most simple Syconoid type.

Stage A (Sycetta stage).—The flagellated chambers are

perfectly straight, unbranched, and radially arranged. They do not touch one another at all, and there is no trace of a dermal cortex, hence there is no enclosed system of inhalant canals, but the water circulates freely between the chambers without any interruption. Sycetta appears to be the only genus in which this most simple condition is retained.

Stage B (Sycon stage).—This stage differs from the foregoing only in the more or less complete fusion of the walls of adjacent radial chambers wherever they come in contact. This results in the formation of more or less well-defined inhalant "inter-canals." The chambers may also branch. In this stage we find most species of the genus Sycon, and we may perhaps also include Sycantha. Those species of Sycon in which a thin pore-bearing membrane covers over the ends of the "inter-canals," as described above, are intermediate between stages B and C.

Stage C (Grantia stage).—The chambers are still elongated and radial, but their distal ends and the ends of the "inter-canals" between them are covered over by a dermal cortex, which contains true inhalant pores, and sometimes a complicated cortical inhalant canal system. In this stage we find Grantia, Grantiopsis, Ute, Synute, Utella, Anamixilla, Sycyssa, Grantessa, Heteropia, Heteropegma, Amphoriscus, and Syculmis.

Stage D (Sylleibid stage).—The chambers are no longer arranged radially around the central gastral cavity, but are still more or less elongated, aud arranged radially around the usually radial exhalant canals. It was this condition, first described by Poléjaeff (**8**), which gave rise to von Lendenfeld's Sylleibidæ (**10, 11**). It is not, however, characteristic of any particular genus, much less of any particular family, but is found in a few isolated species, such as Leucilla uter, Leucandra aspera, and Vosmaeropsis macera.

Stage E (Leucandra stage).—The chambers are small, more or less spherical, and irregularly scattered through the sponge wall; and the inhalant and exhalant canal systems are correspondingly developed. We find this condition in most

species of Leucandra and Leucilla, and presumably also in Lelapia and Leucyssa.

No one of these five stages is very sharply marked off from the stage below it, and the five appear to me to indicate a process of evolution which has actually taken place. Von Lendenfeld (**10**) attributes to the Leuconoid type of canal system an independent origin from the Homocœle type, through his very problematical "Leucopsidæ," without passing through a radiate Syconoid stage at all, although he admits the derivation of the Sylleibid type from the latter. With this view I cannot at all agree. Considering the canal system alone, we have cogent reasons for opposing it, and when we come to discuss the skeleton we shall find others.

III. The Skeleton of the Calcarea Heterocœla.

The Spicules.—So much has been written about the spicules of calcareous sponges, and their variations in minute details of shape are of so little interest from a morphological point of view, that I propose to say very little about them in this place, and only to recapitulate those facts which it is necessary to consider in discussing the arrangement of the skeleton.

In calcareous sponges, whether Homocœle or Heterocœle, three principal types of spicules are met with:

(*a*) Triradiate,

with three rays diverging from a common centre and lying typically in one plane, though frequently curved more or less out of that plane.

(*b*) Quadriradiate,

resembling the triradiate but with an additional ray, known as the apical ray, coming off from the centre in a plane at right angles to the plane of the other three (facial) rays.

In both triradiate and quadriradiate spicules we can distinguish three chief varieties:—(1) Regular, the three facial

rays being all alike, with the angles between them equal. (2) Sagittal, when two of the facial rays or two of the angles form a pair differing in some respects from the remaining ray or angle. In this case the paired rays are termed oral, the odd ray is termed basal, and the odd angle between the oral rays is also termed oral. (3) Irregular, when the three facial rays conform to neither of the above plans.

(*c*) Oxeote,

or uniaxial spicules (oxea), in which there is only a single axis. These spicules vary greatly in details of shape, from symmetrically fusiform, with two sharply pointed ends, to nail-shaped, with one end blunt and swollen.

As in the Homocœla, so in the Heterocœla, the mere presence or absence of one or other of these three types of spicule is of very slight—in my opinion, of not more than specific—value for purposes of classification. With the arrangement of the spicules I believe the case to be totally different, and I find, in the strncture of the skeleton as a whole, characters of family value to the systematist.

The Arrangement of the Spicules.—Here, as in the case of the canal system, we shall find it most convenient to take the various genera one by one and deal with them separately. We shall find that, up to the Grantia stage, the arrangement of the skeleton follows, and appears to be to a large extent controlled by, that of the canal system. But on reaching the Grantia stage the development of a strong dermal cortex introduces new possibilities with regard to the skeleton, which commences to vary independently of the canal system, and branches off along three lines corresponding to the families Grantidæ, Heteropidæ, and Amphoriscidæ. The genus Leucascus appears to occupy an isolated position.

Leucascus (fig. 1).

In this genus (fig. 1) the arrangement of the skeleton is extremely simple, and exhibits no trace of that radial symmetry which is so characteristic of the Heterocœla. It

resembles, on the other hand, the arrangement of the skeleton in such simple Homocœle sponges as Leucosolenia protogenes, excepting that there is a true dermal skeleton in the pore-bearing dermal membrane. In both species of Leucascus (4) the skeleton consists of small, regular triradiates, irregularly scattered in the walls of the elongated chambers and exhalant canals, and in the dermal membrane. In Leucascus simplex these are the only spicules present, but in L. clavatus we find in addition some large, club-shaped, uniaxial or oxeote spicules, partly projecting from the dermal surface.

Sycetta.

Here, in accordance with the arrangement of the canal system, we can distinguish between two main parts of the skeleton,—(1) the gastral skeleton, supporting the wall of the gastral cavity; and (2) the tubar skeleton, supporting the walls of the radial chambers. The gastral skeleton consists of triradiate or quadriradiate spicules, whose three facial rays lie in the thickness of the wall of the gastral cavity, while the apical ray, if one happens to be developed, projects into the cavity. These spicules may be sagittal, as in Sycetta (Sycaltis) conifera (5), and then the basal ray is found to be directed downwards, away from the osculum and towards the base of the sponge, a position which is so constant in this and other genera as to have given rise to the term "basal ray." The tubar skeleton consists exclusively of triradiate spicules, which lie in the thickness of the chamber walls, and which always have the basal ray directed along the length of the chamber and away from the gastral cavity. Hence the oral or paired rays are spread out in a direction at right angles to the length of the chamber, and as several spicules generally lie at the same level, the tubar skeleton forms a series of more or less definite joints or rings, and is hence said to be articulate. This articulate arrangement, however, which prevails in most genera with a Syconoid type of canal system, is usually very irregular.

Sycon (figs. 2—8).

Here, again, we meet with distinct gastral and tubar skeletons, as shown in fig. 2, and the general plan of the skeleton is the same as in Sycetta. Quadriradiates are usually, if not invariably, present in the gastral skeleton, the apical ray projecting freely into the gastral cavity (figs. 2, 4, 7), and doubtless serving as a protection against the ingress of parasites. The gastral skeleton may be very strongly developed so as to form a thick cortex, which may be continued inwards along the exhalant canals, as in Sycon boomerang (fig. 7). Where this is the case—but it is very rare—it seems to me to indicate a folding of the wall of the gastral cavity. The tubar skeleton is always "articulate," and the number of "joints" which it exhibits depends upon the length of the chambers. Usually the spicules composing it are more or less strongly sagittal and triradiate, but occasionally apical rays may be developed, as in S. boomerang (fig. 7). The position of the spicules is always the same as in Sycetta, with the basal ray pointing away from the gastral cavity; where an apical ray is developed it projects freely into the cavity of the chamber. Usually a special set of spicules, known as "subgastral sagittal triradiates" are developed, as is well shown in Sycon Carteri (fig. 2). These have the oral rays extended very widely in the outer part of the wall of the gastral cavity, and thus forming a part of the gastral cortex, while the basal ray is usually very long, and points towards the distal end of the chamber in whose wall it lies. These spicules, or at any rate their basal rays, form the first "joint" of the articulate tubar skeleton, which is commonly a good deal longer than any of the succeeding joints (fig. 2). The subgastral sagittal triradiates may, however, be almost, if not quite, indistinguishable from the ordinary tubar triradiates of the sponge. Usually the three rays of the tubar triradiates do not lie all in one plane, but the oral rays are curved or inclined towards one another, so as to partially embrace the chamber in the thickness of whose wall they lie (fig. 5).

In this genus we always find, at the end of each radial chamber, a more or less dense tuft of oxeote spicules (figs. 2, 3, 6—8). The shape of these spicules varies greatly, and influences in a remarkable degree the character of the surface of the sponge. They may be enormously elongated and project far beyond the surface, which thus becomes covered with a coating of long silky hair, as in Sycon Ramsayi; or their outer ends may be swollen and nail-shaped and project but very slightly, so as to form a dense crust, as in some specimens of Sycon gelatinosum (fig. 6). All conditions intermediate between these two may also be met with. These tufts of uniaxial spicules, which are extremely characteristic of the genus Sycon, doubtless serve to protect the surface of the sponge generally, and also to filter the water before it passes into the "inter-canals." The entrances to the "inter-canals," between the tufts of oxeotes, may also be protected by the basal rays of the more distal tubar triradiates, which may curve outwards from the chamber wall, as in Sycon ensiferum (4).

In Sycon boomerang, as I have already had occasion to point out, we meet with the first indication of a true dermal skeleton distinct from that of the chambers. It consists of a few scattered triradiate and oxeote spicules lying in the thin, pore-bearing membrane, which stretches across and protects the entrances to the intercanals (fig. 8).

In this genus we frequently meet with a more or less specially developed "oscular skeleton," which when present, either in this or any other genus, always consists of a fringe of oxeote spicules, projecting more or less markedly around the osculum. Usually the fringe is vertical, or inclined only at a slight angle to the long axis of the gastral cavity; but occasionally a second, almost horizontal, fringe is developed, which projects almost at right angles all around the base of the first one. A beautiful illustration of such a horizontal fringe is given by Haeckel in the case of his Sycarium elegans (5, pl. lviii), and I have also met with it in a variety of Sycon gelatinosum from Port Jackson. The oscular skeleton is, however, a very variable structure, and of very little import-

ance from the point of view either of the morphologist or the systematist.

Sycantha.

The skeleton of this genus appears to conform exactly to the normal Sycon type, which appears to favour my view that the canal system is but a slight modification of the Sycon type.

Grantia (figs. 9, 10).

In Grantia we find the skeleton built upon the same essential plan as in Sycon, but there is, in addition, a well-developed dermal skeleton lying in the dermal cortex, which covers over the ends of the radial chambers and inter-canals, while we no longer find each chamber surmounted at its distal extremity by a tuft of uniaxial oxea. The gastral skeleton and the articulate tubar skeleton are precisely similar to what we found in Sycon, as will be at once evident on referring to fig. 9, representing the anatomy of Grantia extus-articulata.

The skeleton of the dermal cortex in this genus consists principally of triradiate spicules lying parallel to the dermal surface. These spicules may be sagittal, with the basal ray directed, as in the gastral cortex, towards the base of the sponge. A good example of this arrangement is seen in Grantia labyrinthica (9).

In addition to the triradiate spicules we also find in the dermal cortex of Grantia, in most if not all species, a number of oxeote spicules placed at right angles to the dermal surface. These may either be very small and numerous, as in Grantia extusarticulata (fig. 9), or they may be large and fewer, as in G. Vosmaeri (fig. 10). In the former case they form a kind of crust, and are spoken of by some writers as "mortar-spicules:" their inner ends only penetrate the dermal cortex for a short distance. In the latter case their inner ends may penetrate through nearly the entire thickness of the sponge wall. In no case in this genus do they form tufts at the ends

of the radial chambers, but they are always arranged entirely without regard to these.

In Grantia labyrinthica (9) we find minute surface oxea in the gastral as well as in the dermal cortex. This is a very unusual occurrence, but is paralleled in Ute Spenceri (4).

In Grantia Vosmaeri (fig. 10), where we have a marked shortening of the radial chambers and a corresponding elongation of the exhalant canals, together with a strong development of the mesoderm surrounding them, we find the skeleton in the inner portion of the sponge wall losing its regular radially symmetrical character, and becoming diffuse and scattered. This is an illustration of a general law, that the skeleton of the chambers varies with the chambers themselves, which will be found to hold good throughout the group, with few exceptions. As the chambers lose their radial symmetry and regularity of form and arrangement, so the skeleton loses its radially symmetrical articulate character, and becomes diffuse and scattered. This will be seen to follow as a natural consequence, if we remember that the position of the spicules is to a large extent determined by the position of the layer of mesoderm (in the chamber wall) in which they are developed. Hence it is that irregularity in the skeleton generally accompanies thickening of the mesoderm, for when this is effected the spicules are free to take up a variety of positions which, while embedded in a very thin layer of mesoderm, they are unable to assume.

Grantiopsis (fig. 11).

In Grantiopsis cylindrica (4) we meet with a slight modification of the Grantia type of skeleton, as shown in fig. 11. The dermal cortex is enormously developed, but its skeleton still consists of triradiate and oxeote spicules arranged as in Grantia. In the thin gastral cortex quadriradiates are present as usual, but of somewhat peculiar shape, with small facial and enormous apical rays. The subgastral sagittal triradiates of the ordinary Sycon and Grantia types are here replaced by subgastral sagittal quadriradiates. The position of

these spicules agrees with that of the subgastral sagittal triradiates of Sycon and Grantia, but an apical ray is developed which projects into the gastral cavity, almost in a line with the centrifugally directed basal ray. Hence these spicules are not homologous with the subgastral quadriradiates of some Amphoriscidæ, their position being different.

The articulate tubar skeleton of Grantiopsis cylindrica is very remarkable, owing to the peculiar form of the spicules of which it is composed. These spicules are the most extreme modifications of the sagittal triradiate type which I have ever seen or heard of. They consist almost entirely of the strangely developed centrifugally directed basal ray, which is straight, fusiform, gradually sharp-pointed at the distal end, and at the proximal end provided with a pair of minute, widely divergent, conical teeth, which represent the extremely reduced oral rays. The basal ray measures about 0·3 by 0·008 mm., while the oral rays are only about 0·003 mm. long. The entire tubar skeleton is made up of these spicules and of the basal rays of the subgastral quadriradiates, arranged usually in single series, but with overlapping ends, each series comprising only about three spicules (fig. 11).

Ute (figs. 12—14).

In this genus we find the skeleton arranged as in Grantia, only with the addition of a number of very large oxeote spicules disposed longitudinally in the dermal cortex. The gastral skeleton is exactly like that of Grantia and Sycon, and so also is the tubar skeleton in most species. In Ute argentea, however, there is, as Poléjaeff points out (8), a tubar skeleton composed only of a single joint, i. e. of the rays of the subgastral sagittal triradiates. To this tubar skeleton the term "inarticulate" has been applied, but this appears to me to be a mistake, for it is very different from the so-called "inarticulate" tubar skeleton of the Heteropidæ and Amphoriscidæ. As I shall show later on, however, the distinction between "articulate" and "inarticulate" tubar skeletons is not altogether satisfactory.

The development of the dermal skeleton varies much in different species as regards its extent, but always follows the same pattern. The large longitudinally arranged oxea always form the most conspicuous feature, and frequently give to the surface of the sponge a very characteristic striated appearance. Nearly always, however, triradiates and minute surface oxea are also present, arranged as in Grantia. In Ute spiculosa and U. Spenceri (4) the triradiates are very numerous indeed, while in U. glabra (5), U. argentea, and U. syconoides (4) they are very scarce.

The presence of the longitudinally disposed oxeote spicules in the dermal cortex, although often giving the sponge a very characteristic appearance, is by no means peculiar to the genus Ute, for the same character is found in Leucandra alcicornis (5) and in two species of Homocœla Simplicia, viz. Leucosolenia asconoides (4, 12) and L. uteoides (16); and it is also characteristic of the genus Heteropia (4).

In Ute spiculosa (4), where the mesoderm is very strongly developed, we find, in accordance with the rule laid down above, that the skeleton of the chamber layer is dense and irregular, though showing traces of the normal articulate arrangement in the usually centrifugal direction of the basal rays of the triradiates.

In Ute Spenceri (4) we find one or two slight peculiarities in the skeleton. Thus we find, as in Grantia labyrinthica, a number of minute oxeote spicules on the gastral as well as on the dermal surface; and we find also that the exhalant canals are protected by quadriradiates of special form. The tubar skeleton is distinctly articulate, though becoming confused as it approaches the dermal cortex.

Synute (fig. 15).

In this sub-genus the dermal cortex and its contained spicules are enormously developed, so as to bind together all the individuals of a branching colony into one continuous whole (fig. 15). Here, as in other cases where the dermal cortex is very strongly developed, the cortical triradiates lose

their parallelism with the dermal surface and become irregularly scattered. In other respects the arrangement of the skeleton follows the ordinary Ute plan.

Utella.

In this genus, of which the type species is Haeckel's Sycandra hystrix (5), we find a very remarkable modification of the Grantia type of skeleton, for a layer of longitudinally disposed oxeote spicules is developed in the gastral cortex, instead of, as in Ute, in the dermal one. For a description and illustrations of Utella hystrix I must refer the reader to Haeckel's great work (5).

Anamixilla.

Here, again, the skeleton is very peculiar, as will be seen at once on referring to Polejaeff's drawing thereof (8). The flagellated chambers are elongated and radially arranged. The gastral and dermal cortex, each with its skeleton, is developed as in Grantia. There is, however, no articulate tubar skeleton, although it is important to notice that subgastral sagittal triradiates are still present, as in Ute argentea. The most remarkable feature of this sponge is the presence, in the chamber layer of the wall, of a number of large triradiate spicules, out of all proportion to the size of the chambers and arranged without regard to the direction of these, but generally lying across them and more or less parallel to the dermal surface. It looks as if the chamber layer had first lost the greater part of its own skeleton, all except the subgastral sagittal triradiates, and had then been invaded by large spicules from the dermal cortex.

Sycyssa.

In this very remarkable genus, for our knowledge of which we are entirely indebted to Haeckel (5), the canal system appears to be arranged upon the regular Grantia plan, but we are told that the skeleton is made up solely of oxeote spicules, there being no radiate spicules present of any kind. The skeleton of the gastral cortex consists of (1) a gastral layer of

irregularly arranged bundles of very slender oxea, and (2) a subgastral layer composed of regularly arranged, parallel, longitudinal bundles of large, stout oxea: this is comparable to the similar layer in Utella. The tubar or chamber skeleton is composed of the inner ends of enormous radially arranged oxeote spicules, comparable to those found in Grantia Vosmaeri, but larger and apparently more regularly arranged. The outer ends of these spicules project far beyond the dermal surface. The skeleton of the dermal cortex is composed of (1) a layer of very slender oxea, arranged irregularly but parallel to the dermal surface, and (2) a layer of very slender oxea, arranged radially and forming a kind of pile over the outer surface, as in so many Heterocœle sponges.

This genus, of which the only known species is Sycyssa Huxleyi, from the Adriatic, must be regarded as possessing a very aberrant type of skeleton, and occupying a very isolated position in the group.

Leucandra (figs. 16, 17).

To understand the structure of the skeleton in this genus we must return to that of Grantia, of which it is evidently a modification. We saw how in Grantia Vosmaeri (fig. 10)—where the exhalant canals have begun to elongate and the chambers to shorten, and where the mesoderm is very strongly developed in the inner half of the sponge wall—the skeleton has begun to lose its regular articulate character. The same thing is carried to a much greater extent in Leucandra, where the canal system has lost its symmetry and the skeleton has followed suit (figs. 16, 17). The skeleton of the gastral and dermal cortex, however, retains the same structure as in Grantia; it is only the skeleton of the chamber layer which is affected by the changes in the canal system. Hence we find that in a typical Leucandra (fig. 16) the skeleton of the gastral cortex is made up principally of quadriradiate spicules whose apical rays project into the gastral cavity, while the skeleton of the dermal cortex is made up of triradiates, lying parallel to the dermal surface, and longer or

shorter oxeote spicules usually projecting more or less at right angles therefrom. The skeleton of the chamber layer, on the other hand, consists of irregularly scattered triradiates or quadriradiates.

In some species, e.g. L. phillipensis and L. australiensis, the quadriradiates of the gastral cortex are continued inwards along the larger exhalant canals, which possibly indicates a folding of the wall of the gastral cavity, though I do not think it necessarily does so.

In some species, e.g. L. phillipensis, we find many of the triradiates of the chamber layer retaining their primitive sagittal character, and with the basal ray pointing towards the dermal surface. This may be a reminiscence of the time when they formed part of an articulate tubar skeleton. Subgastral sagittal triradiates may also still be present (figs. 16, 17).

Lelapia.

In this genus of a single species the canal system is unknown, and the skeleton is peculiar. The skeleton of the gastral cortex is composed of ordinary radiates; while that of the dermal cortex is composed of triradiates, quadriradiates, and minute oxea. The skeleton of the chamber layer is composed of large, longitudinally arranged oxea, crossed at right angles by bundles of tuning-fork shaped triradiates whose basal rays point towards the dermal surface. For further particulars I must refer the reader to Mr. Carter's description of the sponge (12), as I have never had an opportunity of examining it myself.

I should, perhaps, mention that Mr. Carter describes the occurrence of small quadriradiate spicules "on the surface," presumably in the dermal cortex. Triradiate spicules are very apt to develop an apical ray, often so small as to be of no conceivable use, and apparently indicating a mere "sport," if one may use the term. Therefore, unless the feature could be shown to be constant, well established, and characteristic, I should not be inclined to attribute any great value to it. It

certainly appears to be a difficulty in the way of utilising the presence of subdermal quadriradiate spicules as a family characteristic in the Amphoriscidæ, but in the latter case the apical rays are so constant, and form, in most cases, such an important part of the skeleton, that we must treat the question from a different standpoint.

Leucyssa.

In the skeleton of this genus we meet with a parallel case to that of Sycyssa, only associated this time (presumably) with a Leuconoid canal system.

The genus is very imperfectly known, and what we do know about it we owe chiefly to Haeckel (5). Two of the three species (L. spongilla and L. cretacea) are described from single specimens, and a third (L. incrustans) is extremely rare. In all, the skeleton appears to be quite irregular, consisting of a felt-work of oxeote spicules. In L. spongilla the spicules are spindle-shaped, straight, and smooth. In L. cretacea they are swollen at one end and pierced like a sewing-needle; and it is interesting to note that similar spicules are found in Leucandra ochotensis (5). In Leucyssa incrustans (Carter's Trichogypsia villosa), a European species which, thanks to the kindness of Mr. Carter, I have had the opportunity of partially examining (in the dry state), the skeleton consists of a very dense, irregular felt-work of spinose oxea.

Grantessa (fig. 18).

In this genus we have an entirely new element introduced into the composition of the skeleton, in the form of subdermal sagittal triradiate spicules. The existence of these spicules in certain species has long been recognised, but their importance from a systematic point of view appears to me to have been under-rated. They never appear, so far as my experience goes, in sponges without a dermal cortex, and they seem to be first introduced as additions to the ordinary Grantia type of skeleton. Thus in Grantessa sacca (4, 11) we find all the parts of a typical Grantia skeleton present,

including the skeleton of the dermal and gastral cortex and a normal articulate tubar skeleton; but in addition to these parts we find also a well-developed layer of sagittal triradiates, whose oral rays are extended in the dermal cortex parallel to the surface, while the elongated basal ray projects inwards for some little distance into the chamber layer. The position of these spicules is thus exactly the reverse of that of an ordinary tubar triradiate, the basal rays pointing in exactly opposite directions in the two cases.

In Grantessa intusarticulata (4), the anatomy of which is represented in fig. 18, we find a precisely similar condition, but the chambers are shorter, and the number of joints of the articulate tubar skeleton correspondingly fewer. In this species, also, it is highly interesting to notice that in the youngest portions of the sponge, nearest the osculum, where the chambers are shortest, the skeleton of the chamber layer consists solely of the basal rays of the subdermal and subgastral sagittal triradiates, so that we have here a typical example of the so-called "inarticulate" tubar skeleton. In other words, we find the two types of tubar skeleton, articulate and inarticulate, existing side by side in the same sponge. In Grantessa poculum, an excellent figure of which, under the name Amphoriscus poculum, is given by Poléjaeff (8), the skeleton is almost exclusively "inarticulate," but I find from my own examination of the sponge that the inner triradiates are not always strictly subgastral, but may be situate some little distance beneath the gastral cortex.

The dermal and gastral skeleton in this genus agrees so closely with that of Grantia as to require no further comment (compare figs. 9 and 18), unless we specially mention the fact that in some species of Grantessa the oxeote spicules of the dermal surface are collected into tufts, which, however, bear no relation to the radial chambers. This character, which is well shown in Grantessa sacca, first gave origin to von Lendenfeld's genus Grantessa (11). I do not believe it one of great systematic value; and I do not hesitate to include in the same genus species like G. intusarticulata and G.

poculum, in which the oxeote spicules are not collected into tufts.

Heteropia.

This genus bears precisely the same relation to the genus Grantessa as Ute does to Grantia, the skeleton being of the ordinary "inarticulate" Grantessa type, but with the addition of large longitudinally arranged oxea in the dermal cortex. For further details I must refer the reader to Mr. Carter's description of Heteropia (Aphroceras) ramosa (15).

Vosmaeropsis (fig. 19).

All three species included in this genus (4) exhibit, like Grantessa and Heteropia, a well-developed layer of subdermal sagittal triradiates; but as the other parts of the skeleton vary somewhat in the three cases, it will be advisable to consider them separately.

Vosmaeropsis macera, the anatomy of which is represented in fig. 19, shows least deviation from the ordinary Grantessa type. There are, however, no quadriradiates in the gastral cortex, but I do not consider this a very important character. The oxeote spicules of the dermal cortex are also very variable in their development, and may be almost, if not quite, absent; but this, again, is not an important character. Otherwise, the skeleton agrees closely with that of an ordinary Grantessa in which no distinct articulate tubar skeleton is developed.

In Vosmaeropsis depressa (4) the skeleton is modified, in accordance with the massive form of the sponge and the arrangement of the canal system. The bulk of the skeleton is made up of fairly large, subregular, or slightly sagittal triradiates, scattered without definite order throughout the thickness of the sponge, many having one slightly longer ray pointing towards the dermal surface. Beneath the dermal surface, but apparently only on the upper surface of the cushion-shaped sponge, is a distinct layer of subdermal sagittal triradiates, with inwardly directed basal rays. The

dermal skeleton is made up principally of subregular triradiates of various sizes, placed horizontally, but with no definite arrangement; amongst these very minute slender oxea are scattered, especially numerous around the osculum.[1] Around the main exhalant canals is a layer of small sagittal triradiates, forming what must probably be regarded as the gastral skeleton. No quadriradiates are present, and I have not detected any special subgastral sagittal triradiates. The skeleton of this sponge bears much the same relation to that of Grantessa as that of Leucandra does to that of Grantia.

Vosmaeropsis Wilsoni is remarkable for the enormous development of the cortex and its contained skeleton on both gastral and dermal surfaces. The skeleton of the chamber layer is like that of Vosmaeropsis macera (fig. 19), but the sagittal triradiates are of unusually large size. A well-developed oscular skeleton, in the shape of a fringe of oxeote spicules, is also present, but this is not a feature upon which much stress need be laid in any case.

Heteropegma (fig. 20).

We have seen how, in Grantessa, the very characteristic skeleton is derived from the ordinary Grantia type by the mere addition of a layer of subdermal sagittal triradiate spicules. The skeleton of Heteropegma is also derivable from the Grantia type by an analogous change, only the subdermal spicules are quadriradiates and not triradiates. Moreover it is important to observe that the subdermal quadriradiates of Heteropegma and other Amphoriscidæ are not modifications of subdermal sagittal triradiates, such as are found in the Heteropidæ. If they were so, we should expect to find the basal ray still pointing inwards towards the gastral cavity, and the additional apical ray lying in a plane more or less parallel with the dermal surface. As a matter of fact, however, the three facial rays of the subdermal quadriradiates lie parallel

[1] Oxeote spicules are sometimes developed around the osculum when they cannot be found anywhere else in the sponge.

with the dermal surface, and it is the apical ray which is directed inwards through the chamber layer. Hence the position of these spicules is quite different from that of the subdermal sagittal triradiates of the Heteropidæ, while in the one case it is the basal ray in the other it is the apical one which plays such an important part in the support of the chamber layer.[1]

The anatomy of Heteropegma nodus-gordii is represented in fig. 20. It will be seen that the dermal cortex is strongly developed, and has the ordinary Grantia structure, except that there are no oxeote spicules. The gastral cortex is extremely thin and its skeleton greatly reduced, consisting of a number of very small quadriradiates arranged in the usual manner. The articulate tubar skeleton is also very much reduced, consisting of very minute spicules, chiefly quadriradiate. This reduction in the skeleton of the chamber layer is partly compensated for by the presence of very large subdermal quadriradiates, whose facial rays are extended in the inner part of the dermal cortex, while the apical ray penetrates through the chamber layer almost to the gastral surface.

The skeleton of the Victorian Heteropegma latitubulata (4) resembles that of H. nodus-gordii, except in certain very minute details of spiculation, the tubar and gastral spicules being still further reduced in size.

Amphoriscus.

In this genus the articulate tubar skeleton, which was still clearly recognisable in Heteropegma, has disappeared, although the radial Syconoid character of the canal system remains. In one species, however (Poléjaeff's Amphoriscus elongatus [8]), the subgastral sagittal triradiates still persist. We always find, as in Heteropegma, a layer of subdermal quadriradiates with inwardly directed apical rays; and we also find, in addition to these, in nearly all species, a layer of subgastral quadriradiates with outwardly directed apical rays.[2]

[1] The subdermal quadriradiates are probably derived from the ordinary triradiates of the dermal cortex by the development of an apical ray. Compare my remarks on the skeleton of Lelapia.

[2] These spicules are not homologous with the subgastral quadriradiates of

Hence the chamber layer is usually supported solely by the apical rays of quadriradiates, which pierce it from opposite directions. In Amphoriscus cylindrus and A. cyathescus (4, 5) we learn from Haeckel that the dermal cortex is composed entirely of the facial rays of quadriradiates, there being no triradiates left. This fact supports the view that the subdermal quadriradiates of the Amphoriscidæ are merely modifications of the ordinary dermal triradiates, which have developed an apical ray without changing their position. In the case of the subdermal sagittal triradiates of the Heteropidæ, on the other hand, the position of the spicules is quite different from that of ordinary dermal triradiates.

Syculmis.

The skeleton of Syculmis synapta (5), the only known species of the genus, agrees in the main with that of Amphoriscus, but is further complicated by the addition of a root-tuft of oxea and anchoring quadriradiates, which is, I believe, without parallel amongst the Calcarea. For further details I must refer the reader to Haeckel's monograph.

Leucilla (figs. 21, 22).

In this genus, again, we always find dermal or subdermal quadriradiates with inwardly directed apical rays. These form the most constant feature of the skeleton, the other parts being somewhat variable in different species. A gastral cortex, constructed as in Grantia, is probably always present, and sometimes, as in Leucilla uter (fig. 21) and L. australiensis (fig. 22), subgastral sagittal triradiates are still recognisable, indicating probably the derivation of the skeleton from the Grantia type. All the triradiates of the dermal cortex may be converted into quadriradiates by the development of a long inwardly directed apical ray, as in Leucilla uter (fig. 21); or some of them may still retain their primitive triradiate character, as in L. australiensis (fig. 22).

Grantiopsis, or of Polejaeff's Grantia tuberosa (8), which are only slight modifications of the ordinary subgastral sagittal triradiates.

Dermal oxea, like those of Grantia, may also be present (fig. 21). The chamber layer is generally further supported by other quadriradiate spicules, which may have a more or less definite subgastral position (fig. 21), or be irregularly scattered through the chamber layer (fig. 22), or by irregularly scattered triradiates, as in Leucilla prolifera (4).

The skeleton of Leucilla (Leucandra) cucumis, Haeckel (5), exhibits a further complication of the Leucilla type. The skeleton of the dermal cortex is made up of an outer layer of triradiate spicules, as in Grantia, and an inner layer of quadriradiates with inwardly directed apical rays. These quadriradiates help to form a framework for a remarkably regular series of subdermal cavities, and are assisted in so doing by a second, deeper layer of quadriradiates with outwardly directed apical rays. Within this deeper layer of quadriradiates comes the chamber layer of the sponge wall, supported by irregularly scattered quadriradiates; and within this comes the gastral cortex, composed of triradiate spicules. In addition to the spicules mentioned, large oxeote spicules are found arranged longitudinally between the dermal triradiates. Illustrations of the skeleton of this remarkable sponge will be found in Haeckel's great work.

Summary.

We find, from our survey of the various genera of Heterocœla, that the starting-point for the development of the skeleton throughout the group (leaving out of account Leucascus, which may have originated independently from the Homocœla) is the radially symmetrical skeleton of Lycetta. This primitive radial symmetry is highly characteristic of the group, and is obviously dependent upon the primitive radial symmetry of the canal system. The first great change in the structure of the skeleton is brought about by the development of a dermal cortex, in which a special skeleton is formed. The skeleton of the chamber layer of the sponge wall now begins to vary. This variation is in some cases obviously dependent upon the gradual change of the canal system from the

Syconoid to the Leuconoid condition. This change brings about a loss of radial symmetry in the chamber skeleton, which is transformed from the regular "articulate" type of Sycetta, Sycon, Grantia, to the irregularly scattered condition of Leucandra. Other modifications, however, also take place, which are obviously not dependent on the variation of the canal system, and which are consequently of the utmost importance for systematic purposes. These modifications consist in the development of subdermal sagittal triradiate or of subdermal quadriradiate spicules, which characterise the two families Heteropidæ and Amphoriscidæ respectively. Both these modifications are first instituted (in the genera Grantessa and Heteropegma respectively) while the canal system still retains its primitive radial symmetry and its articulate tubar skeleton (figs. 18, 20), and in both cases they are retained, while the canal system gradually changes from the Syconoid to the Sylleibid or Leuconoid type, and the primitive articulate tubar skeleton disappears (figs. 19, 22). Thus the primitive centrifugal radial symmetry of the skeleton is lost as the canal system changes from the Syconoid to the Leuconoid type, but in the Heteropidæ and Amphoriscidæ it is to a certain extent replaced by a kind of secondary centripetal radial symmetry, due to the development of subdermal radiates with inwardly directed basal or apical rays. Indications of the primitive radial symmetry are sometimes found in the presence of subgastral sagittal triradiates after all other traces of the articulate tubar skeleton have disappeared. The gastral cortex, as might be expected, is subject to less modification than any other portion of the skeleton, and does not vary to any great extent throughout the group. A striking exception to this rule is, however, found in the genus Utella. Very startling exceptions to the ordinary rules of skeletal structure are found in the genera Sycyssa and Leucyssa, apparently due to the loss of all radiate spicules, and the development in their stead of oxea.

In Leucascus the skeleton appears never to have

reached the radially symmetrical condition, but to have remained in the scattered one found amongst the reticulate Homocœla.

IV.—The Histology of the Heterocœla Calcarea.

All my observations upon the histology of the Heterocœla Calcarea have been made upon spirit-preserved specimens, and I cannot therefore claim for them the same value as attaches to the recent observations of Bidder (**21—24**) and Minchin (**25, 26**), who were able to study the sponges in the living condition. Nevertheless even a cursory examination of my figures 23 to 64 will, I hope, show that there is a good deal of histological detail to be made out even in spirit-preserved specimens of calcareous sponges.

With regard to the classification of the tissues, I still maintain the opinion which I expressed in my memoir on the anatomy of Grantia labyrinthica (**9**)—that is to say, I still follow Schulze (**27**) in considering that the ectoderm of the larval sponge (at any rate in the Calcarea) furnishes not only the epithelium of the dermal surface, but also that of the entire inhalant canal system; that the endoderm lines the remainder of the canal system (including, of course, the flagellated chambers) from the prosopyles to the osculum, and that the mesoderm furnishes all the remainder of the sponge body.

As to the homology of the three layers in sponges with the similarly named layers in higher animals, I do not presume to offer an opinion. It is sufficient for our present purposes that three layers exist in sponges; and as the names ectoderm, mesoderm, and endoderm have come into general use for these layers, and serve admirably to express their relations one to the other, I naturally adopt them in this place.

The Ectoderm.

This forms a single layer of epithelial cells which lines the external surface of the sponge, and also the whole inhalant

canal system, from the dermal pores to the prosopyles. If we consider the manner in which the inhalant canal system of the more highly developed Heterocœla has been derived from the Sycetta condition, by the closing in of a space which originally lay altogether outside the sponge, it is obvious that Schulze's view as to the extent of the ectoderm must be correct.

I also believe that Schulze was perfectly correct in his opinion (7) as to the structure of the ectoderm. In the case of Grantia labyrinthica I stated that the ectoderm resembles exactly what Schulze has described in Sycon raphanus, "consisting of a single layer of flat, polygonal epithelial cells lining the dermal surface of the sponge and the inhalant canal system. These cells are most readily distinguished around the inhalant canals, where they are less obscured by spicules and other mesodermal structures than on the dermal surface. The nucleus is surrounded by the very characteristic granules described by Schulze in Sycandra. In my preparations I have only after some trouble succeeded in making out the boundary lines between the individual cells, and Schulze himself observes that it is remarkable that the boundaries of these cells—sometimes so distinct—are not always clearly visible. Nevertheless I have been able to determine the shape of the cells pretty accurately, and found them to agree precisely with Schulze's drawings" (9).

Since the above was written I have also described and figured very carefully the ectodermal epithelium of a Homocœle sponge, Leucosolenia Wilsoni (1). "It consists of thin, flattened, plate-like cells, polygonal in outline, and each with a swelling in the centre where the nucleus is situate. The cell itself averages about 0·0136 mm. in diameter, and the nucleus, which is very distinctly outlined and more or less spherical (or perhaps somewhat flattened in the same manner as the cell), has a diameter of about 0·0034 mm. Within the nucleus appear a few small, deeply staining granules. Around the nucleus the protoplasm is highly granular, exactly as described by Schulze, while towards the periphery of the cell it becomes gradually hyaline. Adjacent cells are in contact at the edges,

and all together form a single-layered, continuous epithelium over the outside of the Ascon-tube. As a rule, in ordinary preparations, although the nuclei and granules of the ectoderm-cells may be clearly enough visible, it is very difficult to distinguish the outlines."

In writing the above I regret to say that I overlooked Metschnikoff's very precise and clear account (28) of the ectoderm of Leucosolenia (Ascetta), published long before, in which he describes and figures an ectodermal epithelium precisely similar to that previously described by Schulze in Sycon raphanus, and subsequently by myself in Grantia labyrinthica and Leucosolenia Wilsoni. In the same paper (28) Metschnikoff also states that the ectoderm of Leucandra aspera has the same structure.

Metschnikoff's latest views on the subject of the ectoderm in sponges in general are contained in the following very interesting passage from his recent work on "Inflammation" (29). After pointing out that the body of the sponge is composed of three characteristic layers, he observes: "La couche superficielle, ou l'ectoderme, revêt le corps entier de cellules épithéliales plates, limitées entre elles par des contours qui deviennent très nets après l'application d'une solution de nitrate d'argent. Les cellules mêmes sont visiblement contractiles, ce qui s'observe surtout aux bords libres des jeunes individus, où on aperçoit des prolongements amiboïdes appartenant aux éléments ectodermiques. La contractilité de ces cellules joue certainement un rôle dans le phémomène remarquable de l'ouverture des pores nombreux, éparpillés sur la surface de l'éponge entière, et apparaissant entre deux ou plusieurs cellules plates."

Minchin also (25, 26) concludes that the normal condition of the ectoderm in Leucosolenia clathrus, the form specially studied by him, is flattened and plate-like, and he also ventures upon the generalisation that "the contractile elements in all cases are the flattened ectodermal epithelium." The contractile nature of the ectoderm-cells I do not doubt; I believe it to be an important character, and I shall presently

offer some slight evidence of a similar contractility amongst the Heterocœla.

Taking into consideration all the above evidence, I think we may regard it as an established fact that the ectoderm, at any rate in all calcareous sponges, like that of Sycon raphanus, normally consists of flattened plate-like polygonal cells with centrally placed nuclei. I shall presently have to bring forward fresh evidence in support of this view.

A peculiar "flask-shaped" condition of the ectodermal cells has, however, been described from time to time in various sponges, and has recently received a good deal of attention from Bidder and Minchin.

This condition was first described by Metschnikoff (**28**) in an Olynthus form of Leucosolenia, and the peculiarity appears to consist in the nucleus being suspended, as it were, in an envelope of protoplasm from the under surface of the plate-like cell. Bidder (**21**), in criticising my description of the flattened ectoderm of the Homocœla, observes, "Although this form occurs in the Homocœla, it is, in my experience, rare. The typical ectoderm (e. g. Ascetta clathrus) I find composed of onion-shaped gland-cells containing a nucleus and granules, and provided with a usually fine duct, the expanded end of which forms the hexagonal area whose boundaries are, in the case of most sponges, all that has been observed."[1] Bidder identifies his "onion-shaped gland-cells" with the cells above referred to as described by Metschnikoff in an Olynthus form, and gives a list of species in which he has observed this condition. In his later papers he brings forward additional evidence for believing "that in all groups of sponges the flask-shaped epithelium does occur" (**24**). He also identifies (**24**) the "pendent cell body" of his flask-shaped cells with the subdermal gland-cells which have been described by von Lendenfeld in the Keratosa and by myself in Grantia labyrinthica. This identification is probably correct, but I

[1] It appears to me that it is generally much more easy to see the nuclei than it is to see the outlines of the cells.

do not think that Bidder has by any means proved that the "pendent cell body" belongs to the ectoderm at all. It seems quite as reasonable to believe that it is the presence of subdermal gland-cells, in many cases at any rate, which has led Bidder to believe in the general occurrence of flask-shaped or onion-shaped glandular ectoderm-cells. Until we have more light on the subject I prefer to retain my old belief in subdermal gland-cells, but I am quite open to conviction on this point, which, with the material at my disposal, I am incapable of deciding for myself.

The observations of Minchin (**25, 26**) are also very noteworthy in this connection. This author explains the flask-shaped condition as due simply to contraction of the normal, flattened, plate-like cells, and his observations upon Leucosolenia certainly support this view. Subdermal gland-cells do not appear to occur, at any rate as a rule, in the Homocœla, and so far as these sponges are concerned I am inclined to accept Minchin's explanation.

I must now describe what few additional observations I have myself been able to make on the ectoderm of the Heterocœla. These observations support the view that the ectoderm is composed of a single layer of flattened plate-like cells, which retain to a greater or less extent the power of contraction. I believe that the character of this epithelium is, usually at any rate, the same throughout, from osculum to prosopyles, although it is much more difficult to detect on the exposed outer surface of a dermal cortex than on the protected surface of an inhalant canal. I have observed it most satisfactorily in a specimen of Vosmaeropsis Wilsoni (**4**) which I took the precaution to kill with osmic acid. This sponge is provided with a very distinct oscular sphincter, which has much the same structure as that described by Minchin in Leucosolenia clathrus (**25**). It is composed of two layers of epithelial cells with probably a very thin layer of gelatinous mesoderm (ground substance) between them. Both layers of cells are regarded by Minchin in Leucosolenia as ectodermal, and (as a mere matter of convenience, for we must draw the line between

ectoderm and endoderm somewhere) I follow him in this respect, and regard both layers of epithelial cells in the oscular diaphragm of Vosmaeropsis Wilsoni as ectodermal also. As there is no structural distinction between the flattened endodermal cells which line the gastral cavity in Heterocœle sponges and the ectodermal cells, it is impossible to say exactly where one begins and the other ends.

A portion of the epithelium which covers the upper surface of the oscular diaphragm in the sponge in question is represented in fig. 63, drawn from an osmic acid preparation mounted in glycerine. The cells are mostly polygonal, and fit tightly together by their edges. Each has the form of a flat plate, with usually a single nucleus situated in about the centre. The nucleus is small, and in osmic acid preparations looks clearer and more transparent than the surrounding protoplasm. The latter contains numerous granules, larger and more abundant towards the centre of the cell, which are stained very darkly by the osmic acid. The majority of the cells are about as broad as they are long, but some of them are fusiform, like those of the Leucosolenia diaphragm figured by Minchin in his fig. 12. On the under surface of the diaphragm a similar layer of cells occurs, but these are not so deeply stained by the osmic acid, doubtless owing to want of penetration.

I do not doubt that these epithelial cells are contractile, and that their contractility serves to open and close the diaphragm.

If we trace this epithelium from the oscular diaphragm up over the lip of the osculum and on to the outer surface of the sponge, we find that it maintains exactly the same character throughout, covering the whole outer surface of the sponge with a layer of flattened cells. The dermal cortex is penetrated by very numerous inhalant pores (fig. 23), and on reaching the margin of one of these the ectodermal epithelium turns in, and is continued as a lining to the inhalant canal system, a lining which resembles minutely the epithelium of the oscular diaphragm, and the component plate-like cells of which are

extremely conspicuous in sections of osmic acid specimens (fig. 23).

Sometimes in sections of ordinary spirit-preserved material, cut by the paraffin method, the flattened cells of the ectodermal epithelium appear separated from one another by considerable intervals, as is shown in fig. 29, representing a portion of the epithelial lining of the inhalant canal system of Grantessa intusarticulata, and in fig. 59, representing epithelium from a corresponding situation in Sycon Ramsayi. This appearance is evidently due to contraction of the cells, which may still remain connected in places by strands of protoplasm which stretch across from one to the other. Both ectodermal and endodermal epithelial cells appear to be very subject to such contraction, and it appears to me very possibly to indicate a normal contractility in life, such as is described by Metschnikoff (29).

It has been recently maintained by Bidder (23) and Minchin (26) that the prosopyles which pierce the walls of the flagellated chambers are formed each by the perforation of a single nucleated cell; but, while Bidder attributes to these cells an endodermal origin, Minchin regards them as derivatives of the ectoderm. For my own part I am disposed to regard the prosopyles as inter-cellular and not intra-cellular in nature, and Schulze's admirable drawings of the anatomy of Sycon raphanus (7) point to the same conclusion. My own drawings of the anatomy of Grantia labyrinthica show exactly the same condition, and in the very numerous sections of calcareous sponges which I have examined, I have met with no evidence to cause me to doubt the correctness of the view that the prosopyles are simply gaps between cells. Bidder, indeed (21), interprets the remarkable groups of yellow granules which I described in Leucosolenia cavata (1) as perforated prosopyle cells. I do not deny the possibility of this view, but I do not think that the evidence is sufficient to justify it, and, in any case, the occurrence of such structures (whatever they may be) is in my experience very rare even in the Homocœla, and altogether unknown in the Heterocœla, of

which I have examined very numerous species. Therefore, even if we admit that perforated prosopyle cells occasionally occur, there is no reason for believing that this is the usual condition, but quite the contrary. When viewed en face the prosopyles of calcareous sponges usually appear as sharply defined approximately circular spaces (figs. 25, 30). Not infrequently the nucleus of an ectodermal epithelial cell happens to lie exactly on the margin of this space (fig. 30), but I do not think there is any reason for regarding this fact as indicating that the prosopyle itself is of an intra-cellular nature; while sections such as those figured by Schulze in the case of Sycon raphanus, and by myself in the case of Grantia labyrinthica, clearly indicate the contrary.

Before leaving the question of the ectoderm I may conveniently describe in this place a very curious condition which exists in Grantiopsis cylindrica (4). This highly interesting Australian species is known only from a few fragments, probably all belonging to the same specimen. These fragments are, however, in a remarkably good state of preservation, but notwithstanding this fact it is a difficult matter to make out the epithelial cells either on the outer surface of the sponge or in the somewhat complicated inhalant canal system. On the other hand, we find in both these situations a very peculiar layer of minute granules, which, when examined under a Zeiss F objective, presents in surface view the appearance shown in fig. 57, and in sections at right angles to the dermal surface the appearance shown in fig. 56. The granules themselves appear slightly elongated and very thickly and evenly scattered over the surface. Embedded in the gelatinous ground substance of the mesoderm, at some little distance beneath the dermal surface, are found numerous very distinct subdermal gland-cells, connected by thread-like processes with the granular layer, as shown in fig. 56. The granules themselves do not stain with borax carmine, and present, under the Zeiss F obj., a very striking resemblance to bacteria. So great is this resemblance that I submitted a fragment of the sponge to Mr. Thomas Cherry, Demonstrator in Pathology in

the Melbourne University, who kindly made a careful examination of it for me, with the following results. The granules were scraped into a glass, dried, stained with fuchsin, mounted in Canada balsam, and examined under a $\frac{1}{12}$-inch oil immersion objective (Leitz) with a No. 5 eye-piece. We then found that they presented the appearance shown in fig. 58, being usually each somewhat dumb-bell shaped, or composed of two ovoid segments placed end to end, though sometimes the shape was irregular. They averaged about 0·004 mm. in length, and consisted of a fairly darkly stained, sometimes slightly granular body, with a round very darkly staining structure, presumably a nucleus, in each end of the dumb-bell, or, when the shape was irregular, with several such nuclei. It thus appears that, though probably not bacteria, the bodies in question are micro-organisms of some kind which live upon the surface of the ectoderm. Nuclei, presumably belonging to the epithelial cells, are still visible amongst the granules, at any rate in the inhalant canals, and I am inclined to think also on the outer surface of the sponge; but we have here an excellent illustration of the way in which the structure of the ectoderm is sometimes obscured by the accumulation of foreign bodies.

Bidder would possibly interpret the subdermal gland-cells of Grantiopsis cylindrica as representing the true ectodermal epithelium. But they do not look like it (fig. 56). They appear to be but very slight modifications of the ordinary stellate mesodermal cells, and as such they will be described on a future page.

The Endoderm.

I have little to add concerning the histology of the endoderm to what I have already written on the subject. As already stated, I consider as endoderm not only the collared cells which line the flagellated chambers, but also the flattened pavement epithelium which lines the gastral cavity and exhalant canals of the Heterocœla. It is, of course, quite conceivable that this pavement epithelium is formed by an

ingrowth of the ectoderm from the region of the osculum, replacing and, as it were, pushing back the collared cells. I do not, however, consider that there is any reason for supposing this to be the case. In the case of the Homocœla I have shown (1) that the lining of the gastral cavity is not always composed entirely of collared cells, but that the apical rays of the quadriradiate spicules which frequently project into this cavity are clothed with a layer of flattened epithelium. I suggested at the time that this epithelium might possibly be derived from the mesoderm; but, on the other hand, it might with equal justice be regarded as indicating a transformation of collared cells into permanent cells, and we are quite at liberty to suppose that a similar transformation, only on a much larger scale, has taken place in the gastral cavity and exhalant canals of the Heterocœla.

The epithelium which lines the gastral cavity and exhalant canals resembles minutely the ectodermal epithelium which clothes the outer surface of the sponge and lines the inhalant canal system. This will be evident from a comparison of figs. 59 and 60, representing respectively the epithelium from an inhalant and from an exhalant canal of Sycon Ramsayi, the differences which exist between the epithelium in these two situations being obviously very slight and insignificant. Similarly fig. 64 represents the epithelium from an exhalant canal of Vosmaeropsis Wilsoni, which, if we allow for the contraction of the cells due to difference in the method of preparation, is practically identical with the ectodermal epithelium from the same sponge represented in fig. 63. Indeed, unless specially prepared, as with osmic acid, the ectodermal epithelium, as already pointed out, exhibits a precisely similar contraction, and in both cases this is such a constant and well-marked feature that I am inclined to think that it betokens a normal contractility during life. The appearance of the endodermal pavement epithelium, as seen in section, is shown in figs. 27, 28, and 61. The swelling in the centre of the cell, where the nucleus is situated, is frequently very strongly marked, and causes the epithelium to

be very conspicuous in sections taken at right angles to its surface.

In an undetermined specimen of Leucandra, from Port Jackson, the epithelial cells lining the gastral cavity just within the osculum are so much swollen out as to be almost as thick as they are broad, and they also present a vacuolated blister-like appearance, as shown in fig. 32. Lower down in the gastral cavity the epithelial cells have the normal flattened form.

The flagellated chambers of most, if not all Heterocœle sponges, whether they be of the elongated Syconoid or of the rounded Leuconoid form, are separated from the exhalant canals into which they open by very distinct membranous diaphragms (figs. 25—27, 31). When viewed en face these diaphragms have usually the appearance shown in fig. 31, consisting of a thin, transparent membrane, in which are visible spindle-shaped cells arranged concentrically. The actual outlines of the cells I have not succeeded in distinguishing, but their elongated character and concentric disposition are usually clearly indicated by the arrangement of the granules which surround the nucleus. Collared cells are never found on either surface of this membranous sphincter, and whether it consists of one or two layers of cells is a very difficult question, which I have not been able definitely to decide. The concentrically arranged, spindle-shaped cells are obviously muscular, and as such I described them in my memoir on Grantia labyrinthica. In that paper, however, I classed them as mesodermal elements. I am now convinced that they are simply modifications of the cells which line the exhalant canals, comparable to the muscular cells of the oscular diaphragm described by Minchin in Leucosolenia, and by myself in Vosmaeropsis. Fig. 27 shows how the epithelial cells of the exhalant canal are continued on to the surface of the chamber diaphragm. Usually they appear very much elongated in this situation, as shown in fig. 31, but sometimes the elongation is scarcely visible at all, as shown in figs. 25 and 26. Probably the exact form of the cells depends upon their state of contraction. The epithelial cells of the exhalant

canals themselves may exhibit elongation and concentric arrangement around these canals, but not to the same degree as in the chamber diaphragms.

The chamber diaphragms, then, I regard as being composed of endodermal muscular cells, formed by modification of the ordinary endodermal pavement cells. Whether they consist of one or two layers of these cells is doubtful; probably of two layers, with a small amount of gelatinous ground substance between.

It thus appears that, if Minchin is right in regarding the muscular cells of the oscular diaphragm in Leucosolenia as ectodermal, and if Schulze is right in regarding the lining of the exhalant canals in Heterocœle sponges as endodermal, then both the ectoderm and endoderm may give rise to strikingly similar muscular structures. Possibly this may be an argument for regarding the muscular cells of the oscular diaphragm in Leucosolenia as endodermal and not ectodermal; but it appears to me that this is a question which cannot be decided in the present state of our knowledge.

Concerning the remaining endodermal cells, the collared cells which line the flagellated chambers, I have little to say. I may, however, call attention to fig. 25, representing half of a flagellated chamber of an undetermined species of Leucandra, in which Sollas's membrane and the collars of the collared cells are remarkably clearly seen. This figure also shows the exhalant aperture with its membranous diaphragm, and two prosopyles. Whether or not Sollas's membrane is continuous over the prosopyles I have been unable to determine, and it appears to me that the point can only be decided by the examination of living specimens.

I quite agree with Bidder (**21**) that the endoderm is not only multiform, but also "most proteic," as, indeed, was long ago shown by Carter (**30**) from observations on the living Sycon (Grantia) compressum, in which he observed the collared cells becoming amœboid, and moving about the field of the microscope. I am therefore quite prepared to believe that the existence of Sollas's membrane may be only a transitory condition, due to special circumstances, of which we are as yet

ignorant. That it does exist in many cases is beyond dispute, and it is extremely interesting to learn that Bidder (21) has observed in the living Sycon raphanus the coincidence of the flagella of the collared cells with Sollas's membrane, just as I described it in Halichondria panicea (31).

A curious illustration of the polymorphic nature of the collared cells, is afforded by the peculiarly contracted chambers which I described in my memoir on Grantia labyrinthica, and indicated in my drawings of the anatomy of that sponge by the letter *x*. I have now observed the same phenomenon in other Heterocœle sponges—viz. Ute syconoides and Leucandra phillipensis—and am very glad to be able to give some more exact details as to the form and arrangement of the collared cells in these cases. Fig. 24 represents a contracted chamber of Ute syconoides shown in transverse section, and surrounded by four ordinary chambers and four intercanals. It will be seen that the layer of collared cells has shrunk away from the tubar skeleton, drawing the mesodermal tissue after it. The collared cells themselves have become radially elongated, and, owing to their having a smaller area to spread themselves over, very much crowded. It was doubtless this crowded condition which caused me to describe them as being arranged in more than one layer in the case of Grantia labyrinthica. Each cell is more or less pyramidal in form, and the nucleus is situated in the apex of the pyramid towards the lumen of the chamber.

In my earlier paper (9) I endeavoured to explain the occurrence of these contracted chambers (which exhibit a very different appearance from chambers in which contraction has been produced by the action of the preserving fluid) by supposing them to represent old and exhausted chambers in process of dying, and destined to be replaced by the development of new chambers.

Bidder, in his very interesting "Note on Excretion in Sponges" (23), has suggested a different explanation. After describing the accumulation of "spherules" in the bases of collared cells, he adds, "I believe these basal spherules to be

stores of nutritious matter. In Leucandra aspera and Sycon raphanus .... the collar cell, after it has accumulated a certain quantity of spherules in its base, splits off this base by a transverse fission as a non-nucleated mass of protoplasm, which we may term a 'plinth' (fig. 4); the plinth then lies between the nucleated distal part of the cell—the 'column'—and the mesodermal jelly in S. raphanus, or the thin basement membrane which is all that usually divides the epithelia in L. aspera. I have observed in the mesoderm of S. raphanus large wandering cells, which I believe to be generative elements, with pseudopodia attached to these plinths, aud spherules of the same character as the basal spherules, both in the wandering cells and in their pseudopodia. There can be little doubt that they were feeding on ths reserve stores of the collar-cells. The division into column and plinth takes place as a rule at the same time in all or most of the cells of a chamber. The 'columns' or distal parts appear as small, columnar, nucleated cells, provided with a small amount of clear protoplasm, rudimentary collars not united, and flagella (fig. 4A)." Later on, in the same paper, Bidder observes that he interprets the peculiar structures met with by me in Grantia labyrinthica as column-and-plinth chambers violently contracted in alcohol. There is certainly a good deal to be said in favour of this view, and, examining the structures in question in the light of Bidder's remarks, I have seen in Leucandra phillipensis appearances which might be taken as indicating the formation of "plinths." The abundant occurrence of small nucleated cells in the mesoderm which surrounds the contracted chambers, as shown in fig. 24, is certainly suggestive either of the congregation of amœboid cells to feed on the collared cells, or of the collared cells or segments thereof becoming amœboid and wandering away into the mesodermal jelly. The occurrence of such cells in the surrounding jelly is characteristic of the contracted chambers, and their small size appears to me to be an argument in favour of regarding them as metamorphosed collared cells rather than as ordinary amœboid ones.

In Leucandra phillipensis the ordinary amœboid cells are very much larger than the cells in question in the same sponge; in fact, compared to the latter and to the collared cells they are as giants. Curiously enough, I observed one of these large amœboid cells, shown in fig. 50, apparently feeding, by means of pseudopodia, on the collared cells. The chamber to which the collared cells belonged was, however, in the ordinary uncontracted condition, and I could not see any indication of the division of the collared cells into "column and plinth."

On the whole, the peculiar contracted condition, such as is shown in fig. 24, and which occurs in chambers both of the Syconoid and Leuconoid types, is perhaps to be regarded as indicating not so much a process of death as one of rejuvenescence, and this is, after all, but a slight modification of my former view. We may suppose that, after perhaps taking in a large supply of food, the chamber passes into a resting condition. The collared cells then undergo certain changes which are not fully understood, but which finally result in the formation of a new chamber with ordinary active collared cells. The contraction of the chamber during this process appears to me to be normal, and not due to the action of reagents.

The Mesoderm.

The transparent gelatinous ground substance which forms the bulk of the mesoderm in all calcareous sponges, appears to vary only in the extent to which it is present and the proportion which it bears to other parts of the sponge. It is most strongly developed in the dermal and gastral cortex (figs. 10—12). It may also be fairly abundant in the walls of the flagellated chambers, as in Sycon gelatinosum (figs. 3—5), but it is usually very sparingly developed in this situation, so as to be distinctly recognisable only at the points where the chambers touch one another (fig. 13). Hence in very many cases the walls of the chambers appear in thin sections to be made up of two contiguous layers of cells, the collared cells on the inside and the pavement epithelium of the inhalant canals on

the outside. I do not doubt, however, that there is always a thin layer of mesoderm between the two, a fact which is indicated by the development of spicules in the chamber walls. It is certainly continued into the endogastric septa of Grantessa erinaceus, which consist of a layer of gelatinous ground substance, with an occasional stellate cell, covered on each side by a layer of flattened epithelium (fig. 62).

Concerning the spicule sheaths, which are formed by a slight concentration of the structureless mesodermal jelly around the spicules (fig. 53), I have nothing to add.

In my memoir on Grantia labyrinthica I classified the mesodermal cells which lie embedded in the gelatinous ground substance as follows:—(1) Amœboid, (2) Stellate, (3) Glandular, (4) Endothelial, (5) Muscular, (6) Nervous, and (7) Reproductive. I am still prepared to abide by this classification so far as the amœboid, stellate, glandular, and reproductive cells are concerned. The only muscular cells which have yet been observed in calcareous sponges I now regard, as already indicated, as ectodermal or endodermal. The supposed nervous cells I am now extremely doubtful about, in all cases, and I am strongly inclined to think that the appearances described as such are due to the presence of subdermal gland-cells. This point, however, can only be settled by a very careful re-investigation of living material. The endothelial cells I still retain provisionally amongst the mesodermal elements, although I shall presently give reasons for believing that they need not necessarily be regarded as such.

Amœboid and Stellate Cells.—I have observed most beautiful examples of amœboid cells in Leucandra echinata (fig. 33) and L. phillipensis (figs. 48—50). They are found scattered here and there in the mesodermal ground substance between the flagellated chambers, and, in these two sponges, are conspicuous by their enormous size. The amœboid cells are typically distinguished from the stellate cells by their more uniformly granular protoplasm, their larger nuclei, and the absence of the slender, thread-like, radiating processes, which characterise the stellate cells; the latter are very different in appear-

ance from the blunt, rounded pseudopodia which characterise a typical amœboid cell. Nevertheless it is often very difficult to say whether a particular cell should be classed as stellate or amœboid, especially when that cell is of small size; and I am inclined to think that no hard-and-fast line of distinction can be drawn between the two. Thus figs. 39—42 represent a number of cells from the dermal cortex of Leucandra phillipensis, which I personally should class as stellate, but it would be very difficult to prove that they are not amœboid. Similarly fig. 55 represents four mesodermal cells from the dermal cortex of Grantiopsis cylindrica, which may be classified as stellate or amœboid, according to the taste of the observer. For my own part I am inclined to believe that even the most typical stellate cells may be, to a greater or less extent, amœboid, and capable of a certain amount of movement. What appears to be division of the "stellate" cells by fission is very clearly seen in the cortex of Leucandra phillipensis, in which many of the cells have two nuclei, and also show signs of division in the form of the cell body (figs. 39, 40, 42).

Glandular Cells.—These, as I have already pointed out (9), are of two kinds, calcoblasts and subdermal gland-cells. I can only confirm the account of these structures which I gave in the case of Grantia labyrinthica. I have recently observed some very beautiful examples of calcoblasts. They appear to be most easily recognisable on the inner portions of projecting dermal oxeote spicules. Figs. 44—47 represent portions of such spicules from the dermal cortex of Leucandra phillipensis. Each spicule has a single nucleated calcoblast attached to its inner half, and I do not hesitate to regard these cells as the manufacturers of the material of which the spicules are composed. At the same time it will be sufficiently obvious, on comparing figs. 44—47 with figs. 39—42, that the calcoblasts are very similar in appearance to the ordinary "stellate" cells, which are found embedded in the gelatinous ground substance in their immediate neighbourhood.

In Grantiopsis cylindrica I have found very large cal-

coblasts[1] attached to the rays of the large triradiate spicules in the dermal cortex. Three of these cells are shown in figs. 52—54, and their characters certainly justify the assumption that they are but slight modifications of ordinary stellate cells. It appears to me that the calcoblasts, at any rate in the case of large spicules, must be amœboid, for, unless they be so, I cannot understand how the spicules can increase uniformly in thickness. I have already suggested (**9**) that there are probably primary and secondary calcoblasts, the former being mother-cells within which spicules are formed, and the latter cells which apply themselves to the surfaces of already formed spicules and increase the thickness of the latter. The cells represented in figs. 52—54 are probably secondary calcoblasts; those represented in figs. 44—47 may be primary.

Minchin (**32**), in the case of Leucosolenia, found the triradiate spicules to have a nucleus at the extremity of each ray, and a fourth one at the confluence of the rays. As there are four of these nuclei they probably indicate the presence of secondary calcoblasts, for we can hardly suppose that the spicule is originally formed by more than one cell.

We come now to the subdermal gland-cells, which Bidder (**24**) regards as the "pendent cell bodies" of flask-shaped ectoderm-cells. These cells may occur beneath both the gastral and the dermal surfaces of the sponge. In Grantia labyrinthica they are more plentiful beneath the gastral than beneath the dermal surface, a fact which I associate with the peculiar shape of the gastral cavity, which causes its surface to be almost or quite as much exposed as the dermal surface. A situation beneath the dermal surface, however, appears to be their normal one, and they are rare elsewhere. To my description of these structures in Grantia labyrinthica I have little to add. I have found them especially well developed in Grantiopsis cylindrica (fig. 56), where the connection with the surface is very long and slender. In Leucandra phillipensis I have detected them around the inhalant

[1] Compare with these the "conjectural calcoblast," figured by Poléjaeff (**8**) in his Leuconia multiformis.

canals as well as beneath the dermal surface (fig. 43). In all these cases they closely resemble the ordinary stellate mesoderm-cells, and as modifications of such I am for the present disposed to regard them. That they secrete a slime which covers the surface of the sponge in life, I do not doubt.

Vesicular Cells.—I propose this name for certain large rounded cells which occur scattered singly, but in considerable numbers, immediately beneath the gastral epithelium of Ute syconoides. The general appearance of these cells is shown in fig. 37. The body of the cell stains uniformly and fairly darkly with borax carmine. It is not granular, but hyaline. The nucleus is small, darkly staining, and situated at one side of the cell. It appears to have been pushed aside by the accumulation of fluid in the cell body, as in fat-cells. I am not aware that cells of this kind have been noticed before in calcareous sponges, and they appear to me to be most nearly comparable to the "cystenchyme" cells of Silicea and Keratosa—as, for example, Stelospongus (**33**).

Reproductive Cells.—The only reproductive cells upon which I have made any observations are the ova, and these have been so frequently described that I need say little about them here. Figs. 34 and 36 represent typical calcisponge ova, consisting each of a large naked body of highly granular protoplasm, with a large, very sharply defined nucleus and a distinct nucleolus.

In my memoir on Grantia labyrinthica I adduced evidence for believing that the ova, at a certain stage of their existence, migrate through the epithelium of the inhalant canals and hang freely from its surface, so as to be bathed by the inflowing stream of water, and I regarded this as a special contrivance for securing fertilisation. In Ute syconoides I have had the good fortune to observe a very beautiful instance of the manner in which the ovum hangs suspended from the wall of an inhalant canal, as represented in fig. 35. I was particularly glad to obtain this confirmation of my previous observations, as the inhalant canals of Ute syconoides are much more sharply defined and easily recognisable than those

of Grantia labyrinthica, and there is no possibility of doubt as to the exact position of the pendent ovum. Fig. 36 represents an ovum of the same sponge which has presumably been fertilised, and has taken up the normal position for development, just behind the layer of collared cells which lines one of the flagellated chambers, as shown in figs. 5 and 13.

For an account of the spermatozoa of the Heterocœla I must refer the reader to Poléjaeff's work on the subject (34).

Endothelial Cells.—Under this name I have previously described (9) the more or less flattened cells which line, in a single layer, the cavities in which the embryos develop. Fig 38 shows a typical example of an embryo lying in a cavity lined by such cells. Schulze (7) attributes to these cells a mesodermal origin. For the present I adopt this view; but I would like to point out that they may possibly be ectodermal, for it is easy to imagine that an ovum, after migrating through the wall of the inhalant canal, in returning into the mesoderm may push before it a portion of the ectodermal epithelium, from which the embryo capsule might be derived. This, however, is, in the present state of our knowledge, mere speculation.

V.—The Classification of the Heterocœla Calcarea, with Diagnoses of the Families and Genera.

We are now in a position to apply the results of our anatomical investigation to the classification of the group. I have found it necessary to forestall to a certain extent what I have to say here about the classification, in order to satisfactorily arrange my notes on the anatomy, but this was obviously unavoidable. In the present section I propose not only to set forth the classification of the group, and to give brief diagnoses of the families and genera, but also to discuss, so far as appears necessary, the questions of synonymy and nomenclature.

Order HETEROCŒLA, Poléjaeff (8).

Diagnosis.—Calcareous sponges in which the collared cells are confined to more or less well-defined flagellated chambers.

Family 1.—Leucascidæ, Dendy (4).

Diagnosis.—Flagellated chambers very long and narrow, copiously branched; communicating at their proximal ends with exhalant canals, which converge towards the oscula; their blind distal ends covered over by a dermal membrane pierced by true dermal pores, which lead into the irregular spaces between the chambers. Skeleton consisting principally of small radiates, irregularly scattered in the walls of the chambers and exhalant canals, and in the dermal membrane.

Genus 1.—Leucascus, Dendy (4), fig. 1.

Diagnosis.—The same as that of the family.

Remarks.—Leucascus, the sole genus of the family, appears to occupy a very isolated position amongst the Heterocœla, both as regards skeleton and canal system. Its skeleton, as already pointed out, has not yet attained to the typical radiate condition of the Heterocœla, but retains the irregular scattered character of the reticulate Homocœla. The genus might, indeed, be easily confounded with the reticulate section of the genus Leucosolenia (1), but differs in several very important particulars; viz. (*a*) The possession of a distinct pore-bearing dermal membrane, which is found in no Homocœle sponge with which I am acquainted, and is not even developed amongst the Heterocœla until we reach the most advanced Sycon condition. The pseudoderm of many reticulate Homocœla (1) is by no means an homologous structure. (*b*) The absence of collared cells from the exhalant canals, which there is no reason for believing to be of a "pseudogastral" nature, as in my type D of the reticulate Homocœla. (*c*) The flagellated chambers, although, owing to the massive form of the sponge and the absence of a single central gastral cavity, they cannot be truly radial, nevertheless show a marked tendency to become so.

The genus may, perhaps, be best regarded as derived independently from a lowly organised type of radiate Homocœla.

It might, perhaps, be regarded as a degenerate Heterocœle which has lost its radial symmetry, but the facts do not appear to me to warrant this hypothesis.

Family 2.—Sycettidæ, Dendy (4).

Diagnosis.—Flagellated chambers elongated, arranged radially around a central gastral cavity, their ends projecting more or less on the dermal surface, and not covered over by a continuous dermal cortex. Tubar skeleton articulate.

Remarks.—This family is equivalent to van Lendenfeld's (10) two sub-families Sycanthinæ and Syconinæ taken together. As I have already pointed out, his genus Sycantha is probably but a slight modification of the ordinary Sycon type. The name Sycettidæ is adopted partly because the genus Sycetta is the simplest of the three included in the family, and partly to avoid confusion with the much more comprehensive Syconidæ of previous writers, such as Polé-jaeff (8).

Genus 2.—Sycetta (Haeckel [5], emend.).

Diagnosis.—Radial chambers separate from one another, and without tufts of oxea on their distal ends.

Remarks.—This genus, as here maintained, is much less comprehensive than was originally intended by its author (5). Haeckel included in the genus all Syconoid species in which triradiate spicules alone entered into the composition of the skeletons, and thus was obliged to group together such structurally different species as S. primitiva and S. stauridia; while he had to exclude S. conifera simply because some of the triradiates develop an apical ray, and thus become quadriradiate. Von Lendenfeld also confined his diagnosis of the genus to the character of the spiculation (10), but modified it so as to include S. conifera. Poléjaeff (8) merged the genus into Sycon. If, however, we include in the genus only those species which, like Haeckel's Sycetta primitiva and S. (Sycaltis) conifera, conform in skeletal characters and canal system to the diagnosis given above, we shall have left a

very natural and well-characterised genus, comprising only three species, viz. S. primitiva, S. sagittifera, and S. conifera, all of which are described by Haeckel in his great monograph.

Genus 3.—Sycon (Risso, emend.), figs. 2—8.

Diagnosis.—The radial chambers are usually more or less united at places where they come into contact with one another, and they are always crowned at their distal extremities with tufts of oxeote spicules.

Remarks.—The most characteristic feature of this genus is afforded by the tufts of oxeote spicules which crown the distal ends of the radial chambers. In some species, such as Sycon boomerang (4) and S. gelatinosum (4, 5), we find a transition to the genus Grantia, in the presence of a pore-bearing dermal membrane stretched between the distal ends of the radial chambers; but this never forms a cortex which completely covers over the chambers as in the Grantidæ, and it in no way interferes with the characteristic tufts of oxeote spicules.

The genus as here constituted includes most of Haeckel's species of his genus Sycandra (5), but I follow Poléjaeff (8) in giving priority to the old name Sycon. Familiar European examples are S. raphanus and S. ciliatum, and I also include S. (Grantia) compressum, on account of the tufts of oxeote spicules which crown the ends of the chambers. Poléjaeff's genus Sycon is, as already pointed out, more comprehensive, and includes our Sycetta, while von Lendenfeld (10) adopts the genus Sycandra in almost the same sense as Haeckel.

Genus 4. Sycantha, von Lendenfeld (10).

Diagnosis.—Radial chambers united in groups, with freely projecting distal cones surmounted by tufts of oxeote spicules.

Remarks.—It appears to me that the only character which can be relied upon for distinguishing this genus is the grouping

of the chambers. I have already given my reasons for coming to this conclusion in discussing the canal system.

Family 3.—Grantidæ, Dendy (4).

Diagnosis.—There is a distinct and continuous dermal cortex, completely covering over the chamber layer, and pierced by inhalant pores. There are no subdermal sagittal triradiates, nor conspicuous subdermal quadriradiates. The flagellated chambers vary from elongated and radially arranged to spherical and irregularly scattered ones, while the skeleton of the chamber layer varies from regularly articulate to irregularly scattered.

Remarks.—This family does not nearly correspond with any which has hitherto been proposed; for, disregarding differences in canal system, I include therein some genera with a Syconoid and others with a Leuconoid type, and rely upon the structure of the skeleton for thus uniting them. I believe myself to be justified in this course of action by the great variation of the canal system which has been shown to exist in closely related forms and even in the same species, and which I have already discussed in a previous part of this paper.

The family, as here constituted, is distinguished from the Sycettidæ by the positive character afforded by the development of a strong dermal cortex, and from the Heteropidæ and Amphoriscidæ by the negative characters afforded by the absence of subdermal triradiates or quadriradiates. It is very difficult to diagnose the family, which is a very comprehensive one, so as to exclude the Leucascidæ, but the latter present a peculiar combination of skeleton and canal system which is not to be found amongst the Grantidæ, and appear never to have passed, as I believe all the Grantidæ have, through a primitive Syconoid stage with radially symmetrical skeleton.

Genus 5.—Grantia (Fleming, emend.), figs. 9, 10.

Diagnosis.—The elongated flagellated chambers are ar-

ranged radially around the central gastral cavity; they are not provided with tufts of oxea at their distal ends, but are covered over by a dermal cortex composed principally of triradiate spicules, and without longitudinally disposed oxea. An articulate tubar skeleton is present.

Remarks.—The genus Grantia, as here defined, is very nearly co-extensive with the same genus as employed by Poléjaeff (8). It appears to me, however, that the genus must be limited to those species in which no tufts of oxea are developed on the ends of the radial chambers, in order to define it with desirable sharpness from Sycon; for, as I have already shown, we meet with the first indications of a dermal cortex in the latter genus. Hence I cannot agree with Poléjaeff in including Sycon (Grantia) compressum. Nor can I agree with von Lendenfeld's diagnosis (10), for he expressly states that a crown of radial "Thabden" may be present on each chamber, and his diagnosis is so worded as to exclude Haeckel's G. (Sycetta) strobilus and G. (Sycetta) cupula, which, as was recognised by Poléjaeff (8), undoubtedly belong to the genus Grantia.

Sub-genus Grantiopsis, Dendy (4), fig. 11.

Diagnosis.—The sponge has the form of a greatly elongated hollow tube, whose wall is composed of two distinct layers of about equal thickness. The outer (cortical) layer is provided with a very strongly developed skeleton of radiate spicules, and is penetrated by narrow, ramifying, inhalant canals. The inner layer is formed by elongated radial chambers, arranged very regularly side by side. The skeleton of the inner layer is very feebly developed. The tubar skeleton is articulate, and composed of very abnormal sagittal triradiates whose paired rays are greatly reduced.

Remarks.—This genus is obviously only a very special modification of the well-known Grantia type, although at first sight, especially as regards external form, it appears very distinct. The only species known is the Victorian Grantiopsis cylindrica (4).

Genus 6.—Ute (Schmidt, emend.), figs. 12—14, 24.

Diagnosis.—The ends of the elongated radial chambers are covered over by a well-developed cortex, composed in great part of large oxeote spicules arranged parallel to the long axis of the sponge. The tubar skeleton is articulate, or else composed entirely of the basal rays of subgastral triradiates.

Remarks.—This genus I maintain in the same sense as Poléjaeff (8) and von Lendenfeld (10). It now includes five species, viz. the original European type, Ute glabra, Schmidt (17), and the Australian U. argentea, Poléjaeff (8), U. syconoides, Carter (12), U. spiculosa, Dendy (4), and U. Spenceri, Dendy (4). The spiculation of the dermal cortex, upon which the genus is founded, is paralleled in two species of Leucosolenia, L. asconoides, Carter (12), and L. uteoides, Dendy (16); and also in Leucandra (Aphroceras) alcicornis, Gray, and Heteropia (Aphroceras) ramosa, Carter (15). Indeed, this character gave rise to Gray's family "Aphrocerasidæ" (18), which, like other families founded upon an insufficiency of characters, has had to be abandoned.

The genus appears to be a natural one, but is obviously very closely related to Grantia, and, had it not been already well established, I should have hesitated in attributing generic importance to a character which is found in so many very distinct sponges.

Sub-genus Synute, Dendy (13), fig. 15.

Diagnosis.—Sponge compound, consisting of many Ute-like individuals completely fused together, and invested in a common cortex, composed largely of huge oxeote spicules arranged longitudinally.

Remarks.—I at first thought that my Synute pulchella (13) should stand as the type of a new genus, but I have since come to the conclusion that the complete fusion of the Ute individuals, though perhaps unparalleled amongst sponges

with a Syconoid type of canal system, is scarcely a character of generic importance, and I have therefore reduced the species to the rank of a sub-genus.

Genus 7.—Utella, Dendy (4).

Diagnosis.—Flagellated chambers elongated, arranged radially around the central gastral cavity. There are no longitudinally arranged oxea in the dermal cortex, but a layer of oxeote spicules, longitudinally arranged, lies beneath and parallel to the gastral surface. The tubar skeleton is articulate.

Remarks.—This genus was proposed for the reception of Haeckel's remarkable Sycandra hystrix (5), and Schmidt's Ute utriculus (19) may perhaps also be included in it. The genus is obviously a special modification of the Grantia type. It is, as I have already pointed out, very unusual to find oxeote spicules in the gastral cortex of any calcisponge.

Genus 8.—Anamixilla, Poléjaeff (8).

Diagnosis.—Flagellated chambers elongated and radially arranged. There is no special tubar skeleton, the skeleton of the chamber layer consisting of large radiate spicules, arranged without regard to the direction of the chambers, and of the outwardly directed basal rays of the subgastral sagittal triradiates.

Remarks.—The derivation of this remarkable genus from the Grantia type is still indicated by the presence of the subgastral sagittal triradiates. It may be compared to a Grantia in which the ordinary articulate tubar skeleton has been almost entirely replaced by the invasion of large triradiates from the dermal cortex. The only species as yet known is the Australian Anamixilla torresi, Poléjaeff (8), to the account of which given by Poléjaeff I have nothing to add. I have, however, ventured to slightly alter the original diagnosis.

Genus 9.—Sycyssa, Haeckel (5).

Diagnosis.—The flagellated chambers are elongated and

arranged radially around the central gastral cavity. The skeleton consists exclusively of oxeote spicules.

Remarks.—The only known species of this genus is Haeckel's Sycyssa Huxleyi, from the Adriatic (5). The genus occupies a very isolated position. The remarkable skeletal characters upon which it is based have been discussed on a previous page.

Genus 10.—Leucandra (Haeckel [5], emend.), figs. 16, 17.

Diagnosis.—The flagellated chambers are spherical or sac-shaped, never arranged radially around the central gastral cavity, with which (or with the main exhalant canals derived therefrom) they communicate by a more or less complicated exhalant canal system. The skeleton of the chamber layer is composed of irregularly scattered radiate spicules, but it may still present traces of its derivation from a radially symmetrical type, in the presence of a few subgastral sagittal triradiates.

Remarks.—This genus, as here maintained, is still a very comprehensive one, and does not correspond exactly to any which have hitherto been proposed. It includes many species which would fall under Poléjaeff's Leuconia (8), but that author frankly admits that his Leuconia requires sub-dividing; and, moreover, Vosmaer (14) has shown that Bowerbank's name Leuconia, adopted by Poléjaeff, was previously occupied by a genus of Mollusca. I therefore agree with Vosmaer in adopting Haeckel's name Leucandra, but as that genus was based entirely upon the presence of certain forms of spicules, without regard to their arrangement, I cannot accept it in the sense originally intended. On the same principle, I include in the genus Haeckel's species of Leucetta, as I do not believe the mere absence of quadriradiate or oxeote spicules, or both, to be of generic significance. Indeed, I was strongly inclined to adopt the name Leucetta, on grounds of priority, for the genus as now constituted; but considering that Leucandra, as employed by Haeckel and Vosmaer, makes the nearest approach to the genus as now characterised, and considering also that the name Leucetta has been adopted by Poléjaeff

(8) and Vosmaer (14) in a very different sense, I have thought it desirable to make use of the name chosen.

I believe that very probably the genus will still require subdividing at some future date, but it will be an extremely difficult matter to satisfactorily characterise the different subdivisions; the irregular nature of the skeleton appears to me to defy classification. It might be possible to maintain Gray's genus Aphroceras for species like Leucandra alcicornis, in which the dermal cortex is composed chiefly of longitudinally arranged oxea. Aphroceras would then stand in the same relation to Leucandra as Ute does to Grantia; but, unfortunately, we find species intermediate in skeletal characters between the typical Leucandra and the proposed Aphroceras, in which we have large oxea in the dermal cortex, but not arranged with regularity parallel to the long axis of the sponge, nor yet projecting at right angles from the surface. I have already expressed my hesitation in maintaining the genus Ute; and as the genus Aphroceras has not, like Ute, come into general use, I do not care to take upon myself the responsibility of re-establishing it.

Certain species of Leucandra, as already pointed out in dealing with the skeleton, still exhibit traces of descent from a radially symmetrical form, in the presence of subgastral and other sagittal triradiates; and for the present we may conveniently regard the genus as being descended from an ancestral Grantia type, by modification of the canal system and skeleton along the lines laid down in an earlier part of this paper.

The true relations of many species of the genus are obscured by the habit of colony formation by the fusion of many individuals, which gives rise to irregular, massive sponges, as in the case of Synute; but I do not think that we can generically separate these species from those which retain the more primitive condition, with a single central gastral cavity.

My Leucandra phillipensis (4), the anatomy of which is drawn in fig. 16, is a typical example of the simpler section of the genus, while the European Leucandra nivea (5) offers an illustration of the massive colonial habit. Numerous other

examples are given in my synopsis of the Australian Calcarea Heterocœla (4).

Genus 11.—Lelapia (Gray [18], emend.).

Diagnosis.—Canal system unknown. Skeleton of the chamber layer composed of large, longitudinally arranged oxea, crossed at right angles by bundles of tuning-fork shaped triradiates whose basal rays are directed towards the dermal surface.

Remarks.—This genus was first proposed by Gray (18) for certain remarkable tuning-fork shaped spicules described by Bowerbank, but the sponge to which those spicules belonged was then unknown. Carter (12) subsequently described some sponges, collected by Mr. J. Bracebridge Wilson in the neighbourhood of Port Phillip Heads, in which similar tuning-fork shaped spicules were present, and he gave to these sponges Gray's name, Lelapia australis. Whether Mr. Carter's species is really identical with the sponge to which the spicules described by Bowerbank and Gray belonged, must remain an open question. The fact that the spicules in question came from Australia lends an air of probability to the identification, but then similar spicules are known in the fossil condition from deposits as old as the Cretaceans, according to Carter (12). In any case, the Victorian sponges described by Carter (12) under the name Lelapia australis must stand as the types of that species, and the species thus constituted must stand as the type of the existing genus Lelapia.

Unfortunately we know nothing definite as to the canal system of Lelapia australis, although it is to be inferred, from the fact that Mr. Carter places it amongst the "Leucones," that the canal system belongs to the Leuconoid type. The structure of the skeleton as described by Mr. Carter is very peculiar, and it is upon this character alone that the generic diagnosis must, for the present, be based.

The position of the genus in the system of classification is necessarily only provisional, and it can only be finally determined by further research on the canal system. Unfortunately

Lelapia australis is extremely rare, and although Mr. Wilson has for some years past sent me the results of his dredging expeditions, so far as the sponges are concerned, I have never yet had the good fortune to meet with a specimen.

Genus 12.—Leucyssa, Haeckel (5).

Diagnosis.—Canal system presumably of the Leuconoid type. Skeleton composed solely of oxeote spicules irregularly scattered through the sponge.

Remarks.—This is another of those genera of which we know scarcely anything but the skeleton with any degree of accuracy. There can, however, be little doubt that the canal system belongs to the Leuconoid type. The fact that Haeckel included the genus amongst his Leucones, and the irregular character of the skeleton, both point to this conclusion. I have already discussed the relationship of the genus in speaking of the skeleton. For the present it may perhaps best be regarded as derived from a Leucandra-like type by loss of the radiate spicules, and their replacement by oxea. There are three very distinct species included by Haeckel (5) in the genus, viz. Leucyssa spongilla, L. cretacea, and L. incrustans. All these species are extremely rare, so that the probabilities of our obtaining a more exact knowledge of the canal system of the genus are somewhat remote.

Family 4.—Heteropidæ, Dendy (4).

Diagnosis.—There is a distinct and continuous dermal cortex covering over the chamber layer, and pierced by inhalant pores. Subdermal sagittal triradiates are present. The flagellated chambers vary from elongated and radial to spherical and irregularly scattered. An articulate tubar skeleton may or may not be present.

Remarks.—The leading characteristic of this family, by which it is distinguished from all others, is the presence of the subdermal sagittal triradiate spicules, which, to a greater or less extent, replace the primitive articulate tubar skeleton. As regards the canal system, we meet with much the same

series of variations as in the Grantidæ; and the shape and arrangement of the chambers, as in that family, can only be utilised as an aid in diagnosing the genera.

The family is evidently derived from an ancestral Grantia-like type, as is clearly indicated by the retention of the primitive radial arrangement of the chambers, and the articulate tubar skeleton, by the least modified species (fig. 18).

The family, as here maintained, is not nearly identical with any which have hitherto been proposed. No previous writers have drawn that sharp distinction between subdermal sagittal triradiates and subdermal quadriradiates which I have indicated in dealing with the skeleton, and which seems to me to be of the greatest value for purposes of classification.

I have adopted the name "Heteropidæ," not because I regard the genus Heteropia as most typical of the family, but because a family name derived from the principal genus, Grantessa, would be liable to confusion with the name "Grantidæ," already used for the preceding family.

Genus 13.—Grantessa (von Lendenfeld [11], emend.), fig. 18.

Diagnosis.—The flagellated chambers are elongated, and arranged radially around the central gastral cavity. The dermal cortex consists principally of triradiates, and does not contain longitudinally disposed oxea.

Remarks.—In working over the Australian Heterocœla I met with an extensive series of specimens, belonging to at least six species,[1] which evidently formed a very natural assemblage, characterised by essentially the same peculiarities of skeleton and canal system. Most of these species had already been described by various authors under a variety of generic names, viz. Amphoriscus (Poléjaeff), Grantessa (von Lendenfeld), Heteropia (Carter), Hypograntia (Carter), and Leuconia (Carter).

From these names I selected Grantessa for the group of species in question, for the following reasons:—The name Am-

[1] For a list of these species, with synonyms, vide 4.

phoriscus is occupied by a distinct genus of sponges, which ought not to be confounded with the one under consideration; the name Leuconia is altogether out of the question, for reasons already discussed; and the name Grantessa has priority over both Heteropia[1] and Hypograntia.

The genus Grantessa of von Lendenfeld (11), however, is by no means synonymous with the genus here maintained. The original type of the genus is Grantessa sacca, and its author bases the generic distinction upon the presence of tufts of oxeote spicules projecting more or less at right angles from the dermal surface, and arranged without regard to the radial chambers (10). In his description of G. sacca, however, he mentions the presence of "dermal" sagittal triradiates with an inwardly directed basal ray, but he evidently does not consider this character as of generic, much less of family importance. Attributing, as I do, great importance to the presence of subdermal sagittal triradiates, and very little importance to the arrangement of the dermal oxea in tufts (which bear no relation to the radial chambers), I have felt it necessary, while adopting the name Grantessa, to give an entirely new significance thereto.

Genus 14.—Heteropia (Carter [15], emend.).

Diagnosis.—The distal ends of the elongated radial chambers are covered over by a well-developed dermal cortex, consisting principally of large oxea arranged parallel to the long axis of the sponge.

Remarks.—The name Heteropia was first applied by Carter to his Aphroceras ramosa (15), and I therefore consider that species as the type of the genus. The name was also applied by the same author and at about the same time (12) to a number of Victorian sponges, but I have already pointed out that for the latter the name Grantessa must take priority. Indeed, it is perhaps doubtful whether Heteropia ramosa deserves to be generically separated from Grantessa, to which genus it bears exactly the same relation

[1] Heteropia is also in use for another genus.

as Ute does to Grantia. As, however, there appear to be no intermediate stages known between Grantessa and Heteropia, the latter genus appears to have just as much right to stand separately as Ute has, and the same objection does not hold good as in the case of Leucandra and Aphroceras previously discussed. Even should the genus have to be abandoned, still the name "Heteropidæ" may be conveniently retained for the family. The only known species of the genus is H. ramosa.

Genus 15.—Vosmaeropsis, Dendy (4), fig. 19.

Diagnosis.—Flagellated chambers spherical or sac-shaped, never truly radial. Dermal cortex composed principally of triradiates, without longitudinally disposed oxea.

Remarks.—This genus stands in much the same relation to Grantessa as Leucandra does to Grantia. The canal system of Vosmaeropsis macera (fig. 19) affords an excellent example of the "Sylleibid" type which characterises von Lendenfeld's genera, Polejna and Vosmaeria (10); but I have already expressed my opinion that the mere presence of this type of canal system cannot be utilised for purposes of classification, as it is found in several very distinct forms which we cannot unite in defiance of the great structural differences in their skeleton. It might be doubted whether Vosmaeropsis should be generically separated from Grantessa, as this separation depends entirely upon the canal system; but, although I do not consider the distinction between the Sylleibid and Leuconoid types of canal system sufficiently well marked to be utilised for generic purposes, I think we may very conveniently thus utilise the much greater distinction between the Syconoid and Leuconoid types, considering the Sylleibid one, for purposes of classification, as belonging to the Leuconoid division.

I only know of three species which can be included in the genus Vosmaeropsis, viz. V. macera (4), V. depressa (4), and V. Wilsoni (4).

Family 5.—Amphoriscidæ, Dendy (4).

Diagnosis.—There is a distinct and continuous dermal cortex over the chamber layer. Conspicuous subdermal quadriradiate spicules, with inwardly directed apical rays, are present. The flagellated chambers vary from elongated and radially arranged to spherical and irregularly scattered.

Remarks.—In this family, which does not by any means correspond to von Lendenfeld's "Amphoriscinæ" (**10**), the distinguishing characteristic is the presence of subdermal quadriradiate spicules with inwardly directed apical rays. These, as I have already pointed out, are not to be regarded as homologous with the subdermal sagittal triradiates of the Heteropidæ, although they appear to fulfil the same function. We find in this family exactly the same series of variations in the canal system as in the two preceding, and, as before, I have utilised this variation, so far as the Syconoid and Leuconoid types are concerned, for purposes of generic distinction, including the Sylleibid type in the Leuconoid.

The family is evidently to be derived from a Grantia-like ancestral type, by the development of conspicuous and inwardly directed apical rays by the triradiates of the dermal cortex.

Genus 16.—Heteropegma, Poléjaeff (8), fig. 20.

Diagnosis.—The flagellated chambers are elongated and arranged radially around the central gastral cavity. There is a vestigial tubar skeleton of minute radiates. The dermal cortex is very thick, composed principally of triradiate spicules.

Remarks.—This genus is extremely interesting as showing how the primitive articulate tubar skeleton, which is on the verge of disappearance, is gradually replaced by the development of the subdermal quadriradiates (fig. 20). Poléjaeff (**8**) makes no mention of the subdermal quadriradiates in his diagnosis of the genus, although he was well aware of their presence, and I have therefore been obliged to draw up a fresh diagnosis.

The genus was founded for the reception of Heteropegma nodus-gordii from off the Bermudas and Cape York, and

we now also know a species, H. latitubulata (4), very closely resembling the first, from Victorian waters.

Genus 17.—Amphoriscus (Haeckel [20], emend.).

Diagnosis.—The flagellated chambers are elongated, and arranged radially around the central gastral cavity. There is no articulate tubar skeleton, but, in addition to the subdermal quadriradiates, subgastral sagittal triradiates or subgastral quadriradiates may also be present.

Remarks.—In his "Prodromus" (20) Haeckel proposed the generic name Amphoriscus for a very natural group of three species, all characterised by the Syconoid type of canal system and the presence of quadriradiate spicules only in the skeleton. Of the highly characteristic arrangement of the skeleton, with subdermal and subgastral quadriradiates whose apical rays point in opposite directions, his generic diagnosis takes no notice. This arrangement, however, is exhibited by all the species referred by Haeckel to the genus, and affords, to my mind, a much more suitable foundation for a generic diagnosis. In his monograph (5) Haeckel altered the generic name to Sycilla, but retained the old diagnosis ("Sycones spiculis quadricruribus"). He also added a fourth species to the genus.

Poléjaeff (8) retains the genus Amphoriscus, but extends the diagnosis to include species with subdermal sagittal triradiates, which, in my opinion, must be kept quite separate in the genus Grantessa. His Amphoriscus poculum and A. flamma both belong to the genus Grantessa. His A. elongatus, however, must be retained in the genus Amphoriscus, and is, indeed, a very noteworthy species; for, although the articulate tubar skeleton has been lost, the subgastral sagittal triradiates still persist, and have not yet been replaced by subgastral quadriradiates, as in all Haeckel's species of the genus. Von Lendenfeld (10) follows Poléjaeff in including in the genus Amphoriscus species with subdermal sagittal triradiates as well as those with subdermal quadriradiates, while he separates, quite unnecessarily to my

mind, those species which happen to have oxeote spicules as a distinct genus, to which he gives the name Ebnerella.

As examples of the genus Amphoriscus as maintained by me, I may cite all Haeckel's species of Sycilla (5) and Polejaeff's Amphoriscus elongatus (8).

Genus 18.—Syculmis (Haeckel [5], emend.).

Diagnosis.—The flagellated chambers are elongated, and arranged radially around the central gastral cavity. The skeleton of the chamber layer is composed of the apical rays of subdermal and subgastral quadriradiates. There is a root-tuft of oxea and anchoring quadriradiates.

Remarks.—Haeckel's diagnosis (5) of the genus was based entirely upon the spiculation, without regard to the arrangement of the skeleton. As it happened, however, he only knew one species—the remarkable Syculmis synapta—which possesses only quadriradiate and oxeote spicules; and therefore, although it is necessary to alter the generic diagnosis, it is not necessary to make any change in the extent of the genus. Syculmis synapta (5) is evidently only a very special modification of the Amphoriscus type.

Genus 19.—Leucilla (Haeckel [5], emend.), figs. 21, 22.

Diagnosis.—Flagellated chambers spherical or sac-shaped, never truly radial.

Remarks.—This genus occupies a position amongst the Amphoriscidæ, analogous to that occupied by Leucandra amongst the Grantidæ, and Vosmaeropsis amongst the Heteropidæ, being distinguished by the Leuconoid (or Sylleibid) character of the canal system. Leucilla uter, as I have already pointed out, affords an excellent example of the Sylleibid type (fig. 21), while in Leucilla australiensis (fig. 22) we meet with a more typically Leuconoid modification. Even in Leucilla australiensis, however, we often find the flagellated chambers more or less elongated, thus showing how

impossible it is to draw a sharp line of distinction between the two types.

The genus Leucilla was founded by Haeckel (5) for Leuconoid sponges in which the spicules are all quadriradiate, of course without regard to the arrangement of the skeleton. The first species which he describes is Leucilla amphora, and I have taken this as the type of the genus, and constructed the generic diagnosis accordingly. The only other species described by him, L. capsula, also comes into the genus as now constituted. It thus appears that our Leucilla is a much more comprehensive genus than Haeckel's, and this is because we include in the genus species with triradiate and oxeote spicules.

Poléjaeff (8) has attached a very different significance to the name Leucilla, giving to the genus much the same character as von Lendenfeld has given to his family Sylleibidæ; indeed, the latter was founded (11) chiefly upon Poléjaeff's Leucilla. I revert, however, to Haeckel's two species, and make their characters the foundation of the genus.

In my synopsis of the Australian Calcarea Heterocœla (4) I, somewhat unnecessarily I fear, proposed the name Paraleucilla for Haeckel's Leucandra cucumis, which is characterised by the presence of distinct subdermal cavities supported by a somewhat specialised portion of the skeleton of the chamber layer. I now regret having taken this step, the more so as Poléjaeff (8) had previously proposed the name Pericharax for the same sponge. Poléjaeff, however, also included in the genus Pericharax his P. Carteri, which is a very different sponge from Leucandra cucumis, and does not even come within the limits of our family Amphoriscidæ. I do not now think that either Pericharax or Paraleucilla ought to stand as a distinct genus. Poléjaeff's Pericharax Carteri is but a slight modification of the ordinary Leucandra type, while Haeckel's Leucandra cucumis is a similar modification, only rather more marked, of the Leucilla type.

VI. The Origin and Phylogeny of the Calcarea Heterocœla.

No one will probably dispute the now well-established hypothesis that the Calcarea Heterocœla are descended from some one or more ancestral Homocœla. The Homocœla are undoubtedly the more primitive of the two great groups into which Polejaeff (8) divided the calcareous sponges, and the only question which we need discuss is the process by which the Homocœle gave rise to the Heterocœle type.

In my memoir on the organisation and classification of the Homocœla (1) I proposed to divide the sole genus, Leucosolenia, into three sections, according to the nature of the canal system, for even in the simplest of sponges the canal system exhibits a considerable amount of variation. To these sections of the genus I applied the names Simplicia, Reticulata, and Radiata. The Simplicia include such simple Olynthus types as never form colonies, and also those colonial forms in which the whole colony consists of individuals (Ascon-persons) which may branch, but which never form complex anastomoses nor give off radial tubes, so that the individuality of the different members of the colony is always recognisable. In the Reticulata the sponge colony forms a more or less complex network of branching and anastomosing tubes, and it is no longer possible to distinguish the individual Ascon-persons of which the colony is composed. The Radiata include such species as exhibit a radiate structure—the sponge consisting of a single central Ascon-tube, from which other tubes are budded off radially.

In all species of Leucosolenia it is supposed that the whole of the primitive gastral cavity is lined by a layer of collared cells, and that when, as in my type D of the Reticulata, exhalant canals exist which are not lined by collared cells, these canals (pseudogasters) really lie outside the sponge, and are probably formed by upgrowth of the colony around them.

The Olynthus type, consisting of a simple sac lined by

collared cells, and pierced by an osculum and prosopyles, is universally admitted to be the ancestral form of all calcareous sponges. If we look for a more advanced type amongst the Homocœla, from which the Heterocœla may possibly be derived, the radiate section of the genus Leucosolenia at once suggests itself. The type of this radiate section is Leucosolenia tripodifera (1); and Bidder (21) is of opinion that certain other species, notably Leucosolenia Lieberkuhnii, should also be included therein. In L. tripodifera we find a single wide central tube, with a terminal osculum, numerous prosopyles, and a thin wall lined by collared cells—so far a typical Ascon individual. From this tube, numerous radially arranged branches are given off, which themselves branch copiously, and terminate all at about the same level in blind, rounded extremities, which touch one another, and thus form an even surface to the whole sponge. Each of the radial branches repeats, on a smaller scale, the structure of the parent tube, and each is first formed as a hollow bud or outgrowth from the latter, the youngest buds lying at a little distance below the osculum. The radial tubes occasionally anastomose and inter-communicate, like the branches of a reticulate Leucosolenia, but this appears to take place only occasionally; at any rate, it is not a characteristic feature. The skeleton of this sponge consists of rather slender sagittal triradiates and quadriradiates, and of the "tripod" spicules. The latter are confined to the distal extremities of the radial chambers. The sagittal radiates are arranged in a single layer in the walls of the tubes, the quadriradiates with the apical ray projecting into the gastral cavities both of the central tube and its radial branches. As already stated, the spicules are sagittal, and in the central tube the basal rays point towards the base of the sponge; while in the radial tubes the basal rays point towards the blind distal ends, exactly as in the articulate tubar skeleton of Syconoid Heterocœla.

Here, then, we have a form which makes a very near approach, both in canal system and skeleton, to the Sycetta type amongst the Heterocœla, and it is from some such

radiate Homocœlous form as this that the Heterocœla have probably been derived. I do not by any means wish to single out Leucosolenia tripodifera itself as the ancestor of the Heterocœla, for I do not for a moment believe that it is so. The copious branching of the radial tubes, and the fusion of adjacent tubes at the points of contact, indicate a more advanced condition in some respects than is met with in the simplest Heterocœla (Sycetta). The youngest part of the sponge, nearest to the osculum, where the radial tubes are still simple and unbranched, makes a much nearer approach to the hypothetical ancestral form.

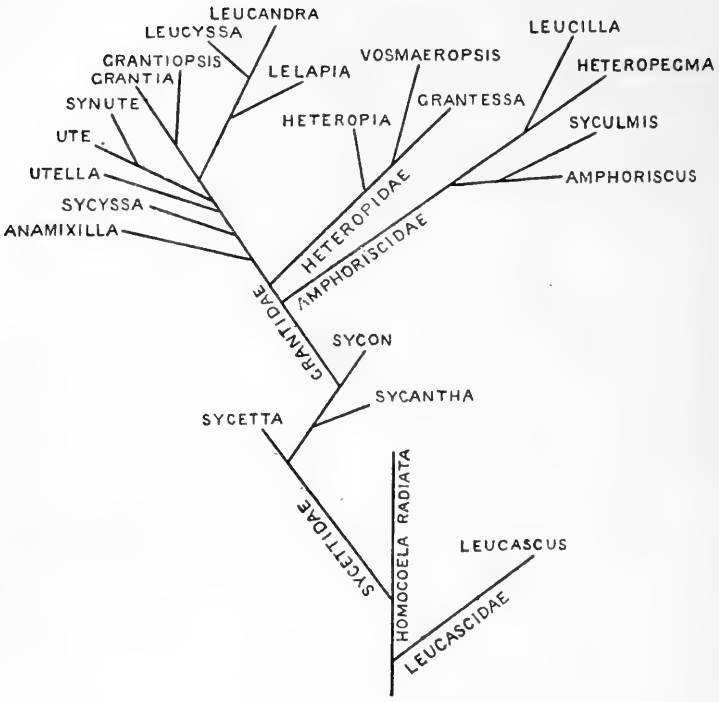
In any case, the simple radiate type of structure, though perhaps no more highly specialised than certain other types met with amongst the Homocœla, appears to form the natural starting-point for all the divers types met with amongst the Heterocœla, with the probable exception of Leucascus, to which I shall refer again later on. The simpler forms of Heterocœla still preserve the radiate character, which becomes at first even more pronounced in the structure of the skeleton. It is also a most important and significant fact, that in the ontogeny of these simple Heterocœla the radial tubes (flagellated chambers) are formed as outgrowths from a central tube, exactly as in Leucosolenia tripodifera. An excellent illustration of this mode of formation is given by Korschelt and Heider (**2**).

I ought, perhaps, in this place to say something about Dr. von Lendenfeld's supposed families "Homodermidæ" and "Leucopsidæ." I have, however, already expressed my views on this subject in an earlier paper (**1**), and those views I still maintain, for I cannot see that there is anything in Dr. von Lendenfeld's observations, including those published in "Die Spongien der Adria" (**10**), to justify us in accepting these families. It would take too long to argue the question here, and I am quite content to refer the reader to Dr. von Lendenfeld's own writings.

I maintain, then, that the ancestral form of the Heterocœla was a simple, radiate, Homocœle sponge, simpler even than

Leucosolenia tripodifera, and that the radiate Heterocœle type was developed from this by replacement of the collared cells of the central gastral cavity by a flattened epithelium and specialisation of the skeleton, a modification which might readily give rise to the primitive Heterocœle genus Sycetta. The manner in which I believe the majority of the remaining genera of Heterocœla to have been derived from a Sycetta-like ancestor, has perhaps been already sufficiently indicated in dealing with the anatomy and classification of the group. The genus Leucascus, however, does not appear ever to have passed through a Sycetta stage, for while the greatly elongated flagellated chambers may indicate by their arrangement (at any rate in some specimens) a certain degree of radial symmetry, the skeleton exhibits none whatever; it is quite as irregular as that of a reticulate Homocœle, or as that of the most modified Leucandra. Considering the nature of the canal system, however, I believe that the irregularity of the skeleton in Leucascus is a primitive condition, and not, as in Leucandra, a secondary one. Possibly Leucascus may be either derived directly from a reticulate Homocœle ancestor, by the formation of a true dermal membrane and dermal pores, or from a very low type of radiate Homocœle, in which the skeleton had not yet acquired any radial symmetry. I am inclined to adopt the latter view.

The opinions as to the origin and inter-relationships of the Calcarea Heterocœla which I have endeavoured to justify in the foregoing pages, may be conveniently summarised in the accompanying diagram, which naturally takes the form of a genealogical tree.



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EXPLANATION OF PLATES 10—14,

Illustrating Dr. Dendy's paper "Studies on the Comparative Anatomy of Sponges."

Note.—The majority of the drawings have been made from paraffin sections of ordinary spirit material, and in most cases a camera lucida has been employed.

In Figs. 1—22 the collared cells are diagrammatically represented by red dots, and the outlines of the spicules are drawn in blue.

Reference Letters.

calc. Calcoblast. *c. c.* Collared cell. *c. g. c.* Central gastral cavity. *ch. di.* Diaphragm of exhalant opening of flagellated chamber. *d. cor.* Dermal cortex. *d. p.* Dermal pore. *ect. ep.* Ectodermal pavement epithelium. *emb.* Embryo. *emb. c.* Embryo capsule. *end. ep.* Endodermal pavement epithelium. *ex. c.* Exhalant canal. *ex. op.* Exhalant opening of flagellated chamber. *fl. ch.* Flagellated chamber. *fl. ch. x.* Flagellated chamber in the contracted condition. *g. cor.* Gastral cortex. *g. q.* Apical ray of gastral quadriradiate spicule, projecting into the central gastral cavity. *i. c.* Inhalant canal. *mus. c.* Muscle cell. *osc.* Osculum. *ov.* Ovum. *pros.* Prosopyle. *s. d. q.* Subdermal quadriradiate spicule. *s. d. s.* Subdermal sagittal triradiate spicule. *s. g. s.* Subgastral sagittal triradiate spicule. *s. m.* Sollas's membrane. *sp.* Spicule. *sp. s.* Spicule sheath (formed from the gelatinous ground substance of the mesoderm). *st. c.* Stellate mesoderm-cell. *t. ox.* Tuft of oxeote spicules at the end of a radial chamber.

PLATE 10.

FIG. 1.—Leucascus simplex. Portion of a vertical section, passing, on the left, through the osculum. Drawn under Zeiss A, ocular 2.

FIG. 2.—Sycon Carteri. Portion of a transverse (horizontal) section, showing three of the radial chambers. Drawn under Zeiss C, ocular 2.

FIG. 3.—Sycon gelatinosum. Portion of a longitudinal (vertical) section, passing through the distal ends of the radial chambers. Drawn under Zeiss C, ocular 2.

FIG. 4.—Sycon gelatinosum. Portion of a longitudinal (vertical) section, passing through the proximal ends of the radial chambers. Drawn under Zeiss C, ocular 2.

FIG. 5.—Sycon gelatinosum. Portion of a tangential section, cutting across the radial chambers. Drawn under Zeiss C, ocular 2.

FIG. 6.—Sycon gelatinosum. Portion of a tangential section of the dermal surface showing the tufts of nail-shaped oxea which crown the distal ends of the radial chambers, and the pore-bearing membrane stretched between them. Drawn under Zeiss C, ocular 2.

FIG. 7.—Sycon boomerang. Portion of a transverse (horizontal) section, showing one much-branched radial chamber. Drawn under Zeiss A, ocular 2.

FIG. 8.—Sycon boomerang. Portion of a tangential section of the dermal surface, showing the tufts of oxea which crown the distal ends of the radial chambers, and the pore-bearing membrane (containing a few spicules) stretched between them. Drawn under Zeiss C, ocular 2.

PLATE 11.

FIG. 9.—Grantia extusarticulata. Portion of a transverse (horizontal) section. Drawn under Zeiss A, ocular 2.

FIG. 10.—Grantia Vosmaeri. Portion of a longitudinal (vertical) section. Drawn under Zeiss A, ocular 2.

FIG. 11.—Grantiopsis cylindrica. Portion of a transverse section. Drawn under Zeiss A, ocular 2.

FIG. 12.—Ute syconoides. Portion of a transverse (horizontal) section. (The majority of the spicules have been dissolved out by the action of acid alcohol, but the large oxea of the dermal cortex are shown cut across.) Drawn under Zeiss C, ocular 2.

FIG. 13.—Ute syconoides. Portion of a tangential section cutting across the radial chambers. Drawn under Zeiss C, ocular 2.

FIG. 14.—Ute syconoides. Portion of a tangential (longitudinal) section, passing above through the dermal surface and below through the dilated distal ends of the radial chambers. Drawn under Zeiss A, ocular 2.

PLATE 12.

FIG. 15.—Synute pulchella. Portion of a transverse (horizontal) section, showing the central gastral cavities of three Ute individuals, each surrounded by radial chambers and all together invested in a common cortex. Drawn under Zeiss A (with the bottom lens removed), ocular 2.

FIG. 16.—Leucandra phillipensis. Portion of a transverse (horizontal) section. Drawn under Zeiss A, ocular 2.

FIG. 17.—Leucandra australiensis. Portion of a transverse (horizontal) section. Drawn under Zeiss A, ocular 2.

PLATE 13.

Fig. 18.—Grantessa intusarticulata. Portion of a transverse (horizontal) section. Drawn under Zeiss A, ocular 2.

Fig. 19.—Vosmaeropsis macera. Portion of a transverse (horizontal) section. Drawn under Zeiss A, ocular 2.

Fig. 20.—Heteropegma nodus-gordii. Portion of a transverse section. Drawn under Zeiss A, ocular 2.

Fig. 21.—Leucilla uter. Portion of a vertical section. Drawn under Zeiss A, ocular 2.

Fig. 22.—Leucilla australiensis. Portion of a longitudinal (vertical) section. Drawn under Zeiss A, ocular 2.

PLATE 14.

Fig. 23.—Vosmaeropsis Wilsoni. Portion of the cortical inhalant canal system, as seen in a transverse section of a specimen killed with osmic acid. The space occupied by the dermal cortex, between the inhalant canals, is left blank, and the pore-bearing dermal surface is shown in perspective. Drawn under Zeiss C, ocular 2.

Fig. 24.—Ute syconoides. Portion of a tangential section cutting across the radial chambers, showing a chamber in the contracted condition, surrounded by four ordinary chambers and four inhalant canals, portions of which only are drawn. Drawn under Zeiss E, ocular 2.

Fig. 25.—Leucandra sp. One of the rounded flagellated chambers cut in half. One row of collared cells, with collars united by Sollas's membrane, is seen round the margin, while the observer looks down upon the exhalant opening of the chamber and two prosopyles. Drawn under Zeiss F, ocular 2.

Fig. 26.—Leucandra sp. Exhalant aperture of another chamber from the same specimen as Fig. 25, surrounded by the membranous chamber diaphragm, in which the nuclei and granules of muscle-cells are seen. Drawn under Zeiss F, ocular 2.

Fig. 27.—Grantessa intusarticulata. Portion of a section through the gastral cortex and proximal end of a radial chamber. On the right a portion of the chamber diaphragm, marking the junction of the radial chamber with the exhalant canal, is seen in section. On the left a portion of the inner end of an inhalant canal is seen. Drawn under Zeiss F, ocular 2.

Fig. 28.—Grantessa intusarticulata. Portion of a section through the gastral cortex, showing two of the endodermal pavement cells in section and a stellate cell embedded in the gelatinous ground substance of the mesoderm. Drawn under Zeiss F, ocular 2.

Fig. 29.—Grantessa intusarticulata. Three contracted ectodermal

pavement cells from the lining of an inhalant canal. Drawn under Zeiss F, ocular 2.

Fig. 30.—Grantessa intusarticulata. Portion of the wall of a radial chamber looked down upon, showing a number of contracted collared cells, and between them a prosopyle, with the nucleus of an ectodermal pavement cell on its margin seen at a slightly higher focus than the collared cells. Drawn under Zeiss F, ocular 2.

Fig. 31.—Vosmaeropsis macera. Exhalant opening of a flagellated chamber, surrounded by the membranous chamber diaphragm containing muscle-cells. Drawn under Zeiss F, ocular 2.

Fig. 32.—Leucandra sp. Small portion of a vertical section through the region of the osculum, showing four of the blister-like epithelial cells which line the gastral cavity in this region. Drawn under Zeiss F, ocular 2.

Fig. 33.—Leucandra echinata, var. An amœboid cell from the mesoderm. Drawn under Zeiss F, ocular 2.

Fig. 34.—Leucandra echinata (?). An ovum from a cavity in the mesoderm. Drawn under Zeiss F, ocular 2.

Fig. 35.—Ute syconoides. Section across an inhalant canal (intercanal), showing an ovum suspended from its wall. Drawn under Zeiss F, ocular 2.

Fig. 36.—Ute syconoides. An ovum from behind the wall of a radial chamber. Drawn under Zeiss F, ocular 2.

Fig. 37.—Ute syconoides. Two vesicular cells from beneath the epithelium of the gastral cortex. Drawn under Zeiss F, ocular 2.

Fig. 38.—Grantessa intusarticulata. Section of an embryo lying in a cavity lined by epithelial cells (the embryo capsule). Drawn under Zeiss F, ocular 2.

Figs. 39—42.—Leucandra phillipensis. Stellate mesoderm-cells from the dermal cortex. Drawn under Zeiss F, ocular 2.

Fig. 43.—Leucandra phillipensis. Subdermal gland-cell beneath the wall of an inhalant canal, which is cut through on the right. Drawn under Zeiss F, ocular 2.

Figs. 44—47.—Leucandra phillipensis. Portions of four oxeote spicules from the dermal cortex, with calcoblasts attached. Drawn under Zeiss F, ocular 2.

Figs. 48—50.—Leucandra phillipensis. Amœboid cells from the mesoderm between the flagellated chambers. The one shown in Fig. 50 appears to be feeding by means of pseudopodia upon the collared cells of a flagellated chamber. Drawn under Zeiss F, ocular 2.

Fig. 51.—Leucandra phillipensis. A group of retracted collared cells. Drawn under Zeiss F, ocular 2.

FIGS. 52—54.—Grantiopsis cylindrica. Calcoblasts from the dermal cortex. In Fig. 52 the calcoblast is lying upon a ray of a large triradiate spicule. In Fig. 53 the spicule is partially dissolved by the acid alcohol, and the spicule-sheath is visible. Drawn under Zeiss F, ocular 2.

FIG. 55.—Grantiopsis cylindrica. Four small mesoderm-cells from the dermal cortex. Drawn under Zeiss F, ocular 2.

FIG. 56.—Grantiopsis cylindrica. Three subdermal gland-cells, each connected by a long, slender process with the granular dermal surface. Drawn under Zeiss F, ocular 2.

FIG. 57.—Grantiopsis cylindrica. Small portion of the dermal surface. Drawn under Zeiss F, ocular 2.

FIG. 58.—Micro-organisms from the dermal surface of Grantiopsis cylindrica. Drawn under Leitz $\frac{1}{12}$, oil immersion.

FIG. 59.—Sycon Ramsayi. Contracted epithelium from an inhalant canal. Drawn under Zeiss F, ocular 2.

FIG. 60.—Sycon Ramsayi. Contracted epithelium from an exhalant canal. Drawn under Zeiss F, ocular 2.

FIG. 61.—Sycon Ramsayi. Sections of two epithelial cells from an exhalant canal. Drawn under Zeiss F, ocular 2.

FIG. 62.—Grantessa erinaceus. Contracted epithelium of an endogastric septum, formed by outgrowth of the gastral cortex into the gastral cavity. Drawn under Zeiss F, ocular 2.

FIG. 63.—Vosmaeropsis Wilsoni. Epithelium from the upper surface of an oscular diaphragm. From an osmic acid specimen mounted in glycerine. Drawn under Zeiss F, ocular 2.

FIG. 64.—Vosmaeropsis Wilsoni. Contracted epithelium from an exhalant canal. Drawn under Zeiss F, ocular 2.

Some Points in the Origin of the Reproductive Elements in Apus and Branchipus.

By

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With Plates 15 and 16.

The mode of generation of the reproductive elements and their relation to the cells of the parental tissues, is a problem which has always made heavy demands on the labours of microscopical investigators, whether zoological or botanical; and although an immense literature has grown about the subject since Flourens thought embryos were formed "tout d'un coup," at the moment of the fusion of the sexual elements, it may be safely asserted that not until the last few years has any very definite knowledge been acquired, and only in a limited number of cases has it yet reached any high degree of accuracy.

In a certain number, however, we do know what is actually done during the origin of these cells; and this knowledge is a priceless gift to the biologist, as there is little doubt that the apprehension of karyokinesis, both in its relation to the "Reductions-Theilung" and the ordinary division of somatic cells, has brought him face to face with the actual mechanical expression of hereditary transmission and the problems connected with it.

The modus operandi of the forces which bring about these changes, however, or any serious attempt to ascertain whether they be modifications of ordinary physical phenomena at all, or whether the whole "fleeting show" of attractions, repulsions, and nuclear metamorphoses must be looked upon as something

outside the physical domain, as ordinarily understood, is still a fundamental, though quite legitimate problem for microscopical inquiry.

In attempting to obtain a clear conception of the premises from which we have to start in such inquiries, the once absorbing question of the nature of the wide structural differences often apparent in the male and female cells will be found to have lost, if not much of its importance, at any rate most of its original characters. The conjoint labours of Hertwig,[1] Ishikawa,[2] vom Rath,[3] and others, as well as the curious observations of Weismann concerning the non-specialisation of certain spermatozoa, have completely changed the scenes in this direction; and it is matter for rejoicing that the shifting, at any rate in this particular, tends towards a simplification in the gradual banishment of apparent difference in such elements, and of their associated mechanical complexity, to the rank of a purely physiological importance.

Opinion to-day is almost unanimous that the ova and spermatozoa are strictly similar objects, that even the most modified spermatozoon still carries about with it the dwarfed representatives of cell structure; and our knowledge of its development is sufficiently advanced to recognise in the head the reduced nucleus, the kytoplasm in the tail; and lastly, it appears probable from Hermann's,[4] and more especially from Fick's[5] investigations, that the hitherto enigmatical "Mittel Stück" is in reality nothing less than the attraction sphere.[6]

[1] "Vergleich der Ei und Samenbildung bei Nematoden. Eine Grundlage für celluläre Streitfragen," 'Archiv f. mikroskop. Anat.,' Bd. xxxvi, 1890.

[2] 'Studies of Reproductive Elements.'

[3] 'Archiv mikro. Anat.,' Bd. xl, p. 102.

[4] 'Archiv für mikros. Anat.,' Bd. xxxiv, Tafel 3.

[5] "Ueber die Befruchtung des Axolotleies," 'Anatomischer Anzeiger,' vii, pp. 818—821.

[6] The results of a re-examination of the facts of Mammalian spermatogenesis have shown that the centrosomes are incorporated in the spermatozoa in the position of the Mittelstück of Amphibia, while a portion of the archoplasm is applied to the pointed extremity of the head. Field has shown that the archoplasm "Nebenkern" of Echinoderms is incorporated together with the centrosomes as the Mittelstück.

Although both the eggs and the spermatozoa are cells, and similar cells, the final karyokineses which produced them are different from the preceding divisions in the cells of the genital epithelium, whether male or female. As is now well known, this change in the divisional phenomena appears in the extrusion of the polar bodies in the egg, and the "Reductions Theilung" in the spermatozoa. The existence of these phenomena constitutes the empirical though important ground for several well-known theories concerning the physiological value of the "Reductions Theilung" as a preparatory balancing of the "hereditary substance" in two cells, whose fusion forms the starting-point of a succeeding generation.

Nearly all the readings of this riddle actually offered, have sprung from one of two sources: either they have come back upon us as the new-clad ghosts of Balfour's famous interpretation of the polar bodies; or have accepted as sufficient explanation of the facts, Weismann's theoretical conceptions of the necessity of a reduction in the quantity or the quality, or both, of the hereditary substance (chromatin).

The continuous processes of assimilation and growth in the resting cells of any tissue, although beyond our actual scrutiny, offer nothing antagonistic to the generally adopted notion, that they are the result of the passive influence of an immensely complicated structure, operating under certain rather limited conditions. But the final dissolution of these conditions, when assimilation and growth in the individual can presumably go no further,[1] and their re-establishment under more favorable circumstances, is quite another matter.

In this procedure, the essential material constituents of the cell are accurately halved and separated, and we may presume that the complicated mechanical basis of the resting cell's activity, or at any rate the power to return to it at some future time, goes with them. What are the means by which this material distribution is effected? According to more recent

[1] Herbert Spencer suggests that cell division becomes a necessity in virtue of the continual decrease of the absorptive area proportionally to the growth of the cell.

investigation, an essential agent seems to be two centres of an alternately attractive and repulsive nature (centrosomes); but concomitantly with, and independently of, their operation, other and no less important changes accrue within the cell, perhaps most notably, the evolution of the fine chromatic reticulation of the resting nucleus into a limited number of chromosomes at the nuclear periphery, their number being constant for any particular species—a fact making them of great practical importance in all questions relating to heredity.

Now I wish to call attention to some points in the spermatogenesis of Branchipus, which may appear to throw much light on portions of these successive stages; and although it is at present hopelessly inadequate to illuminate the greater problems which arise from it, I have taken extra trouble to be sure of my ground here, because I conceive that the existing theoretical solutions of these problems must one day find powerful confirmation or the reverse, in a true appreciation of the character of the processes which underlie the karyokinetic metamorphosis.

Spermatogenesis.

The male gland in Branchipus is a rather straight biramous tube extending up the tail, and a short distance further up the body. If spermatozoa are free in the lower portion all the stages of spermatogenesis are visible as we pass up. The difference in phase amongst the cells may be taken to represent the zones of Hertwig, van Beneden, and Julin.[1]

The spermatocytes, however, break away from the walls in groups (fig. 21), their individual components being all in the same phase. But as this phase, characteristic of each group as a whole, is not often similar to those on either side of it, we

[1] "Nouvelles recherches sur la fécondation et la division mitosique chez l'ascaride mégalocéphale," 'Bullet. de l'Académie Royal de Belge,' 3me sér., c. xix, 1887. "Befruchtung und Theilung des thierischen Eies," 'Morph. Jahrb.,' 1875.

cannot strictly divide the tube into ascending zones. When the ordinary somatic division has come to an end spermatogenesis sets in among the cells lining the hollow of the tube. These somewhat minute elements rapidly increase in size, and their nuclei pass into a spirem, with a peculiar and characteristic grouping of their chromatic elements all on one side, just as in Hermann's beautiful figures of spermatocytes in the salamander (fig. 3).[1]

Sometimes before, and always during the course of these changes, bodies answering to the centrosomes in all peculiarities except their number, which is abnormally great, make their appearance in the angular mass of protoplasm at the bases of the characteristic cells represented in figs. 1—4, *a*.

Merely for the sake of clearness, and to keep these bodies out of confusion with the true and enormous centrosomes appearing later, as well as to separate them from another type of body, to which I shall have to refer at length, I have provisionally collected these bodies under the term pseudosomes.

As the spermatogenesis proceeds, the lop-sided chromatic arrangement of the spirem rapidly gives place to ten chromosomes, all arranged on the nuclear periphery, and these ten chromosomes in turn become transversely constricted to form the well-known dumb-bell elements (figs. 8—12), so that we have ten double or twenty single chromosomes, which rapidly arrange themselves in the disc-like equatorial plate seen in optical section (fig. 11).

At this period of the metamorphosis (Flemming's metakinesis) a number of most remarkable bodies make their appearance, more or less exclusively related to the cell periphery, but connected one to another and to the inner group of chromosomes by fine strands, which remain uncoloured by reagents; and, as their relation to these fine threads suggests the nodal points in a net, I have termed them dictyosomes (figs. 11—13, *d*).

The constriction between the dumb-bell-like heads of the chromosomes becomes more and more pronounced, and they

[1] 'Arch. f. mikros. Anat.,' Bd. xxxvii.

ultimately separate, passing in opposite directions towards the relatively colossal centrosomes now occupying the spindle apices (figs. 12, 17, 19, *c*, *d*). I have separated the dictyosomes from the centrosomes, not because they appear to be in any way essentially distinct, but because they originate at a later period in the division, and the two sets of structures might otherwise be confused.

Respecting the relation between the pseudosomes, centrosomes, and dictyosomes I shall speak later on.

The two nuclear groups now separate as in fig. 14, and the first reduction division is completed. The small elements thus formed never again regain the character of a resting cell,[1] but there are appearances of irregular division, resulting in the formation of two excessively small spheroidal bodies, each presumably containing the equivalent of five chromosomes (figs. 15, 16). This procedure must be looked upon as constituting the second "Reduction Theilung," and the resulting elements are the mature spermatozoa.

With the above broad facts of spermatogenesis kept well in view I proceed to a more minute description of the successive stages of the karyokinesis related to it, more especially with a view to determining the nature of the bodies I termed dictyosomes and pseudosomes in the previous description, and which at first seemed to appear, disappear, and reappear in a quite bewildering fashion. The original small spermatocytes are similar in all essentials to the least specialised elements of the somatic tissues.

When stained with orange, gentian violet, or hæmatoxylin, after treatment with Hermann's or Flemming's fluid (the best results were obtained from a combination of gentian violet and orange), the somewhat triangular cells[2] present a fine reticulate appearance, both within and without the nucleus. The meshes of this reticulum are of fairly equal size in both cases (fig. 1), and a close examination leaves no doubt that the appearance (at any rate in these cells) is produced by a vast number of

[1] Compare vom Rath and Ishikawa, loc. cit.

[2] Compare vom Rath's figs., loc. cit.

clear globules, kept apart by some non-miscible intervening fluid;[1] in fact, the whole might fitly be described as a foam structure, or "Schaumplasm" of Bütschli.

I have attempted to give some idea of this appearance in fig. 1, a resting spermatocyte just previous to its division, but the result is not nearly so impressive as I could wish.

Nuclear stains affect to a certain extent the intervening fluid throughout the whole cell, and the stain appears to be related to excessively fine granules suspended in a clear plasma. These cyto-microsomes do not appear to be "varicosities" of the kytoplasmic strands between the globules, but the stain appears to affect microsomes suspended in this intervening fluid. The whole darkened nuclear area suggests a condensation of this staining material, possibly by its own cohesion.

Outside the nucleus there are usually to be found, on the side where there is most cell body, and where vom Rath represents the centrosomes in the resting spermatocytes of Gryllotalpa, those dark points, whose appearance corresponds in everything but number with the centrosomes as ordinarily understood, and which I collected in the more general description under the term pseudosomes (figs. 1—6, *a*). No archoplasm is apparent round them, and a close examination suggests that they are simply the expression of a collection of the above staining material (microsomes) in the angular spaces between the spheroids, producing the reticulate appearance (figs. 1—9).

Careful search will, as I have said, often raise the number of these bodies as high as six or eight. The more we look the more difficult it becomes to separate the pseudosomes from the less conspicuous interspaces of fluid between the globules; both appear to pass insensibly into each other. The appearance and relation of the more conspicuous are very striking, as observation of their subsequent behaviour left no doubt on my mind that they were intimately bound up, if not with the origin, at any rate with a remarkable increase witnessed in the centrosomes ultimately occupying

[1] When sufficiently high powers are used the appearance is almost identical with the coarse vacuolation in the ectosarc of Amœba and other protozoa.

the apices of the spindle figure. It will, however, be well to advance the description of the mitosis a little before discussing this point. The first nuclear differentiation appears at one side of the nucleus as a colourless spot (fig. 2), which grows, driving the chromatic network before it to one side (fig. 3). The individual chromatin bands become shorter and thicker in proportion to this displacement, and nearly all the fine strands of "linin" disappear from this area, or, in other words, the spheroids fuse one with another, the fusion being produced by the substance of the clear globules breaking through the walls of intervening fluid one into another. In fact, this fusion spreads just as in soap froth the larger bubbles grow at the expense of the smaller, and the continuance of such a process results in the chromatin being thrown to one side in the form of a crescent (fig. 3), its threads are naturally thickened in proportion to their displacement, and the curious initial figure, which so much struck Hermann in the spermatocytes of the salamander, appears to be a necessary consequence of an intra-nuclear fusion in Branchipus.[1]

As the intra-globular fluid (with its staining granules) is between the adjacent spheroids, or in any single instance is peripherally disposed towards them, it follows that if the fusion continues until the whole nucleus consists of one or of a small number of spheroids, the intervening staining chromatin will be, as it practically is, all on the periphery.

Secondly, the fewer the globules, the larger and fewer the angular spaces between them (figs. 4, 5), and consequently the more deeply staining intervening matter appears as a limited number of chromosomes connected by fine striæ (linin); their actual number will naturally depend on the size of the spheroids compared with that of the nucleus.[2]

A great deal of importance has been attached directly

[1] 'Arch. für mikroskop. Anat.,' Bd. xxxvii, pp. 569—582.

[2] I do not mean to maintain that there are no other controlling factors in the formation of a definite number of chromosomes; this cannot be the case on account of the wide difference in the size of the nuclei of tissues of the same animal. At the same time we have no knowledge of the reticulum as related to different cellular dimensions.

or indirectly to the number of the chromosomes by all the more recent investigators, and this factor in their origin (in Branchipus) fully bears out the assumption that they are the visual expression of the primary constitution of the cell to which they belong. Nor is this all; for if we believe, as we have every reason to believe, that the character of the nucleus is the determining factor of the nature of the cell's activity, that curious variation in the number of the chromosomes in cells of closely allied species would be more intelligible; for although the frothy structure of the nucleus might be actually or closely similar, a very slight difference in the cellular dimensions would, provided the foam structure remained the same, materially alter the number of the spaces between the globules, and consequently the number of the chromosomes. It is at the same time apparent that this number, as well as the general nuclear characteristics, oscillate within narrow limits for the same species.[1]

It is interesting to note in this connection that the characters of nuclei, in Arthropods and Annelids, have much in common. They nearly all present the peculiar ball-like chromosomes during metamorphosis, just as they tend to form a reticulate nucleus when at rest. In fact, we might say such nuclei constitute an Annelidean nuclear type.

Again, the characters of the Mammalian nuclei are very constant, but they nevertheless differ in minor details even from those of the Amphibia. In fact, the difference between these two latter is as small as that between them both, and the former, is great. The comparative study of nuclei is well worthy of more minute attention; suffice it, however, at the present moment to point out that such generalisations would have weight in our conceptions of heredity.

Of the regular occurrence of a peculiar intra-nuclear fusion in Branchipus the appearances leave no doubt, or that it is primarily instrumental in bringing about the conversion of the

[1] In connection with this see Valentine Häcker, "Die heterotypische Kerntheilung im Cyklus der generation Zellen," 'Berichte der Naturforschenden Gesellschaft zu Freiburg,' Bd. vi, 1892, pp. 160—188.

fine chromatic network of the resting nucleus first into the lop-sided figure described by Hermann, and probably has a good deal to do with the origin of the ten chromosomes on the nuclear periphery (figs. 4—8). But the initial impulse which starts such a fusion is an entirely different matter. This might rise from a variety of causes, from a gradual increase of internal pressure caused by osmotic action, or it might be produced by some change in that polarity supposed to exist between the centrosomes lying close to its exterior; and it is curious to note in this connection that the fusion in Branchipus does start from that side where the most marked pseudosomes exist (figs. 3, 4, 6, *a*, *a*), and, if we may put the same interpretation on the metamorphosis of other cells, Hermann's, vom Rath's, and possibly Flemming's figures would be in complete accordance with such a view. I very much doubt, however, if either of these suppositions will be found to be the explanation of the origin of the fusion in the first instance. But, if once started, we have seen that the nuclear metamorphosis during karyokinesis, from the resting stage up to that at which a limited number of chromosomes exist on the periphery, is, to a certain extent, the logical consequence of its progress.

I have arranged the succeeding description in the light of this conception because, since it has helped us thus far, we might pre-suppose it useful in the elucidation of other karyokinetic phenomena; and, unless I have done very indifferent justice to the appearances before me, this supposition should be fully justified.

The ten ellipso-spherical chromosomes (figs. 5, 7) which have arisen from an irregular transverse splitting, or, rather, running into drops of the thickened chromatic network, after it was brought by the progressive fusion to the nuclear periphery, become rapidly constricted in the middle to form the dumb-bell figures characteristic of these and many other Arthropod nuclei (figs. 7—12).

Each cell now contains ten double or twenty single chromosomes, i. e. double the ordinary number (figs. 7, 10, 12); and it is interesting to compare such nuclear figures and their origin

with others, like those of Salamander, in which the chromatin is arranged in a succession of bands or irregular annuli, set more or less transversely to the long nuclear axis. These appearances would be produced by a similar fusion of globules, in such a manner that a few diaphragm-like membranes of the intervening fluid with its staining microsomes were left across the long axis of the nucleus. In such a case the chromatin would inevitably be arranged as it always is in the re-entrant solid angles. Interesting artificial reproductions of the nuclear figures may be seen by watching the growth of bubbles, and I have given in fig. 18 some drawings of the ultimate configurations produced by the growth of bubbles in a fine froth. The lines of foam left as bands along the position of the ruptured walls would represent the chromatic loops, and it will be seen that they show a marked tendency to contract into more or less rounded bodies.

The existence of nuclei in groups of four or five, each with their ten dumb-bell chromosomes, gives a very striking appearance to the testes of Branchipus (fig. 21); and while the condition characterises one of the longest phases of the whole nuclear division, its final metamorphosis occurs with the utmost rapidity, the cells appearing as if transformed by magic into a complete spindle figure. Intermediate phases are, however, to be found, and it appears that the fusion or running together of the globules continues, breaking through the old nuclear boundary at several points into the surrounding kytoplasmic network (fig. 8), so that the clear mass of nuclear plasm appears to spread out on all sides (figs. 8, 11, 19). The result of this is that chromosomes are at last left hanging in a clear central space by a few irregular strands of this kytoplasmic network, into which the fusion has not yet broken (figs. 10—12, 19). These irregular strands are ultimately reduced to fine threads (figs. 11, 12, 17, 19), and their peripheral extremities are related to dark bodies which can be nothing but the pseudosomes of which I have already spoken in an earlier phase of the metamorphosis (figs. 10, 11, 12, *da.*). These pseudosomes appear now to have

increased in size somewhat, their relation to the spaces of the intra-globular network being more pronounced, and we are naturally led to the conclusion that such an increase is brought about by the massing of the staining material in these angular spaces, owing to the progressive fusion tending to lessen their number and increase their size, just as it did with respect to the chromatin within the old nuclear limits.

Proportionately to the extension of this fusion, the tension along the achromatic lines, on which the chromosomes are suspended, becomes greater as the dark points (pseudosomes) at their peripheral extremities retreat with the vanishing achromatic network and its contained microsomes towards the cell's circumference (figs. 10—12, 19).

If we now try to realise what is actually taking place, it will become apparent that the traction towards the periphery through these points (pseudosomes) along the achromatic threads, and ultimately upon the chromosomes themselves, will tend to set itself along some axis across the nuclear figure as a whole, and the points (pseudosomes) chosen will be those on opposite sides which have, so to speak, the best foothold in the periphery. The remaining points (pseudosomes) will tend to glide as they do (figs. 9, 10, 12) towards the extremes of such an axis, and a spindle figure will be finally set up (figs. 9, 12, 22, 23). From the figures just referred to, it will be apparent that the coalescences of the points of attachment of the distal extremities of the achromatic fibres (pseudosomes) become marked out as centrosome-like bodies which travel away towards the cell's circumference, and finally come to rest on its extreme margin (figs. 11, 22, 23). In other words, these centrosomes are virtually derived from a fusion of some of the pseudosomes, and these were in turn seen to originally correspond to the angular spaces in a network exterior to the nucleus.[1]

In cells a trifle more advanced than those represented in the preceding figures showing (fig. 19) the area of clear

[1] Professor Farmer has kindly shown me some preparations of Lilium which give exactly the same bundles of fibres related to separate granules, any one of which might be individually considered as a centrosome.

fluid produced by the massing of the original diffuse staining material within the nucleus into the small space of the chromosomes, by the process I have described—it will be seen that this space, which in the first stage of the spindle figure represents the nuclearplasm, and retains the spherical character of the original nuclear contour, has become very much enlarged (figs. 11, 12, 23), not only in the direction of the spindle axis, but laterally all round, so that it appears as a continually increasing irregular area, occupying by far the greater part of the cell's substance. Round this irregular space a rind of the original kytoplasmic reticulum still remains (figs. 11, 12, 19, 23), and it will be noticed that at the junction of this rind and the clear fluid within (fig. 19) a number of small staining points exist, related to the angular spaces between the clear globules and the non-miscible intervening fluid.

These bodies grow continually, and their size marks the progress of the fusion of the clear central mass of fluid with the similar constituents of the peripheral rind, just as the thickening of the chromatin bands was the measure of the fusion proceeding within the original nuclear limits.

They continually stain more and more deeply with orange and gentian violet, as the diffuse staining material dispersed through what remains of the kytoplasmic network is swept before the progress of the fusion into nodal points until, simultaneously with its extension through the whole cell, they are left as some twenty conspicuously dark bodies regularly arranged on the periphery (figs. 13, 17). Close examination reveals, however, that the fusion is not really complete, but that fine achromatic threads connect these bodies one to another (figs. 13—19) and to the inner group of chromosomes in the manner described in an earlier part of my paper—a fact which led me to devise the term dictyosome as expressive of these peculiar relations.

It will be seen that these dictyosomes appear at a definite point in the karyokinetic metamorphosis, viz. the later phases of the spindle figure; and the cells which present these conditions are comparatively large spherical bodies, which have

become so much altered in their refractive characters that one is reminded of Flemming's words when describing a similar change witnessed in the dividing cells of the salamander:

"Bekommt man unwillkürlich den Eindruck, als sei die Zelle während ihrer Theilung durch und durch mit einer besonderen Substanz durchtränkt oder—um mich vorsichtiger auszudrücken—als besitze sie durch und durch eine besondere physikalische oder chemische Beschaffenheit."

This change appears in the spermatocytes of Branchipus to be the direct result of the collecting of the primarily diffuse staining material of the resting nucleus into ten chromosomes, and of that existing in the kytoplasm without into distinct chromatic bodies (dictyosomes). Both these changes are apparently due to a progressive fusion or running together of the clear globules which, begun within the nucleus, formed the chromosomes on its surface, and extending, swept the diffuse staining material of the cell body together into some twenty dictyosomes on its circumference. In this brief and necessarily crude manner I hope to have made the main drift of the investigation up to this point clear. I have dealt with the development of the chromosomes in Branchipus, and shown reason to believe that it is in a measure dependent on a fusion of the globules which give rise to the reticulate appearance. The progress of such a fusion would produce the one-sided figure described by Hermann in the spermatocytes of the salamander, and tend ultimately to form a limited number of chromosomes all on the nuclear periphery; and we have seen that during these changes there exist in the resting spermatocytes those dark points (pseudosomes) whose appearance corresponds in everything but number with the centrosomes of previous authors.

We have seen also that the fusion producing such wide changes in the nucleus spreads beyond it, leaving the chromosomes suspended to the pseudosomes. These pseudosomes retreat with the remnant of the original network as it vanishes towards the periphery, and in connection with this motion an axis tends to be set up round which the spindle figure gathers,

while at its apices some of the pseudosomes coalesce to build up the colossal centrosomes.

Lastly, just as the fusion within the nucleus brought about the massing of the chromatin into a limited number of chromosomes, so also the extra-nuclear fusion operating in the same way upon the sparse staining material of the kytoplasm, without the nucleus ultimately collects this, into chromatic bodies in the angular spaces between the enlarged globules. They first appear as an irregular cloud on the outskirts of the fusion (fig. 23, *d*), grow enormously in size, and acquire a regular distribution on the cell periphery. They still, however, remain connected one to another and to the inner group of centrosomes by fine threads; the fact that they thus form, as it were, the nodal points in a net, suggesting the term dictyosome as expressive of this peculiar relation. It will, moreover, have become apparent from the description that there is no genetic distinction between the pseudosomes, centrosomes, and dictyosomes, and my sole reason for using the two new terms is their successional appearance.

In thus bringing into prominence the existence in Branchipus of a veritable "Schaumplasm" and its inter-activities, I would observe that I do so with no predisposition to utilise Bütschli's conception of such structure as a fundamental interpretation of some of the phenomena of karyokinesis, either in this or any other case, but rather the reverse. Nevertheless the observation that a foam structure is intimately bound up with the phenomena of karyokinesis on the one hand (even in a single type) must materially enhance the value of Bütschli's ingenious hypothesis that it is sufficient to account for the amœboid activities of protoplasm on the other.

A very natural objection to the conclusion I have stated may arise out of the apparent whittling process to which it subjects the centrosomes, resolving these bodies into nothing more than the irregular staining material between the globules of a protoplasmic froth.

I wish, however, while concluding this part of my paper, to point out that such an objection is only apparent, and not real.

In a former essay, while discussing the meaning of the difference in the component parts of the spheres apparent in the works of Flemming, Hermann, van Beneden, Boveri, and others, I remarked, "Comparison between the spheres and their constituent parts in various animals might appear pedantic, and, in the present state of our knowledge, unnecessary, if it were not that some of these parts are probably, as we have seen, the fleeting expression of metamorphic phenomena; while others (such as the central body), though dividing, retain their characteristics unimpaired;" and I have ventured to repeat this as showing that the great pioneers of this phase of cytology had already hunted the all-important part of the sphere down to the narrow limits of the centrosome. And the fact that in Branchipus six or eight bodies indistinguishable from one another exist at first, and that these afterwards fuse to augment the size of the two actually chosen to occupy the spindle apices, does not prevent anyone from regarding these two of the six or eight, as endowed with special properties if he pleases, nor does their relation to the interglobular spaces affect the point in any way that I can see. Whether two of these bodies are really to be regarded as different from the rest is a point on which at present I offer no opinion.

Comparison with the Ovigenesis.

It will be seen that the spermatogenesis of Branchipus corresponds in the main with that described by vom Rath in Gryllotalpa, and that the reticulum has disappeared in the ultimate division altogether (fig. 16). Now if, as I have shown, there is reason to believe the reticulum in this particular instance is a mechanical factor in portions of the karyokinesis, all possibility of such phenomena will come to an end with the complete fusion of the clear globules, and there is thus a definite reason why the subdivision goes thus far and no farther, at any rate for a time.

In the ovigenesis proper—that is, in the metamorphosis among those cells which directly produce the eggs—there is nothing special; but among the cells subsidiary to this process,

many points are worthy of attention. Scattered through the egg mother-cells are numerous groups and rows of nuclei, obviously of a different character from those destined to form the eggs (figs. 20, 21, 43, &c.). These nuclei are very irregular in outline, and of a fine reticulate appearance. They show numerous figures of direct division (figs. 20, 43). At the same time it is quite easy to establish a gradational series extending from the true egg, forming nuclei on the one hand to the irregular akinetically dividing elements on the other, the latter class being always intimately and actually concerned in the secretion of a peculiar slimy substance (fig. 44); and this slime in turn is ultimately worked up in the lower portion of the tube to form the ornamental egg-case, so that although in the egg formation in Branchipus the primitively similar genital cells (male ova) diverge along two ways, one leading through successive karyokinesis to the final eggs, they ultimately both co-operate in the perpetuation of the species by the rest being bodily transmuted into the ornamental case in which the eggs are laid. This duality in the ovarian elements is interesting in the sense that it offers a precise parallelism to the dualism caused in the spermatic apparatus by the presence of the akinetically dividing foot-cells, over whose significance so much controversy has at times been raised. In Branchipus the foot-cells are more regularly arranged than the above akinetically dividing elements in the ovary. At the upper end of the gland they occur at intervals of about ten cells in all directions, and, true to their female homologues, are more numerous as we descend towards the genital aperture. Apart from the function of the foot-cells no one can be in doubt as to their homology with the above akinetically dividing elements of the ovary; and the fact that the latter are intimately bound up with the formation of the slime that makes the egg-case (slime-cells) seems to me to remove all doubt from vom Rath's theory, that in the spermatogenesis they are concerned in the secretion of a fluid in which the spermatozoa are suspended. The key to the whole position seems to lie in the observation of La Valette St. George, that the mulberry-shaped masses of the spermatocytes in Blatta are

produced from one cell, whose residual moiety remains, acquiries distinct characters from the rest, and is not converted into the spermatozoa. From such a starting-point we may see our way through a gradual evolution to meet the physiological necessities of the case, to the complex reproductive apparatus in Branchipus, where two different kinds of cells exist in both sexes, one to form the eggs or spermatozoa, and one to form the case or fluid in which these bodies are respectively suspended or enclosed.

Whether akinetic division is really wholly related to the foot-cells in animals is a controverted question, but from what I have seen in Branchipus (figs. 20, 21) and elsewhere, I am inclined to believe that it is not wholly restricted to these elements, but that there is a general tendency towards the two methods in the two kinds of cells.

To recapitulate, it will be seen then—

I. That in Branchipus the observations bear out the general law as to the similarity of the male and female cells, and that their own specific peculiarities are physiological in origin, having no morphological significance.

II. The derivatives of the primitive genital cells (male ova) are of two kinds, one transformed directly into the reproductive elements, the other into the egg-case or into the fluid in which the spermatozoa are suspended. Karyokinesis is the method of procedure in the one—akinesis in the other.

III. That the divisional phenomena of these cells are intimately related to a protoplasmic structure, which might be fitly described as "Schaumplasma," and one of the initial physical impulses towards metamorphosis is a fusion of some of the intra-nuclear globules; and a considerable portion of the complicated karyokinetic figures, with their centrosomes, pseudosomes, and dictyosomes, appear to be the logical as well as the actual consequence of the continuance of this process.

With the foregoing results of observation as a basis of comparison, I made a close examination of the ovigenesis in Apus. Unfortunately the male of this species is practically unknown, ten thousand having been collected without a male appearing

in a single instance. This renders the chances of proper fertilisation very rare, and, as all the specimens are equally prolific, we must look either to hermaphroditism or parthenogenesis as the means by which the embryonic development is started. The hermaphrodite character has recently been ascribed to the genital gland of various species of Apus, and certainly the appearances which have come under my notice favour this view.[1] It is, however, immaterial to the line of inquiry I have adopted, which method of reproduction actually obtains. The genital gland is an irregular tube with numerous diverticula branching out on all sides. The cells lining the main tube and its numerous ramifications are excessively minute columnar bodies (figs. 24, 31, and 35—37), and the whole appearance is far more like that of an Invertebrate intestine than a reproductive gland. Each of the epithelioid cells contains a small peculiar nucleus (fig. 36), whose position in the rod-shaped mass of protoplasm it dominates, varies in concert with all the nuclei of the same diverticulum. The nuclei oscillate backwards and forwards from the extremities of the cells nearest the lumen of the gland to those nearest the basal membrane bounding at its periphery. When in the former position, such protoplasm as remains between them and the actual glandular cavity is seen to be rapidly degenerating into masses of slime (fig. 31); and, just as in the case of Branchipus, this slime is ultimately worked up into an ornamental egg-case. When the nuclei have translocated themselves towards the bases of the cells, the slime has broken away in streaks and globules, and many nuclei are seen subdividing themselves into groups, from whose derivatives the nuclei of the future eggs are formed (figs. 25, 27—29, 31).

It is thus obvious, that for some reason or other an economy has been effected in the reproductive apparatus of Apus, and that there is no such permanent differentiation between slime and egg-producing cells as is apparent in Branchipus, but that

[1] For an account of this see the description of Siebold's results in the 'Klassen und Ordnungen des Thier Reichs,' pp. 960—962. Also H. M. Barnard's 'Apodidæ.'

the same type of nuclei having gone down to the lumen of the gland and, so to speak, performed their dirty work themselves, travel back again to the more peripheral regions, proceeding by a series of extraordinary divisions to instal themselves directly as the nuclei of the eggs. During these migrations the nuclei retain their peculiar character little changed. In all the genital cells, the chromatin is aggregated into one or two nucleoli (figs. 24, 30, 36), constituting a nuclear type which represents the extreme term in a series, whose mean would be represented by the intestinal nuclei of Carcinus, Idotea, and others described by Frenzel;[1] where the chromatic substance is still, to a certain extent, distributed through the mass of the nucleus, although some may be aggregated into massive nucleoli.

In Apus there is no vestige of colouring matter outside the single chromosome which occupies its centre (fig. 36), the substance of which is so dense and refractive, that it appears like a red lens suspended by one or two colourless threads from the hollow sphere of the nuclear membrane. The intervening space is entirely filled by a perfectly clear nuclearplasm.

If such nuclei are at the periphery of the gland, and the egg formation is about to begin, one of these single chromosomes is seen to elongate just as the nucleolus in Frenzel's "Nucleoläre Kernbulbirung" become constricted in the middle, and finally separate into two halves, the nuclear membrane being but slightly elongated in the direction of the fission (figs. 36—41). The two derived chromosomes may in turn divide at right angles to the first separation axis, and a nucleus with four chromosomes results (figs. 27, 29, 34, 35), whose membrane is seen to be gradually tucked in at four intermediate points, and at fig. 27 a final cross-shaped differentiation can be made out between the indentations.

Along these lines the four quadrants ultimately separate, giving rise to four nuclei, each with a single chromosome (fig. 29).

Such groups of four nuclei are always associated with the

[1] 'Arch. für micro. Anat.,' Bd. xxxix.

egg formation in Apus, and are seen in all stages bulging out the membrane of the gland into the body-cavity. But it is by no means the rule that these tetrad groups are formed in this manner from one cell. In some cases two nuclei in adjacent cells divide, and the derivatives nearest the periphery divide again to form the group (fig. 25), or the group formation may proceed in a much more irregular fashion out of one or two secondary divisions of adjacent cells (fig. 27).

The dividing chromosomes within the nuclei at times present the very curious appearance represented in figs. 34, 35. It will be here seen, that between the separating, more darkly stained portions stretches a stainless band, which again suggests that the stain only affects particles suspended in a clear fluid, and that this fluid is non-miscible. Moreover it would seem that these particles tend to run together into chromatic drops, leaving a clear fluid (paranuclein).

The initial impulse, whatever it may be, which gives rise to the groups of four chromosomes, and is ultimately concerned in the formation of the egg, is seen also to affect the surrounding nuclei, which divide in the same peculiar manner again and again, until they form a narrow stalk connecting the original group of four with the cavity of the gland (fig. 42). The extreme minuteness to which this subdivision is carried will be seen (fig. 33), where it will be observed that all trace of cell membrane is fast disappearing. The minute, free, nuclear elements then left spread over the surface of the tetrad group as a thin protoplasmic membrane, in which they rest without dividing walls of any kind (fig. 42, *a*).

During all this multiplication of the nuclei, the character of their division remains precisely the same. In every division the single chromatic ball passes through the metamorphosis represented in figs. 36—41, the nuclear membrane contracting until two precisely similar nuclei are left in the place of one.

It will be obvious that this method of procedure, though on the face of it approaching akinetic or direct division, is in reality very different from the process as it appears in Branchipus, or in the other forms in which it has been described. In

all these the resting reticulate nucleus never passes out of that condition, but is constricted into two portions, each retaining its original character. It does, however, as above stated, bear considerable likeness to the "Nucleoläre Kernbulbirung" described by Frenzel. This latter mode of division is also normal to the intestinal cells of Apus. It will, moreover, be admitted that it bears a superficial resemblance to the fragmentation seen in leucocytes. But the division in these elements is certainly merely a shortened-up karyokinesis, accompanied by centrosomes and an archoplasmic metamorphosis, while no such structures are apparent in either intestinal or genital cells of Apus.

In the sense that no spindle or related parts are apparent in these cells, their division approaches akinesis. In the sense that all the chromatin is gathered into a single chromosome it approaches karyokinesis. Monomeric (or division by single chromosomes) is the best term I can devise for this method of nuclear fragmentation, although of its actual affinities I am still in considerable doubt.

It is well known that in many plant forms, such as the Myxomycetes, the karyokinesis, although not absent, is passed through in a reduced condition, and is apparently, exclusively related to spore formation; and within the last few days Professor Farmer has drawn my attention to a very remarkable mode of spore formation, which he has found in certain liverworts of Ceylon, in which it is with the utmost difficulty that the apparently akinetic formation of the tetrads can be shown to be in reality a quadripolar karyokinesis; and, further, it seems generally agreed that such simplification is a reduction from, and not an antecedent of, the more complex karyokinetic division phenomena.

Consider also the nuclear division in the Protozoa themselves. It is now known that karyokinesis of a more complex order, accompanied by an enormous number of chromatic bands, is normal to some Rhizopods, while in the more specialised and less primitive Ciliates, this phenomenon is restricted to the micronuclear elements, being in them so much reduced,

that it is not without difficulty that it can be recognised at all.

Such evidence seems to me to favour the conclusion that the monomeric division in the genital cells of Apus is the most extreme term known in a progressive modification of the more primitive karyokinesis through such forms as Frenzel's "Nucleoläre Kernbulbirung," in which the spindle, and the breaking up of the chromatin into bands or globes, as well as the resting reticulum, have long since disappeared.

There seems some probability in the assumption, then, that owing to the introduction of a peculiar method of reproduction in Apus (parthenogenesis or hermaphroditism), the divisional phenomenon has exhibited a corresponding change, that the cells of the genital gland are all alike, and can function both in slime or egg formation as opportunity arises.

The egg nuclei take origin from nuclei containing a single chromosome, but they ultimately develop a coarse chromatic reticulum with an external attraction sphere or archoplasm (fig. 42, *b*), and appear much like an enlarged somatic cell.

How such a modification of the original type has arisen it is not, perhaps, very difficult to see. In sexually produced species, the nuclei intended for fusion must, so to speak, balance one another; and if karyokinesis is the original method of procedure, any tendency in an individual to infringe this rule in the origin of its reproductive cells would quickly tend to be eradicated from the race on account of the wide abnormalities it produced. But a parthenogenetic or hermaphrodite species might please itself as to the manner in which it evolved its reproductive elements, so long as these contained the premises necessary to the proper development of the individual.

DESCRIPTION OF PLATES 15 & 16,

Illustrating Mr. J. E. S. Moore's paper on "Some Points in the Origin of the Reproductive Elements in Apus and Branchipus."

PLATE 15.

a = Pseudosomes. *c* = Centrosomes. *d* = Dictyosomes. *da* = Stages intermediate between dictyosomes and pseudosomes.

Figs. 1—5.—Stages in first division, from the resting cells to the formation of chromosomes. Flemming's fluid and gentian violet. *a*. Pseudosomes.

Figs. 6 and 7.—Formation of the chromosomes.

Fig. 8.—Cell showing the breaking down of the nuclear membrane.

Figs. 9 and 10.—Two spermatocytes, with nearly the same stage of early spindle figure. *da*. Bodies intermediate between pseudosomes and dictyosomes.

Fig. 11.—Transverse optical section of equatorial plate; fine strands of protoplasm connecting the chromosomes to the exterior network.

Fig. 12.—Cell a little later, in which the centrosomes have appeared.

Fig. 13.—Older cell, in which the spindle figure seen in section is breaking down, and in which numerous bodies have appeared on the surface dictyosomes.

Fig. 14.—Final division of same.

Fig. 15.—Secondary cell.

Fig. 16.—Spermatozoa.

Fig. 17.—Group of three spermatocytes in division, with numerous (*d.*) dictyosomes and (*c.*, *c.*, *c.*) centrosomes.

Fig. 18.—Figures produced by the growth of bubbles in a fine froth.

Fig. 19.—Spermatocytes, showing the breaking down of the nuclear area.

Fig. 20.—Foot-cell in akinetic division.

Fig. 21.—Portion of spermatic epithelium, with various stages of spermatogenesis. *F. c.* Foot-cells. *Sp.* Spermatocytes and spermatozoa.

Fig. 22.—Spermatocytes in division, showing the extreme position of the centrosomes.

Fig. 23.—Ditto, showing the early formation of the dictyosomes round a spreading clear space.

PLATE 16.

FIG. 24.—Portion of epithelium of the female gland of Apus.

FIG. 25.—Single cell with nucleus in partial division.

FIG. 26.—Ditto.

FIG. 27.—Irregular formation of tetrad group.

FIG. 28.—Element with four nuclei.

FIG. 29.—Tetrad formed from a single cell.

FIG. 30.—Three nuclei of a tetrad, showing micronucleoli (*a*).

FIG. 31.—Two tetrad groups in relation to surrounding cells.

FIG. 32.—Cell with dividing nucleus.

FIG. 33.—Portion of nuclei of tetrad, showing the minute subdivision of the nuclei.

FIGS. 34 and 35.—Method of division occurring in same nucleoli.

FIGS. 36—41.—Stages of division.

FIG. 42.—Tetrad group, the upper cell being the future six "Nebenkern." *a*, *a*, *a*. Nuclei which have spread over the circumference of the group.

FIG. 43.—Group of cells from the spermatic gland of Branchipus.

FIG. 44.—Ditto, ditto, from the female gland of Branchipus, showing the relation of the akinetically dividing elements to the formation of slime.

All the preparations, except where otherwise stated, were fixed in Flemming's fluid and stained with gentian violet.

Notes on the Peripatus of Dominica.

By

E. C. Pollard, B.Sc.Lond.

With Plate 17.

A NUMBER of specimens of Peripatus from Dominica, West Indies, have recently been handed over to me for description by Professor Ray Lankester, to whom I owe my best thanks for intrusting me with this material. The specimens were collected by Mr. Ramage in Dominica, and sent, some alive, some preserved in alcohol, to Professor Lankester.

I had, in all, eighty-six individuals, of which fourteen had been opened in salt solution before preserving, whilst the rest were preserved whole.

Size.—There are considerable differences in size; the length, measured without the antennæ, varying from a minimum of 17 mm. to a maximum of 50 mm.

The males are, as a rule, much smaller than the females, and they are also much less numerous. Out of thirty-nine specimens in which I determined the sex only eight are males, and of these the largest is only 25 mm. in length.

On the other hand, a good-sized female measures 42 mm.

There are one or two apparent exceptions to this generalisation, one of the females being only 17 mm. and another only 19 mm. long; but from the fact that I have not found any males longer than 25 mm., and also that the majority of the

females are considerably larger than this, I am inclined to regard the small females as not yet full-grown.

Colour.—My observations as to the colour of this Peripatus have been made entirely upon specimens preserved in spirit.

The general colour of the body is a reddish brown dorsally, with a diffuse longitudinal streak of a darker shade extending down the centre of the back. The median dorsal line is marked by a well-defined narrow band still darker in colour. Ventrally the colouring is much paler, being a light grey or greyish yellow. The colouring of the legs on their dorsal and ventral surfaces corresponds with the colours of the dorsal and ventral body surfaces.

The antennæ are of a dark red-brown shade, with their terminal enlargements much lighter, almost flesh-coloured.

This colouring obtains, with slight individual variations, for all the specimens with one exception. In this unique case the dorsal surface is piebald, with a pale straw-colour and a reddish brown. The brown is disposed as a broad collar, and as two lateral bands just dorsal to the legs; the band on the right side, however, is only present in the posterior region of the body. There is a white median line dorsally. The ventral surface and the legs are pale yellowish white. The antennæ are dark red-brown, with their knobbed terminations pale yellow or whitish.

This specimen is small, and appears to be a young form in which the pigment is as yet not completely developed, or it may possibly be an abnormality.

Ridges and Papillæ of the Skin.—As in the other neotropical species of Peripatus, the ridges of the skin are continued right across the dorsal median line.

The papillæ of the ridges are arranged in a single file of larger ones, or two or three smaller ones occur abreast.

As in P. Edwardsii (Blanchard), there are accessory ridges extending across the dorsal median line, but not reaching far on either side of it; and also, as in P. Edwardsii, the diagonal lines which occur in the Cape species, breaking the surface into lozenge-shaped areas, are absent.

Many of the papillæ, both on the legs and body, are divided into two main portions, a basal and a more distal part. Of these the basal portion is cylindrical in form, thus agreeing with the species from Caracas, and differing from the Demerara species, in which the basal portion of a papilla is conical (fig. 1).

The papillæ vary considerably in shade, some being much lighter than others, and to this is due the speckled appearance of the skin.

Jaws.—Fig. 2 shows that the jaws are very similar to those of P. Edwardsii (4, figs. 25, 26).

The outer blade is provided with a large main tooth, and a smaller but still well-marked secondary one. On the inner blade there is a large main tooth and seven or eight smaller ones, of which the first is closely approximated to the main one, and is considerably larger than the remaining six or seven, from which it is separated by a wide diastema.

Antennæ.—The papillæ on the rings of the antennæ are arranged in several rows.

Ambulatory Appendages.—Only one specimen of Peripatus from Dominica has been previously described, and the authorities differ as to the number of legs possessed by it. Professor Jeffrey Bell states that there are thirty pairs (1), whilst Mr. Sedgwick considers that there are only twenty-nine (4).

In my specimens the number of ambulatory appendages varies from twenty-five to thirty pairs, the great majority having twenty-nine.

The relationship between the number of appendages and the sex of the individual is interesting.

Out of thirty-nine specimens in which the sex was ascertained only eight are males, and each of these is possessed of twenty-five pairs of legs only.

Of the thirty-one females which I have examined, two are possessed of twenty-six pairs of ambulatory appendages, one of twenty-eight, twenty of twenty-nine, and six of thirty; whilst I am uncertain as to the number possessed by the two remaining specimens.

Of the remaining two, one at least possessed more than twenty-seven pairs, but the exact number in both cases is doubtful, since the specimens had been mutilated.

The numbers given above are perhaps better realised when arranged in a tabular form, thus:

8	specimens	with	25	pairs	of	ambulatory appendages.	All males.
2	„	„	26	„	„	„	Both females.
1	„	„	28	„	„	„	Female.
6	„	„	30	„	„	„	All females.
67	„	„	29	„	„	„	Of these 20 were opened, and all found to be females.
2	„	„	an uncertain number of legs.				Both females.

The male, therefore, seems to be always possessed of twenty-five pairs of ambulatory appendages; whilst the female, with one doubtful exception, has always more than twenty-five pairs.

There are four foot-pads ventrally on each of the ambulatory appendages (fig. 3), with the exception of those of the last pair, which are possessed of two pads only (fig. 4).

At the distal extremity of the foot, close to the claws, there are three primary papillæ, two on the anterior margin of the foot and one on the posterior; but the basal papillæ are absent.

The foot-groove, which in P. capensis extends on to the body surface as far as the median ventral line, is in the Dominican form continued only a very short distance on to the ventral surface.

There are no white papillæ on any of the ambulatory appendages.

In his description of specimens of Peripatus from Guiana and Dominica, Mr. Sclater (3) mentions the occurrence of a "bladder-shaped appendage" attached to the foot-grooves. Such a vesicle-like structure is very obvious on the legs of some of my specimens, but it appears to be due simply to an extroversion of the lining of the groove.

Apertures.—The anus is situated posteriorly between the legs of the last pair.

The generative aperture in both sexes is found ventrally between the legs of the penultimate pair.

The segmental organs of most of the appendages open into the foot-groove close to the junction of the leg with the body. On the fourth and fifth pairs, however, the aperture is situated on a papilla between the proximal and third foot-pads (fig. 5).

Internal Anatomy.—Male Generative Organs (fig. 6). —The vas deferens of each side is extremely short. Each passes under the nerve-cord of its own side, and the two then unite to form a very long coiled ductus ejaculatorius.

A pair of accessory glands are present, but these are much shorter than those of the other South American forms which have been described.

The accessory glands open out independently on either side of the anus, as in P. Edwardsii, not into the ductus ejaculatorius, as described for P. capensis.

In figs. 10 *a*—*c*, three sections through the testis are figured. Of these the most anterior (fig. 10 *a*) is through the prostate (*pr.* in fig. 6), and shows simply a mass of large nuclei surrounded by a single layer of flattened epithelial cells. The layer of epithelium round the nuclei of the prostate is a point to be noticed, since Gaffron (2), in his description of P. Edwardsii, states that there is in this region no epithelial covering to the cells, which are simply enclosed in a thin muscular sac. Gaffron, in fact, makes this a distinction between the prostate (Schlauchhoden) and the testis proper (Blasenhoden). This is well seen in his figure (Taf. xxiii, fig. 46), and is clearly described in his own words:—"Der bedeutsamste Unterschied zwischen Schlauch und Blasenhoden ist jedoch das Vorhandensein eines characteristichen Epithels im letzterem. Während der dünnhäutige Sack des Schlauchhodens gleichmässig und dicht angefüllt ist mit grossen Zellen, die sich nicht zu einem geschlossenen Wandungsepithel anordnen, liegt der eben beschriebenen Blasenhodenmuscularis innen ein sehr regelmässiges polygonales Pflasterepithel."

It is possible, however, that in P. Edwardsii an epithelial covering to the prostate is also present, but the flattened cells lying close upon the large nuclei below as seen in fig. 10 *a* may have escaped notice.

The next figure (fig. 10 *b*) is from a section through the actual testis (*te.* in fig. 6). The contents of the testis in this region consist of numerous large nuclei and whisps or bundles of spermatozoa. There is a considerable space between these and the epithelium, probably due to shrinkage. The epithelial wall is well seen, and is similar to that described and figured in P. Edwardsii by Gaffron. The cells are higher and the nuclei rounder than in the epithelium of the prostate.

More posteriorly still we get numerous cross-sections of the coiled duct of the testis (fig. 10 *c*). The walls of this duct are composed of cells with large round nuclei, and within its cavity are numerous spermatozoa. The whole coiled mass is surrounded by a layer of flat epithelium, and this is continued over the straight portion of the vas deferens, where we have accordingly two layers of epithelial cells,—an inner layer of fairly tall cells with round or oval nuclei, and an outer layer of flattened cells with rod-like nuclei.

In no region of the testis have I found a muscular covering external to the epithelium.

Female Generative Organs (figs. 7—9).—On each oviduct is situated a globular receptaculum seminis (*R. S.*) containing spermatozoa, and possessed of double ducts as in the other neotropical forms. Between the receptacula and the ovary are a pair of sac-like appendages (*R. Ov.*), the so-called receptacula ovorum of Kennel, but I have been unable to make out a vesicle at their distal ends.

Comparison with other Neotropical Species.—The Peripatus of Dominica, P. Dominicæ, resembles the other neotropical species in—

i. The possession of four spinous foot-pads.

ii. The position of the generative aperture between the legs of the penultimate pair.

iii. The division of the primary papillæ into two portions.

iv. The absence of the dorsal white line.

v. The arrangement of the teeth on the inner blade of the jaw, there being a considerable gap between the first minor tooth and the rest.

vi. The presence of the receptacula ovorum and seminis on the oviducts.

vii. The unpaired portion of the vas deferens is of great length and much coiled.

viii. The number of legs is not constant.

Compared with P. Edwardsii, to which the Dominican species is most nearly allied, we find the following special points of agreement:

i. There are two foot-pads only on the legs of the last pair.

ii. The male has a smaller number of legs than the female.

iii. The basal part of the primary papillæ is cylindrical in both.

iv. The jaws and arrangement of the teeth are similar in the two.

v. There is in each a pair of accessory glands opening on each side of the anus.

The chief differences between the two are—

i. The number of ambulatory appendages; of these there are 29 to 34 pairs in P. Edwardsii, and 25 to 30 in P. Dominicæ.

ii. The white tubercles which are present on some of the legs in P. Edwardsii are not found in the species now described.

Compared with P. Trinidadensis.—The Dominican species differs much more from P. Trinidadensis. Besides the difference in the number of ambulatory appendages (P. Trinidadensis having 28—31 pairs), the two forms differ in the number of teeth on the inner blade of the jaw, the Trinidad species having a much larger number than the Dominican. Moreover in the Trinidad Peripatus the basal portion of the primary papillæ is conical, whilst in the Dominican form it is cylindrical.

Compared with P. torquatus.—P. torquatus is possessed of a much larger number of legs than the Dominican form, having 41 or 42. The colouring is also strikingly different, P. torquatus being characterised by a bright yellow band behind the head on the dorsal surface.

Bibliography.

Complete lists of the literature referring to Peripatus have already been published by Mr. Sclater and Mr. Sedgwick, and therefore the following list includes only the papers to which special reference is made in the above description.

1. Bell, F. J.—"Note on a Peripatus from the Island of Dominica, West Indies," 'Ann. Mag. Nat. Hist.,' 5th ser., xi, 1883.

2. Gaffron, E.—"Beiträge zur Anatomie und Histologie von Peripatus," 'Zool. Beiträge' (Schneider), Bd. i, 1885.

3. Sclater, W. L.—"Notes on the Peripatus of British Guiana," 'Proc. Zool. Soc. London,' 1887.

4. Sedgwick, A.—"A Monograph on the Species and Distribution of the Genus Peripatus (Guilding)," 'Quart. Journ. Micr. Sci.,' New Ser., xxviii, 1888.

EXPLANATION OF PLATE 17,

Illustrating Miss E. C. Pollard's paper, "Notes on the Peripatus of Dominica."

Fig. 1.—Primary papilla from skin of Peripatus.

Fig. 2.—Inner and outer blades of jaw.

Fig. 3.—Leg of Peripatus, showing four foot-pads.

Fig. 4.—Last leg of specimen with twenty-nine pairs, possessed of two foot-pads only.

Fig. 5.—Fourth leg, showing aperture of nephridium between the third and proximal pads.

Fig. 6.—Male generative organs. *Ac. gl.* Accessory gland. *D. Ejac.* Ductus ejaculatorius. *N. C.* Nerve-cord. *Pr.* Prostate. *Te.* Testis.

Fig. 7.—Female generative organs. *Lig.* Ligament of attachment. *N. C.* Nerve-cord. *Ov.* Ovary. *R. S.* Receptaculum seminis. *R. Ov.* Receptaculum ovorum. *Ut.* Uterus.

Fig. 8.—Enlarged view of ovary and receptacula. Reference letters as in Fig. 7.

Fig. 9.—Diagram of the same.

Fig. 10.—Transverse sections through the testis.

10*a*.—Through the prostate.

10*b*.—Through the testis proper.

10*c*.—Through the coiled duct of the testis. *Ep.* Epithelium.

Outlines drawn with Zeiss, oc. 4, obj. B; and details filled in with Zeiss, oc. 2, obj. D.

Fig. 11.—Transverse section through vas deferens.

Studies on the Protochordata.

By

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With Plates 18—20.

II.

The Development of the Neuro-hypophysial System in Ciona intestinalis and Clavelina lepadiformis, with an Account of the Origin of the Sense-organs in Ascidia mentula.

(With Plates 18 and 19.)

While following the metamorphosis of the larva of Ciona intestinalis last year (1892) in the Zoological Station at Naples, I noticed several peculiarities in the behaviour of the nervous system which apparently could not be reconciled with the account relating to Clavelina which was given by Éd. van Beneden and Charles Julin in their work on 'Le système nerveux central des Ascidies adultes et ses rapports avec celui des larves urodèles.' The first assumption to be made was that the conditions might be different in Ciona from what they were in Clavelina, as I had already perceived how much the general development of Clavelina was modified in the direction of a compression of the ontogenetic processes. But on making preparations in toto of larvæ of various ages of Clavelina lepadiformis I could see, as far as the hypophysis is concerned, nothing at all like the appearance figured by the above-named authors in their pl. xvii, fig. 11 ('Archives de Biologie,' t. v, 1884). Seeliger's figures of the larvæ of Clavelina are much more accurate in this respect (see I, 31).

The results, moreover, to which my friend Dr. Johan Hjort of Christiania had come in his investigations as to the development of the hypophysis and ganglion in the buds of Botryllus, determined me to study the same question in the case of the metamorphosing larvæ of Ciona intestinalis and Clavelina lepadiformis more closely than I had at first intended. Dr. Hjort had the kindness to show me his preparations and to make me thoroughly acquainted with his results, a preliminary account of which has appeared in the 'Zoologischer Anzeiger' (No. 400, 1892).[1]

Hjort's results are of unusual interest, as they place the contrast between the organogeny in the larva and in the bud respectively of Botryllus in the clearest possible light.

1. Closure of Neuroporus and Origin of Sense-organs in Ascidia mentula.

For the earliest stages of the nervous system after the fusion of the medullary folds I examined the embryos of Ascidia mentula, as they are much more transparent than those of Ciona.

Very soon after the commencement of the curvature of the embryo within the follicle, the curvature being initiated and necessitated by the outgrowth of the tail, the neuroporus, as was correctly described by Kowalevsky (I, 21), closes. It will be seen later that this primary closure of the neuroporus in the Ascidians is only temporary, and does not occur in Amphioxus; while what may be called the secondary or final closure occurs in both the Urochorda and the Cephalochorda.

After the first closure of the neuroporus has taken place, the nervous system of the Ascidian embryo consists of a perfectly closed tube lying immediately below the epidermis, and containing a lumen which is slightly dilated anteriorly, the neurenteric canal having been obliterated at a somewhat earlier stage (Pl. 18, fig. 1). In fig. 1 is represented an optical sagittal section of an embryo of Ascidia mentula at the stage in which the first trace of the sense-organs appears, in

[1] In the same number a preliminary note on my own results appeared.

the form of a number of pigmented granules which are deposited in the interior of certain cells of the dorsal wall of the cerebral portion of the medullary tube. Without entering into a detailed histological account of the sense-organs I will confine myself to a few points in which I can to a certain extent supplement the classical work of Kowalevsky.

The origin of the sense-organs in point of time seems to underlie a certain amount of variation. Kowalevsky describes the otolith as arising first in Phallusia mammillata. In the embryo from which fig. 1 was drawn the eye was the first to appear, being represented at this early stage by scattered rounded pigment granules lying in several—four or five—of the cells in the dorsal wall of the cerebral vesicle, while in fig. 3 the otolith and eye appear simultaneously. The otolith, as well as the eye, first appears in the form of a number of scattered pigment granules very similar to those which go to form the eye, but rather larger, and differing from the latter in their being confined to one cell, while, as I have just mentioned, the pigment granules of the eye are distributed among several cells. Kowalevsky would appear not to have seen the eye granules at their very first origin. He says (loc. cit., p. 117), "Am Grunde der Zellen des hinteren abgesetzten Theils der Blase [i.e. cerebral vesicle] erscheinen sehr feine Pigmentkörner," and he figures them (Taf. xii, fig. 31) outside the cells which form them. It is possible that this may be their mode of deposition in the species studied by Kowalevsky—viz. Phallusia mammillata.

The granules which belong to the eye have at first essentially the same character and nearly the same size as those which go to form the otolith, being scattered throughout the body of the cells in which they lie (fig. 1); but as they increase in number they become very much smaller, and then lie entirely at the inner free extremities of the cells (figs. 3—5). The otolith granules do not tend to increase in number, but retain their original size until they fuse together (fig. 5).

The otolith cell or otocyst lies immediately in front of and adjacent to the eye-cells, and, in fact, forms primarily one of

the same series of cells with the latter. This exact primary relation of the otocyst to the eye-cells was not observed by Kowalevsky, and it is interesting as showing that two such different organs as an eye and an ear can arise in the same way—namely, by a deposition of pigment—from one and the same sense-tract.

Next begins the migration of the otocyst, which was discovered by Kowalevsky. This migration is not an active one on the part of the otocyst, but goes hand in hand with a change in the histological character of the wall of the cerebral vesicle, and is therefore, as in so many other cases of change in the position of organs, a result of the relative differential growth of parts. This histological change in its turn is correlated with the expansion of the original slight anterior dilatation of the nerve-tube into a spacious vesicle. Successive stages in this expansion of the cerebral vesicle are shown in figs. 1 and 3—5. In fig. 4 the otocyst is seen to have separated itself from its previous contact with the cells of the optic region, and now lies at a lower level in the right dorso-lateral region of the cerebral vesicle. In fig. 5 the interval between the otocyst and the eye has further increased itself by the reduction of the wall of the cerebral vesicle in this region to a thin and apparently structureless cuticle, the reduction in bulk being accompanied by an increase in extent.

In this way, therefore, by a local thinning out or cuticularisation of the wall of the cerebral vesicle the otocyst is shifted from its primary dorsal position to its secondary position on the floor of the cerebral vesicle. The migration of the otocyst, therefore, occurs concurrently with the reduction to a thin membrane of part of the wall of the cerebral vesicle. The mode in which the otocyst becomes transported from the dorsal to the ventral wall of the cerebral vesicle was not made quite clear by Kowalevsky, who says (loc. cit., p. 117), "Die vordere pigmentirte Zelle, welche wir vermuthlich als einen Gehörapparat gedeutet haben, schiebt sich von der rechten Wand der Blase nach unten, so dass sie auf den Boden der Blase kommt." From this description it might very natur-

ally be supposed that the migration of the otocyst was an active one, whereas, as we have seen, it is really passive.

In Clavelina the migration of the otocyst is also effected by relative growth, but occurs at a very early stage before the cuticularisation of the wall of the cerebral vesicle (Pl. 2, figs. 25 and 29—31).

Meanwhile, in Ascidia mentula, the eye-cells collect themselves together (figs. 4, 5), and finally, as is well known, come to occupy the posterior right-hand corner of the cerebral vesicle (fig. 2).

After the sense-organs have taken up their definite positions, the stomodæum forms by a median dorsal invagination of the ectoderm (fig. 2), and shortly afterwards a communication is established between the base of the stomodæum and the branchial sac. At the stage at which the mouth breaks through, Kowalevsky described the formation of an opening between the cerebral vesicle and the stomodæum. I have looked for this opening repeatedly in the tadpoles of Ciona intestinalis, Phallusia mammillata, and Ascidia mentula, but have not been able to see it; and, in fact, I will go so far as to say, in confirmation of Kupffer (8), that the opening as described by Kowalevsky does not exist in the tailed larvæ.

At a later stage, after the commencement of the metamorphosis, a corresponding opening is actually formed, the relations of which are essentially the same as those of the pore described by Kowalevsky, though with certain important differences; but, nevertheless, Kowalevsky's actual observation was, according to my account, erroneous. Thus in the tadpoles of the above-named simple Ascidians there is no communication whatever between the cavity of the cerebral vesicle and the stomodæum.

On the other hand, as we shall see, in the tadpole of Clavelina there is such a communication, which was, however, denied by van Beneden and Julin. We have, therefore, the curious circumstance that what Kowalevsky asserted in the case of Phallusia mammillata was contradicted by van Beneden

and Julin in the case of Clavelina; and although it might appear at first sight that somebody must be right, it turns out in fact that all are wrong. I have stated above that a communication between the cavity of the nervous system and stomodæum is effected in the simple Ascidians at a considerably later stage than that described by Kowalevsky. Remembering the general abbreviation in the development of Clavelina, to which attention has already been directed, we should expect to find that this communication would become established at a much earlier stage in Clavelina than, for instance, in Ciona, and this is exactly what happens.

2. Origin of the Neuro-hypophysial System in Ciona intestinalis.

Stage I.—In transverse sections through a young tadpole which has, by convulsive movements of its tail, succeeded in bursting the egg-follicle, and so entered upon the brief free-swimming phase of its existence, we find that quite anteriorly a minute portion of the cavity of the cerebral vesicle is separated off from the main cavity, and appears in section as an independent lumen in the thickness of the wall of the cerebral vesicle on its left side (fig. 6). A section or two behind this the small lumen in question is seen to communicate freely with the cerebral cavity (fig. 7), and farther back still there is nothing more of it to be seen but simply the plain wall of the vesicle (fig. 8).

Thus already at this stage a portion of the cerebral vesicle has begun to be constricted off in the form of a tube, at present ending blindly in front, and communicating behind with the main cavity of the vesicle. It is of a very small extent, and lies at present entirely in the thickness of the wall of the vesicle; in fact, it is only with the utmost pains that it can be detected at all at this stage. Attention may be drawn here to the cupule of the otolith shown in fig. 7 in the ventral wall of the cerebral vesicle. The otolith itself has become separated from the cupule which normally carries it, in the process of cutting. A distinct nucleus-like body is to be seen in the interior of the

crescent-shaped cell. Kowalevsky says that the nucleus disappears entirely, and the whole cell becomes strongly refractive. The latter is of course true, but the nucleus apparently does not vanish, although it is impossible to see it in the fresh state.

I should add that I have examined sections of these early stages taken in three planes, but have never found an opening from the neural tube into the stomodæum in the free-swimming larva.

Stage II.—Fig. 9 represents a transverse section through the region of the cerebral vesicle in a larva of the stage immediately preceding fixation. The shape into which the wall of the branchial sac is thrown by the pressure of the superincumbent expanded cerebral vesicle should be noted. The epithelium of the dorsal wall of the branchial sac is very flat as compared with that of the lateral walls into which it gradually passes on either side. The ventral groove of the branchial sac in fig. 9 is not the endostyle, as follows clearly from a comparison of the actual position of the endostyle as seen in surface views (cf. figures accompanying I); but it is possible that Kowalevsky mistook it for a continuation of the endostyle—a mistake which is not difficult to make if larvæ of a later stage are not examined for comparison, since, after the withdrawal of the tail, both the enteric and the body cavities undergo a general distension, which renders the internal structure and the topographical relation of parts much clearer.

With regard to the cerebral vesicle, the chief point of difference between this and the preceding stage lies in the fact that the tube which we saw embedded in the thick wall of the vesicle, in the form of a minute cul-de-sac, communicating at its hinder end with the cavity of the vesicle, has now begun to set itself off more distinctly from the rest of the wall of the vesicle, forming a considerable prominence slightly to the left of the middle line. The process of constriction by which the tube, or, as it may at once be called, the neuro-hypophysial canal, comes to be entirely separated from the cerebral vesicle has therefore now commenced. It can be noticed in fig. 9 that the nuclei in the neighbourhood of the tube in question

have begun to arrange themselves in such a way as to give plainly the appearance of the lumen being surrounded by a distinct epithelium. Fig. 10 shows the free communication between the tube which is being constricted off from the cerebral vesicle, still ending blindly in front, and the cavity of the vesicle itself. The rudiment of the neuro-hypophysial tube or canal has at this stage slightly shifted its relative position from that which it occupied in the preceding stage, having approached more nearly to the dorsal middle line. This shifting can readily be understood by a comparison of fig. 9 with fig. 6, from which it will be seen that during the transition from the preceding stage to the one now under consideration the wall of the cerebral vesicle has become drawn out to a thin membrane in the left latero-ventral region of the vesicle. It will be remembered that the migration of the otocyst was directly traceable to a similar local thinning out of the wall of the vesicle.

Stage III.—This is the stage at which the communication between the cavity of the nervous system and the base of the stomodæum at the point of junction between the stomodæum and the wall of the branchial sac is effected (see fig. 2 as to depth of stomodæal invagination).

The tube, whose constriction from the wall of the cerebral vesicle we have been following, has now separated itself entirely from the latter (fig. 11), and has meanwhile acquired an opening into the stomodæum (figs. 12, 13). The cerebral vesicle itself has entered upon the process of histolytic disintegration which eventually leads to its entire disappearance.

Thus the neural tube, of which the neuro-hypophysial canal, so called on account of its later destiny, is merely a continuation, now opens into the stomodæum; but the opening is a perfectly simple one at present, and no appreciable evagination from the wall of the stomodæum can be demonstrated. Later on an evagination does possibly take place, and the opening which we see at this stage appears to be carried somewhat further back. The hypophysis has not yet differentiated itself from the nervous system.

What, then, is this pore leading from the stomodæum into the neural tube? My answer is that it is the neuroporus. We have already mentioned the fact, which was originally determined by Kowalevsky, that the anterior opening to the exterior of the medullary canal closed up before the formation of the mouth. We now see that some time after the formation of the mouth—in fact, as soon as it begins to be functionally active and to take in water, which does not occur until after the fixation of the larva the neuroporus opens out again, but this time into the stomodæum.

The primary closure of the neuroporus was therefore only temporary, and comparable to what occurs in the case of many blastopores and other organs which become temporarily solid—such, for instance, as the œsophagus of the Selachians. Whatever may be the actual cause of the temporary closure of the neuroporus in Ascidians, it is perfectly plain that its persistence through the period during which it is closed would be of no service to the embryo or larva, because the development up to the time of the formation of the mouth takes place inside the egg-follicle, and when the mouth does appear it does not at first open directly to the exterior, but is covered over by the so-called testa.

This is a quite different state of things from what we find in Amphioxus, where the neuroporus does not undergo this temporary primary closure; but then the embryo of Amphioxus leaves the follicle precisely at the stage in which, with the above-named simple Ascidians, the neuroporus closes. In Clavelina the neuropore remains open somewhat longer than in the simple Ascidians mentioned above.

Fig. 13 represents a sagittal section through a young fixed Ciona of this stage, drawn with a low power to explain the topographical relations of the various parts. The neural tube, or neuro-hypophysial tube, as it may now be indifferently called, is seen to open into the buccal cavity in front. Fig. 12 shows a portion of the neuro-hypophysial tube from the same section, but drawn under a much higher power (Zeiss J, water immersion). It commences anteriorly as a well-marked

tube lined by a columnar epithelium, the lumen of which, however, becomes irregular as it proceeds backwards, where, at the point marked *g* in the figure, its dorsal wall is seen to consist of a mass of cells in place of a well-defined epithelium.

In fact, this is the first stage in the formation of the cerebral ganglion of the adult (fig. 12, *g*).

Beneath the neuro-hypophysial tube lie the remains of the cerebral vesicle of the larva, now filled with histolytic residue.

Stage IV.—A sagittal section through the neuro-hypophysial tract of a young Ciona, about the time of the budding off of the two intermediate stigmata from the two primary, as already described by me, is shown in fig. 14. The section is slightly oblique. The ganglion has attained a much greater development than in the preceding stage, and, indeed, seems to have been exceptionally large in this particular specimen.

The greater part of the lumen of the primitive neural tube has become obliterated, and the nervous system is now for the greater part of its extent solid, with a lumen, however, still persisting in front, which opens anteriorly through the mediation of a funnel-like dilatation, which has possibly arisen in part by evagination from the stomodæum, into the stomodæal region of the branchial sac.

Figs. 15—19 are taken from an extremely instructive series of transverse sections through the central nervous system of a rather older individual than that from which the sagittal section of fig. 14 was obtained. Starting from behind, we have in fig. 15 a section through the now solid nerve-cord, which is continued into the "cordon ganglionnaire viscéral" of van Beneden and Julin. Advancing gradually from behind forwards, we come to fig. 16, where the transverse section has an irregular contour, and in its lower portion a very distinct lumen can be seen, while dorsally it consists of a solid proliferation from the dorsal wall of the neuro-hypophysial tube, the nuclei being arranged peripherally, while the central portion of the solid mass shows the first indication of the later characteristic "Punktsubstanz." In fig. 17 the transverse section has a very regular and characteristic 8-shaped outline,

the lower half of the 8 containing a lumen, while the upper half is solid. In the next more anterior section (fig. 18) the upper division of the 8 predominates over the lower, the lumen of the latter being still of small diameter—in fact, rather smaller than in the preceding sections; while still farther in front (fig. 19) the lumen, which is perfectly continuous all along, has attained a relatively large diameter, while the superjacent solid portion of the ganglion is correspondingly small, and is distinct from the dorsal wall of the subjacent canal. In fact, fig. 19 represents a section through the funnel-like terminal dilatation of the neuro-hypophysial canal spoken of above, which may be called the hypophysial funnel; and the portion of the ganglion involved in the section is its anterior extremity, which has come to overlap the posterior portion of the funnel (cf. the figures of young individuals of Ciona accompanying "Studies," &c., No. I).

Attention may be drawn to the ciliated prominence in the wall of the branchial sac behind and below the hypophysial opening in fig. 14. This is the epibranchial ridge (epibranchial groove of Julin), which is grooved in many adult forms. It is directly traceable to the projection caused in the dorsal wall of the branchial sac by the pressure of the distended cerebral vesicle in the larva (cf. figs. 9 and 12).

Stage V.—The description which follows applies to the relations of the neuro-hypophysial system in young immature adults.

Anteriorly the duct of the hypophysis expands into the large funnel-shaped dilatation, which in its turn opens into the branchial sac at the end of a papilliform prominence, which projects boldly into the branchial chamber, and is continuous with the epibranchial ridge referred to above. Fig. 20 shows a section taken a short distance behind the branchial opening of the hypophysis, and passing through the anterior dilatation, above which are seen two cerebral nerves. Fig. 21 is drawn from a section posterior to that of fig. 20, and shows a great decrease in diameter of the lumen of the hypophysis, while still farther back the lumen becomes reduced to a minimum

(fig. 22). This temporary obliteration of the lumen of the hypophysis at this point and at this period of the development seems to be a constant feature, and extends over one, or at most two sections of a thickness of about 7 μ. The lumen then opens out again (fig. 23), and in the posterior region of the hypophysial tube, which now lies closely applied to, but at the same time distinct from, the ganglion, glandular tissue is seen to be developing from its ventral wall[1] (fig. 24). Here and there the peripheral nuclei of the ganglion are absent in the region where the hypophysis is in contact with the latter (fig. 24).

We see, therefore, that the hypophysis and the ganglion, which have been gradually differentiating themselves from the common neuro-hypophysial tube, have at last separated entirely from one another by a completion of the constriction of which we saw the commencement in the preceding stage (fig. 17).

From the appearances presented I am disposed to believe that the anterior portion of the hypophysis, including the funnel-shaped dilatation and the duct, as far as the above-mentioned point of reduction of the lumen, is derived from a secondary evagination from the stomodæal region of the wall of the branchial sac; while the division of the hypophysis which lies behind that point, and from which the gland is developed, represents the tube derived by constriction from the cerebral vesicle of the larva in the way described above. The original opening, therefore, of the neuro-hypophysial tube into the branchial sac has on this supposition been carried backwards by a secondary outgrowth from the stomodæum. It is difficult to bring other than circumstantial evidence in support of this view, but it may be possible to test its truth on a future occasion. Meanwhile this seems to be a reasonable explanation of the local and temporary obliteration of the lumen which divides the proximal from the distal or glandular portion of the hypophysis.

[1] Seeliger ('Jenaische Zeitschrift,' xviii, p. 100) described the hypophysis-gland in Clavelina as arising by the aggregation of free mesoderm-cells. He does not, however, commit himself unreservedly to this view.

In Ciona, therefore, the cerebral ganglion of the adult arises by proliferation and constriction from the dorsal wall of the neuro-hypophysial tube.

3. Origin of the Neuro-hypophysial System in Clavelina lepadiformis.

The description given above as to the origin of the neuro-hypophysial system in Ciona, together with that which I am about to give for the same system in Clavelina, will be found to be considerably at variance with the results obtained by Seeliger and van Beneden and Julin in the case also of Clavelina.

My observations were at first entirely confined to Ciona, and led me to the conclusion, judging from the very explicit account, accompanied by numerous figures, of van Beneden and Julin (loc. cit.), that the mode of development of the parts in question must be different in Clavelina. But when I came to study the origin of these structures in the latter form to enable me to make a definite comparison with Ciona, it turned out that the relations above described for Ciona were not only essentially the same in Clavelina, but were very much easier to determine, on account of the larger size of the object.

The stage which van Beneden and Julin took as their point de départ was much older than that which I shall now commence with. In fact, their first stage was that at which the larval nervous system had already attained the climax of its development.

Stage I.—The stage from which I find it is desirable to start in describing the future development and fate of the nervous system of Clavelina is a very young embryo, with the anterior neuropore still open to the exterior; no mouth, no atrial involutions, no pigment in the brain, and before the migration of the otocyst.

A transverse section through the neural tube, some distance behind the neuropore, is shown in fig. 25, Pl. 11. The part of the neural tube extending between the region through which

this section is taken and the neuropore has in transverse section an approximately round contour, and is quite simple. Its lumen, which more anteriorly is reduced to a minimum, gradually widens out until it becomes a transversely elongated slit, as shown in the section figured. A large cell in the dorsal wall of the neural tube in fig. 25 can be identified as the otocyst, although at present it contains no pigment.

The nerve-tube has therefore not yet commenced to swell out in its anterior region into the remarkably voluminous cerebral vesicle which appears later. At this stage it is chiefly desired to call attention to the fact that at a region considerably removed from its anterior extremity the neural tube, though still simple, possesses a transversely elongated lumen.

Stage II.—In embryos belonging to this stage the nerve-tube still opens in front to the exterior by the neuropore. No mouth is present, but the atrial involutions have put in their appearance, in the form of the two well-marked longitudinal grooves which I have previously described (No. I). No stigmata have broken through. This stage also marks the first appearance of pigment in the brain, while the otocyst has attained its ventral position. In fig. 30 an intermediate stage in the migration of the otocyst is shown, its position there being lateral, on the right wall of the cerebral cavity.

Figs. 26—29 represent transverse sections through the cerebral portion of the nervous system of an embryo belonging to Stage II. Fig. 26 passes through the neuropore, and was drawn with a higher power than the succeeding figures of this series. In fig. 27 the section is taken a little behind the neuropore, and the regularity of the circumference of the neural tube is disturbed on the right side (left of the figure) by a bluntly-pointed protuberance, which becomes still more prominent as we pass backwards in the series. Meanwhile the neural tube begins to show a tendency to divide itself transversely into two portions; and, in fact, when we reach the point from which the section shown in fig. 28 was taken, we find that here the neural tube is double, and possesses in this region two distinct lumina.

This double character of the neural tube only extends in this stage through two or three sections. Anterior and posterior to this region it is a simple tube with a single lumen (figs. 27 and 29). Of the two halves of the neural tube in fig. 28, that on the left side (right of the figure) retains approximately its present shape, and forms part of the future hypophysis; while the other (right) division of the neural tube, which is at present rather smaller than its neighbour, becomes enormously distended in the later stages, and is converted into the spacious cerebral vesicle.

We see, therefore, at this stage the first commencement of the separation of the hypophysis from the rest of the larval nervous system taking place entirely independently of any evagination from the wall of the stomodæum, which, indeed, does not yet exist. It is to be noted also that the formation of the hypophysis commences here at a much earlier stage than in the case of Ciona, a fact which is in thorough keeping with the general character of the development of Clavelina, to which I have already alluded.

The neuro-hypophysial tube decreases considerably in diameter in the later stages, owing to the absorption of the yolk with which its cells are at first filled (cf. figs. 28, 31, and 40).

Stage III.—At this stage the lumen of that portion of the neural tube which will give rise to the cerebral vesicle is commencing to enlarge (fig. 31). The neuropore is closed, but there is still no mouth. The lumen of the neural tube in front has a more or less round outline, but widens out transversely behind until, as in the preceding stage, but now in a more pronounced way, it becomes divided into two. This condition is shown in fig. 31, from which it will be seen that the two portions of the neural tube have now reversed the relative dimensions which they held in the preceding stage,—the one on the right side, namely, the one that will become, and, in fact, is becoming, the cerebral vesicle, and which contains the otocyst, being considerably larger than the other division of the tube, which, as we have already seen, is the rudiment of the hypophysis. The section drawn in fig. 32 lies slightly poste-

rior to the region from which fig. 31 was taken, and shows again the posterior communication between the two portions of the neural tube. In comparing figs. 31 and 32 with figs. 28 and 29 of the preceding stage, it will be seen that the hinder opening of the hypophysial portion of the neural tube into that division of it which corresponds to the later cerebral vesicle lies somewhat farther backwards in the present stage; whereas in fig. 29 the two halves of the neural tube are in open communication in the region of the otocyst, in fig. 31 they are distinct from each other in this region, and their lumina unite more posteriorly (fig. 32). This fact illustrates the gradual constriction of the hypophysial tube from the neural tube, which is taking place from before backwards.

As we trace the series of sections backwards it is found that the lumen of the nerve-tube gradually becomes again narrower, showing that its expansion in the transverse direction is confined to a particular region, namely, the region from which the hypophysis takes its origin.

Stage IV.—At this stage the larva possesses a mouth. Fig. 33 is a section taken through the cerebral region of the neural tube of a young larva of this stage at the time of the first formation of the mouth. It would seem that almost immediately after the mouth has broken through, a communication is established between the neuro-hypophysial canal and the cavity of the branchial sac at the base of the stomodæum. This communication is at first a perfectly plain one, and not involved with any evagination from the wall of the branchial sac.

As to the region of the branchial sac into which the neuro-hypophysial canal opens, it is only reasonable to suppose that it corresponds to the base of the stomodæal involution. This also follows from a comparison between the depth of the stomodæal invagination before the actual perforation of the mouth and the level at which the communication between the neuro-hypophysial canal and the branchial sac becomes effected at a later stage (cf. Pl. 10, figs. 2 and 13).

It is possible that in Clavelina, as in Ciona intestinalis,

a secondary infolding of relatively inconsiderable extent takes place from the wall of the branchial sac—i. e. from the base of the stomodæum,—and carries the original branchial opening of the neuro-hypophysial tube farther inwards in the same way as I have suggested above for Ciona.

Van Beneden and Julin, on the contrary, whose account of the origin of the hypophysis differs essentially from that which I am giving, speak of the entire hypophysis, including the glandular portion of it, as arising from an endodermic diverticulum of the branchial sac, and Professor Kupffer (**10**) has recently seized on this statement to confirm him in his opinion that the so-called hypophysis of the Ascidians is really nothing of the kind, but merely a "Kiemendarmdrüse." The whole development of the Ascidian hypophysis, however, obviously opposes itself to such a view.

To return then to fig. 33, we see here a further progress in the distension of the cerebral vesicle, while the section also shows the posterior opening of the hypophysis into the vesicle. The division of the primitive neural tube into two does not extend to its anterior extremity, but the whole of that portion of the neural tube which in the previous stages lay between the point at which the neuro-hypophysial constriction commenced and the neuropore becomes bodily taken up in the service of the hypophysis, and at its front end comes to open into the base of the stomodæum as described above.

Stage V.—At this stage the cerebral portion of the medullary tube has assumed its definite vesicular character with the accompanying local thinning out of its wall, which has been already referred to in the case of Ciona. The contrast between the cerebral vesicle and the hypophysial tube in point of size is now very great (figs. 34, 35). The latter here appears in the form of a minute lumen in the thickness of the cerebral wall just as we found it in Ciona.

Figs. 34—37 are taken from a series of transverse sections which show very clearly the way in which the lumen of the hypophysis opens posteriorly into the cerebral vesicle. In fig. 37, the most posterior of the sections drawn, there is no trace

whatever of the hypophysis at this stage. The branchial or stomodæal opening of the hypophysis and its cerebral opening may be conveniently referred to as its anterior and posterior openings respectively. The posterior opening of the hypophysis now occurs in the region of the cerebral vesicle which contains the eye—that is, still farther back than in the preceding stage. Figs. 38—42 represent a series of sections through the cerebral vesicle of a larva which shows the hypophysis in a rather more advanced stage of development. It now projects from the wall of the cerebral vesicle, and has a definitely tubular appearance. Fig. 38 shows the branchial or anterior aperture of the hypophysis, while fig. 42 shows its posterior communication with the cavity of the cerebral vesicle. It should be noted that the cerebral vesicle expands in every direction, not only laterally, but also in a longitudinal direction, so that its anterior wall projects far beyond its previous limit, and so comes to lie side by side with the anterior opening of the hypophysis, where its wall, as shown in fig. 38, consists almost entirely of a thin and apparently structureless membrane.

Stage VI.—At this stage the hypophysis no longer opens into the cerebral vesicle sensu stricto, but its posterior opening has been carried back by progressive constriction to the region of the prominent ganglionic enlargement of the ventral wall of the neural tube (figs. 45 and 46) which lies between the cerebral vesicle and the anterior extremity of the notochord, and which has been accurately described by van Beneden and Julin. As described by the latter authors, this ganglion eventually becomes absorbed and disappears entirely, leaving the superjacent neural tube in the shape of a solid cordon ganglionnaire viscéral. After this stage the posterior opening of the hypophysis becomes closed, and only the anterior opening into the branchial sac persists.

Fig. 43 represents a section taken slightly posterior to the anterior opening of the hypophysis, and serves to illustrate the general topographical relation of the parts. In fig. 44 a very important point is illustrated—namely, the origin of the

definite cerebral ganglion on the left side (right of the figure) of the cerebral vesicle just above the hypophysis tube. The continuity of the lumen of the latter can be traced in the clearest manner from the anterior branchial opening to the section under consideration. The extreme posterior termination of the hypophysis was rather difficult to make out at this stage, and between the sections drawn in figs. 44 and 45 there seemed to be an interruption in the continuity of the lumen. This probably is an indication of the eventual closing up of the hypophysis posteriorly.

As to the actual origin of the cells which compose the permanent cerebral ganglion, it is undoubtedly correct to say that they proceed, together with the hypophysis, from the cells which form the left dorso-lateral portion of the wall of the cerebral vesicle (cf. figs. 34—37). This becomes especially obvious by the study of such a series of sections as that from which figs. 43—46 were taken. In the hinder region of the cerebral vesicle the boundary line between the hypophysis and the developing ganglion was by no means so distinct as it is in fig. 44.

It is clear from the above description that in Clavelina the formation of the permanent ganglion commences at a relatively much earlier stage than it does in Ciona—in fact, before the atrophy of the cerebral vesicle; and we see, further, that it is from the beginning a solid structure. Van Beneden and Julin appear to have mistaken the developing hypophysial tube for the developing ganglion. Some of their figures coincide fairly closely with some of mine, but they have interpreted them totally differently, and, it must be added, to a large extent erroneously. They agree with Seeliger in saying that the hypophysis, including for their part emphatically its glandular portion, is entirely derived from an evagination from the wall of the branchial sac, to which they give the name of the "cæcum hypophysaire," which applies itself against the cerebral vesicle, but never communicates with it. My observations show conclusively that this is quite wrong.

Van Beneden and Julin say (loc. cit., p. 353), "Le cerveau de l'adulte procède du cul-de-sac cérébral." But their "cul-de-sac cérébral," which they suppose to be entirely transformed into the adult ganglion, is no other than my neuro-hypophysial canal; and although, as we have seen, the brain of the adult does proceed from the same epithelial tract as the latter structure, yet it is perfectly distinct from it to the extent that the lumen of the "cul-de-sac cérébral" is and continues to be throughout the lumen of the neuro-hypophysial canal. Evidently the true origin of the ganglion of the adult escaped the attention of the Belgian authors. For the rest, I may repeat that their fig. 11, planche xvii, which would appear to be clear enough to dispel any doubt as to the origin of the hypophysis, is to me, in that regard, quite unintelligible, and I have been unable to duplicate it in any of my preparations. Possibly in the figure in question it is merely the funnel-shaped anterior dilatation of the hypophysis which has been drawn, its posterior narrower continuation having eluded observation.

In face of the above statements I was much surprised to read in an interesting note on the eyes and subneural gland of Salpa, communicated to a recent number of the 'Zoologischer Anzeiger' (No. 409, Jan., 1893) by Maynard M. Metcalf, the following lines:—"The ganglion of Salpa is homologous with the visceral portion of the larval Ascidian nervous system. Van Beneden and Julin have shown that the dorsal wall of this portion of the Ascidian tadpole's neural tube proliferates cells which become the ganglion of the adult, while the thickened ventral wall of the same region gives rise to the subneural gland." It is sufficiently clear from what I have said above that this statement must rest upon a complete misapprehension on the part of the author as to the results arrived at by van Beneden and Julin. This is what they say about the subneural gland (loc. cit., p. 350):—"En un point de son trajet [i. e. of the cæcum hypophysaire] sur un petit nombre de coupes et seulement dans sa partie antérieure on constate que le plancher du tube épithélial [i. e. the hypophysis-tube which for them is derived entirely from an evagination of the wall of the

branchial sac] s'est développé en un petit amas de cellules ; c'est là l'ébauche de la glande hypophysaire."

A communication between the cavity of the central nervous system and that of the branchial sac in the Tunicata has been observed in several other cases by previous authors—thus by Ganin (**2**) in the case of Didemnum (Diplosoma) gelatinosum, Keferstein and Ehlers (see Uljanin, **20**) in the case of Doliolum, Kowalevsky (**7**) for Pyrosoma, Salensky (**14** and **15**) and more recently Metcalf (**12** and **13**) for Salpa, Lahille (**11**) and Hjort (**5**) for Distaplia magnilarva, and Hjort again for Botryllus.

From the observations of these authors, together with those which I have recorded above, we may conclude that in all the Ascidians the lumen of the hypophysis is in all cases at first in direct communication with the lumen of the central nervous system. And this forms the great difference, but at the same time a very suggestive and instructive difference, between the development of the hypophysis in the Ascidians and in the higher Vertebrates. In the latter the lumen of the oral portion of the hypophysis does not come into communication with the cavity of the infundibulum, and this permanent separation of the two parts of the hypophysis cerebri in the higher Vertebrates may be compared with the temporary obliteration of the lumen between the proximal and distal portions of the hypophysis which I have described above for Ciona.

Julin's (**6**) and Balfour's ('Comp. Embryol.,' vol. ii, p. 437) suggestion of the homology of the subneural gland and dorsal tubercle taken together of the Ascidians, with the pituitary body of the higher Vertebrates, founded on anatomical considerations, and especially worked out in great detail by Julin, may be considered as being borne out fully by the facts of development as described above. In the Ascidians, as in the higher Vertebrates, the hypophysis cerebri consists of a neural portion and an oral or stomodæal portion. The neural portion of the hypophysis in the higher Vertebrates is the infundibulum or processus infundibuli, and in the Ascidians it may be that this is represented by the subneural gland. The

oral portion in the Vertebrates is the pituitary body, and in the Ascidians the proximal portion of the hypophysis, including the dorsal tubercle.

III.

On the Position of the Mouth in the Larvæ of the Ascidians and Amphioxus, and its Relation to the Neuroporus.

(With Plate 20.)

In the first of these "Studies" I have quoted a sentence of Kupffer, in which he says that the pronounced dorsal position of the mouth in the Ascidian tadpole is occasioned by the presence of what I have called the præoral lobe, which contains the anterior body-cavity. But in Balanoglossus, where an homologous anterior body-cavity or proboscis-cavity is present, the mouth is ventral. So that, according to this point of view, what causes the mouth to be dorsal in one case causes it to be ventral in another.

The way out of this dilemma is found as soon as the fact is recognised that the anterior body-cavity has nothing to do per se with the position of the mouth, and that at least in the groups of the Protochordata (Cephalodiscus, Balanoglossus, Tunicata, Amphioxus) the dorsal or ventral position of the mouth does not affect the homology of organs which lie in front of it, for the reason that there is every evidence to show that the anterior body-cavity in all these forms is not a truly median structure, but has, either actually or virtually, a bilateral origin.

In Balanoglossus, as shown by Bateson, the anterior body-cavity arises at first as a perfectly median archenteric pouch. It becomes, however, in the course of the development, incompletely divided into two by the formation of a mesenchymatous septum, in which lie the so-called heart, notochord, and proboscis-gland. But perhaps the strongest evidence of the essential bilaterality of the proboscis-cavity of Balanoglossus is, that while in most forms there is only one proboscis-pore, namely,

on the left side, in B. Kupfferi, as is well known, there are two such pores—a right and a left.

Returning to the question of the dorsal position of the mouth in the Ascidian tadpole, I have on a previous occasion (see this Journal, vol. xxxii, N. S., 1891, pp. 214—217) put forward the suggestion that the lateral position of the mouth, and consequently the unilateral position of the gill-slits, in the larva of Amphioxus, was due to the mouth having been shifted from a primitively dorsal to a lateral position by the secondary forward extension of the notochord (see Pl. 12). Any attempt to account for this position of the mouth on principles of utility to the larva would be futile, because it only occurs during the period in which the larva is pelagic. On the other hand, when the young Amphioxus begins to burrow in the sand at the bottom or near the shore, frequently lying on its side on the sand, the mouth has already become median, anteriorly directed and ventral. The observations which I have been able to make as to the relations existing between the mouth, hypophysis, and nervous system in the Ascidians have raised the above view as to the origin of the asymmetry of the larva of Amphioxus in my mind from the rank of a tentative suggestion to that of a demonstrated fact.

In the Ascidians, as we have seen, the neuropore opens, or more correctly reopens, at first directly into the stomodæum. Later on there is some reason for supposing that an evagination occurs from the stomodæum which carries the original neuropore further back.

In Amphioxus the neuropore opens for a long time directly to the exterior in the dorsal middle line, and then later an invagination of the epidermis occurs, which carries the neuropore some distance inwards. This invagination gives rise to the so-called "olfactory pit" of Kölliker, or "Flimmergrube" of Hatschek, and into its base, as shown by Hatschek, the nerve-tube at first opens by the neuropore. Eventually the neuropore becomes closed, and the olfactory pit is then a ciliated cul-de-sac abutting against the anterior end of the nerve-tube.

Thus the so-called olfactory pit of Amphioxus bears precisely

the same relation to the neuropore as the dorsal tubercle does to the neuropore in the Ascidians (cf. fig. 14, Pl. 1). The only conclusion to be drawn from this is that Kölliker's olfactory pit in Amphioxus is homologous with the proximal portion of the hypophysis duct in the Ascidians, while the glandular portion of the hypophysis is unrepresented in Amphioxus.

Hatschek (I, 16), if I understand him aright, has curiously enough suggested that the dorsal tubercle of the Ascidians—that is, the opening of the hypophysis duct into the stomodæum—is homologous with the præoral pit of Amphioxus; while the glandular portion of the Ascidian hypophysis, or the "Neuraldrüse," would be homologous with the olfactory pit (Flimmergrube) in Amphioxus, the two portions of the hypophysis being in the latter separated from one another by the notochord. Judging from a recent publication (3), in which Hatschek makes the præoral pit of Amphioxus a gill-slit, he would seem to have somewhat modified his original view, which was based largely on observations made by Herdman (4) on Ascidia mammillata, in which, while confirming Julin's discovery that in this species the neural gland, besides having the usual duct running anteriorly to communicate with the pharynx by the dorsal tubercle, has also a number of short funnel-shaped apertures into the peribranchial cavity, he adds that in two specimens examined by him the duct of the hypophysis had no opening into the pharynx, the dorsal tubercle being entirely absent. Herdman, therefore, suggested that the dorsal tubercle and neural gland represent originally distinct structures, which in most Ascidians have acquired a secondary communication with one another. This view, which receives only the slenderest support from the facts intended to establish it, is obviously untenable in the light of what has been said above as to the origin of the respective structures.

In 1870, several years before Ussow discovered the continuity of the dorsal tubercle of the Ascidians with the subneural gland, Ganin, in studying the development of Didemnum (Diplosoma) gelatinosum, found that the cavity

of the central nervous system communicated directly with that of the branchial sac, and said (2, p. 515), "Somit ist die Flimmergrube [dorsal tubercle] der Ascidien am ehesten mit dem Geruchsorgane [olfactory pit] des Amphioxus zu vergleichen."

Schimkewitsch (16) has recently put forward the same opinion in that he says, "Der vordere Neuroporus [of Balanoglossus] entspricht der Flimmergrube der Amphioxus-Larve (Hatschek) und dem Flimmerausgang der Neuraldrüse der Tunicaten (Julin)."

I consider it, therefore, well established by all this more or less concurrent testimony that the hypophysis of the Ascidians is represented in a simplified form by the olfactory pit of Amphioxus, both structures communicating during a longer or shorter period of the development with the cavity of the central nervous system by means of the neuropore. But while in the Ascidians the hypophysis opens into the mouth-cavity, in Amphioxus it opens dorsally to the exterior, and is separated from the mouth by the notochord.

In Amphioxus the mouth has not merely been forced by the forward extension of the notochord to forsake its primitive dorsal position, but it has also, ipso facto, lost its primitive relation to the hypophysis, by which name we may now designate the olfactory pit of Amphioxus.

The relation of the mouth to the hypophysis is a remarkably close and constant one throughout the whole of the Vertebrate series. There are, however, as might be expected, some exceptions to the general rule. One of these exceptions is the well-known case of Petromyzon, where the hypophysis, as shown by Dohrn (1), Scott (17), and Kupffer (9), arises approximately in the normal position for the Craniata, and is then secondarily carried round to the dorsal middle line by the enormous development of the upper lip which grows out between the hypophysial involution and the stomodæum.

Another exception is met with in the case of Amphioxus, where it is not the hypophysis which has been carried away from the mouth, but the mouth which has been separated by

the secondary forward extension of the notochord from the hypophysis. In Petromyzon the whole process can be observed, while in Amphioxus only part of it, as the notochord grows forward at a very early stage before the formation of the mouth.

I take it for granted, therefore, that the mouth of Amphioxus was primitively dorsal, and the prime reason of its being dorsal was, not the presence of a præoral lobe or anterior body-cavity, but the fact that in the common ancestor of the Urochorda and Cephalochorda the mouth stood in intimate relation with the neuroporus, probably through the intermediation of a ciliated funnel or hypophysis. This conclusion would suit very well with the views of Sedgwick (19) and van Wijhe (21) as to the primitive respiratory function of the neural canal, water entering it by the neuroporus which opened into the mouth, and leaving it by the neurenteric canal.

As to the position of the mouth in the higher Vertebrates, it is obvious, supposing the above considerations to be correct, that it has, so to speak, been pushed round to its present ventral or subterminal position by the cranial flexure. This was first suggested in part by Sedgwick in his well-known paper on the "Origin of Metameric Segmentation," although the mouth of Amphioxus, whose final ventral position is due to an entirely different set of causes, was left out of consideration. He says (18, p. 77), "With a slight change in the shape of the anterior end of the body of the Ascidian larva in Kowalevsky's figure, the mouth would be removed from what we call the dorsal (neural) to what we call the ventral (abneural) surface. This would involve a flexure of the anterior end of the neural canal, and, I think, gives a clue to the phylogenetic meaning of the cranial flexure."

As for the higher Vertebrates, my friend Mr. H. B. Pollard had the kindness to show me in Naples some of his preparations of Teleostean embryos, in which it could readily be seen that the hypophysis was morphologically dorsal with reference to the nervous system, its actual ventral position being due to its having been carried round the front of the head by the cranial flexure, just as the optic nerves are morphologically the

first pair of nerves, as pointed out by van Wijhe. This point is of great importance, and is very strong evidence in favour of the view that the hypophysis of Amphioxus (i. e. Kölliker's olfactory pit) occupies a primitive position, which in the higher Vertebrates has been shifted to the ventral median line by the cranial flexure.

Returning to the Protochordata, it follows from what has been said above that the mouth occupies a more primitive position and exhibits more primitive relations in the Ascidian tadpole than it does in Amphioxus. In the larva of Amphioxus, however, the mouth occupies an intermediate position between that of the Ascidian larva and that of the adult Amphioxus. Some of the stages in the migration of the mouth from a dorsal to a ventral position have, in fact, been preserved to us in the ontogeny of Amphioxus. The mouth of the adult Amphioxus occupies the same position as that of the craniate Vertebrates, but gets there by totally different means. It is extremely interesting to note that there is more than one way in which the primitive position of such an apparently stable organ as the Vertebrate mouth can become altered, namely, either by the cranial flexure or by a forward extension of the notochord.

Thus we find that in the case of the mouth of Amphioxus and the higher Vertebrates we have almost identical topographical relations, established by widely divergent methods. A similar instance is afforded by the hypophysis, which opens to the exterior in the dorsal middle line in both Amphioxus and Petromyzon, but primarily in the former and secondarily in the latter form.

A question may arise as to the actual way in which the mouth of Amphioxus could have been originally forced aside from its primitive position by the advance of the notochord. The probability is that the actual oral opening was never displaced by the notochord. But the change from a dorsal to a lateral position of the mouth in the larva of Amphioxus could be, and undoubtedly has been, effected by a change in the order of its appearance.

The time or order of formation of certain organs seems to be very generally subject to a great deal of variation. I have previously described some such variations in the case of the secondary gill-slits of Amphioxus, and similar instances are very easy to find. I therefore suggest that either a slight delay in the formation of the mouth, or an acceleration in the anterior development of the notochord—probably the latter,—introduced in the first place as a variation and subsequently becoming a fixture, was the method by which the perforation of the mouth at a point other than the primitively dorsal one was rendered possible.

The above observations and considerations all tend to show that the primitive vertebrate mouth, before the cranial flexure had become an established feature of the vertebrate ontogeny, had a dorsal or a dorso-terminal position.

In view of this conclusion a genuine difficulty is presented by the position of the mouth in Balanoglossus, where it is from the beginning ventral. This difficulty cannot be fully met at present, but it may be well to point out that the intermediate stage between a ventral mouth as found in Balanoglossus, and a dorsal one as it occurs in the Ascidian tadpole, would be arrived at by a form in which, by a reduction of the præoral lobe, the mouth came to occupy a terminal position. Supposing it possible to conceive a common ancestor for all the Protochordata, it would seem to be probable that it had a terminal mouth. For from such a situation the mouth could be made to assume either a definitely dorsal or ventral position, according to circumstances, as soon as the paired head-cavities co-operated to produce the peculiar features and proportions of a præoral lobe.

Appendicularia is a form in which, together with the reduction, and indeed apparent absence in the adult of any trace of a præoral lobe—a state of things brought about by the purely pelagic life which has been acquired by the organism—the mouth has come to occupy a terminal position, and thus shows us that, under certain circumstances, the topographical relations of the mouth which I have just predicated for the

ancestor of the Protochordata could exist, and, moreover, in a free-swimming animal.

In Sagitta, again, we have a pair of head-cavities which are very possibly homologous in a certain way with the head-cavities of Amphioxus, but which do not occur in such a way as to produce a præoral lobe, and therefore do not prevent the mouth from holding its anterior terminal position. The præoral lobe or proboscis of Balanoglossus, as well as its homologue which I claim to have identified in the Ascidian larva, represents a pair of head-cavities analogous to those that occur in Sagitta—although I do not wish to assert a genetic relationship between the former and the latter—which, however, have acquired such a mode of development as to produce by their fusion a large median lobe in front of the mouth. The præoral lobe, however, while standing in the way of a terminal mouth, does not, as I have said above, determine whether the mouth shall be dorsal or ventral. That is dependent on other circumstances, such as the mouth coming into important relations to the central nervous system.

In Balanoglossus and in the Ascidians the two head-cavities do not appear as such distinctly paired structures in the ontogeny of the individual as they do in Amphioxus. And in the latter case, as is well known, they do not fuse together, but remain distinct, one of them undergoing hypertrophy and giving rise to the præoral body-cavity and the other to the præoral pit.

This hypertrophy of the head-cavities in the forerunners of the Protochordata necessitated a change in the position of the mouth, and a removal from its primitive situation at the anterior terminal extremity of the body. Along the line of descent which led to Balanoglossus the mouth migrated along the ventral side of the body, and along the line of descent that led to Amphioxus and the Ascidians the mouth passed along the dorsal side, but in all cases the identity of the head-cavities and of the mouth remained unaffected.

I have thus shown a possible means of explaining the discrepancy between the primitive position of the mouth in the

Ascidian tadpole and the larva of Amphioxus and in Balanoglossus, which may at least serve as a working hypothesis. My main object has been to point out, that the fact that the mouth lies dorsally or ventrally has nothing to do with the homology of the præoral body-cavity in all the forms in question. The homology between the præoral cœlom of Balanoglossus and the head-cavities of Amphioxus was urged very strongly by Bateson, but it is most important to remember, as has been repeatedly pointed out, that the mouth of Amphioxus, although ventral in the adult, has, as I think beyond a shadow of a doubt, descended from a primitively dorsal position in the neighbourhood of the neuropore. In other words, the mouth in Amphioxus originally possessed the same topographical relations as it does in the Ascidian tadpole; and if the præoral body-cavity of Amphioxus is homologous with the corresponding structure in Balanoglossus, so is the præoral body-cavity of the Ascidian tadpole. The above considerations all tend to establish the accuracy of my identification of the latter structure.

The diagrams on Pl. 12 will place the whole question here discussed in the clearest possible light. From these diagrams it will be at once seen that the mouth of the larva of Amphioxus occupies an intermediate position between that which it holds in the Ascidian larva and in Balanoglossus, but I am very far from meaning to suggest that phylogenetically it represents an intermediate stage between these two extremes. On the contrary, it certainly does not. There is no evidence whatever to suppose that the mouth of Balanoglossus has migrated from a dorsal to a ventral position. As has been said above, it is probable that both the mouth of the Ascidian and that of Balanoglossus have attained their present situation from an ancestral terminal position.

Errata.

In No. I of these "Studies on the Protochordata" one or two trifling lapsus calami, which I had not the opportunity of correcting in the proof, crept into the text. On p. 348, re-

ferring to the pyloric gland of Ascidians and the cæcum of Amphioxus, it is stated that they both lie on the left side. While they are of course essentially median outgrowths of the alimentary canal, the cæcum of Amphioxus usually lies for the greater part of its extent to the right of the pharynx. Attention may, however, be drawn to Schneider's observation ('Beit. zur vergl. Anat. und Entw. der Wirbelthiere,' Berlin, 1879, p. 17, foot-note) that he often found it on the left side of the pharynx.

Finally, on p. 336 (eight lines from bottom of page) "Trigeminus" should read "Facialis."

Addendum.

Since the above contributions were sent into the press, several new publications relating to kindred subjects have appeared.

1. A. Pizon, in a "Note additionnelle" appended to his long treatise on the Blastogenesis of the Botryllidæ (see 'Annales des Sciences Nat.,' 7me série, t. xiv, p. 374, et seq.), expresses doubt as to the accuracy of Hjort's and my results, and states his own opinion that in the larvæ of Botryllus, and in the buds of many other forms, "l'organe vibratile est toujours un diverticule de la vésicule endodermique primitive."

2. Hjort ("Über den Entwicklungscyclus der zusammengesetzten Ascidien," 'Mitth. Zool. Stat. Neapel,' x, pp. 584—617, Taf. 37—39) gives a detailed account of his researches on the budding of Botryllus and the metamorphosis of the nervous system of Distaplia. In the latter case his observations agree in the most satisfactory manner substantially with mine on Ciona and Clavelina.

3. Davidoff ("Über den 'canalis neurentericus anterior' bei den Ascidien," 'Anat. Anz.,' viii, pp. 301—303) objects to the identification of the subneural gland of the Ascidians with the hypophysis of the Vertebrates, and agrees with Kupffer that the latter is homologous with the Tunicate mouth.

4. Van Wijhe ("Ueber Amphioxus," 'Anat. Anz.,' viii, pp. 152—172) agrees with me in regarding the club-shaped gland

as a modified gill-slit, but thinks that its antimere is the larval mouth which he calls the Tremostoma. This he homologises with the left spiracle of Selachians. The Tunicate mouth is for him represented in Amphioxus by the pre-oral pit, which he calls the Antostoma.

For the rest, van Wijhe records some most important observations on the peripheral nervous system and on the musculature of Amphioxus.

5. W. Salensky ("Morphologische Studien an Tunicaten: I, Ueber das Nervensystem der Larven u. Embryonen von Distaplia magnilarva," 'Morph. Jahrb.,' xx, pp. 48—74) appears to come to similar results to those already published by Hjort with regard to the neuro-hypophysial system of Distaplia. He also comes to a conclusion on which I have dwelt in the foregoing pages in connection with Ascidia mentula. Salensky finds likewise in Distaplia "dass alle Theile der Sinnesblase: Retina, Linse, Pigmentschicht und Otolithenzelle durch die Differenzirung einer und derselben Epithelschicht der primitiven Gehirnblase enstehen."

6. W. K. Brooks ("Salpa in its Relation to the Evolution of Life," 'Studies from the Biological Laboratory, Johns Hopkins University,' vol. v, No. 3, Baltimore, 1893).

At the conclusion of this otherwise interesting memoir, Prof. Brooks devotes several paragraphs (pp. 199—201) to a criticism of my "Studies on the Protochordata" (No. I, 'Quart. Journ. Micr. Sci.,' vol. xxxiv, Jan., 1893).

In the body of his memoir, Professor Brooks develops, with great elaboration, the view that "the chordata type was evolved under purely pelagic influences," and that Appendicularia is the direct descendant and somewhat modified living representative of this pelagic archetype.

Then, referring to my work, he says (p. 199), "While the author seems to agree with me in rejecting Dohrn's view that the Tunicates are degenerated fishes, he holds that the Ascidians exhibit, during their development, certain features of resemblance to other primitive chordata which are not exhibited by Appendicularia; and he believes that these characteristics prove

that the Ascidians are more closely related than Appendicularia to these protochordata.

"The features upon which he lays most emphasis are these:— I. The endostyle is at first vertical and pre-oral; II. The organ of fixation is a pre-oral lobe, and its cavity is the pre-oral or anterior body-cavity; and III. The first four primary stigmata of Ciona intestinalis are developed from one primitive gill-slit. . . . .

"I cannot believe that students of the Tunicata will regard the first and second of these arguments as entitled to the least consideration." After this very decided expression of opinion, Professor Brooks goes on to say, "It has long been known that the endostyle of Ascidian larvæ is at first vertical or at right angles to the long axis, and it is so figured and described by Seeliger; but the relative position of organs is so much influenced by changes in other organs that we cannot attribute a phylogenetic significance to the position of the endostyle." It was certainly so described and figured by Seeliger for Clavelina, and as a tribute to the excellence of his description I quoted a considerable portion of it verbatim in my paper (loc. cit., pp. 331, 332).

The point on which I insisted, however, was that in the larva of Ciona, a simple Ascidian whose development in comparison with that of Clavelina is remarkably uncompressed, the endostyle behaved in the way stated by me, and not as described and figured by Kowalevsky in the case of a closely allied simple Ascidian. It is not a light thing to impeach Kowalevsky's accuracy, and I considered it important to call attention to the actual relations of the endostyle in the larva of the simple Ascidians, which had not been done before.

With regard to the second half of the above-quoted paragraph, I will merely point out that the primary position of the endostyle in the larva is that which it holds prior to the changes in the arrangement of the other organs, in which it is subsequently involved.

The method by which the endostyle attains its secondary and final position has nothing whatever to do with the question

as to whether its primary position has a phylogenetic significance. The remarkable constancy of the latter and its analogy with Amphioxus would seem to indicate that it has.

Professor Brooks says (p. 200), "Willey's observations add nothing to Seeliger's excellent account of the organ of fixation" (except to show that it behaves very differently in Ciona from what it does in Clavelina, the differences being of such a nature as to affect very sensibly the morphological interpretation of the structure); "and he gives no reason for holding that it is a pre-oral lobe, except that it contains loose mesenchyme-cells derived from the two lateral mesodermic bands." This is a complete misrepresentation. What I chiefly relied on in forming my opinion as to its morphological value was its topographical relations. It is the barest statement of the facts of the case to say that it is a lobe, that it is pre-oral, and that its cavity is the anterior and pre-oral portion of the body-cavity. Under these circumstances I confess my inability to understand how the suggestion as to the possibility of this pre-oral lobe being genetically related to a similarly placed structure in Amphioxus can, on any pretence, be regarded as not being entitled to the least consideration. The presence of loose mesenchyme-cells in place of a lining epithelium was emphasised by me as a necessary evil common to the rest of the body-cavity. Professor Brooks, however, argues as follows:—"This (*i.e.* the presence of loose mesenchyme-cells) is equally true of other parts of the body-cavity, and there is no more evidence that the organ of fixation is a pre-oral lobe than there is that it is homologous with the jaws and teeth of sharks."

"How much," I ask—"how much consideration is this argument entitled to?"

"If," continues Professor Brooks, "it is a pre-oral lobe, it is a ventral one, and it cannot be compared with the dorsal one of such protochordata as Balanoglossus and Amphioxus." I venture to think that the reflections urged in the foregoing contribution, No. III, will demonstrate that here Professor Brooks has fallen into an egregious error. The primary

topography of the mouth in the larvæ of the Ascidians and Amphioxus belongs to one and the same category, while that of the mouth of Balanoglossus belongs to quite another category. If it is desirable to speak of bilateral structures as being dorsal or ventral, the pre-oral lobe of Amphioxus is, palingenetically, ventral and not dorsal.

In view of the numerous divergent opinions which have recently been expressed with regard to the correspondence of parts in the Protochordata and Chordata generally (Kupffer, Hatschek, van Wijhe, Davidoff), it is obvious how much depends on a correct estimate of the asymmetrical mouth of the larva of Amphioxus.

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EXPLANATION OF PLATES 18, 19, and 20,

Illustrating Mr. Arthur Willey's paper, "Studies on the Protochordata."

Letters for Plates 18 *and* 19.

ant. p. Anterior opening of neuro-hypophysial canal into branchial sac. *at.* Atrial involution. *br. s.* Branchial sac. *cer. ves.* Cerebral vesicle. *cer. ves. res.* Histolytic residua of cerebral vesicle. *e.* Eye, or eye-tract. *ect.* Ectoderm. *end.* Endostyle. *ent.* Entoderm. *ent. c.* Enteric cavity. *ep. r.* Epibranchial ridge. *g.* Cerebral ganglion. *gl.* Subneural gland. *hyp. f.* Funnel of hypophysis. *int.* Intestine. *m.* Mouth. *mes.* Mesoderm. *n. c.* Neural canal. *nch.* Notochord. *n. hyp.* Neuro-hypophysial canal. *n. p.* Neuropore. *ot.* Otocyst, or otolith. *post. p.* Posterior opening of neuro-hypophysial canal into cerebral vesicle. *st.* Stomodæum. *t.* Remains of tail. *vac.* Vacuolar spaces in cells of optic region. *visc. g.* Visceral ganglion (Rumpfganglion of Kowalevsky).

PLATE 18.

Figures 1—5 relate to Ascidia mentula, and were drawn from living object with camera lucida.

Fig. 1.—Optical section of young embryo with closed nervous system, showing first appearance of pigment-granules of eye in dorsal wall of brain. Zeiss, 3, C.

Fig. 2.—Older embryo, to show depth of stomodæal invagination. Behind cerebral vesicle is seen the right atrial involution. Zeiss, 3, C.

Fig. 3.—Cerebral vesicle of young embryo (a trifle older than that shown in Fig. 1), to show primary relation of otocyst to eye-tract. Zeiss, 4, D.

Figs. 4 and 5.—Cerebral vesicles of somewhat older embryos in optical section, to show stages in the migration of the otocyst. Zeiss, 3, D.

Figures 6—24 relate to Ciona intestinalis.

Figs. 6—8.—Transverse sections through cerebral vesicle of newly hatched larva. Fig. 6 shows the neuro-hypophysial canal in left wall (right of figure) of vesicle. Fig. 7 shows its communication with cavity of vesicle. Fig. 8 shows cerebral vesicle posterior to neuro-hypophysial region. Zeiss, 3, E.

Fig. 9.—Transverse section through an older larva of the age of that figured in I, Plate XXX, fig. 1 (this Journal, Jan., 1893).

Fig. 10 shows posterior communication of neuro-hypophysial canal with cerebral vesicle—farther back than in Fig. 7. Zeiss, 3, E.

Fig. 11.—Transverse section through cerebral vesicle of newly fixed larva. Shows disintegration of the vesicle, and complete separation of neuro-hypophysial canal. Zeiss, 3, E.

Fig. 12.—Sagittal section through neuro-hypophysial system of same stage as preceding, showing first appearance of adult ganglion as a proliferation in the dorsal wall of the tube. Zeiss, 3, J, water immersion.

Fig. 13.—Entire section, of which Fig. 12 was a part, to show topographical relations. Zeiss, 3, C.

Fig. 14.—Sagittal section through more advanced neuro-hypophysial system. Posteriorly in the region of the ganglion the section is tangential. The nervous system proper has become solid. Zeiss, 3, J, water immersion.

Figs. 15—19.—Transverse sections through neuro-hypophysial system, slightly older than preceding. Fig. 15 is the most posterior section, and passes through the hinder portion of the ganglion or the anterior extremity of the "cordon ganglionnaire viscéral," which is now solid. Figs. 16—18 show the ganglion developing from the dorsal wall of the neuro-hypophysial canal. In Fig. 18 the remains of the eye are involved in the section. Fig. 19 passes through the anterior extremity of the ganglion, which overlaps the funnel of the hypophysis. Zeiss, 4, J, water immersion.

Figs. 20—24.—Transverse sections through the hypophysis and ganglion of young immature adult. Fig. 20 is the most anterior section, passing through funnel of hypophysis and two cerebral nerves. Fig. 21 is taken slightly further back, and Fig. 22 passes through the point at which the lumen of the hypophysis is temporarily obliterated by mutual approximation of the cells forming its wall. Fig. 23 shows the lumen widening out again posterior to this point, and finally Fig. 24 shows the origin of the glandular portion of the hypophysis by cell-proliferation from its ventral wall in its posterior portion. Zeiss, 2, J, water immersion.

PLATE 19.

All the figures on this plate relate to Clavelina lepadiformis, and all represent transverse sections.

Fig. 25.—Stage I. Through cerebral region of very young embryo, of an age corresponding to that shown in Plate 18, fig. 1. Shows transversely elongated lumen of neural tube in this region. 3, D.

Figs. 26—29.—Stage II. Fig. 26, through neuropore; 3, J. Fig. 27, just behind neuropore. Fig. 28, through neuro-hypophysial region. Fig. 29, posterior to this. 2, D.

Fig. 30.—Intermediate between Stages I and II, showing otocyst in right (left of the figure) wall of cerebral vesicle. 3, D.

Figs. 31 and 32.—Stage III. Fig. 31, through region of otocyst, shows increase in size of cerebral vesicle. Fig. 32 shows the communication between the hypophysial and cerebral portions of the nervous system. 3, D.

Fig. 33.—Stage IV. Shows opening of neuro-hypophysial canal into still larger cerebral vesicle, between the region of the otocyst and of the eye. 3, D.

Figs. 34—37.—Stage V. Series showing relation of neuro-hypophysial canal to cerebral vesicle at this stage. Fig. 37 passes through the vesicle behind the posterior opening of the canal. For the anterior opening into the branchial sac in this larva see I, Plate XXXI, fig. 28. 2, D.

Figs. 38—42.—Similar series through somewhat older larva, showing the neuro-hypophysial canal from its anterior opening into the branchial sac to its posterior opening into the cerebral vesicle. 2, C.

Figs. 43—46.—Stage VI. Fig. 43 shows an entire section slightly posterior to the anterior opening of the neuro-hypophysial canal, and in front of the atrial cavities (cf. I, Plate XXXI, fig. 29); 2, D. Fig. 44 is a most important section, and shows the origin of the adult ganglion in company with the neuro-hypophysial canal from the left (right of the figure) latero-dorsal wall of the cerebral vesicle. Figs. 45 and 46 pass through the region of the visceral ganglion, which later becomes absorbed. 2, J.

PLATE 20.

Letters for Plate 20.

p. l. Præoral lobe. *p. p.* Præoral pit or proboscis pore. *n. p.* Neuropore. *m.* Mouth. *end.* Endostyle. *n. c.* Neural canal. *nch.* Notochord. *gl.* and *h.* Proboscis-gland and heart of Balanoglossus.

Fig. 1.—Diagram of anterior portion of an Ascidian larva (e. g. Ciona) about the time of fixation. The features possessed by the larva at the stages immediately prior to and after fixation are thrown into one diagram.

Fig. 2.—Diagram of anterior region of larva of Amphioxus.

Fig. 3.—Similar diagram of Balanoglossus (compiled from Bateson).

Observations on the Development of the Head in Gobius capito.

By

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With Plates 21 and 22.

Introduction.

When attempting to pursue some studies on the Comparative Anatomy of the head in Teleostei, I became aware that it would be desirable to understand the development of some form. Gobius capito showed itself to be the most suitable, and therefore in this species I have investigated and described stages of development of the brain, mouth, and mesodermal structures. The work has been carried out during occupation of the Oxford table at the Naples Zoological Station.

My grateful acknowledgments are due to the University of Oxford and to my college, Christchurch, for funds, and to the members of the staff of the Naples Station for their constant kindness, extending frequently to questions other than those of material and reagents.

Material and Technique.

Gobius capito lays its eggs in the month of March at Naples, at which time they can be brought in in unlimited quantities by the fishermen. The embryos are enclosed in a tough shell, spindle-shaped, with pointed ends somewhat like a grain of barley. The eggs are 4—5 mm. long, yellowish in colour, and are regularly affixed to rocks and bits of pottery,

&c., by one end—hundreds together. Apparently they are deposited by preference on fragments of tufa, with the colour of which they match. Kept in aquaria the young fish hatch out at about the twenty-fifth day. Very possibly they grow faster in their natural habitat. I found that the best way to obtain the embryos ready for section-cutting was to snip off the free projecting ends of the egg-shells with scissors, and quickly shake the embryos out into dilute nitric acid or picro-sulphuric acid (Kleinenberg). Afterwards when wanted the embryos may be removed from the yolk in alcohol. I at first employed the methods of Henneguy (**6**), removing the embryos from the yolk before complete hardening in alcohol, but by these methods they are very liable to be deformed. Sections were made by the Thoma microtome, and usually 5 μ in thickness. For convenience I used Kastschenko's "Beschneider" (**12**) in order to obtain good ribbons of sections. The models were made after Strasser's method (**23**).

Brain.

One finds by experience that, in order to understand the disposition of the various organs in the Teleostean embryo, an exact comprehension of the form of the brain at the separate stages is necessary. The development of the brain in the trout has been described by Rabl-Rückhard (**19**), who investigated the embryos as opaque and transparent objects under the microscope. Goronowitsch (**4**) studied the early stages of the brain in the salmon by means of models. My own observations have been made by models and projections, and though in the main I have but to confirm what Goronowitsch says, yet Gobius shows some not uninteresting variations from the salmon, and, moreover, some description is necessary in order later to explain the disposition of the mesoderm and mouth.

Fig. 1 represents the brain of Gobius in the first stage viewed from the side. Scarcely any flexure is visible apart from the general curve of the embryo. As shown by transverse sections, the extent dorso-ventrally is far greater than from side to side, the former being perhaps on the average

double the latter. The figure shows that the brain of Gobius at this age bears considerable resemblance to the brain of a human embryo of the third week as depicted by His (7), except that the fish does not possess the sharp curve in the region of the mid-brain. The chorda extends as yet only to below the cerebellum, and the central cavity of the brain is but partially formed. This cavity appears first in the region of the optic stalks, and generally in the ventral half of the neural cord.

Fore-brain, mid-brain, and hind brain are already indicated by slight constrictions. In the hind brain can be distinguished cerebellum and medulla oblongata. The mid-brain already shows the preponderance characteristic of Teleostei. It is slightly moulded to the shape of the eyes, which appear to be relatively larger in Gobius than in Salmo. The fore-brain differs somewhat from that of other Teleostei, and shows some peculiarities which deserve special attention. Ventrally a bulging of the floor indicates the commencement of the infundibulum. Behind this point, as will be shown later, the premandibular mesoderm is continuous across the middle line.

In front of the infundibulum is a constriction where the optic chiasma comes to lie subsequently. The foremost ventral portion of the brain is a rounded prominence which gives rise laterally to the optic stalks and eyes. At the extreme anterior end the wall passes inwards to form with the corresponding upper portion of the brain a well-marked indentation. The nasal organs lie above and in front of the optic stalks, both nose and eye being as yet directed outwards and slightly upwards. That portion of the brain lying between the nasal organs is the region of the corpora striata. Figs. 5 and 6 represent sagittal and transverse sections of the anterior end of the brain. In fig. 6, whose position is indicated in the diagram (*a*, *a*, fig. 2), both upper and lower portions of the brain are cut in section where they separate to form the above-mentioned indentation. The upper portion, the corpus striatum, is oval in section, the long axis being dorso-ventral. The lower is more rounded. In the corpus striatum

the central cavity is only indicated by the disposition of the cells, and near the centre are seen several cells characteristically in mitotic division. In fig. 5, which is approximately in a median vertical plane, the corpus striatum and lower extremity of the brain are seen to be separated by a fairly well-defined line extending from the indentation to the median cavity between the optic stalks. The ectoderm in this section is artificially deformed. It should continue over the yolk exactly abreast of the indentation. The characteristic mitoses are again seen near the central cavity. Where the latter is fully formed—that is, in the immediate region of the optic stalks—it is seen to be partially occupied by structures (*Gp.*) which Henneguy (6) terms "globules parablastiques," but which Goronowitsch regards as cell débris due to the manner of formation of the central cavity. The same structures are seen in section in fig. 7, *b*.

The account here given confirms the observation of Goronowitsch made on the salmon, that the neural axis ends approximately between the optic stalks. The indentation in Gobius can but be regarded as a rudimentary neuropore.

The brain at this first stage is approximately in the same condition as the salmon brain of the sixteenth day.

On the following day the brain of Gobius shows the same characters, but with a slight general advance. The eyes are becoming more rounded, and the optic stalk is being separated from the eyes by a distal constriction. The lens is formed and cut off from the ectoderm. Thus the development of the eyes in Gobius proceeds more rapidly than in the salmon. No choroidal fissure is as yet formed. The indentation which marked the neuropore is less perceptible, though the exact point where it occurred is still to be determined. The cavities of the brain are well formed, and the vertical extent is less in proportion to the breadth. The corpora striata, which in the previous stage, as shown in sections (figs. 6, 7), were only part of the dorso-lateral wall of the neural tube, are now well-marked thickenings, pushing the optic stalks downwards, and giving rise to a slight cranial flexure.

On the third day important changes have taken place. There is a considerable cranial flexure, inasmuch as the optic stalks are distinctly ventral. The eye has rotated correspondingly, and a choroid fissure has been formed. The nose is turned downwards and forwards. The pineal organ is a small solid knob. The cavity of the fore-brain is formed, and, as Goronowitsch states for the salmon, is a dorsal outgrowth from the cavity. The mid-brain has become very broad, and possesses a large cavity, which, however, is being invaded by the tori longitudinales, which appear as thickenings of the dorso-lateral wall. The cavity of the medulla oblongata shows the well-known beaded constrictions. The infundibulum is large, and its cavity is narrow but deep. As yet there is no indication of lobi inferiores or of the formation of the saccus vasculosus. Behind the infundibulum the isthmus and base of the cerebellum are raised from the yolk, leaving a space occupied by loose mesodermic tissue. The wall of the brain from the pineal organ to the infundibulum—that is, the region of the "vordere Endnaht" of His—is epithelial in the median line.

At this stage nerve-fibres are first differentiated at the periphery of the brain.

Fig. 4 represents a model of the brain at the fifth day. The model has been cut in halves at the middle line in order to show the relation of the cavities. Viewed externally it shows practically the same characters as are depicted by Goronowitsch for the salmon embryo of the thirtieth day.

Posteriorly, to the left in the figure, the medulla oblongata is cut in section. The conditions of the first day have been completely reversed, in that the brain in this region is broader than deep. The fourth ventricle is roofed in by thin membrane. The roofing membrane passes into the cerebellum, which at this stage passes near the middle line insensibly into the mid-brain. The cavity of the mid-brain is now mainly filled by the massive torus longitudinalis. In the figure the commissures present at this stage are represented in red. Where the mid-brain passes into the fore-brain the posterior commissure occurs.

In front of it is situated the pineal organ, which has a slight cavity. Below the pineal organ lies the cavity of the fore-brain; below the latter, the massive corpus striatum. The cavity of the fore-brain is continuous with the recessus opticus. A slight distance in front of the ventral limit of the recessus opticus is seen the anterior commissure; behind it the optic chiasma, for the optic nerves are formed between the third and sixth day, dating from the first day described. The infundibulum is seen to be very large. Below it the hypophysis is already formed and in situ. The cavity of the infundibulum shows two smaller cavities proceeding slit-like outwards. The anterior becomes later the cavity of the saccus vasculosus. The posterior is the cavity of the lobus inferior. The axis of the brain-cavity may clearly be seen to be bent like a shepherd's crook, and to terminate in the region below and behind the anterior commissure. Examining a transverse section in the region of the mid-brain, a very clearly marked crucial lumen is seen to divide the brain into four quadrants. The upper laterals evidently correspond to the "Flügelplatten" of His, and the lower laterals to the "Grundplatten." The nucleus of the oculo-motor nerve lies in the lower lateral quadrants, and the upper forms the torus longitudinalis.

The characteristic crucial lumen may be traced continuously forward round the curve of the cranial flexure in such a manner as to indicate that the brain axis ends at the above-mentioned point. In a model of the cavities these relations are especially clear. Thus in its morphological disposition the corpus striatum corresponds in the fore-brain with the torus longitudinalis, while the wall of the infundibulum corresponds with the region which gives rise to the oculomotorius in the mid-brain.

The wall of the infundibulum, however, does not give rise to motor nerves.

The position of the neuropore and termination of the neural axis in Vertebrates has been the subject of great discussion. The various views have been well summed up by Kupffer in a

recent paper (**14**) on the development of the sturgeon. Von Baer, Dursy, and His regarded the infundibulum as the terminal point. Reichert, Kölliker, and Mihalkovics sought the point in the region of the optic stalks. Goette concluded that the pineal organ represented the neuropore. Van Wijhe found that in birds the last point of connection of the ectoderm and brain representing the neuropore lay at the middle of the sac of the fore-brain.

Orr (**17**) found that in the frog the "anterior medullary fold" representing the end of the floor of the neural canal lay above the optic chisma in later stages. Kupffer himself describes in the sturgeon a very definite structure, the lobus olfactorius impar, at first hollow, and communicating with the exterior, and in all respects resembling the neuropore of Amphioxus; and from the agreement of his conclusions with those of Van Wijhe he regards the question as settled for all Vertebrates.

In Teleostei, however, as we have seen, there is no definite structure corresponding to the lobus olfactorius impar. The wall of the brain passes continuously over the spot, and only comparatively late (fig. 4, *x*) can it be determined which region is identical with that structure. In Teleostei the neuropore and end of neural axis seem to have been situated at the level of the optic stalks, and below the subsequently formed anterior commissure. This conclusion is confirmed by the exact observations of Orr on the frog.

In his latest writings (**8** and **9**) His speaks of the neuropore as a "Nabelartige Unterbrechung der vordere oder frontale End-nath." Further he says, "The total extent of the neuroporic cleft stretches in all craniate Vertebrata from the position of the basilar ridge through the region of the subsequently formed recessus infundibuli, chiasma, and recessus opticus, between the nasal organs and along the lamina terminalis to its dorsal extremity." Considering the several conflicting and yet exact observations, this view of His seems the only reasonable one to take.

Mouth and Hypophysis.

The mouth and hypophysis in Teleostei formed the subject of Dohrn's first two studies on the ancestral history of the Vertebrate body. He stated that the mouth appeared first as a pair of lateral openings of the alimentary canal to the exterior, and that the hypophysis arose as a bilateral outgrowth of the upper wall of the alimentary canal in front of the endodermal pouches which form the mouth.

This account was criticised by Hoffmann (**10**), who described the hypophysis as developing from a thickening of the lower layer of the ectoderm in front of the mouth opening—that, in fact, in Teleostei, just as in other Craniata, the pituitary body is an organ derived from the epiblast of the stomodæum. Subsequently Dohrn, in his fourth "study," modified his first views to the effect that there exists an ingrowth of ectoderm.

I find that Professor Dohrn's figures are correct, except as far as the interpretation of the endoderm is concerned. Examination of earlier stages gives the true explanation. With Hoffmann's figures and statements my own conclusions on Gobius do not agree, though from individual pictures by Henneguy (**6**) and Goronowitsch (**4**) the development in Salmo appears to be the same as in Gobius.

Dohrn's account was confirmed by Miss Platt (**18**) for Batrachus tau. On the other hand, all doubleness of origin of the mouth is denied by McIntosh and Prince (**16**) for pelagic Teleostei. Nor, according to the same authors, is a stomodæum or involution of epiblast present.

Figs. 6—10 represent sections through the mouth and hypophysis regions of my first stage of Gobius capito embryos. Fig. 2 gives a diagrammatic view of the general relations.

Examining first fig. 9, whose position is indicated on the diagram by the line *d—d*, it is seen that the section is somewhat oblique, cutting the eye on the left near its posterior edge, and on the right nearer the centre of the lens. In the middle the brain is cut in section between the mid- and fore-brain, where it is most compressed from side to side. Below the eye, on

the left, occurs a considerable ingrowth of ectoderm, which represents the maximum ingrowth of the mouth at this stage. At this spot it cannot be perceived exactly how far the ectodermic ingrowth extends. In fig. 10, which represents a section 25 μ further posteriorly (5 sections), the limits can be sharply defined, and the ectodermic ingrowth is seen to be connected only with the ectoderm of the skin by a neck, while towards the middle line it touches another solid mass of cell, representing a solid forward growth of endoderm.

In fig. 9 ectoderm and endoderm are fused. Where the cells border on the yolk they form a more or less definite layer. In all the sections anterior to that represented in fig. 10 this layer can be perceived, and it separates brain and mesoderm from the yolk. It is very distinct on the right hand in fig. 9. It can be seen to pass insensibly into the external ectoderm, which forms the lens of the eye. In the same figure the outer layer or "Deckschicht" of the ectoderm has been separated from the lower layer. Under the centre of the eye no marked solid ingrowth of the ectoderm occurs.

Fig. 8 is a drawing of a section further forward through the posterior limit of the optic stalk. Its position is indicated on the diagram by the line *c*. Here, again, is seen solid ectoderm (*Hyp.*), occupying a portion of the angle between the eye and brain. Further forward (fig. 7, on the left side) the ectoderm is indistinguishable from the mesoderm surrounding the eye. This mesoderm extends forward below the eye-stalks. At the anterior end of the embryo the thickening of ectoderm (*Hyp.*) is continuous with the ectoderm of the nose.

From these observations one may learn that the embryo at its anterior end is separated from the periblast and yolk by continuous ectoderm, one cell thick in the median region, and that two paired thickened ingrowths occur, the anterior proceeding inwards from the region of the nose to below the optic stalk, and the posterior proceeding directly towards the middle line below and behind the eye. The anterior ingrowth, which is to a certain extent continuous with the ectoderm of the nose, gives rise, as we shall see later, chiefly to the hypophysis; while

the posterior thickened ingrowth, which is also continuous with the ectoderm of the lens, gives rise to the portion of the mouth lying in the region of the articulation of the jaws.

Considering now the endoderm at this stage, it is found that the alimentary canal is only tubular in the auditory region of the head. The lumen extends anteriorly to a short distance behind the termination of the chorda dorsalis, then passes outwards to open at the only gill-slit formed as yet. This gill-slit is the hyoid, or the aperture behind the subsequently formed operculum. By German authors it is termed the "Kiemendeckelspalte." In front of the chorda the outlines of the endoderm are almost impossible to discover, but it appears to extend forward as a solid layer, becoming in the median line very thin and unilaminar. Laterally, however, in front a more distinct prolongation meets the posterior ingrowth of ectoderm, and fuses with it, as shown already in figs. 9 and 10. Curiously enough, when this prolongation meets the ectoderm it also proceeds upward, and fuses with the mesoderm surrounding the eye.

In fig. 2 these conditions are represented diagrammatically. On the right of the figure only the brain and eyes and a portion of the premandibular mesoderm are drawn. On the left, ectoderm and endoderm are intact. The embryo is supposed to be viewed from the ventral side, that is through the yolk. The edge of the ectodern, where it passes over the yolk, is cut in section. The blue tint shows its extent. Towards the centre of the embryo, where the ectodermic layer is one cell thick, it passes without demarcation into the corresponding endodermal layer. The dotted blue line indicates the extent of the above-described thickened ingrowth of the ectoderm. This figure also shows how these ingrowths are moulded to the shape of the brain and eyes. Consequently the doubleness of origin must probably be regarded merely as an embryonic feature without any phylogenetic significance.

The endoderm is tinted yellow. Posteriorly beneath the chorda the lumen is indicated, and the dotted line proceeding to the hyoid cleft (*Hy.*) shows the communication of this

lumen with the exterior. The dotted yellow line (which towards its anterior limit must be regarded as lying below the blue line) represents the upward prolongation of endoderm, which fuses with the mesoderm surrounding the eye (fig. 2,*).

Figs. 3 and 11 show the condition two days later. Fig. 11 represents a sagittal section passing through the eye, the lens at its anterior border—the mid-brain and ear. The eyes and brain are now separated from the yolk by various organs. The mouth and hypophysis ingrowths are still solid, but at this stage they are no longer two separate involutions. The posterior portion is surrounded by condensing mesoderm, which forms upper and lower jaws, and is separated from the yolk not only by the forward-growing lower jaw, but also by the pericardium and its cavity, which are in process of extension forwards. From the eye the united ingrowths are separated by the mesoderm of the upper jaw, and by the suborbital ganglion of the lateral line. The ectoderm is specially thickened at the angle where it passes over the yolk, beneath the choroid fissure in fig. 11.

In the same section are seen the mandibular artery (*Bl. v.*) behind the lower jaw, the rudimentary hyomandibular cleft (*H. M.*), and the hyoid cleft (*Hy.*).

Following the same series of sagittal sections towards the middle line, the ectoderm is found to be perfectly continuous to the middle line. Beneath the infundibulum two layers of cells may be distinguished, the upper consisting of cubical cells closely packed together, and the lower of much flattened cells, which form a flat epithelium next to the yolk. This lower layer was the only one present in the first described stage. The cells of the layer bordering on the infundibulum can but be derived from the anterior thickened ingrowth of the previous stage. They are the cells destined to form the hypophysis.

Transverse sections put the conclusions derived from this series of sagittal sections beyond doubt.

The ectoderm of the angle of the mouth has fused indistinguishably with the endoderm, which also now gives rise to a

rudimentary hyomandibular cleft (*H. M.*, fig. 11). As in the former stage, the lumen of the alimentary canal does not extend further forward than to the level of the hyoid clefts. The T-shaped lumen is constant for about four days of development.

Fig. 3 is a diagrammatic representation of the conditions at this stage. Ectoderm, endoderm, and mesoderm are tinted, as in fig. 2, and the brain and other organs, which are represented as being solid, divided in the middle line so as to show to some extent the vertical relations. The view is, as in fig. 2, from the ventral side.

The ingrowth of ectoderm is seen to start from below the recessus opticus, choroid fissure, and posterior region of the eye, and it is now throughout of more uniform thickness than in the earlier stage. As stated above, the ectoderm is more condensed below the infundibulum, and is thicker where it is embraced by the maxillary and mandibular processes. In the figure the maxillary process is indicated by the dotted line. The yellow dotted line represents the rudimentary hyomandibular gill-pouch. Ectoderm and endoderm pass into one another without demarcation.

On the fourth day the hypophysis becomes more rounded, though still more elongated than in its later stages, and at the same time it is becoming cut off from the mouth. The cells of the ectodermic ingrowth which forms the mouth are arranging themselves in layers along the upper and lower jaws, so as to give rise to a central cavity. An epithelium continuous with the epithelium of the hollow alimentary canal becomes differentiated from behind forwards. In subsequent stages the hypophyis becomes separated from the mouth, and a membrane is found to surround it. Thus the condition depicted in the majority of Professor Dohrn's figures is arrived at.

Mesoderm.

The origin, extent, and fate of mesodermic structures are by no means easy to determine in Teleostei, on account of the fusions of the three layers and the indefiniteness of the cells.

No head cavities seem ever to be present in Gobius, though in Syngnathus a premandibular cavity occurs. Fig. 9 shows the premandibular mesoderm fairly defined on the right side (*Pmd. mes.*). In this section it passes continuously across the middle line below the brain, to join the corresponding mass on the opposite side. This is the bridge of mesoderm, so well known from Selachian embryology, which lies behind the infundibulum; at its median side and in front the premandibular mesoderm fuses with the mesoderm surrounding the eye. One point of fusion is shown on the left side in Fig. 9. A similar phenomenon is described by Kupffer (**13**) for Petromyzon. This author considers the cells lying round about the eye to be chains of nerve-cells.

Behind the eye, layers in the mesoderm cannot be distinguished (fig. 10) at this stage.

The mesoderm surrounding the eye and corresponding mesoderm in other regions has been made the subject of special investigation by Goronowitsch (**5**). As far as my researches go they confirm his statements. This mesoderm is derived, as it seems, from the "Ganglienleiste," or "neural ridge" of Marshall, whose rôle, according to Goronowitsch, has been entirely mistaken by many embryologists. At the same time the fusions which occur between this mesoderm and the premandibular mesoderm make it difficult, at least in Teleostei, to decide whether "sclerotomic" elements derived from the premandibular mesoderm may or may not take part in the formation of the skeletal structures which arise from this tissue much later.

The position of the premandibular mesoderm is shown in the diagram fig. 2, which also shows the extent of the cœlom, which, as is well known, forms the pericardial cavity. The cœlom actually borders on the mouth involution—that is, it extends almost to below the eye. The somatopleure (*So. pl.*) is thin, but the splanchnopleure forming the inner pericardial wall is thickened. On the third following day the premandibular mesoderm is taking its position round the eye so as to form the eye muscles, which later are supplied by the oculomotorius.

The bridge of mesoderm still persists behind the infundibulum (fig. 3) in such a manner as to lead one to suppose that formerly the eye muscles extended across the middle line, possibly when no cartilaginous skeleton was as yet formed.

The pericardial cavities have extended to the middle line and fused, and the heart is formed, though as yet solid (fig. 3).

The development of the pericardial walls in Teleostei seems to me to have an important bearing on some theories of the Vertebrate head. According to Van Wijhe (24), in Selachii the cavities of the visceral arches, whose walls give rise to the muscles of the gills and jaws, are continuous with the pericardial cavity, but not (at least in the posterior arches) with the cavities of the somites. Therefore, concludes Van Wijhe, the muscles of the gills and jaws, which are voluntary, are homologous with the muscles of the intestine, which are involuntary, and not with the body muscles, which are voluntary. Van Wijhe further makes this one of the cardinal points for the interpretation of the nerves. In Teleostei the somites are, as is well known, solid, and the cœlom with its walls, the somatopleure and splanchnopleure, is sharply defined. Now the cœlom in Teleostei is perfectly continuous far forward (fig. 2), but its walls, the somatopleure and splanchnopleure, the "Seitenplatten" of Van Wijhe, do not give rise to the gill and jaw muscles. Yet it does not seem possible to deny the general homology of the gill and jaw muscles in Selachii and Teleostei.

The Jaws.

Fig. 11 shows the first condensation of mesoderm around the mouth, and it is seen that one Anlage gives rise to skeletal elements of both upper and lower jaws. The condensation of mesoderm extends forward above the ectodermic ingrowth of the mouth some little distance below the eye. Below the mouth the lower jaw at this stage extends only a very slight distance forward, though further towards the middle line. In fig. 3 the structures are represented schematically. The dotted line extending forward below the eye

shows the region of condensation of the mesoderm. In later stages the tissue at the point of deepest ingrowth of the ectoderm of the mouth becomes less compact, so that then two Anlagen may be distinguished. The upper grows forward, and, with the differentiation of cartilage, gives rise to trabeculæ, intertrabeculæ, and the tissue underlying the maxilla, as described by Stöhr (**22**). The lower gives rise to the lower jaw. I hope to make the subsequent modifications which give rise to the adult conditions the subject of a special study. The fact that both upper and lower jaw structures arise as one paired Anlage at the angle of the mouth appears to have interest in view of the theories as to the presence and number of gill-bars having relation to the mouth.

Summary.

1. The neural axis in Gobius terminates at a point near the optic stalks, precisely as stated by Goronowitsch for the salmon. An indentation and the disposition of the cells at this spot indicate a rudimentary neuropore. In later embryos a characteristic crucial lumen, which may be traced continuously from behind forwards, gives another indication of the termination of the axis, and also shows that the corpora striata belong to the upper part of the wall of the brain.

2. Mouth and hypophysis arise as solid ingrowths of ectoderm. That the earliest and maximum ingrowths are independent and paired is probably an embryonic feature due to the mode of development of the brain and eyes. Nothing has been observed to indicate that the mouth was ever anything but a mouth.

3. Skeletal structures of both upper and lower jaws arise from one condensation of mesoderm round the ectodermic ingrowth of the mouth.

4. The jaw muscles do not arise from the "Seitenplatten"—a point of considerable theoretical importance.

Naples,
March, 1893.

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EXPLANATION OF PLATES 21 AND 22.

Illustrating Mr. H. B. Pollard's paper, "Observations on the Development of the Head in Gobius capito."

Lettering of Figures.

Aud. Ear. *Bl. v.* Blood-vessel. *C. str.* Corpus striatum. *Cb.* Cerebellum. *Ch.* Chorda dorsalis. *Ch. fiss.* Choroid fissure. *Ch. opt.* Optic chiasma. *Comm. ant.* Anterior commissure. *Comm. post.* Posterior commissure. *Ect.* Ectoderm. *End.* Endoderm. *Fb.* Fore-brain. *Gp.* Cell débris (Glob. parabl.—Henn.). *H. M.* Hyomandibular cleft. *Hy.* Hyoid cleft or bar. *Hyp.* Hypophysis. *Inf.* Infundibulum. *L. inf.* Lobus inferior. *M.* Mouth. *M. obl.* Medulla oblongata. *Mb.* Mid-brain. *Md.* Lower jaw. *Mes.* Mesoblast. *N.* Nose. *Np.* Neuropore. *Opt.* Eye. *P.* Pineal organ. *Pc.* Pericardial cavity. *Pmd. mes.* Premandibular mesoderm. *R. opt.* Recessus opticus. *S. orb.* Suborbital ganglion (i. e. buccale). *Sacc. vasc.* Saccus vasculosus. *So. pl.* Somatopleure. *Sp. pl.* Splanchnopleure. *T. l.* Torus longitudinalis. 4 *Vent.* 4th ventricle.

Fig 1.—Model of brain at the first stage, viewed from the side. The nose is partially represented.

FIG. 2.—"Schematic" representation of brain—ectoderm (blue), mesoderm (red), and endoderm (yellow)—viewed from below. First day.

FIG. 3.—"Schematic" representation of brain. Third day. The structures are represented also as cut in halves in the middle line vertically.

FIG. 4.—Model of brain at fifth subsequent day. Left half viewed from inside. Commissures represented in red.

FIG. 5.—Sagittal section of anterior end of embryo of first day. (× 450 approx.)

FIG. 6.—Transverse section through same region. Slightly oblique. (× 450 approx.)

FIG. 7.—Transverse section through optic stalks. Slightly oblique. (× 450 approx.)

FIG. 8.—Transverse section behind optic stalks. Slightly oblique. (× 450 approx.)

FIG. 9.—Transverse section through the eyes. Slightly oblique. (× 450 approx.)

FIG. 10.—Transverse section behind the eye. Slightly oblique. (× 450 approx.)

(Figs. 6—10 are from the same embryo.)

FIG. 11.—Sagittal section through eye and ear. Third day.

On the Head Kidney of Myxine.

By

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With Plate 23.

In a former number of this Journal Professor Weldon has given an account of the structure of the pronephros of Bdellostoma (9), but, so far as I know, that of Myxine is undescribed, excepting for some observations made by Professor Wilhelm Müller (8). Professor Weldon has very kindly furnished me with some specimens of Myxine glutinosa, in order that I might examine the head kidney. I should like here to express my thanks to him for this kindness, and also for advice and assistance given me throughout the investigation, which was conducted in his research laboratory in University College, London.

The head kidneys of Myxine are situated towards the anterior part of the body, a little behind the external gill-slit, and a little in front of the fore-end of the mesonephron. Each is an elongate organ, showing on the surface a much convoluted tuft of tubules (fig. 1, *p. t.*), the pronephric tubules of Müller, and projects into the pericardial cavity just beneath the heart, being also connected with the anterior extremity of the post-cardinal vein. I have been unable to trace any connection between the pronephros and the segmental duct.

Of the material upon which I have worked, some individuals contained large ova, whilst others did not, and the pronephros of an animal containing ova differs in many respects from that

of one without. I will describe first the anatomy of an organ from a Myxine in the latter condition.

Examined by means of transverse sections, the head kidney is found to be in very intimate relation with the post-cardinal vein (fig. 2). The greater part of it is actually lodged in the vein, whilst the more superficial tubules are embedded in the vascular wall, or lie free in the pericardial cavity. At the posterior end a glomerulus is present (fig. 2, *gl.*), extending along the inner side of the head kidney for about one fourth of its length. Posteriorly it is enclosed in a sheath of its own, but towards the front end this becomes indistinct, and the glomerulus tissue is interwoven with that of the pronephros. The organ seems, however, to be in a state of reduction, and the vascular supply cannot be clearly made out from any sections. The tubules, which are caught in section in every direction, open into the pericardium on the one hand by means of funnels (fig. 2, *f.*), and on the other are connected ultimately with a large duct (fig. 2, *c. d.*), the central duct (9), which, just as has been shown for Bdellostoma, sometimes divides into two or three great anastomosing branches, and then again becomes single.

In Myxine, however, as Müller states, the central duct gives off, on the side away from the tubules, outgrowths containing glomeruli (fig. 4, *c.*). In sections towards the hinder end of the organ I find that the glomerulus may, for a short distance, quite fill up the central duct, so that no lumen is visible, and then this reappears a few sections further on (fig. 2, *gl. d.*). Blood-vessels pass across the vein to break up inside the capsule into the characteristic capillary loop. The efferent vessels return to the capillary network between the central duct and the wall of the vein (fig. 2, *c. n.*).

The central duct does not reach to the anterior extremity of the organ ; it stops short, and only a bunch of tubules projects forward. The walls of the duct consist of tall columnar cells of granular protoplasm, each containing a large oval nucleus of coarser granulation (figs. 3 and 6). The cells have rather a ragged appearance at their fore-edge; this is emphasised by an

aggregation of the protoplasmic granules, which seem to be cast off from the cells, along with the mucus apparently excreted by them. Externally a well-defined basement membrane (9) gives support to these cells.

The walls of the tubules differ little from those of the main duct. The columnar cells are not as tall, but they consist of the same granular protoplasm, with the same large nuclei and the same jagged look at the free edges. They also rest upon a basement membrane, but they show distinct traces of ciliation throughout the whole length of the tube.

The lumen, which always contains more or less mucus, decreases somewhat in diameter towards the nephrostome, and here the columnar cells are continued in to the flat cells of the pericardial epithelium. In between the tubules are numerous blood-capillaries. As in Bdellostoma, the peripheral tubules are aggregated into lobuli, which are invested with pericardial epithelium; or, again, isolated tubes may be provided with a separate investment (fig. 7). In these cases also the capillaries are always present, lying beneath the epithelium and around the tubes.

Sections through the pronephros of the other Myxine present a very different appearance. The anterior tubules for their whole length, and also the distal region of the others, are unchanged, but as these more posterior tubes reach their inner extremity their character is much altered. The lumen is diminished in diameter, or even obliterated, and the tall columnar cells are no longer recognisable: some have become much attenuated—in fact, fibrous, with a dwindled nucleus; whilst others have a greatly swollen nucleus, surrounded with a small layer of protoplasm. A section through this region of a tubule is shown in fig. 8. Within the vein no definite central duct or glomerulus can be made out. The position occupied by these structures in specimens, as already described, is here taken up by a mass of tissue, consisting of a reticulum of protoplasm, whose fibres are nucleated, and in whose meshes are small cells with small nuclei, and larger cells with very large, round, and deeply staining nuclei (fig. 7). Blood-

vessels enter the tissue and break up into fine capillaries, which are caught in section in every direction. The blood-capillaries surrounding the tubes are still distinct, and so is the pericardial epithelium encircling the outermost parts; but that connected with the inner regions, as also the vascular wall, where it is much involved in the branching tubes, is considerably altered. Where at all recognisable the pericardial epithelium has lost its flat delicate appearance, and has become thickened and heavy-looking : the wall of the vein is also considerably thickened and much broken.

In sections of head kidneys of any stage I find a small quantity of lymphatic tissue (4, 8) lying external to the wall of the post-cardinal vein; but though in the later stages it comes into close relation posteriorly with this "central mass," I do not think the two are connected in origin. The "central mass" would appear clearly to be derived from the breaking down of the central duct of earlier stages.

Grosglik has already pointed out (4) that the recorded observations on the pronephros of Myxine (8) and Bdellostoma (9) were probably made on developing, and not on mature specimens. A study of my sections confirms this view, and also the opinion that the Myxinoid head kidney is undergoing reduction.

In the younger stages the organ consists of numerous tubules opening at one extremity into the pericardium by means of funnels, and at the other into a main duct, in connection with which are glomeruli.

As the animal reaches maturity a change takes place, beginning at the hinder end of the organ and working forwards, affecting first the central part and the ends of the tubules adjacent. The change consists in the formation of a mass of tissue much resembling lymphatic tissue, which would appear to result from the breaking down of the walls of the ducts, the constituent cells furnishing the fibrous groundwork of the new tissue, and also cells with large nuclei, which I am inclined to regard as formative cells, since in the most reduced organs I have examined they have

disappeared, their place being taken by many smaller cells. The whole tissue is richly supplied with blood-vessels provided by the vascular supply to the glomeruli and the capillary network into which this breaks up, and answers with great exactness to the description of the mass of lymphatic tissue in the anterior region of the Ganoid excretory system given by Balfour (1).

It would seem, therefore, that the head kidney of Myxinoids may be regarded as a stage in the phylogenetic reduction of this organ—a reduction which continues in the Pisces until the tubular structure entirely disappears.

As regards the relations of this organ with the supra-renal bodies, the absence of nerve-structures would seem at first sight to exclude the possibility of any connection between the two, which, indeed, is the view advanced by Emery (3). A consideration of the subject, however, shows that this is not an insuperable objection.

In his description of the supra-renals of Elasmobranchs, Balfour[1] states that these organs are derived partly from the mesoblast and partly from the sympathetic ganglia, and that in fact the two constituents remain distinct in this group throughout the life of the animal; whilst Mitsukuri has demonstrated the compound origin of these organs in the Mammalia (6).

It may, therefore, be concluded that the pronephros in Myxine represents the mesoblastic part of the supra-renal bodies, which have been shown by Professor Weldon (10) to be derived from the anterior part of the mesonephron in the higher Vertebrata.

[1] 'Comparative Embryology,' vol. ii, p. 664.

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EXPLANATION OF PLATE 23,

Illustrating Miss J. W. Kirkaldy's paper, "On the Head Kidney of Myxine."

b. c. Blood-corpuscles. *b. m.* Basement membrane. *b. v.* Blood-vessel. *c.* Capsule. *c. d.* Central duct. *c. n.* Capillary network. *c. w.* Capillary wall. *f.* Funnel. *gl.* Glomerulus outside the vascular wall. *gl. d.* Glomerulus of the central duct. *l.* Lumen of tubule. *m.* Mucus. *m. s.* Anterior end of mesonephron. *n.* Nucleus. *p. c.* Pericardial epithelium. *p. t.* Pronephric tubule. *s.* Cilia. *w. d.* Wall of central duct. *w. v.* Wall of vein.

Fig. 1.—Head kidney of Myxine glutinosa.

Fig. 2.—Diagrammatic longitudinal section.

Fig. 3.—Transverse section through the head kidney to show the relations of the central duct and the pronephric tubules.

Fig. 4.—Transverse section, showing glomerulus.

Fig. 5.—Glomerulus enlarged.

Fig. 6.—Longitudinal section through a funnel, showing the columnar animal, to show the central mass.

Fig. 7.—Transverse section through the head kidney of a more mature epithelium passing into the pericardial epithelium.

Fig. 8.—Tubule from the same organ, with modified epithelial cells.

Report on a Collection of Amphioxus made by Professor A. C. Haddon in Torres Straits, 1888-9.

By

Arthur Willey, B.Sc.Lond.

This collection, comprising some ninety specimens of Amphioxus, was obtained by Professor Haddon between the months of September, 1888, and January, 1889, inclusive, and was placed by him in the hands of Professor Lankester, who kindly entrusted it to me for examination.

The specimens were in a good state of preservation, and a careful study of them has revealed several facts of interest.

They were taken from different localities (Flinder's Entrance, Mabinag, &c.), at depths varying from six to thirty fathoms, the sea bottom being here, according to Professor Haddon's records, largely composed of fine broken shells.

They all belong to one species of Branchiostoma,[1] namely, B. cultellum = Epigonichthys cultellus, Peters.

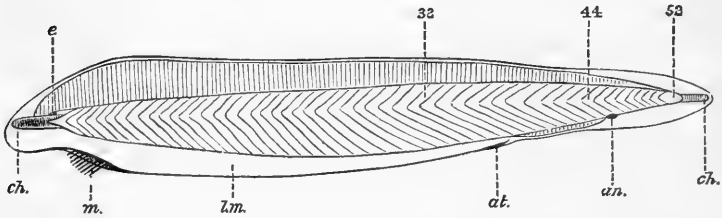
This species can be distinguished at a glance by the great development of the dorsal fin, which presents a striking appearance on account of the unusual height to which it projects above the body. The height of the fin gradually increases from behind forwards, reaching its climax in front in the region of the seventh and eighth myotomes. The distinctive

[1] As it is not only out of the question, but undesirable, to relinquish the name "Amphioxus," although "Branchiostoma" has the priority, the latter, following the precedent set by Günther, is retained as the systematic name of the genus, while the former may be regarded rather as a colloquial name.

characteristic of the dorsal fin is accurately portrayed in the figure of the species given by Peters; but he erred seriously in asserting the absence of a caudal fin, and in describing the anus as occupying a median ventral position.

Günther[1] perceived this error on the part of Peters sufficiently to justify him in releasing the species from the cumbrous genus to which the latter author had assigned it.

As a matter of fact, the caudal fin and anal opening have the same relations here as in the European species, only the former structure is much thinner and more delicate in B. cultellum, and can be practically annihilated by unfavorable reagents.



Explanation.—Branchiostoma cultellum from left side. *ch.* Anterior and posterior extremities of notochord. *e.* Eye. *m.* Mouth. *at.* Atriopore. *an.* Anus. *l. m.* Left metapleur.

Continuity of right metapleur with mesial ridge of ventral fin is indicated, as is also the lateral position of anus and the well-marked caudal fin.

In many of Professor Haddon's specimens, particularly in the sublimate and osmic acid preparations, the caudal fin, which is perfectly hyaline and of extreme tenuity, is very finely preserved, and in all cases the anus lies on the left side.

The number of myotomes in B. cultellum is about fifty-

[1] Günther (**3**, p. 33), criticising the account given by Peters, says, "The position of the vent is, at least in some of our specimens, rather lateral than median. Whether these differences are owing to the better state of preservation of our specimens, or related to the difference of locality, I am not prepared to decide." Professor Haddon's collection decides this point in favour of the former explanation of the differences in question.

two, but it appears to vary within narrow limits, namely, from fifty-one to fifty-five. Günther formulates them with reference to the position of atriopore and anus as follows:

$$32 + 10 + 10 = 52,$$
$$\text{or} \quad 31 + 11 + 10 = 52.$$

As the position of these apertures stands in no causal relation to any particular myotomes, it is difficult and perhaps impossible to give an unvarying statement with reference to this point.

In one or two instances I have counted as follows:— $32 + 12 + 8 = 52$, the anus being placed at the base of the forty-fourth myotome (cf. accompanying figure). In another case, in which I counted fifty-five myotomes, the formula was $33 + 11 + 11 = 55$. Still, in several other instances I have counted as many as fifty-four myotomes.

The average length of the specimens in Professor Haddon's collection may be placed at 2·5 cm., although several individuals measured upwards of 3 cm., two of them attaining a length of nearly 3·5 cm. Peters stated the length of his specimens, which came from Moreton Bay, to be 2·3 cm. Beyond a few measurements Peters gives no numerical data whatever, and no account of the internal organs.

There are from twenty-four to twenty-seven ventral fin-chambers between atriopore and anus. External examination from the ventral aspect gives rise to the impression that the ventral fin in this species contains paired fin-rays, such as are known to occur in B. lanceolatum. This optical effect is due to the fact that the caudal fin is continued forwards as a mesial ridge below the ventral fin-chambers, and so produces a double appearance in the latter in a surface view of the ventral aspect. As in B. lanceolatum, the fin-chambers are always single median spaces; but while in the former each of them coutains a pair of gelatinous fin-rays suspended from its dorsal wall, in B. cultellum the ventral fin-chambers are destitute of fin-rays.

The absence of ventral fin-rays has recently been also observed by E. A. Andrews in a new species of Amphioxus

from the Bahamas; but in this case the author adds the curious fact that the ventral fin space is also absent. In this respect, therefore, the new species differs essentially from B. cultellum, in which separate fin-chambers are emphatically present in the ventral fin.

In B. lanceolatum the right and left metapleural folds gradually decrease in size behind the atriopore, and, while converging together, finally die out on either side of the mid-ventral line below the anterior portion of the ventral fin. In B. cultellnm this is not the case. Here the left metapleural fold gradually dies away behind the atriopore, whilst the right fold does not die out, but is continued behind the atriopore into the mesial ridge, which lies below the ventral fin-chambers, and represents, as mentioned above, the forward continuation of the caudal fin.

This behaviour of the right metapleur has also been previously signalised by Andrews in the Bahama species.

In the latter, again, according to Andrews, the buccal cirri are "smooth and united by the hood membrane for the greater part of their length." In B. cultellum, on the other hand, the cirri are distinctly free throughout their entire length, except, of course, at their bases, which are embedded in the margin of the oral hood; at the same time they appear to be without those papilliform prominences which are a well-known feature in the European species.

I have been unable to detect an olfactory pit in B. cultellum, but will not undertake to say that it is always absent. It appears, however, according to Andrews, to be absent in the Bahama species.[1]

[1] That this is a distinct species is shown once for all (in the absence at present of the detailed illustrated description which I am informed by Professor Andrews will shortly appear in vol. v of the 'Studies from the Biol. Lab. Johns Hopkins University') by the formula of the myotomes as given by Andrews, viz. 44 + 9 + 13 = 66. Length 13—16 mm.

[Since the above was in type Andrews' memoir has appeared in the 'Bulletins of the Biol. Lab. of the Johns Hopkins University,' vol. v, No. 4, under the title "An Undescribed Acraniate: Asymmetron lucayanum."—EDITOR].

One of the most remarkable features in the internal organisation of B. cultellum is the fact that the gonads occur as a unilateral series of pouches confined to the right side of the body.

Singular to say, it agrees in this respect also with the Bahama species, and it should be clearly pointed out that the first discovery of this curious form of asymmetry in Amphioxus is due to Professor Andrews. The fact of its occurrence also in B. cultellum, which is a distinct species with a very different geographical distribution, is of some interest.

In view of what we know as to the asymmetry of the larva of Amphioxus, I cannot, however, agree with Andrews in regarding the above species as being generically distinct from the European species, although it might perhaps be legitimate from a systematic point of view to create a new sub-genus for it.

I have paid particular attention to this asymmetry of the gonads in B. cultellum, and find that it occurs invariably. A great number of specimens in the collection were mature, and they were all examined with great care. In some cases so large were the gonads, extending from one side to the other across the middle line, that a casual glance would lead to the impression that they were paired in the usual way, but a dissection or a careful examination with a lens always revealed their unilateral disposition.

All observations were controlled by transverse sections, and in those which passed through the greatly distended gonads the pharynx was found to be pressed tightly against the left wall of the atrium, almost the entire available space being usurped by the gonads.

In view of the fact of the unilateral disposition of the gonads, both in B. cultellum and in the species from the Bahamas, it is important to note that often in specimens of Amphioxus from the Mediterranean the gonadic pouches of the right side can be observed to preponderate greatly over those of the left side, as if the hypertrophy of the former led to the reduction or incipient atrophy of the latter. I am not aware that this observation has ever before been recorded.

In B. cultellum, as well as in the Bahama species, the predominance of the right gonads over the left has been accentuated to such a degree that the latter have been entirely lost. Whether or not rudiments of the left gonads appear at any time in the development remains to be decided.

Such instances of unilateral asymmetry as that described above are always of interest, since they are obviously cenogenetic deviations from the normal, which can in a measure be satisfactorily accounted for.

Since, according to Boveri's observations, the development of the reproductive organs of Amphioxus takes place after the metamorphosis of the larva, it is evident that the occasional partial asymmetry of the gonads (in respect of size) which I have noted above in the Mediterranean species, and the complete asymmetry of the gonads in B. cultellum, &c., must belong to a different order of phenomena from the remarkable asymmetry of the pharynx in the larva of Amphioxus. Moreover, Boveri has shown that the perigonadial cœlom is a derivative of the myocœle, and the myotomes are not involved in the larval asymmetry.

It would seem, in fact, that the absence of the antimeres of the right gonadic pouches in the Bahama species and in the Australian species is due to considerations of economy of growth and accommodation to a limited space. Provision being made in the economy of the organism for a certain bulk of gonads, the onus of this can either be shared equally by the two sides, or a greater proportion can be assigned to one side, or finally the entire mass can be confined to one side. It is difficult to give a priori a reason why it should always be the same side which is affected by this unequal growth.

The above would seem to be the correct mechanical explanation of the asymmetry in question, the hypertrophy of the gonads of one side necessarily leading to the final atrophy of those of the other side. Whether it is correlated physiologically with any greater locomotor activity on the part of the species in which it occurs must remain an open question.

According to Andrews the Bahama species "swims free in the evening, both at Bimini and in Nassau Harbour."

There is thus every reason to suppose that the above-described unilateral asymmetry of the gonads in certain species of Amphioxus belongs, broadly speaking, to the same category as, for example, the well-known asymmetry of the female genital glands and the lungs of snakes, in which the respective organs of the right side usually predominate over their antimeres on the left side, the latter being often rudimentary; and, again, the female reproductive organs of birds, in which the left ovary and oviduct predominate over the right, the latter being either absent or rudimentary.

There are seventeen to twenty unpaired gonadic pouches in B. cultellum. When there are as many as twenty the first one lies at the base of the 9-10th myotome. In one specimen, taken from a bottle labelled "Mabinag, Oct. 24th, 1888," there were quantities of free ova in the atrial chamber derived from the discharge of the anterior eight or nine gonadic pouches, while the nine posterior pouches still remained intact. Another specimen, in which the hypertrophy of the unilateral gonads was carried to an extraordinary pitch, was taken on Dec. 24th, 1888.

This, therefore, may be taken to indicate the time of spawning of B. cultellum, which is somewhat later than is the case with the Mediterranean species. In fact, it would appear as though the spawning of B. cultellum commences at about the time of the year at which that of B. lanceolatum ceases.

I am not aware of any observations on the habits of B. cultellum, but the special elaboration of the dorsal fin would seem to point to the fact of its being, like the Bahama species, an active swimmer.

As for the significance to be attached to the continuity of the right metapleur with the mesial ridge of the ventral fin, it seems to show that the metapleural folds or ridges are, after all, structures of the same nature as the median longitudinal ridges which constitute the fins of Amphioxus. If the meta-

pleura had been entirely different in their nature from the median fins it is not very likely that one of them would have undergone concrescence with the ventral fin. As shown by Lankester and Willey, the metapleural folds arise as solid longitudinal thickenings of the integument, which are at first largely ectodermic in origin (the ectoderm-cells assuming a columnar character), while the cutis subsequently takes part in their formation. Eventually a lumen (schizocœle) appears in the ridges.

The right metapleuron is in advance of the left in order of appearance, and in front of the pharyngeal region of the larva it curves sharply inwards towards the middle line, in which it gradually dies away on the ventral surface of the snout.

There is, in fact, no essential difference between the mode of origin of the metapleura and of the median fins as integumentary ridges, and it is possible that in the above-mentioned cases, in which the right metapleuron is continuous with the ventral fin (*i. e.* the mesial ridge in connection with it), they actually arose in continuity in the first instance.

If, then, it is necessary to admit the intrinsic similarity in the nature of the metapleural folds and the median fins of Amphioxus, we are led back to the theory of Thacher with reference to the origin of the paired limbs of Vertebrates.

Balfour, as is well known, was the first to discover the continuous lateral fin-ridges of the Selachian embryo in 1876; and it is curious to note, in the light of what has just been said as to the ectodermic thickenings which prelude the formation of the metapleura in Amphioxus, that they also (*i. e.* the Selachian fin-ridges) consist in longitudinal thickenings of the epiblast. From his observations on the embryonic development of the Selachians, Balfour came to the conclusion "that the limbs are the remnants of continuous lateral fins;" but he did not suppose that the continuous lateral fins were represented in Amphioxus.

At about the same time, and quite independently, Thacher was led by observations on the adult forms and on the skeleton of Selachians and Ganoids, &c., to a belief in the homodynamy

of median and paired fins. As to their phylogeny, he said, "As the dorsal and anal fins were specialisations of the median folds of Amphioxus, so the paired fins were specialisations of the two lateral folds which are supplementary to the median in completing the circuit of the body. These lateral folds, then, are the homologues of the Wolffian ridges in embryos of higher forms."

Shortly afterwards Mivart also came independently to the conclusion that the paired and azygos appendages of Vertebrates were fundamentally of the same nature. Subsequent palæontological researches have only confirmed this view.

The point, however, which is at issue on the present occasion is whether or not the primitive continuous lateral fins are represented by the metapleural folds of Amphioxus. From what has been said above there seems to be good reason to expect that this question will sooner or later be answered by the consensus of morphologists in the affirmative.

Reference should be made here to an interesting feature in the geographical distribution of Branchiostoma, which does not appear to have received sufficient attention.

The areas of distribution of the species of Branchiostoma are, as a rule, separated from one another by such wide intervals of space that it is extremely surprising to find an instance of the overlapping of two specific areas. Such an instance apparently occurs in the Torres Straits.

According to Dr. Günther specimens of Branchiostoma Belcheri were obtained by Dr. Coppinger from the sea around Prince of Wales Island, Torres Straits, while during the same voyage (H.M.S. "Alert") B. cultellum was obtained from the neighbouring Thursday Island. B. Belcheri, Gray, which is characterised chiefly by the presence of sixty-four or sixty-five myotomes, and is more elongated than B. lanceolatum, was first obtained by Sir E. Belcher, during the cruise of H.M.S. "Samarang," from the coast of Borneo.

Professor Haddon does not appear to have obtained any specimens of Amphioxus from Prince of Wales Island; but the fact that his large collection, taken from several different

stations in Torres Straits, does not contain any other species than B. cultellum is noteworthy, and it would be a matter of interest to determine the exact relations to one another, and limitations in the distribution of the two species of Branchiostoma which have been recorded from the Torres Straits.

A great deal of work has still to be done in connection with the geographical distribution of Amphioxus, and this not only with regard to its distribution on the face of the earth, as to which we can hardly hope for a speedy settlement of the question, but even as to its distribution in more restricted provinces, as, for example, the coast of Europe. Although there is only one species, there are undoubtedly several varieties of European Amphioxus which differ from one another in point of size. Thus the Messina Amphioxi average larger than those from the Gulf of Naples, while both of these varieties would appear to come far short of that found on the coast of Brittany, which is said to attain a length of no less than 8 cm.

An extensive series of measurements of European Amphioxus from different localities would be certain to yield important results

It is worthy of note that differences in size among individuals or species of Amphioxus are not causally related to the number of myotomes. A single instance will suffice to illustrate this point. The Bahama species, according to Andrews, has sixty-six myotomes, with a length of only 13 to 16 mm. B. lanceolatum has sixty myotomes (sometimes fifty-nine and sometimes sixty-one), with a length of 4 to 6 and even 8 cm.

The relation of size to physical or organic environment is a subject for investigation.

In conclusion it may be said that Professor Haddon's collection, which I have had the privilege of examining, has enabled the specific characters of Branchiostoma cultellum to be definitely ascertained, and has brought to light several interesting features in its organisation.

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The Orientation of the Frog's Egg.

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With Plates 24 and 25.

I.

The classical experiments of Pflüger on the segmenting frog's egg, and the important conclusions drawn by Roux from a study of the same egg, have made it very desirable to have an accurate knowledge of the relation existing between the early segmenting egg and the position of the embryo with respect to the egg.

The interpretation of certain embryos in which the blastopore has failed to close, recorded by Roux and Hertwig, will likewise depend on the normal position of the embryo on the egg. Pflüger, Roux, and Hertwig have come to the conclusion that the embryo forms over that portion of the unsegmented egg which is normally directed downwards, i. e. over the white hemisphere. Schultze supports the old view, that the embryo lies on the upper or black hemisphere.

Pflüger based his conclusion on the evidence obtained by actually following the dorsal lip of the blastopore in its migration over the white hemisphere. Roux based his conclusion on evidence obtained by destroying definite portions, both of the segmented and unsegmented eggs.

Hertwig's conclusions were based on the evidence furnished by certain abnormalities, while Schultze's conclusion rests on a study of normal development.

It seemed to me at first, from a study of eggs developing normally, that it was impossible that the embryo should lie entirely over the white hemisphere. Schultze pointed out that Roux's earlier results are contradictory in themselves, and I had reached the same conclusion from a careful reading and re-reading of Roux's earlier papers. I was prepared, therefore, to find some truth in each view, and expected to find the embryo forming partly over the black, partly over the white hemisphere. I was then not a little surprised to find that our studies led to the conclusion that the embryo is formed over part of the white hemisphere of the egg. In the main point, therefore, I am in agreement with Pflüger and Roux, although not entirely so, for I hope to be able to show the extent of the white hemisphere of the unsegmented egg, covered by the blastopore, to be somewhat different from that affirmed by Pflüger and Roux.

Our work in relation to the orientation of the embryo has covered the ground somewhat more extensively than that of any previous author, since we have made use of the methods employed by all of them.

Our results will be considered under three headings:

1st. Normal development and location of blastopore.

2nd. Results obtained by injury to definite portions of the early embryo.

3rd. Results obtained from embryos whose development had been modified by artificial means.

A word of personal explanation ought to be added. The senior author is responsible for Sections I, III, IV, and V of the present paper. The work recorded in these was done in the spring of 1893.

Section II is the record of the results obtained by Umé Tsuda while a student in the Biological Laboratory of Bryn Mawr College. This work was done during the winter of 1891–2; the account written in the spring of 1892. Only

very slight alterations have been made in this portion preparatory to publication.

II.

In studying a series of eggs of the early stages of Rana temporaria from the segmentation period to the beginning of the formation of the blastopore, a few points in regard to the peculiar development of the pigment and the orientation of the dorsal lip of the blastopore have been noted, and are here given briefly.

The eggs, which had been previously hardened and preserved in 80 per cent. alcohol, were studied chiefly by surface views with a dissecting microscope. The study of the segmentation of the early stages only verified previous accounts. The first cleavage furrow divided the egg into two equal parts; the second is at right angles to it; the third or horizontal furrow is much nearer the upper or pigmented pole, thus forming four small pigmented cells in the upper and four large cells in the lower hemisphere.[1] The four cells of the upper half then each divide, thus forming eight cells; but the division from this point becomes quite irregular, both in the upper and lower halves of the egg. I found a number of eggs in what clearly seemed a twenty-four cell stage—eight cells in the lower and sixteen in the upper; but I could not verify the fact in the living egg.

A curiously abnormal egg of eight cells was found where the horizontal or third furrow was entirely wanting, and the furrows of the next division, which would normally have divided the egg into sixteen cells, had cut through from the upper pole, reaching down about two thirds of the distance to the lower pole. On sectioning the egg eight nuclei were found corresponding to the number of segmentation furrows. No

[1] I have found a number of probably abnormal eggs from one lot in which the first furrow divided the egg into unequal parts, one large and the other much smaller. Also a number of eggs of the four-cell stage, where neither the first nor second furrow had met at the lower pole.

nuclei were found in the yolk portion of the lower half of the egg, which would normally have been separated from the upper cells by the third furrow.

In addition to the division of the cells of the segmenting egg from the surface a delamination of the cells begins about the thirty-second cell stage. The horizontal and vertical sections at this stage show elongation of the nuclei at right angles to the plane of division. In the sixty-fourth cell stage the delamination can be easily seen to have taken place by the dissection of an egg under a hand-lens or a dissecting microscope.

A careful study of the segmentation of the cells around the lower pole in the advanced segmentation stages has shown that the greatest irregularity exists.

In many cases, however—and I have reason to believe in nearly all cases,—the cells lying nearest the lower pole, and especially the four cells which are around the point where the first and second furrows intersect, remain larger than the surrounding cells. In the later stages of many eggs I have distinctly made out four cells, which I think are without doubt the ones grouped around the lower pole. Figs. VII, VIII, IX, where cells marked *b* and *a* are much smaller than the surrounding ones, might seem to oppose such a conclusion; but in the later stages, at the time of the formation of the blastopore (figs. X, XI, XII), there is a certain regularity in the grouping of the larger size cells around the point which, from other indications, I should judge to be the lower pole; and hence I believe that such a cell as cell *a*, fig. VIII, though smaller at this particular stage than the cells surrounding it, does not develop so rapidly later on. I have not been able to section the eggs at these stages to find out what relation the real size of the cells bears to the apparent size from surface views. At present I see nothing against the hypothesis that the portion of the egg around the lower pole in the late as well as in the early segmentation stages is the most retarded portion of the developing egg. The group of cells that remain largest always bear a certain relation in position to the pigment that leads me to

believe that this is undoubtedly so, and as yet I have seen no indication that would tend to a contrary conclusion.

It has been noted that with the splitting off of cells from the upper corner of the yellow cells of the lower hemisphere new ectoderm-cells are formed, and it had been generally supposed that with this growth a continuous formation of pigment took place, the black pigment gradually growing down over the whole egg. I have found that the growth of pigment does not in any way correspond to the growth of new ectoderm-cells, but, on the contrary, there seems to be a great variation in the amount of pigment found in various lots of eggs of the same stage procured at different times. Some eggs of the two- or four-cell stage have the pigment covering more than two thirds of the egg, while others at this point of development have only a black cap of pigment extending down to the third furrow. However, all the eggs of the same stage from one cluster, and hence laid by the same frog, are alike in the quantity of pigment. The amount of pigment in the egg seems a variation dependent on conditions previous to the beginning of segmentation, and due to individual difference in the adult frog. There is, of course, some formation of new pigment in the later segmentation stages, and a most marked and rapid change in the amount of pigment formed at the time of the first appearance of the blastopore.

In all the eggs, from the earliest stages up to the blastopore, there is one marked peculiarity of the pigment. There is always a greater deposit of pigment on one side of the egg than on the other; and if we judge the exact position of the lower pole from the crossing point of the first two segmentation furrows, we find that the pigment is not only denser, but it comes down much nearer to the lower pole on one side than on the other. As this was found to be the case in greater or less degree, and in some eggs very markedly, in all the stages up to the beginning of the blastopore, and, moreover, in eggs procured at several different times and places, I judged it could not be an accidental variation.

I have tried, therefore, to find out—

I. Whether the pigment bore any fixed relation to the first furrow, and by this to follow out approximately the first furrow in the later stages.

II. The relation of the dorsal lip of the blastopore to the pigment and to the first furrow.

I have examined a large number of eggs in the early stages, in order to ascertain the exact appearance of the pigment, and to compare it with the later stages. When the egg is looked at on the under and non-pigmented side, with the lower pole turned uppermost, the line of pigment, extending, as it does, nearer to the lower pole on one side than on the other, has a crescentic outline. The pigment zone or band is not usually visible on the opposite side (see fig. VI). The same crescent-shaped appearance of the pigment is easily followed in the later stages up to the formation of the blastopore, at which time there is a rapid growth of pigment downward towards the lower pole. In order to ascertain what relation the first furrow bears to this crescent-shaped band of pigment, I examined 119 preserved eggs, as well as a number of living ones, in the stage when the second furrow had begun to come in from the upper pole and was about to meet the first furrow on the lower side. I made my observation on the eggs just before the two sides of the second furrow had met and intersected the first furrow at the lower pole, but when they were near enough to meeting, so that the point of the lower pole could be approximately judged. In this way I was able to distinguish the first furrow from the second, which would not have been possible after the second cleavage was completed, and at the same time I could guess approximately the position of the lower pole, the meeting-point of the first and second furrows.

The first furrow does not seem to cut the pigment zone bilaterally; nor does it, on the other hand, always divide the egg into a lighter and a darker half. Out of the 119 eggs examined, in 76 cases the first furrow cut through a little to one side of the central point of the crescent, only approximately dividing the pigment.

Figs. A, B, and C show diagrammatic views of the lower

pole of different eggs. The line *m n* represents the first furrow, and *a, b, c, d,* the four cells formed by the first and second segmentation furrows. In Fig. A the dotted line *x y* passes through the centre of the crescent-shaped pigmented area, and divides the egg symmetrically. The first furrow lies to the right of it.

FIG. A.

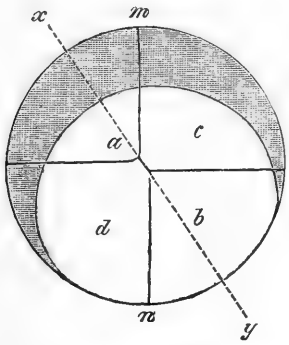
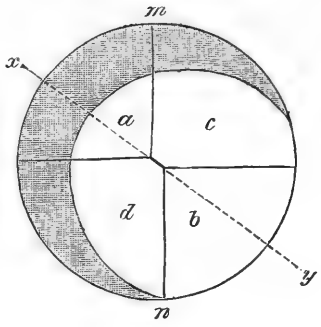
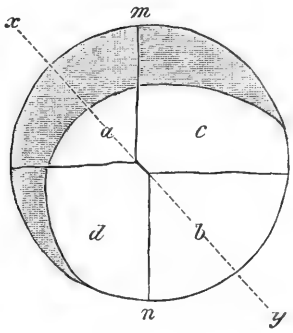


FIG. B.



In 30 cases out of 119 the second furrow seemed to divide the pigment more equally (see Fig. B), and the second furrow is a little to the left of the imaginary line of symmetry *x y*.

FIG. C.



In the remaining 13 cases the pigment seemed to extend as much on one side as on the other, and the line *x y*

in this case would be as near to the first furrow as to the second (Fig. c). It will be seen on examining the above diagrams that the apparent variation of pigment in the three cases depends on very slight differences. A little shifting of the pigment or an addition to one side or the other would change Fig. A to Fig. C or B. Judging by numbers, A would seem to be the more typical one.

One thing remains obviously unchanged in all the eggs. Of the four cells into which the egg is divided, one cell, *a*, has always the greatest amount of pigment; and cell *b*, which is opposite to it on the other side of the egg, is the lightest. This is true in every case, and these two opposite sides can be distinguished in eggs far advanced up to the end of segmentation. The cells marked *c* and *d* are intermediate, being neither so dark as *a* nor so light as *b*.

The pigment on the darker side of the egg not only extends much farther down, but I have observed on dissecting the egg in the upper hemisphere that in some cases the pigment extends inward almost to the centre of the egg (blastula), and to about twice the depth of the opposite side.

The interesting point in connection with the two opposite, the darker and the lighter, sides of the egg, on which I have dwelt at such length, is that the less densely pigmented half of the egg very early in the segmentation shows signs of a more rapid development and growth than the darker and pigmented side. This is true of the cells of the upper hemisphere as well as the lower. Moreover it is on the side that shows this advance in development that the dorsal lip of the blastopore makes its appearance.

I first noticed the unequal growth of the cells in an egg of about ninety-six cells, sixty-four in the upper and thirty-two in the lower half. There was a decided retardation of one side of the egg. In later stages the lighter half seems often two stages ahead of the other side. Figs. IV, V, show the unequal development in the early stages. Figs. IV, V (camera drawings), are surface views of an exceptionally fine egg, in which the unequal development is seen to what might appear an ex-

aggerated degree. Very few eggs at this early stage in the development show such a marked difference of the two halves. In some eggs of this stage the segmentation seems equally advanced on both sides, but these are rather the exception than the rule. I have never found a single case in any stage where the pigmented side was in any way in advance of the lighter side. On the contrary, the reverse is true in almost every egg towards the close of segmentation, and in most cases a superficial glance will reveal the fact plainly.

Sections of the egg parallel to the third furrow show the cells smaller over one hemisphere than over the opposite, and prove at least that there is an unequal development of two sides of the egg, and that the difference which exists between them is no superficial one.

A careful examination of the early blastopore stages of the egg with reference to the pigment and to the unequal development shows conclusively that the blastopore makes its first appearance on the less pigmented and further developed side of the egg, and, moreover, at a short distance only from the group of large cells around the lower pole.

I have examined over a hundred eggs at this stage, and my best observations have been made on eggs in which the development of the yolk-cells, as compared with the rest of the egg, was retarded, so that the outlines and size of the cells, as well as the unequal development of the two sides, could be plainly seen by surface views with a dissecting microscope. In some eggs it was very easy to follow out the outline of the yolk-cells around the lower pole after the formation of the blastopore, though the development was often too far advanced to make this out satisfactorily. But wherever I could follow out the cells it was plain that the region around the blastopore was in advance of the opposite side. In most cases it is difficult also to orient the egg as regards the pigment after the appearance of the blastopore, though I had a number of specimens where this could be done. Towards the close of the segmentation period pigment rapidly forms over

the area where the blastopore is about to appear, so that a line of dark pigment is distinctly seen in sharp contrast to the lighter cells lying next to it. The change is so rapid that it is often impossible to orient the sides of the egg. In some cases, however, when the blastopore has only just appeared, and before the pigment increases to any extent, it is easy to see that the blastopore is forming on the previously lighter side of the egg, as well as on the side which is segmenting most rapidly. In spite of the dark pigment formed just above the blastopore there is often a distinct light area on one side of the arc. This area probably corresponds with the cell marked *b*, the least pigmented cell, which lies opposite to the centre of the pigment crescent and opposite to the dark cell, *a*, in the diagrammatic figures *A*, *B*, *C*.

Figs. x—xiii are views of favorable specimens, and show distinctly a cluster of large cells, presumably those around the lower pole. Fig. x is before the appearance of the blastopore. The crescent-shaped area of pigment is distinctly seen, the pigment coming much nearer the group of large cells on one side than on the other. It is on this pigmented side that the cells are largest. In the centre are a large cell and three smaller ones, which probably are the four cells nearest the lower pole. The unequal segmentation is also shown in fig. xi, where the blastopore has already formed on the side where the cell division is more advanced. In fig. xii there are four cells distinctly larger than the surrounding ones, between which probably run the first and second furrows. It will be noted how much nearer the pigment approaches these cells on the side marked *m* than on the opposite side, where the blastopore appears. To the right and left of the blastopore the pigment is less dense than on the opposite side, though it is rapidly forming just above it. If it is granted that the four cells are around the lower pole, and that this is the point where the first and second furrows intersect, the exact relation of the blastopore to the lower pole can be easily ascertained. Fig. xiii is a side view of the same egg, in which the position of the supposed lower pole is shown. It is very near

the line of pigment on one side, as we should expect, while the blastopore on the opposite side is less than one third of the distance from the lower to the upper pole.[1]

It has been almost conclusively proved by previous experiments and observation that the plane of the first furrow in the case of the frog divides the egg into halves corresponding to the right and left sides of the embryo; and this study of the blastopore does not contradict, but would tend to confirm the fact. Although the arc of the blastopore is often not opposite the centre of the crescent of pigment (as it is in fig. XII), this is easily accounted for by the distribution of the pigment as shown in Figs. A, B, C (text). If we suppose the second furrow rather than the first to cut through the centre of the crescent (Fig. B), we should have the pigment much as in fig. XI, allowing for the formation of some new pigment.

III.

The eggs of two species of frogs were used for the experiments recorded below. Eggs of Rana temporaria were found on the morning of March 25th. These had not as yet segmented. The eggs of another species (not determined) were brought to the laboratory on April 4th. These had just begun to segment. Since much of the experimental work was done on these eggs, it was first necessary to find out whether the facts recorded in the last section were also applicable here.

A study of representative stages showed the same distribu-

[1] It has been thought by some investigators that the blastopore formed much higher up in the egg, but it needs only a superficial study to show that this at least is impossible. The cells within the blastoporic ring are non-pigmented and yolk-cells. A study of the surface view of the early stages shows that the pigment from an early stage often extends down on one side two thirds of the side, and on the other one half of that side. The blastopore forms lower down than the pigmented area, and this would make it at least halfway down the egg from the upper pole, and much below the plane of the third furrow. We see in fig. XIII that if the lower pole, as I have attempted to show, is marked by the large cells the blastopore appears below even the equator of the egg.

tion of pigment as found in the eggs of Rana temporaria, but the eccentricity in its distribution in respect to the axis of cleavage was greater than in eggs of Rana temporaria. The egg looked at from above (with one pole of the axis turned directly upwards) showed on one side a distinct white crescent, as seen in fig. 1.

The most interesting fact is that in the thirty-two-celled stage a very decided irregularity of the segmentation spheres of the upper portion of the egg is to be found.

This is readily seen in the three figures of the same egg drawn in figs. I—III. The first of these (fig. 1) shows the egg from above; eight cells lie along a line (four on each side) that corresponds presumably to the first cleavage plane. These upper eight cells are all approximately the same in size in this egg. The eight cells forming the zone around the egg below the upper eight, and which are sister cells with the latter, show a difference in size on opposite sides of the egg, as shown in figs. II, III. The difference may be seen from above, but still better by a study of the opposite (lateral) sides of the egg.

The lighter side of the egg is shown in fig. III, in which the border line of black pigment extends only for a short distance over the side of the egg. The dark side of the egg is drawn in fig. II, and here the pigment extends much further into the lower hemisphere. On the light side of the egg (fig. III) the cells of the second and third zones are smaller than the corresponding cells on the opposite side of the egg (fig. II). Unfortunately the four-, eight-, and sixteen-celled stages of these eggs were not preserved, so that I am unable to say how far back this difference in the two sides may be present.

This led to a re-examination of the eggs of R. temporaria. Here I found that at the eight-celled stage in most eggs one of the four upper cells is somewhat smaller than its upper vis-à-vis. It was also found that this smaller cell is the cell nearest to the highest point reached by the white crescent; therefore it must have come from that cell (now) of the lower pole that contains the least pigment. At the sixteen-celled stage those cells on the side of the egg nearest the upper limit

of the white are also smaller than those opposite to them. Sections of the hardened eggs, made with a scalpel through the plane of these smaller cells and their opposites, showed that these cells are not only smaller superficially, but in the third dimension as well.

Undoubtedly, then, from the eight-celled stage onwards the distribution of larger and smaller cells on the dark and light sides of the egg is present, and I have been able to push back a step farther the differences noted for the later stages in the preceding section.

Whether or not a still more careful examination of very favorable material would find the same difference present in the four-celled stage I am unable to say, but it seems not improbable that such a difference exists.

A study of the method of gastrulation of the egg of the unknown species shows that the first traces of the blastopore appear on the light side of the egg within the white cells. Presumably the pigment has here also extended farther over the sides of the egg than at first. The outlines of the cells in the region of the blastopore are at first polygonal. Dark pigment appears in the walls of the cells, producing the dark line seen in surface view. Certain of the cells pull in from the surface, leaving only their outer small pigmented ends exposed. These cells subsequently pull in all together to form the beginning of the archenteron by invagination. The cells dorsal to the blastopore become narrow and elongated from above downwards. The light cells, below the point of invagination, retain their polygonal outline.

The changes that take place in the overgrowth of the dorsal lip of the blastopore will be recorded below. First let us examine the embryo when first outlined on the egg. Sometimes the outlines of the medullary folds may appear before the yolk-plug has entirely disappeared—at other times not until after this change has taken place. Careful measurements of the embryo at this time show that the embryo anterior to blastopore covers in length about one third of the periphery of the egg. The relative length of the embryo

to the egg is shown in Pl. 25, fig. 5. In some cases the embryo measures a little more than one third of the periphery, in others a little less. Very quickly after the appearance of the medullary folds the embryo increases in length, and the proportions of the egg change, so that in order to determine accurately the length of the medullary folds as compared with the egg they must be measured when their outlines are just indicated by darker pigment.

The suckers appear in front of the medullary folds, and arise at about the same time: a dark line of pigment marks their position. This crescentic line of pigment—the beginning of the suckers—is not quite halfway around the egg from the blastopore, i. e. it is nearer to the dorsal lip of the blastopore than to the ventral.

Experimental Investigation.—Loss of time and material was caused at first by attempts to do experiments that proved to be impossible; also many results were valueless, because the eggs experimented upon were not watched continuously. I cannot too strongly emphasise this point, that unless each egg is carefully followed, from the moment of injury to the time of preservation, the results become uncertain and of little value. I have seen an injured point completely heal over, and the extra-ovate of Roux plough a long furrow over the surface of the developing egg; consequently any conclusion drawn from the end result without a knowledge of the intermediate stages would lead to error, and I cannot but think that some of Roux's earlier experiments that seem to be so contradictory may have been caused by some such changes.

Futile attempts were made to remove as much as half the yolk and protoplasm from the fertilised egg. Such eggs collapsed completely. Equally unsuccessful were attempts to add the yolk removed, by means of a hypodermic syringe, to another egg.

At the two-celled stage, just as the four-celled stage was beginning, attempts were made to suck out with a syringe all of the protoplasm from one hemisphere, in order to determine

whether the remaining hemisphere would develop a perfect half-sized embryo or half an embryo. Many eggs went to pieces, both during and subsequent to the operation. Others partially rounded up and continued to develop, but the greater number of these died later. The few embryos that formed the medullary folds were very imperfect, but, as each egg stuck had not been carefully followed during the stages of segmentation and gastrulation, I hold these results to be valueless. They show, however, I believe, the possibility of carrying out the experiment successfully.

In several eggs at the eight-celled stage one of the black cells was killed by pricking, so that its contents ran out. Such eggs developed, and in the blastular stage defects were found in the black hemisphere. In other eggs one of the white cells was stuck, and, later, defects were found either in the white hemisphere or just within the edge of the dark area. In these cases no record was kept of the position of the particular cell removed; hence the results are of little or no value, and I think the same statement will apply to the similar experiments of Roux. Now that it seems to be possible to recognise, even in the eight-celled stage, the relationship between particular blastomeres and definite portions of the later embryo, more successful results ought to be obtained.

The consistency of the yolk in the eggs of the two species is different. That of R. temporaria is more fluid, and the egg collapsed more easily than in the other case. Owing to this difference the eggs of the unknown species were far more favorable for experiment, and the following results were made on these eggs.

In order to determine the extent of overgrowth of the lower pole by the blastopore a large number of experiments were made by slightly sticking the white cells below the blastopore. By using a very fine and sharp needle an exceedingly small injury could be made, so that only a few small cells protruded from the surface of the egg. These, however, gave a definite landmark for orientation. The determination of the extent of overgrowth by injury to the lower cells has a great advan-

tage, it seems to me, as compared with the more common method of injuring the upper or black cells.

Owing to the great thickness of the lower wall of yolk-bearing cells there is no chance of breaking into the segmentation cavity or archenteron. As the white cells seem to be the more passive cells during development, injury to them has less serious consequences for the developing embryo.

The eggs were stuck at the time when the blastopore first appeared, and a sketch made in each case to indicate the distance of the point of injury from the blastopore. A series of these eggs were prepared in which the injuries were at varying distances from the dorsal lip of the blastopore that had just appeared. A series of figures were drawn from time to time to show the relations between blastopore and point of injury. Moreover duplicates of each lot were followed. Inasmuch as all the experiments gave similar results, I think any doubt as to abnormality caused by the operation is removed.

If the egg (embryo) be turned with its white area uppermost at the time when the blastopore first forms, so that the blastopore just appears above the horizon, it will be found that the white area does not cover quite a hemisphere of the egg. A border of dark pigment appears around the periphery of the white, as shown in outline by Pl. 25, fig. 9. The primary pole of this white area (hemisphere) lies not quite in the centre of the white, but nearer to the side where the blastopore has appeared, as shown in figs. 1, 3, and 4. The "centre" of the white area does not, therefore, correspond with the "lower pole."

The experiments here recorded were made on Rana, sp.?

The × shows the point where the egg was stuck.

Experiment I (figs. 10—12).—Egg in which the blastopore had just appeared. Pricked at 4 p.m. on opposite side of white area, i. e. nearly a hemisphere away from blastopore. At 8 p.m. the blastopore has become more arched, and the distance between the point injured and the dorsal lip of the blastopore is much less than at first. The dotted line running out from the ends of the blastopore marks the rather sharp

line of separation of the black and white, and also indicates the subsequent line of invagination of the remainder of the blastopore. It will now be seen (fig. 11) that the point of injury lies just outside of the pigmented line. At 12 midnight the blastopore had grown much smaller (fig. 12), and the point of injury was outside of the blastoporic rim. The point of injury is now at less than half its former distance from the dorsal lip of the blastopore.

Experiment II (figs. 13, 14).—Egg at blastula stage, had been kept overnight on ice to retard rate of development. At 9 a.m. the blastopore had appeared. Egg stuck in white at a point not quite so far from the blastopore as in the last case. At 4.30 p.m. the outlines of the whole blastopore to be seen, but the point of injury, as before, is still outside of the blastoporic rim, and is nearer to the dorsal lip of the blastopore than at first.

Experiment III (figs. 15, 16).—In this egg the blastopore appeared at first as a vertical pigmented line (fig. 15), which soon extended laterally into the usual crescent. The point of injury was nearly the diameter of the egg from the blastopore. At 4.30 p.m. (fig. 16) the crescentic blastopore was much nearer to the point of injury. Later stages not followed.

Experiment IV (figs. 17—19).—Stuck at 8 a.m. at far edge of white, fig. 17. Blastoporic crescent already formed. At 4.30 p.m., fig. 18, dorsal lip of blastopore much nearer to defect. At 8 p.m. circular outline of blastopore present, and defect lies just within the edge of the blastopore.

Experiment V (figs. 20, 21).—Stuck at 4 p.m. quite near to the blastopore. Blastopore had already formed a crescent. At 8 p.m. the dorsal lip of blastopore had nearly reached the defect.

Experiment VI (figs. 22, 23).—In this experiment the lower pole was not stuck until the circular outline of the blastopore was formed (fig. 22). The dark line of the crescent marks the dorsal lip of blastopore. The dotted line marks

the boundary line, between black and white, ready to invaginate. The injury was made nearly in the centre of the blastoporic plug, somewhat nearer to the dorsal lip. In a later stage, when the yolk-plug is smaller, the defect still lies near the centre of the yolk-plug (fig. 23). It seems relatively a little nearer to the dorsal lip than at first. The blastopore, therefore, must close in after its circular outline is formed at a nearly equal rate from all points. In this case the injury was so small that the overgrowth of the blastopore could not have been in the least retarded.

The experiments recorded above are taken from a series of twenty-one recorded cases, and will serve as types for the rest. All the results point unmistakably to the conclusion that there is an overgrowth of the lower white cells by the lips of the blastopore. Moreover the experiments show the extent of overgrowth of the blastopore and the relative amount of overgrowth of the dorsal and ventral lips respectively.

Examining the results more in detail, we find, if we assume the point of injury to be a fixed point, that the dorsal lip of the blastopore moves over the white to the extent illustrated in fig. 24. This figure is made from data of fig. 10, &c., keeping the point of injury in the same position. The diameter of the circle (representing the outline of the egg) equals 27 mm. The distance between the blastopore and the injury equals 24 mm. From 4 p.m. to 8 p.m. the dorsal blastoporic lip has moved through 8 mm., and is therefore now 17 mm. from the defect. At 12 p.m. the distance travelled through since 8 p.m. is 7 mm. The dorsal lip is now 10 mm. from the defect. So far the blastopore has passed through 15 mm. of the 24. As the defect lies outside of the point of closure of the blastopore by 2 mm., the blastopore now measures 8 mm. Assuming that from this time onwards the blastopore grows together at an equal rate towards its centre, the dorsal lip will pass over about 4 mm. more of the white. In this time the dorsal lip has moved through 20 mm. of the white area. The ventral lip has passed through 4 mm.

The region in front of the blastopore covered by the over-

growth (20 mm.) is less than the diameter of the circle (27 mm.). Comparing this with the length of the medullary folds when they first appear, the area overgrown is found to be somewhat less in length. If we deduct from the length of the embryo the thickness of the medullary folds at their anterior border, we find that the length of the two regions corresponds almost exactly. In other words, the connection around the anterior end of the medullary folds lies just in front of the point where the blastopore first formed, and the area overgrown by the dorsal lip equals the length of the medullary folds between the anterior connection and the blastopore.

A few corrections should be made; the measurements just given apply only to the flat surface, while the embryo lies over a spherical surface. As the measurements of the overgrowth and the measurements of the embryo are both projections into the same plane, no gross error will come into the calculation. The rate of overgrowth is not quite the same in all the observations, but approximately so. Even the extent of overgrowth is variable, and we have seen that the length of the embryo formed is also variable.

The first overgrowth of the dorsal lip of the blastopore is more rapid than the later growth ; that is, the approach to the point of injury is faster at first. After the blastopore has completed its circular outline the process of overgrowth (or withdrawal) of the yolk-plug is much slower.

I have assumed the point of injury to be the fixed point, and the approach of the blastopore to be due to the movement of the latter. We might have assumed that the overgrowth was due to a forward movement of the whole of the white area passing beneath the blastoporic lip. The end result would be the same in either case, the process different. It is not an easy question to decide, but to any one following the process in the living egg it will be clear that the change is due to the movement of the blastopore lips, and not to the white area. The condition of the cells in the white area points to a relative stability and inertness, while the reverse is true for the dorsal lip of the blastopore. The method of invagination of the

anterior, lateral, and posterior edge of the blastopore points to the same conclusion.

I believe, however, that the details of the actual process of concrescence of the blastopore has not as yet been accurately worked out. The migration of cells that takes place during the process has not been determined. Whether or not the dorsal lip rolls in as it grows over, or whether its exposed edge always carries the same cells, has not been shown. Both experimental and structural evidence must be brought to bear on the problem before its solution will be possible.

A series of ten experiments were made by sticking the embryo (when the blastopore first appeared) at the apex of the black pole. Other experiments involved sticking at the apex of the black and white in the same egg. The latter experiment ought to settle the question as to what portion was the active agent in the overgrowth. Unfortunately the experiments did not give satisfactory results, nor were the results uniform. Injury to the delicate roof of the segmentation cavity may have helped to produce poor results. Failure to find in the later stage the point injured, shifting of the extraovate if large, and the difficulty of determining the exact apex, may all have had a hand in the matter. Only two such embryos are drawn, although other as definite records were also obtained.

Experiment VII (fig. 5).—Egg when blastopore had just appeared was stuck at apex of black pole. When the medullary folds appeared the injury was found on the ventral surface of the body, as shown by the × in the figure. The defect was at about equal distances from the anterior end of the medullary folds and from the blastopore.

Experiment VIII (fig. 6).—Injured as in last. Defect appeared at point 180° from blastopore, therefore some distance in front of the anterior end of the medullary folds.

Both of these results show that the embryo does not form over the black pole, but why in these cases the defects are at such different distances from the blastopore I do not know.

Review of Literature.

There are certain statements made in the papers of Pflüger, Roux, Schultze, and Robinson and Assheton that bear directly on the results given above. Pflüger records that in one set of eggs the blastopore first appeared at 6 a.m. At 11 a.m. the blastopore was broader, with the corners turned down. The blastopore had left the equator of the egg and approached to the lower pole. At 12.30 p.m. the blastopore was semicircular, and had pushed further towards the lower pole. At 1 p.m. it was circular, and now it lay at the opposite point of the white hemisphere from which it had started.

An examination of the relation of the pigment shows that the egg as a whole has had no part in this overgrowth of the lower pole, i. e. no rotation of the whole egg has taken place.

At 2.15 p.m. the yolk-plug was smaller, and the blastopore has continued to move in the same direction. At 4.15 the blastopore is narrower still, and its diameter equals about one eighth the diameter of the egg; it has moved even further, and is in the region of the equator of the egg, but at the opposite point of the equator from which it started in the morning.

These observations point conclusively to the view that "the opening of Rusconi passes from a point on the equator lying in the meridian of the egg over to the opposite point of the equator through the lower white hemisphere, and the egg-axis during the period has not changed its position."

The arc travelled is not quite 180°, but is certainly more than a right angle,—variable, however, in different eggs. The overgrowth is due to a process of invagination.

From 4.15 p.m. till 7.45 p.m. the egg as a whole rotates in the opposite direction along the same meridian. Due to this true rotation more than one half of the (new) upper hemisphere is covered by those cells that overgrew the blastopore, and which therefore have a lighter colour than the cells of the primary upper hemisphere. From this clearer portion in front

of the anus of Rusconi develops the anlage of the central nervous system.

Pflüger adds, "In order to avoid a misunderstanding I must say that I do not by any means believe that the whole anlage of the central nervous system is a derivative of the white hemisphere. Since the lighter substance of the white hemisphere is directly continuous with the lighter substance of the black, it is possible that the anterior portion of the medullary plate corresponding to the brain, and even to the upper portion of the neck, may form in the black hemisphere."

There are two statements only in the foregoing account from which I should dissent. In the first place it seems reasonably certain that the blastopore does not originate in the equator of the egg, but at some distance below it. In the second place Pflüger believes that the blastopore, as it encroaches on the yolk-plug, moves as a whole further along the meridian of migration. This means that after the ventral invagination of the blastoporic rim has formed, the ventral lip still moves upwards towards its nearest equatorial point. This migration of the whole blastopore is stated in the text, and is definitely shown in the series of diagrams drawn to illustrate the process of overgrowth.

I have attempted to show that the overgrowth of the dorsal lip itself is sufficient to account for the length of the medullary folds; also that the posterior lip of the blastopore, after its formation by invagination, closes by a forward growth. There is, therefore, no evidence for such a migration as Pflüger supposes, and if the circular blastopore after its formation does move further upwards it must be due to a slight rotation of the egg as a whole in this direction. But the statement that it does continue to move must be re-examined in living eggs.

It is difficult to give any adequate summary of Roux's results. In his later papers he is not always consistent with his earlier views. Schultze's damaging criticism of some of Roux's earlier conclusions Roux has not answered, although

he has ably replied to other parts of the criticism at great length. It is needless, however, to criticise Roux without repeating his experiments. This, no doubt, will come in time, and it is somewhat surprising that so little has been done by other workers along the lines laid down by Roux. We may here confine our criticism to those points that are connected with the present ground covered. We may pass over the experiments of Roux in which one of the first eight cells was killed in order to determine its position in relation to the embryo. These experiments, as Schultze says, contain direct contradictions.

In Roux's paper published in the 'Breslauer Aerztliche Zeitschrift,' No. 6, March 22nd, 1884, it is stated, "Eine weitere hierher gehörige Beobachtung machte ich an den Eiern vom Wasserfrosch (Rana esculenta, s. viridis). Bei dieser Species stellt sich die Eiaxe nicht senkrecht sondern der Art schief ein, dass bei der Ansicht von oben neben dem hier braunen oberen Pol an einer Seite noch ein mondsichelförmiger Saum des hier gelbweissen unteren Poles zum vorschein kommt. Die erste Furchungsebene steht wie bei R. fusca senkrecht, ist aber so orientirt, dass sie dieses Bild symmetrisch theilt, wies blos möglich ist, wenn sie zugleich durch den höchsten Punkt der gelben Randsichel und durch die schief stehende Eiaxe hindurch geht. Durch die schiefe Einstellung der Eiaxe zur Richtung der Schwerkraft wird hier also auch schon die Richtung der ersten Furchungsebene und mit ihr die Richtung der künftigen Medianebene des Embryo noch vor der Theilung bestimmt.

"An diesjährigen Eierstockeiern von Rana escul. sah ich trotz der noch mangeln den Entwickelungsfähigkeit schon diese Einstellung beim Schwimmen im Wasserglas eintreten. Sofern die gleiche Einstellung reifer Eier im Wasser sich nach der Befruchtung nicht ändern, würde hier also schon im unbefruchteten Eie die Lage der Medianebene und das Oral und Aboral neben dem Dorsal und Ventral bestimmt sein; womit alle Hauptrichtungen des Embryo bereits vor der Befruchtung gegeben wären."

Schultze maintains the same view for the brown frog, but Roux, in a later publication, denies this for this species.

From Roux's conclusions, published in the 'Archiv für Mikros. Anat.,' vol. xxix, 1887, the following quotation is taken:

1. The unfertilised frog's egg has determined one main axis of the median plane of the embryo. This results from the bipolar arrangement of the yolk material, and corresponds to the direction of the egg-axis passing from the black to the white pole, i. e. to a ventro-dorsal direction of the real, a cephalo-caudal direction of the virtual, embryo.

2. From the innumerably different meridional planes which can pass through the egg-axis, that one corresponds to the median plane of the embryo that lies in the direction of the copulation of the two pronuclei.

3. The plane of copulation of the pronuclei is not in any predetermined meridian, but may be determined by localised [artificial] fertilisation.

4. The side of the egg where the sperm enters forms the ventro-caudal side of the embryo; the opposite side corresponds to the dorso-cephalic.

In the body of the same paper Roux says that his experiments show conclusively that there does not exist a latent bilateral construction of the frog's egg. He further adds that in this year he "was fortunate enough for the first time to follow out with success the process of fertilisation in Rana esculenta, and to observe that in this species a peculiar typical change of position of the egg-axis takes place, i. e. the black hemisphere sinks down 20°—30° towards the side of entrance of the spermatozoon."

Hence the first line of cleavage that passes through the upper pole of the egg and the line of the entering spermatozoon would also pass through the apex of the white crescent. Logically no fault can be found with this ingenious explanation; but how explain Roux's earlier observation, that unfertilised eggs of Rana escul. also show the white crescent? Moreover the distribution of the pigment in the unfer-

tilised normal egg seems to be such as not to allow a secondary orientation described by Roux. While, therefore, we cannot deny or refute Roux's statement at present, it seems to me that this point must be carefully examined by other workers before its acceptance will be possible.

In 1888 Roux records the results of new experiments "with improved methods" to determine the relationship of the embryo to egg-axes. If the blastula were injured at the apex of the black pole the defect was found on the ventral side of the embryo. Roux says that he had previously found that if the blastula was stuck at the equator on the blastopore side the defect appeared in the middle of the medullary folds, and he had concluded that the head half of the embryo was formed in the upper half of the egg, i. e. the embryo was placed vertically. The researches of this year (1888) show that this defect was not a primary phenomenon, but that it represented a later change where a "reparation" had taken place.

Roux injured the first anlage of the dorsal lip of the blastopore, and found the defect to lie in the cross-connection at the anterior end of the medullary folds. Injury to the blastula or young gastrula at one side of the equator produced a defect in the middle of the medullary folds. Injury to the young gastrula at a point opposite the gastrula crescent produced defects in the caudal region. Injuries in the middle of the white area gave no defect in later embryo.

These experiments of Roux's are of great importance, for if true they show the method by which we must regard the blastopore to be closed. I shall return to this in the final section when speaking of the general problem of concrescence.

Roux concludes that the embryo lies over the lower hemisphere, and that the dorsal lip of the blastopore moves through 170°. His figures ('Anat. Anzeiger,' 1888) show the head end of the embryo near that point of the equator at which the embryo first appeared. The anterior connection of the medullary folds lies just above the equator upon the black hemisphere. From this region the embryo stretches over the lower pole for 170°.

In these figures I believe Roux represents the early embryo as extending over too great an extent of surface of the sphere. Moreover, it seems, as I have said, most probable that the blastopore does not start at the equator of the egg, but some distance below that circle.

Schultze's conclusion that the embryo lies over the black hemisphere may be dismissed, as it is completely contradicted by well-determined facts.

Finally, Robinson and Assheton make certain statements as to the method of closure of the blastopore that call for notice. Apparently at the outset they 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 pole of the ovum,—that is, in the floor of the segmentation cavity." Again, they say, "For during the formation of the blastopore the epiblast does not grow over the yolk-cells enclosing them by a process of embolic invagination." This statement is intended to apply rather to the extension of the epiblast over the sides of the embryo, and as such is perfectly correct. But, in addition to this process of delamination, there is a decided and extensive overgrowth, as we have seen, of the dorsal lip of the blastopore enclosing the yolk embolically. Further, 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.

They continue, "According to some former accounts, to which we have made reference above, the anus of Rusconi has been said to diminish in size by the gradual coming together of each portion of the blastoporic rim simultaneously. This we believe to be incorrect. The anus of Rusconi gradually diminishes in size by the concrescence of the ventral part of the lateral lips." In their diagrams, to show the method by which the ventral lip of the blastopore comes together, they show the right and left sides applied to one another, and subsequently fused. Later they say, "We infer . . . . that the

anus of the frog, although apparently a new perforation, is really a reopening of the temporarily closed portion of the original blastopore." Now I doubt exceedingly whether concrescence of the ventral lip of the blastopore takes place in any such way as the authors' diagram indicates. The cells from the sides may come later to the middle line, but not by a process of apposition of the latero-posterior walls of the blastopore. Rather the cells stream or migrate to the median line below the surface, while the surface grows continuously from behind forward. Hence we cannot speak of a reopening of the blastopore, as nothing was left behind to reopen; but we must speak of a perforation at the point where the blastoporic lips first began to concresce in a sense different from that used by the authors.

IV.

Roux and Hertwig have given accounts of embryos in which the blastopore had failed to close. Roux figures an embryo with a hemisphere of white exposed, and the embryo lying as a thickened zone around the border line between the black and white hemispheres. Hertwig has not figured such extensive exposures of yolk, but describes stages with varying amount of blastopore unenclosed.

I attempted to produce such embryos artificially, and after a great number of attempts that gave no favorable result found at last a method that made it possible to produce such embryos at will.

Embryos in which the blastopore had just appeared were put into the following solutions, with the results recorded:

Hydrochloric acid, $\frac{1}{20}$ per cent.	Died.
Sodium hydroxide, $\frac{1}{20}$ per cent.	Some died, in others the blastopore closed.
Corrosive sublimate, $\frac{1}{20}$ per cent.	Died.
Curari (weak solution)	Nearly normal.
Quinine, ·02 gr. to 500 c.c. H_2O	Normal.
Morphine ,, ,,	Nearly normal.
Strychnine (only partially dissolved)	Normal.
Alcohol, 10 per cent.	Developed very little.
,, 5 ,,	More than last, but died (unclosed blastopore).
,, $2\frac{1}{2}$,,	Developed partially (closed).
Sodium chloride (3 grms. to 500 c.c. H_2O = $\frac{1}{4}$ strength of sea water	Gave the desired result. Blastopore open.
,, ,, $\frac{1}{8}$,, ,,	Normal.
,, ,, $\frac{1}{2}$,, ,,	Died.

Of the solutions given only one gave the desired result, although the alcoholic solutions seemed to have a similar tendency. The series in which ·6 per cent. salt was used produced the embryos to be described below. This happened both for the frog's and the toad's eggs, and was repeated with similar results. Success depends on using exactly the right amount of salt. Too much kills; too little does not affect the embryo. To ensure success a series of trials should be made approximating to the ·6 per cent. solution.

In a second series of experiments the recent suggestion of Herbst was followed. Embryos were placed in solutions of salts of barium, calcium, sodium, and potassium. Three sets of each solution were used, one stronger, one weaker, and one the same strength as the ·6 per cent. solution.

Although in some of these solutions embryos with large blastopores were produced, no particular relation was found between the formation of abnormalities and the series of compounds used. The best results were again in the sodium chloride.

Fig. XIV shows an embryo as seen from in front. A narrow pigmented line marks the position of the suckers. Between this and the white a thick fold of ectoderm marks the anterior end of the medullary folds. The fold continues on each side

along the border line between the black and white hemispheres. These lateral medullary folds can be traced for only a short distance.

Between the anterior connection of the medullary folds and the white is found a small plate of ectodermal cells. The same embryo seen from below is shown in fig. xv. We see that the extent of white exposed corresponds to the whole of the lower white area,—in fact, to somewhat more of the lower hemisphere than would be finally enclosed by the normal blastopore; for in the normal egg the far edge of the white, where it shades off into the black, does not normally become involved in the closure of the blastopore. This was shown definitely in the experiments made by sticking the lower pole, and is corroborated by the fact, that in these abnormal embryos the far side of the large blastopore contains much more pigment than does the ventral surface of the yolk-plug of the normal embryo. Hence any statement as to the extent of the white closed over by normal embryos, based on these abnormal embryos, will give an erroneous conclusion. This, I believe, Roux has drawn.

The embryos produced in the salt solution may be examined at each stage of their development, and the exact method by which the blastopore forms be followed out. This gives a decided advantage over the haphazard finding of embryos already formed. In watching such embryos one sees that the blastopore extends from its point of origin differently in these embryos than in normal embryos. Instead of the corners of the blastopore extending downwards to produce a deep crescent or horseshoe-shaped outline, they extend laterally around the border line between black and white. Hence results, I believe, a more extensive enclosure of the lower hemisphere than under normal conditions.

In fig. xvi is drawn another embryo, differing from the last only in the greater extent of the medullary plate lying in the black hemisphere. This is due without doubt to the greater extent to which the dorsal lip of the blastopore has crept over the white. Figs. xvii and xviii are drawings of embryos where the overgrowth of the dorsal lip has been carried farther

than in the last case, so that not only the anterior connection, but also the anterior end of the medullary folds, lie on the black portion of the egg. This embryo shows conclusively that the extent of closure of the blastopore is far more than the normal, for if the embryo really had covered so much of the sphere as the whole of the white area, and as much of the black as the anterior end now occupies, it would have covered nearly two thirds of the sphere, and not one third, as in the normal embryo.

Fig. XIX is from an embryo in which the dorsal lip of the blastopore has grown over the lower pole to the extent indicated by the medullary folds. When looked at from below—i. e. with the white area turned up—we see still a large exposure of white, but the posterior extension of the blastopore is not completed, again verifying the statement made above, that the exposure of the white is in these eggs abnormally extensive.

In fig. XX is drawn the posterior end of an embryo much more advanced than the last. Quite a large exposure of yolk is present, but not nearly so much as in the other cases. Anterior to the blastoporic plug the medullary folds have met to form a closed tube. Posterior to the blastopore, as seen in the figure, a deep groove is present, and this groove is formed by the posterior medullary folds. The ventral lip of the blastopore has, therefore, grown over the white to the extent indicated by the medullary folds. Whether this forward growth of the ventral lip is unusually extensive I do not know, nor have I any records to show whether in the earlier stages so much of the white was enclosed as in the preceding cases; but, judging from the length of the embryo and from other facts, I think we may safely conclude that the area enclosed was less.

Other embryos, with still less exposure of white, need not be figured at present; Hertwig's description seems to cover such cases.

Serial sections were made through these embryos. In the embryo shown in figs. XIV, XVI, &c., sections add little knowledge to that formed from surface views. The most noticeable

structure is the large archenteron that begins at the edge of the black in the mid-dorsal line, and extends as far forwards as the level of the suckers. The medullary folds in these embryos have not as yet rolled in to form the (half) nerve-cords. Longitudinal section of the embryo drawn in fig. XIX shows that at the ventral lip of the blastopore only a very slight depression is present.

Conclusion.—His supposed concrescence of the Vertebrate embryo to take place by apposition of the sides of the germ ring, and due to this process the embryo grew posteriorly.

Balfour believed this to be untrue, and that the posterior end of the embryo grew in length by a process of intussusception in front of the last segment of the body.

Roux's experiments by sticking the border between black and white point directly to a process of concrescence of some sort. If Roux's experiments are accurate we must suppose that the cells that will later form the central nervous system are already laid down along the black-white border. These cells must come to the middle line as the blastopore gets smaller. The closing of the blastopore from before backwards would then be due not to a backward extension of all of the material of the dorsal lip over the yolk, but would take place by new tissue coming up to the middle line from the sides and placing itself with or behind the cells already present in the dorsal lip.

I should not regard this, even if it took place, as apposition in the strict sense of the word, nor is it intussusception in Balfour's sense. It would not be intussusception because new tissue is coming in continually from the sides to mingle or mix with the cells already present and multiplying in the dorsal lip of the blastopore. Nor would it be apposition in His's sense, because the lateral borders of the blastopore are not laid down side by side, since the blastopore does not close by actual apposition of its lateral rim. I shall not here attempt to formulate a theory of overgrowth, but merely to point out the apparent bearing of the evidence furnished by this experiment of Roux. It will, I think, be possible to de-

termine experimentally exactly the process that takes place in the dorsal lip of the blastopore, and then we shall be prepared to formulate more definitely a theory of overgrowth.

Bryn Mawr, Pa., U.S.A.,
May 22nd, 1893.

DESCRIPTION OF PLATES 24 & 25,

Illustrating Messrs. T. H. Morgan and Umé Tsuda's paper on "The Orientation of the Frog's Egg."

Figs. iv, v, vi, vii, viii, ix, x, xi, xii, xiii, Rana temporaria.
Figs. i, ii, iii, xiv, xv, xvi, xvii, xviii, xix, xx, Rana, sp. ?

PLATE 24.

Fig. i.—View of 32-celled stage from above.
Fig. ii.—View of same stage from dark side.
Fig. iii.—View of same stage from light side.
Fig. iv.—View of blastula (about 150 cells), dark side.
Fig. v.—View of same, light side.
Fig. vi.—Four-celled stage, lower pole; *M*—*N*, first furrow.
Fig. vii.—About 100-celled stage, lower pole.
Fig. viii.—About 100-celled stage, lower pole.
Fig. ix.—Earlier stage than last.
Fig. x.—View of lower pole of egg at end of segmentation.
Fig. xi.—Early blastopore stage. Pigment has somewhat shifted its earlier distribution, lower pole.
Fig. xii.—Early blastopore stage, lower pole.
Fig. xiii.—Early blastopore stage, side view.
Fig. xiv.—Embryo with unclosed blastopore, seen from in front.
Fig. xv.—Same from below.
Fig. xvi.—Embryo with unclosed blastopore, seen from in front.
Fig. xvii.—Embryo with unclosed blastopore, seen from in front.

FIG. XVIII.—Same as last, side view.

FIG. XIX.—Embryo with large blastopore, seen from dorsal side.

FIG. XX.—Embryo with large blastopore, seen from behind.

PLATE 25.

FIG. 1.—Diagram normal egg, lower pole.

FIG. 2.—Diagram same egg, side view.

FIG. 3.—Diagram normal egg, lower pole.

FIG. 4.—Diagram same egg, side view.

FIG. 5.—Diagram to show defect × produced by sticking apex blastula.

FIG. 6.—Diagram to show defect × produced by sticking apex blastula.

FIG. 7.—Diagram normal embryo to show length of medullary folds, side view.

FIG. 8.—Diagram same embryo to show length of medullary folds, dorsal view.

FIG. 9.—Diagram to show lower white area when blastopore appears.

FIG. 10.—Diagram egg stuck opposite edge of white from blastopore, see text.

FIG. 11.—Diagram same, later stage, see text.

FIG. 12.—Diagram same, later stage, see text.

FIG. 13.—Diagram egg stuck far from blastopore, see text.

FIG. 14.—Diagram same, later, see text.

FIG. 15.—Diagram egg stuck far from blastopore, see text.

FIG. 16.—Diagram same, later, see text.

FIG. 17.—Diagram egg stuck nearer to blastopore, see text.

FIG. 18.—Diagram same, later, see text.

FIG. 19.—Diagram same, later, see text.

FIG. 20.—Diagram egg stuck near blastopore, see text.

FIG. 21.—Diagram same, later, see text.

FIG. 22.—Diagram egg stuck centre of early blastopore, see text.

FIG. 23.—Diagram same, later.

FIG. 24.—Diagram with injury taken as a fixed point × to show relative advance of dorsal lip of blastopore.

On the Fossil Mammalia from the Stonesfield Slate.

By

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With Plate 26.

Ever since the discovery, made some eighty years ago, of Mammalian remains in the Stonesfield Slate near Oxford, these fossils have excited the interest of naturalists, and have been the subject of much discussion amongst geologists and palæontologists both in England and abroad. Nevertheless something still remains to be described in the few specimens which exist; and, while one of them has not yet been figured at all, others have been only inaccurately represented. I therefore propose to write a short history of each fossil, as far as it is known, giving figures when necessary; and to sum up the most important results which have been reached with regard to them by previous authors, together with some remarks as to the bearing of the facts ascertained by a careful study of the teeth belonging to these remains on the general question of the origin and homology of the cusps of Mammalian teeth.

Besides the fragment of the multituberculate form Stereognathus, which I shall mention later, there are only twelve undoubted fossil Mammalian remains from the Stonesfield Slate at present known; ten of these are lower jaws, two are limb bones. Six of the fossil jaws are in the Oxford Museum, one in the York Museum, one in the private collection of

Mr. Parker of Oxford;[1] the two remaining jaws and the limb bones are in the British Museum.

Through the kindness of Professor Green and of Professor Lankester, who placed the Oxford fossils in my hands for the purpose of displaying them in a museum case in a manner more worthy of their interest and value, I have had the opportunity of examining and handling our six specimens. I am much indebted to Dr. Henry Woodward for allowing me to examine the two British Museum fossils, and to Mr. Parker for lending me his. To the authorities of the Museum at York I must express my thanks for lending me the excellent specimen in their keeping; but more especially to Professor Lankester, who spared himself no trouble in obtaining for me this privilege, and who has further given me much help during my researches.

It may here be mentioned that I have been able, by carefully working away the matrix with sharp needles under Zeiss's dissecting microscope, to expose new cusps, and in some cases new teeth, in five of the jaws described. My figures, which I have endeavoured to make as faithful as possible, differ, therefore, considerably from those previously published.

The formation from which these fossils were obtained belongs to the Lower Jurassic period; whence the great interest attached to them, for at the time of their discovery they were by far the earliest known remains of warm-blooded Vertebrates, —being, in fact, the first Mesozoic Mammalia obtained. Since then, as is well known, remains of a few fossil Mammalia have been found both in England and elsewhere in strata belonging to the Triassic age; as, for instance, Microlestes in England, Dromatherium in America, and Tritylodon in South Africa.

The two fossil limb bones mentioned above have been figured and described by Professor H. G. Seeley (29). As, unfortunately, they afford no clue to the relationship of the animals whose jaws are described below, there being no proof

[1] Mr. Parker also has in his possession a toothless fragment of a jaw which may perhaps be Mammalian.

that these bones belonged to any of them, I need only mention that Seeley considers that they are "limb bones indicating a generalised insectivorous type, modified from a Monotreme stock in the direction of the Marsupial plan."

Genus Amphitherium.

Four of the fossil jaws appear to belong to this genus. Three of these, two of which are type specimens, are in the Oxford Museum; the fourth is in the British Museum.

Amphitherium Prevostii, Blainville.

Type specimen, Pl. 26, fig. 1.

A left ramus of the lower jaw, seen from the inside; in the Oxford Museum.

In 1824[1] Dr. W. Buckland, the well-known geologist, first announced the discovery of the remains of mesozoic Mammalia in his paper 'on Megalosaurus' (5). He there mentions "two portions of the jaw of the didelphys or opossum," which he refers "to this family on the authority of M. Cuvier, who has examined it." From Mr. W. J. Broderip we learn that these rare fossils were obtained as follows:—"An ancient stonemason living at Heddington . . . made his appearance in my rooms at Oxford with two specimens of the lower jaws of mammiferous animals, embedded in Stonesfield slate. . . . One of the jaws was purchased by my friend Professor Buckland, who exclaimed against my retaining both" (4). The fossil purchased by Broderip himself is the type specimen of Phascolotherium (see below); Buckland's fossil is the type specimen with which we are now concerned. He placed it in the Ashmolean Museum, whence it has come with the other specimens of the Buckland collection to its present home, the Oxford University Museum. The exact date at which it was purchased I have been unable to ascertain for certain; it was probably about 1814: the date given by Zittel is 1812 (32), but I know not on what authority.

Cuvier, who visited Oxford in 1818, says of these fossils

[1] Not 1823, as is generally stated to be the case.

that "lors d'une inspection rapide que j'en pris à Oxford, en 1818, [ils] me semblèrent de quelque Didelphe;" and adds in a note that the jaw of Amphitherium "est celle d'un petit carnassier dont les machelières resemblent beaucoup à celles des Sarigues, mais il y a dix dents en série, nombre que ne montre aucun carnassier connu" (8). This note was written after the examination of some careful drawings of Buckland's fossil and of the type specimen of Amphilestes (see below) sent to him by Prévost, who was then travelling in England.

These announcements of the discovery of Mammalian remains in stone belonging to the Mesozoic age created a great sensation amongst the palæontologists of the time, and it was not for more than twenty years afterwards that the opinion of the great French naturalist was generally accepted. Some contended that the fossil did not really belong to the slate, others that the strata in which they were found were not of the Mesozoic period; while others, again, urged that the jaws were those of a reptile, or even of a fish. All doubt having been set at rest with regard to these points, it will not be necessary to enter here in detail into the arguments used on either side.

Prévost, in 1825, on his return from England, where he had carefully examined the specimen of Amphilestes now at York, and "le fameux Didelphe" in Buckland's collection, published the first detailed description and figure of this the type specimen of Amphitherium (25). He describes the teeth as having tricuspid crowns, and two distinct roots in alveoli, concluding that the fossil was Mammalian in confirmation of Cuvier. As to its relationship, Prévost considered that it was probably "un mammifère carnassier insectivore qui pouvait offrir quelque analogie avec les Didelphes, mais qui appartiendrait à un genre inconnu."

Agassiz, in 1835, mentions the Stonesfield fossils in a short note (1). He considered that the remains were not sufficient to allow of a certain determination of their affinities, but drew attention to the resemblance of the teeth (especially those of Phascolotherium described below) to those of certain

seals possessing tricuspid molars. Owen mentions (**20**) that Agassiz proposed the name Amphigonus for Amphitherium, in the German translation of Buckland's Bridgewater Treatise (which I have not seen).

Dr. Buckland, in 1836, gave a rough figure of this jaw, together with enlarged drawings of two of the teeth (**6**).

Two years later M. de Blainville published his "Doutes sur le prétendu Didelphe de Stonesfield" (**2**), in which he tried to prove that the fossil in question, of which he reproduced Buckland's figure, belonged to a reptile. In this paper he laid considerable stress on the fact that "une portion de mâchoire inférieure, rapportée de Stonesfield par M. Brochant de Villiers et ses élèves MM. Elie de Beaumont et Dufrénoy, et qu'on avait supposée appartenir au même Didelphe," had been proved to be reptilian, and accepted as such even by Cuvier. Blainville mistook the mylohyoid groove,[1] well marked in our specimen, for a suture indicating that the jaw was of a compound structure: a similar mistake was made in the case of the other jaws. He proposed the generic name Amphitherium, which has since been generally adopted.

Buckland then took with him to Paris the type specimen of Amphitherium and the second fossil of the same species now in the Oxford Museum, which will be described below. He showed these to M. Valenciennes, who made a careful study of them, publishing a detailed account mainly confirming the results of Cuvier and Prévost, in which the name Thylacotherium Prévostii is proposed (**31**). Unfortunately Blainville was not convinced, as he did not see the fossils, for he tells us that "le jour où M. le docteur Roberton voulut bien m'inviter à passer la soirée chez lui avec M. Buckland, je partais pour la campagne" (**3**). He therefore only brought forward "Nouveaux doutes" (**3**), in which we learn that "M. Buckland lui-même a exposé le problème et les pièces sur lesquelles il repose à l'investigation des naturalistes allemands reunis en congrès à Fribourg, en Brisgaw, au mois de septembre dernier" (1838). In his contention as to the saurian

[1] For a full discussion of the mylohyoid groove see Osborn (**14**).

character of these remains, Blainville was opposed in Paris by Duméril (9) and Geoffroy Saint-Hilaire (27), whilst in England he was supported by Professor Grant (11) and by Ogilby. The latter took up a more impartial position, and considered that they were not justified by the evidence in pronouncing whether the fossils were mammalian or reptilian, arguing in favour of saurian affinities that the molars and premolars could not be distinguished, that the canine and incisors occupy five twelfths of the dental line, that the incisors (in the type Phascolotherium) are nearly in the same straight line as the grinders, and that the condyle is below the level of the crown of the teeth (13).

On returning to England, Buckland entrusted to Owen these "bones of contention," as the latter calls them, for the purpose of making an exhaustive study to ascertain their true nature. In 1838 Owen read two papers on this subject before the Geological Society (18), and in 1842 his full treatise was published with carefully executed figures (19). Owen drew attention to such Mammalian characters as the convex articular condyle, the broad, high, and curved coronoid process, situated immediately in front of the condyle and resembling that of the opossum, and the separate angle inflected as in Marsupials and some Insectivora. He then described the teeth in detail, comparing the six molars of Amphitherium to those of Didelphys, and the four premolars to those of Didelphys and Talpa. For the first time Owen definitely pointed out that the molars of these animals closely resemble each other, belonging to the type of tooth now known as the tritubercular-sectorial. "An interesting result of this examination is the observation that the five cusps of the tuberculate molares [of Amphitherium] are not arranged, as had been supposed, in the same line, but in two pairs placed transversely to the axis of the jaw, with the fifth cusp anterior, exactly as in Didelphys, and totally different from the structure of the molares in any of the Phocæ, to which these very small Mammalia have been compared" (18). It is surprising, after this, to see Professor Osborn, fifty years later, claiming the "discovery" of tritubercular molars in Amphitherium (15).

In subsequent works Owen again describes and figures this jaw (**20, 21, 23**); a "diagram" of it is given by Professor Phillips in his 'Geology of Oxford' (**24**).

In 1888 Professor H. F. Osborn published his interesting memoir on the 'Mesozoic Mammalia of the Old and New Worlds,' in which he treats of the Amphitherium jaws (**14**). Unfortunately the description of the molars is quite erroneous, owing to the author's considering that the cusps were all in the same line. "The fact is," says Osborn, "these crowns of the molars consist of an elevated anterior and median cusps, followed by a low posterior heel, and with an internal cingulum rising into the low cusp on the inner face of the median cusp." At this time Osborn had only seen drawings and published figures of the Oxford jaws, but shortly after, during a second visit to England, he examined them himself, and corrected his mistake in a subsequent publication (**15**), finding the external cusp described half a century before by Owen.

I have nothing to add to these descriptions, excepting the fact that Owen was wrong in considering the median projections in molars 4 and 5 as being the external cusp (which he found in molars 2 and 6); they are simply the surface where the internal cusp has been broken off, and I have exposed in these two teeth the external cusp itself, which was previously hidden (see figure).

The second specimen in the Oxford Museum (Pl. 26, fig. 2) is a left ramus, seen from the inside.

Valenciennes was, I believe, the first to mention this fossil (**31**), which was one of the two brought over to Paris by Buckland in 1838. The French author, however, erroneously considered it was "de la même espèce que celle décrite et figurée par M. Broderip, son Didelphys Bucklandi" (Phascolotherium).

Owen figured and described it correctly (**19—21, 23**) as a left ramus containing one molar behind, separated (by a gap wide enough to allow for four molars) from two entire and two

broken premolars.[1] In front of these Owen detected the sockets for six teeth.

Osborn has lately (15) discovered a young molar emerging from the jaw just behind and within the posterior molar.

The British Museum specimen (Pl. 26, fig. 4) is a portion of the right ramus, inner view.

This fossil has been partially figured by Osborn in his first treatise (14), who took it to belong to a left ramus; but in his later paper (15) he recognised its true character by the presence of the "double internal cusps [of the molars], by the cingulum upon the premolar, and by the faint mylohyoid groove near the lower border," adding in a note that "it would be well to run the risk of injuring one of these molars to expose the external cone."

The jaw, which is figured here complete and in detail for the first time, although much broken is really the most instructive of the four specimens extant of this genus, as regards the structure of the molars. In front are the traces of four teeth, followed by an entire premolar. The latter possesses a laterally compressed crown bearing one large cusp, a very small anterior cingulum cusp, and a posterior heel. On the whole this tooth is very similar to the premolars of the other Amphitherium jaws. The molars, of which there are five remaining, when I first examined them displayed only the two nearly equal and pointed cusps of the inner margin, and a simple low posterior heel (as shown in the figure by the third and fourth molars); by carefully clearing off the matrix I have exposed the large external cusp (protocone) in the first, second, and fifth molars (see woodcuts, figs. 1 and 2). In the latter tooth I have also exposed the full extent of the posterior heel, which is seen to rise on the external margin into a pronounced cusp; the internal cusp is hardly distinguishable from the general margin of the heel at its postero-internal angle. The heel is somewhat narrower than the body of the tooth at the

[1] The posterior premolar bears a small internal cusp (= the deuterocone of Scott ?).

level of the more anterior external cone, and a "basin," if it can be said to exist at all, is small and shallow as far as I have been able to ascertain.[1] It should be noticed that this

Fig. 1

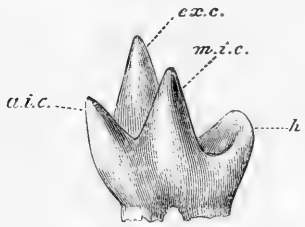

Fig. 2.

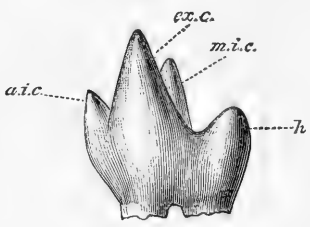

Fig. 1.—Inner face of a molar of the right ramus of the lower jaw of Amphitherium Prevostii.

Fig. 2.—Hypothetical representation of the outer face of the corresponding molar of the left ramus. *a. i. c.* Anterior internal cusp (paracone). *ex. c.* External cusp (protocone). *h.* Heel. *m. i. c.* Median internal cusp (metacone).

The teeth are represented as if entirely freed from the matrix.

is the first complete tooth of the tritubercular-sectorial type which has been seen in the Stonesfield fossils.

Amphitherium Oweni, Osborn.

The type specimen in the Oxford Museum; a right ramus with the external surface exposed (Pl. 26, fig. 3).

The type and only specimen of this species, obtained with the other jaws from Dr. Buckland's collection, was found probably about 1845. It was first described and figured by Owen in his 'History of British Fossil Mammalia' in 1846 (**20**), and subsequently in his Monograph (**23**) and Odontography (**21**) under the name of A. Prevostii. This specimen, perhaps the most perfect of all the Stonesfield jaws, shows very clearly the angle which, according to Owen, exhibits a degree of inflection which is "less than in any of the known Marsupialia, and does not exceed that of the Mole or Hedgehog" (**20**). He describes the six posterior molars as quinquecuspidate, but

[1] Judging from other jaws, we may presume that the heel is, if anything, better developed in the anterior molars.

these teeth are (at all events at the present time) so mutilated that it is scarcely possible even to recognise their tritubercular character. The outer cusps have been entirely broken away, and only traces of the two inner cusps and the posterior heel remain.

An outline figure of this fossil has been given by Phillips (**24**). Professor Osborn in his first paper (**14**) separated this specimen from the genus Amphitherium with the name of Amphitylus Oweni, under the impression that the molars were tricuspidate.[1] There can now be no doubt that this view is erroneous, and that the teeth are really, as Owen described them, of the Amphitherium pattern. Zittel (**32**) follows the latter in including it in the species A. Prevostii; but I have retained it as a separate species, chiefly on account of some considerable differences exhibited between the shape of the jaw of this fossil and that of the specimens described above. The coronoid process is straight above and more pointed at its posterior extremity; the condyle is more slender, the notch between it and the coronoid process being more pronounced; the angle is rather larger, and produced farther back. The premolars, as exemplified by the third and only entire one in this jaw, differ somewhat from those of A. Prevostii; the cusps are more rounded, the main cusp is situated not near the centre of the tooth, but well forward, and the swelling on the fangs, also rather large in the molars, is strongly developed. The molars are unfortunately too broken to compare in detail with those of the previous species.

The question of the lower dental formula of this genus has been purposely left until after the description of all the specimens. Owen, who evidently saw the second Oxford Amphitherium (Pl. 26, fig. 2) in a more perfect condition than that in which it now is, gave the formula i. 3, c. 1, pm. 6, m. 6 (**19**), describing eight sockets in front of the anterior broken premolar. There can be little doubt, however, that he overestimated the number of teeth anterior to the four premolars. In front of these can still be seen the broken roots of what was

[1] Osborn has since changed his mind, and now, I believe, includes this specimen in the genus Amphitherium.

probably a small premolar, and the next two sockets are most likely those of a double-fanged canine, such as we find in many other mesozoic Mammalia. Four incisors account for the anterior sockets. On the other hand, the type specimen (Pl. 26, fig. 1) has six molars, and four molars beyond which the jaw is broken; so that on combining the two we get the formula i. 4, c. 1, pm. 5, m. 6.[1] The dentition of A. Oweni conforms perfectly to this; we find four incisors, a double-fanged canine, followed by eleven teeth, of which five were probably premolars and six molars. This is also the conclusion reached by Osborn (**15**) for the dental formula of Amphitherium.

Genus Phascolotherium.

There are three jaws belonging to this genus—the type specimen in the British Museum, a specimen in the Oxford Museum, and one in Mr. Parker's collection. They are all placed in one species.

Phascolotherium Bucklandi, Broderip.

The type specimen, a right ramus with the inner surface exposed, in the British Museum.

This is the fossil mentioned above as having been obtained by Mr. Broderip, together with the type specimen of Amphitherium, about 1814. It must, therefore, be the other of the "two portions of the jaw of the Didelphys" which Buckland tells us were seen by Cuvier (**5**). This jaw was lost for a time, but, on being found again, was described and figured by Broderip in the 'Zoological Journal' of 1828 (**4**). He there described the teeth as consisting of seven grinders, one canine, three incisors, and the alveolus of a fourth, naming the jaw Didelphys Bucklandi.

It has been figured by Buckland (**6**), by Blainville (**2**), by Owen (**19—21, 23**), and lastly by Osborn (**14**).

[1] The second Oxford specimen is the only jaw which shows definite signs of having had five premolars; A. Oweni affords no certain evidence in this respect.

In his writings on this jaw Owen describes the rounded and sweeping outline of the lower margin, the wide recurved coronoid process resembling that of the "zoophagous Marsupials," the notch between the coronoid process and the condyle being especially like that in Thylacinus. Other resemblances he also finds to Thylacinus, namely, in the inflected angle and the molars, whilst the condyle is said to be more like that of Dasyurus and Didelphys. A well-marked mylohyoid groove is present, and the dental foramen is far forward, as in other related Mesozoic mammals. Owen claims that the "molars" (grinders or cheek-teeth) of Phascolotherium resemble those of Thylacinus in number (seven in both cases) and in shape, both possessing three main cusps in a line and two accessory fore-and-aft cingulum cusps, very small in the case of the living Marsupial. He could distinguish no difference between premolars and molars, and looked upon the grinding teeth as being in a simple condition in which the two varieties were not differentiated.

All observers have noticed the peculiar pitting of the surface of the grinders of Phascolotherium.[1] Osborn, in his first paper, also held the view that the seven grinders could not be distinguished into premolars and molars: "A close study of m. 1 shows that it possesses in miniature the characteristic features of the other molars, three cusps and a basal cingulum" (14). In the 'Additional Observations' (15), after examining all the specimens of this species, he writes, "The *first* tooth behind the canine has a main cusp like that of the posterior molars, and an internal cingulum horizontal and rising in two points, instead of showing the sweep downwards and backwards which is so characteristic of premolar cingula. The accessory cusps are either covered with matrix or broken off. . . . . The chief interest lies in the main cusp [of the second tooth], which is loftier and more pointed than the protocone

[1] It is interesting to notice that the teeth of Phoca barbata, which resemble them so closely in shape (as pointed out by Agassiz, 1), also exhibit a pitting of the surface, which might lead one to believe that they are both adapted to some similar kind of food.

of the *third* tooth, which in turn has all the characteristics of a molar." Osborn concludes that the formula may be provisionally written i. 4, c. 1, pm. 2, m. 5. In assigning this formula to Phascolotherium I entirely agree with Professor Osborn, and can only say that the difference between the first two grinders and the succeeding molars is perhaps even more marked than he has described.

The Oxford Museum specimen; a left ramus, seen from the inside (Pl. 26, fig. 8).

This specimen has been figured in outline by Phillips (**24**) and mentioned by Osborn (**15**). Mr. Lyddeker, in the British Museum catalogue (**12**), erroneously referred it to the genus Amphilestes.

Behind are four molars in very good preservation, showing the marked cingulum rising in the two internal points characteristic of the genus; in front and behind the cingulum forms small, sharp cusps. I have lately exposed from the matrix an entire incisor, and as far as possible the sockets of the other teeth which are missing. This jaw is, therefore, now of some use in making out the dental formula. Posteriorly we have the four molars already mentioned, and immediately in front of these are two sockets, presumably belonging to the first molar. Then come two pairs of sockets, of which the anterior is much the smallest, in which were the two premolars. In front of these, again, is the large alveolus of the canine, preceded by two sockets for two incisors. Next comes the incisor recently brought to light, which in shape is stouter at the base, more closely resembling the incisors of Thylacinus than seem to do those of the type specimen. Beyond this tooth the jaw is slightly damaged; the first incisor has probably been broken away. The dental formula of this jaw would then agree with that given by Osborn for the type specimen, and adopted here.

Mr. Parker's specimen; a portion of the right ramus with the inner surface exposed (Pl. 26, fig. 9).

This, the third and last specimen of Phascolotherium, is the

hinder portion only of the ramus, with three molars in situ. It has been figured in outline by Phillips (24), and mentioned by Osborn (15). As the latter notes, it is remarkable for the great development of the coronoid process. The articular condyle is slender and very well preserved; and the broken angle, which I have recently cleared from the matrix, can be clearly discerned. From the fractured surface we may conclude that the angle was very much inflected, and exceedingly thin and flat near its point of attachment; unlike the same process in living Marsupials, it was situated entirely behind the dental foramen. In front of the teeth are the impressions in the matrix of the two anterior molars.

Genus Amphilestes.

Of this genus there are three specimens, included in one species, two of which are in the Oxford Museum, and one in the museum of the Philosophical Institution at York.

Amphilestes Broderipii, Owen.

The type specimen in the museum at York; a left ramus with the inner surface exposed (Pl. 26, fig. 5).

Valenciennes was the first to mention this fossil in 1838. He says, "Une autre mâchoire, que je crois être de cette dernière espèce [Phascolotherium Bucklandi], fait partie du cabinet de M. Sykes" (31). The Rev. H. Sykes presented it to the museum in which it now rests. Valenciennes, "d'après le dessin qui a été envoyé par M. Phillips à M. Cuvier," mistook it for a right ramus seen from the outside. Owen, in the same year, described it briefly under the name of Amphitherium Broderipii,[1] giving an elaborate but in some respects erroneous and misleading figure (19), which has since been frequently copied in his own and other writers' works (20—23). In this, and in all his subsequent figures, Owen represents the angle of the jaw as separate and hardly inflected (as in Amphitherium), and the coronoid pro-

[1] Owen remarks that it appears to be closely allied to Phascolotherium.

cess as angular and small. Phillips (**24**) and Osborn (**14**) followed him in this; but in his second paper Osborn recognised the fact that the angle is "precisely as in Phascolotherium" (**15**),—that is to say, it is inflected and confluent with the condyle.

Owen described the molars with their three high-pointed cusps in a line (much more pointed than those of Phascolotherium), their well-developed internal cingulum rising in two fore-and-aft cusps, and one internal median cingulum cusp. The premolars have no cingulum, and possess one main median cusp with a small cusp in front and behind. There are five molars in situ behind, separated by a gap for one tooth from three premolars; in front of these all the teeth are missing except one incisor, which I have exposed from the matrix.

The jaw is much damaged posteriorly, but the impression of the condyle and coronoid process is still visible in the matrix. The process is seen to resemble in shape that of Phascolotherium, although it is relatively smaller.

The first specimen in the Oxford Museum; a left ramus with its outer surface exposed, reversed (Pl. 26, fig. 6).

Professor Phillips first figured this jaw in outline in his 'Geology of Oxford' (**24**), probably soon after its discovery. By mistake he put one more molar in the figure than the number exposed in the fossil.[1] Osborn also mentions this specimen (**15**).

The coronoid process, condyle, and angle are unfortunately broken. I have exposed an entire fifth molar behind the four molars previously visible, the broken base of an anterior incisor, and have rendered what remains of the other incisors more distinct. The molars have no cingulum on the outer surface, but the sharp lateral cingulum cusps are well seen, especially the hinder one.

The second specimen in the Oxford Museum; a portion of the right ramus seen from the outside (Pl. 26, fig. 7).

[1] Apparently this eminent and keen-sighted geologist "could see through a stone wall" better than most people, for the fifth molar which he thus figured has now been brought to light, twenty-two years after (see figure here given).

This fossil has never been figured before; it was probably obtained about 1875. The coronoid process and the condyle have been broken; there are eight consecutive teeth present—five molars and three premolars. These teeth resemble in every particular those of the foregoing specimen.

The dental formula of this genus has hitherto been very difficult to settle, but the working out of the new teeth and of the sockets of the missing teeth has rendered the task easier.

Owen, who only studied the York specimen, assigned to it the formula i. 3, c. 1, pm. 6, m. 6 (23). He considered that the tooth missing between the molars and premolars had been a molar. In front he made out sockets for the three additional premolars, the canine, and the incisors. However, these so-called sockets were mere undulations of the edge of the matrix, not corresponding to the true sockets which I have now exposed. Mr. Lyddeker (12) gives the probable formula as i. 4, c. 1, pm. 4, m. 7, which was adopted by Osborn in his first paper (14). After personally examining the three specimens Osborn gives the formula "with considerable certainty as follows:—i. ? 3, c. 1, pm. 4, m. 6" (15). He tells us that "the Oxford specimens show that there were but *six* molars instead of seven. . . . . In fact, one . . . . specimen . . . . shows but five molars. If this specimen be adult, as seems improbable, it may represent a new genus transitional between Amphilestes with six molars and Triconodon with four" (15). I must confess that I am quite unable to see how one specimen with five molars and another with four (at that time) can even without "certainty" lead to the adoption of the formula m. 6. The ingenious speculation as to the intermediate genus seems to be quite unnecessary when we now know that all the existing specimens possess five molars, and show no signs of having had more or fewer. The premolars are more difficult to deal with. If we measure the distance between the last molar[1] and the first premolar in the first and second Oxford specimens we find that the eight consecutive teeth occupy exactly the same space

[1] In these measurements I have found it convenient to measure from the middle of one tooth to the middle of another.

(10 mm.) in both cases. Measuring now the space occupied by the eight posterior teeth in the York specimen (including the gap as one tooth), we find that it is the same as in the others. There can, therefore, be little doubt that the missing tooth was a premolar, the fourth. Returning to the first Oxford specimen—in front of the three premolars is a space with two sockets, which were evidently occupied by the first premolar, still present in the York jaw. In front of the first premolar we have a region which must be carefully compared in both fossils. As this region in the York specimen is broad and flat, and cannot well be seen from the side, I have given a figure of it more from above (Pl. 26, fig. 5 *a*). The new and only incisor present appears to be the fourth, and three sockets are visible in front of it. Between the incisor and the first premolar are two large sockets, somewhat difficult to account for. If the first of these represents a missing canine, and the second a small premolar, we would have one more post-canine tooth in this jaw than in the Oxford specimen (see above). On the other hand, if they both belong to a large double-fanged canine,[1] we would then apparently have only three incisors in the Oxford jaw, assuming that here also the canine occupied two sockets.[2] On examining the latter specimen closely it is seen that the anterior extremity of the jaw is broken; moreover the distance from the extremity to the second premolar in the York Amphilestes is 2 mm. longer than in the Oxford specimen. There is, then, no great difficulty in supposing that in the latter the first incisor has been broken away.

Provisionally the formula of Amphilestes Broderipii may therefore be written i. 4, c. 1, pm. 4, m. 5.

As for the systematic position of these Stonesfield fossils, the remains are too scanty to allow us to form any very definite opinion. Owen considered Phascolotherium to be

[1] Double-fanged canines are not uncommon amongst Mesozoic Mammalia.

[2] It is to be noticed that in both the jaws these sockets touch one another, whilst there is a small space on either side of them. Should the York fossil prove to have possessed ten teeth behind a single-fanged canine, it would have to be separated from the Oxford specimens.

marsupial and nearly related to Thylacinus; Amphitherium, on the other hand, he places nearer the Insectivora, and in one work (**20**) puts it actually in that group. Lydekker classes all these Stonesfield Mammalia in one family, the Amphitheriidæ (including Amblotherium, Achyrodon, and Peramus); but there can be no doubt that Amphitherium should be widely separated from the triconodont group (Phascolotherium and Amphilestes). Osborn includes the latter with Triconodon and its allies in one group, the Triconodonta; Amphitherium he places with the living Polyprotodont Marsupials in the group Trituberculata, which also includes Amblotherium and its allies. Zittel adopts this arrangement, which certainly seems to be the best and safest yet proposed.

Stereognathus ooliticus, Charlesworth.

The only specimen of this interesting Multituberculate form has been purchased for the Museum of Practical Geology (London), where it now rests. This fossil, a fragment of a jaw containing three molars, came from the collection of the Rev. J. Dennis, of Bury, and was originally described by Charlesworth in 1854 (**6***a*) as a piece of the lower jaw with molars having "six similar cusps arranged in two rows" (transverse). Three years later Owen described and figured Stereognathus in detail (**21***a*). The teeth, with their three longitudinal rows of two cusps, he considers somewhat resembled those of certain Ungulata, concluding that they belonged to a "diminutive form of the great Ungulate order of Mammalia." Marsh (**12***a*) suggested that the fossil belongs to the upper jaw, as no Multituberculate teeth of the lower series are known to possess more than two longitudinal rows of cusps. The specimen is, however, too fragmentary to enable us to come to any definite opinion on this point; and its exact systematic position, therefore, must remain somewhat uncertain. It is generally classified in the family Plagiaulacidæ (**14**, **32**).[1]

[1 I take the opportunity of Mr. Goodrich's publication to record once again the existence of another Stonesfield jaw, which I obtained from a

On the Primitive Mammalian Molar.

Before closing this paper I should like to make a few remarks with regard to the "Tritubercular theory," which has been so zealously put forward by several eminent American palæontologists, and which has been so generally accepted in Europe. There are two important questions involved in the discussion: firstly as to the character of the primitive mammalian molar; secondly as to the origin and homology of the particular cusps. As before, only the teeth of the lower jaw will here be dealt with. Professor Osborn, in his illustrations of the theory of the origin of the tritubercular molar, has made large use of the Mesozoic mammals found in England; one can therefore stand on firm ground while criticising his conclusions and his interpretations of the facts.

school-fellow thirty-two years ago. I took the specimen to Professor Huxley at Jermyn Street, who cleared it from matrix and came to the conclusion that it was a second example of Stereognathus. The jaw was a fragment, but there were four molars present instead of three, the number in the type specimen. My first appearance in the field of scientific literature was as the author of a letter to the 'Geologist,' vol. iv, 1861, recording the discovery of this jaw. Much to my annoyance at the time my signature appeared as that of a Mr. E. Ray, residing at the town of Lanbeater. Whether such a town exists, or whether the name was a pure invention on the part of the printer's devil, I have never ascertained.

Further disappointment fell on me in connection with this jaw. Before Huxley had had time to figure or describe it, I took it home for a few days in order myself to make a lithograph of it. The anguish and despair of a schoolboy on finding that he had broken to powder the treasure the detection of which had been the pride of his life may be imagined. The crowns of the molars, so carefully cleared from the matrix by Huxley, were rubbed from the specimen—wrapped though it was in cotton wool—in my pocket. I carried back the now mutilated and comparatively worthless specimen to Jermyn Street, and speechless placed it in Huxley's hands, who was only a little less grieved than I was. It was put aside in some cabinet in the "den" then tenanted by the Naturalist to the Survey, and has never been seen since, though searched for some twelve years later. There is a possibility that in the course of time it may turn up either at Jermyn Street or at the College of Science, South Kensington, whither my revered friend and master—for so he became from the day when he took my Stonesfield jaw in hand—migrated in 1870.—E. Ray Lankester.]

It is well known that Osborn (**16, 17**), Cope (**7**), and other advocates of the theory assume that the primitive mammalian molar was represented by a simple "reptilian cone," the "protoconid," forming the Haplodont type of tooth. The protoconid subsequently acquired a cusp in front and behind ("paraconid" and "metaconid" respectively), giving the Triconodont type. This tooth with three cusps in a line would then have become converted into the Tritubercular type of molar by the movement outwards of the median large protoconid, and inwards of the anterior paraconid and posterior metaconid. A small posterior "heel" then became developed, which subsequently formed an external cone, the "hypoconid," and an internal cone, the "entoconid," yielding the Tritubercular sectorial type of molar seen in most orders of Mammalia.

Let us now examine the facts. If the primitive mammalian molars were simple cones, we should expect to find a gradual approximation to this condition on comparing the more specialised living mammals with the more primitive, the later fossils with the earlier. But, as a matter of fact, we find on the contrary that these simple molars are only seen in such highly modified forms as the Cetacea, and that no early mammalian fossils whatever have been yet discovered possessing them, whilst multituberculate forms increase in number the lower we search. Dromatherium and Microconodon, animals having teeth with one large and several accessory cusps, presumed to be intermediate between the Haplodont and the Triconodont types, are possibly not mammals at all, but reptiles; at all events, they differ so widely in structure from any known mammal, living or extinct, that they can afford little certain information on this question. The Reptilia themselves cannot be said to support the theory, for the most mammalian of all known reptiles, Galesaurus planiceps, has molars with three distinct cusps. On the other hand, the most reptilian of all living mammals, Ornithorhynchus, has multituberculate teeth. Dealing next with the Triconodont type of molar, we find here again that it does not occur amongst primitive forms, but in

the highly developed Carnivora, and amongst the more specialised Phocidæ. These animals possess molars of a perfect Triconodont pattern of obviously secondary origin.

Another point on which Professor Osborn lays great stress is the origin of the Tritubercular from the Triconodont type by the assumed movement outwards of the protoconid (the median cusp), the paraconid and metaconid occupying the inner angles of the "primitive triangle" so formed. Phascolotherium, Spalacotherium, and Amphitherium represent, according to that author, three stages in the process. However, I can see no traces of the beginning of such a movement in the first species. Spalacotherium has simple tritubercular molars in which the heel is not developed,[1] somewhat similar (as observed by Mr. Lydekker, **12**) to those of the golden mole, Chrysochloris inaurata, and leading perhaps to the Stylodon type. As for Amphitherium, the molar shows no trace whatever of being more primitive with regard to this supposed movement of the protoconid; the heel, although not very large, is quite normally developed,[2] like that of many other Mesozoic, Tertiary, or recent forms.

The examination of existing forms only confirms these results: cusps may disappear, and cusps may arise, but the relative position of the protoconid and the two inner cusps is always essentially the same. Embryology also shows that in the development of molars the cusps arise in those positions which they will occupy in the adult tooth (Topinard, **30**; Röse, **26**).

Mr. W. B. Scott, in a quite recent paper (**28**), has clearly shown that in the premolars the protoconid remains in place while another cusp, the "deuterocone," is formed on its inner

[1] There is no evidence that the teeth of Spalacotherium are of a primitive intermediate type, even should they prove to have been evolved as suggested by Osborn. The angle of the jaw of this genus has always been described as inflected and confluent with the condyle; one specimen in the British Museum, No. 47,799, shows that it was really separate and little inflected, if at all.

[2] The small cusp figured by Osborn in the molar of Amphitherium in front of the paraconid (**17**) does not exist in any of the specimens.

posterior surface. So averse, however, is he to damaging Osborn's theory, that he is actually driven to the conclusion that similar cusps in precisely the same situations in the premolars and the molars are not homologous, but of entirely different origin![1]

Another argument—perhaps the strongest of all—against the assumption that the Triconodont tooth represents a stage in the evolution of the Tritubercular sectorial type is afforded by the consideration of the occurrence of the latter in the various orders of Mammalia, and the probable phylogeny of the Ditrematous mammals. That the Tritubercular sectorial type was that of the lower molars of the ancestors of all the placental mammals can scarcely be doubted in the face of the mass of evidence collected by Cope, Osborn, Schlosser, and others. All the various forms of molars met with amongst the different orders, as adaptations to special food or methods of feeding, can be referred back to this primitive type.[2] Now the Tritubercular sectorial molar occurs also amongst the Marsupials; we must, therefore, conclude that the common ancestor of both Placentals and Marsupials possessed teeth of this type; for we cannot assume without evidence that this complicated yet definite pattern arose independently in the two cases: the resemblance between the arrangement of the cusps in these teeth in the two groups is not vague and general, but definite and detailed.

We have, on the other hand, conclusive evidence that the Triconodont type of tooth has independently arisen in at least two widely separated groups, namely, the Phocidæ and Carnivora, and the carnivorous Marsupials (Phascolotherium, Thylacinus, &c.); in the former certainly, and in the latter most probably, by the reduction of the cusps of the primitively Tritubercular sectorial molar.

[1] Speaking of the Insectivora, Scott naïvely remarks that "in this group, strange to say, the oldest member yet discovered exhibits the most complicated premolar structure" (28).

[2] Dr. Forsyth Major (10) does not favour this view. However, even in the teeth he figures, traces of the Tritubercular sectorial plan may be easily detected.

The evidence is, therefore, strongly in favour of the view that the common ancestor of marsupial and placental mammals had teeth with many cusps of the Tritubercular sectorial pattern. What, then, was the pattern of the molars of the ancestors of both Monotremes and Ditremes? As yet we can give no definite answer to this question; but one thing seems extremely probable, namely, that they were of an indefinite multituberculate pattern, which gave rise, on the one hand, to the elaborate multituberculate teeth,[1] and on the other to the Tritubercular sectorial. Thus the development of two longitudinal rows of three cusps would give rise to the type of lower molar common amongst the Multituberculata; the fusion of the two anterior of these cusps or the loss of one would yield the Tritubercular sectorial tooth common amongst the Marsupials and Placentals; while the loss of the inner cusps would result in the formation of a Triconodont molar. The conclusion reached is, therefore, that the primitive mammalian molar bore a crown with several cusps.

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EXPLANATION OF PLATE 26,

Illustrating Mr. E. S. Goodrich's paper "On the Fossil Mammalia from the Stonesfield Slate."

Reference Letters.

a. Angle. *a. c.* Articular condyle. *c.* Canine. *c. p.* Coronoid process. *i.* incisor. *m.* Molar. *m. g.* Mylohyoid groove. *pm.* Premolar.

Fig. 1.—Left ramus of Amphitherium Prevostii, inner surface exposed. Type specimen in the Oxford Museum. × 5.

Fig. 2.—Left ramus of Amphitherium Prevostii, inner surface exposed. Second specimen in the Oxford Museum. × 5.

Fig. 3.—Right ramus of Amphitherium Oweni, outer surface exposed. Type and only specimen in the Oxford Museum. × 5.

Fig. 4.—Right ramus of Amphitherium Prevostii, inner surface exposed. In the British Museum. × 5.

Fig. 5.—Left ramus of Amphilestes Broderipii, inner surface exposed. Type specimen in the York Museum. × 5.

Fig. 5a.—Anterior portion of the same fossil seen more from above. × 5.

Fig. 6.—Left ramus of Amphilestes Broderipii, outer surface exposed. First specimen in the Oxford Museum. To facilitate comparison the figure has been reversed. × 5.

Fig. 7.—Right ramus of Amphilestes Broderipii, outer surface exposed. Second specimen in the Oxford Museum. × 5.

Fig. 8.—Left ramus of Phascolotherium Bucklandi, inner surface exposed. In the Oxford Museum. × 3.

Fig. 9.—Right ramus of Phascolotherium Bucklandi, inner surface exposed. In Mr. Parker's collection. × 3.

The lines above the figures indicate the actual length of the fossils.

A Polynoid with Branchiæ (Eupolyodontes Cornishii).

By

Florence Buchanan, B.Sc.

With Plate 27.

A SINGLE specimen of an interesting Polychæte was presented to the British Museum a short time ago by Mr. V. H. Cornish, of the cable-ship "Mirror," who had obtained it off the mouth of the river Congo. It was shown to me by Professor Bell, who was good enough to suggest that I should describe it, and Dr. Günther has kindly sanctioned my doing so.

The worm is evidently a Polynoid belonging to the sub-family Acoëtidæ. It is remarkably large even for that sub-family, the specimen, although incomplete, measuring over a foot in length, its breadth exceeding one and a half inches, and its depth being nearly half an inch. It is, unfortunately, somewhat mutilated, the alimentary canal being torn out, so that the pharynx, which is a characteristic feature of the group, cannot be diagnosed. The head and greater number of segments present are, however, complete externally, and while showing clearly the genetic position of the worm, present also characters of interest only slightly developed in other members of the group, and which therefore have not hitherto received sufficient attention.

Before describing the worm itself I think it will be advisable to review briefly the characteristics of the sub-family, and to enumerate the few known species belonging to it, especially as

most of them are described in scattered journals, and some have been overlooked by later writers on the group.

The Acoëtidæ may be defined as elongate Polynoids, with the elytra alternating regularly with dorsal cirri throughout the body, except for the second and third pairs which are on consecutive segments, the 4th and 5th respectively. The dorsal surface of the body is generally transversely grooved, the grooves being very fine and close together, often quite obliterating the segment boundaries. The prostomium bears two large pupillated eyes, generally on well-developed peduncles; there may be in addition smaller eyes or pigment spots behind them. There is a single median prostomial tentacle and a pair of lateral ones: the former is sometimes rudimentary or even absent; when present it springs from the posterior part of the prostomium. The paired prostomial tentacles are also occasionally absent; when present they generally arise from the ventral surface of the prostomium. There is a pair of palps, usually very large and well developed. The parapodia of the buccal segment have moved forward so as to lie in front of the mouth; they consist each of a basal part bearing two peristomial tentacles corresponding to the dorsal and ventral cirri of the other parapodia, and sometimes also bearing chætæ. The parapodia of the following segments are either uniramous or biramous with the notopodial lobe very small; each one contains a much-coiled dorsal chætal sac, the "spinning gland" of Eisig (6), producing numerous exceedingly long, fine, silky capillary chætæ, which probably help to form the tube in which the creature lives; occasionally, however, the sac may be shorter, and the chætæ produced in it more like ordinary chætæ, projecting from the sac instead of being kept inside it. There is a median ventral longitudinal ridge protecting the nerve-cord; it is bounded on each side by a deep furrow, and widens in front just behind the mouth. The pharynx is exsertile, papillose on the anterior margin; the jaws large and horny, armed with two central and many lateral teeth.

The known species of the sub-family are only fifteen in number, and of most of these only single and incomplete speci-

mens have been seen; only two, the Mediterranean Polyodontes maxillosus and the Northern Panthalis Oerstedi, have been found by more than one observer, but even they are not very abundant. I will enumerate the species in the order of their foundation:

1817.—Polyodontes maxillosus (Ranzani), Audouin and Edwards. Mediterranean.

Described and figured by Claparède (4), who gives its synonymy and refers to previous descriptions and figures.

1832.—Acoëtes Pleei, Audouin and Edwards. Martinique.

Single specimen, described and figured by the founders (2), and further described by Quatrefages (15). Grube (8) refers it to the genus Polyodontes.

1841.—Polyodontes Blainvillei (Costa), Claparède. Mediterranean.

Single specimen, imperfectly described by Costa (5), who calls it a "Sigalion." Referred to the genus Polyodontes by Claparède (4).

1855.—Polyodontes gulo, Grube. Red Sea.

Single specimen, described and figured by the founder (8).

1855.—Eupompe Grubei, Kinberg. Near Guayaquil. Single specimen.

Panthalis Oerstedi, Kinberg. British and Scandinavian coasts.

Panthalis gracilis, Kinberg. Near Rio Janeiro. Single specimen.

All three described (11 and 12) and figured (12) by their founder.

1855.—Acoëtes lupina, Stimpson. South Carolina.

Imperfect description by Stimpson (16).

1876.—Eupanthalis Kinbergi, McIntosh. Adventure Bank.

Described and chætæ figured by the founder (13).

1877.—Panthalis bicolor, Grube. Congo.
Two specimens differing greatly from one another, described but not figured by Grube (**9**).

1878.—Panthalis melanotus, Grube. Philippine Islands.
Panthalis nigromaculata, Grube. Philippine Islands.
Described but not very well figured by Grube (**10**).

1885.—Eupompe australiensis, McIntosh. Off Cape York, Australia.
Described and figured by the founder (**14**).

1887.—Euarche tubifex, Ehlers. Off Carysfort Reef, West Indies.
Described and figured by founder (**7**).

1887.—Eupompe indica, Beddard. Mergui Archipelago.
Described and head figured by the founder (**3**).

The new species which may now be added to the list bears most resemblance to the Polyodontes gulo described by Grube (**8**), and it has, indeed, certain characters in common with it in which they both differ from all the other known species. Like P. gulo and no other member of the group there are no long well-developed palps,[1] and the eye peduncles are lateral instead of being anterior, and fused with the sides of the prostomium, thus giving the prostomium a very broad appearance (figs. 1 and 2). The paired prostomial tentacles, when present, in all other Acoëtidæ with pedunculate eyes, arise from the ventral surface of the prostomium, or rather from the base of the anteriorly placed eye-stalks, and just in front of the palps (cf. fig. 10). Here and in P. gulo there are two small tentacles springing from the anterior (and slightly ventral) surface of the prostomium, which probably represent them (figs. 1 and 2, *t.*). Behind these (fig. 2) and springing from the base of the laterally placed eye-stalks are two other very minute tentacles, which probably represent the palps of

[1] McIntosh does not mention the palps at all in his Eupanthalis, but I conclude that he would have done so had they been greatly reduced in size or absent.

the other species although extremely reduced. Their relation to the eye-stalks suggests for a moment their homology with the paired prostomial tentacles ("antennæ" of authors) rather than with the palps, but if this were the case we should have not only to regard the palps as altogether absent, but we should also have to explain the presence of an extra pair of prostomial tentacles in front with no homology in other forms. A comparison of the arrangement of the different prostomial appendages[1] in the sessile-eyed forms shows that there also, as in P. gulo and the new worm, the paired prostomial tentacles arise close to the anterior edge of the prostomium, while the only other paired prostomial appendages, the palps, arise close behind them and are developed to their usual extent.[2] I think, therefore, that we may conclude that the relation of any of the prostomial appendages to the eye-stalks is a secondary one, while their relation to the prostomium is constant.[3] The parapodia of P. gulo are not figured, but from the description they seem to resemble in arrangement those of the new species.

The only characteristic points of difference between P. gulo and the new worm is that while in P. gulo there is no trace of a median prostomial tentacle here there is one, although only a very rudimentary one; and that the few dorsal papillæ on the parapodia of P. gulo, some of which are described as elongated to cirri, are here enormously developed and very numerous and arborescent, resembling in appearance the branchiæ of other Polychætes. Both these points, however, seem to me to be only of specific importance, since they are characters which vary also in other members of the group. While the other characters of the prostomium, so much alike in these two species, but differing so markedly from all the other forms, seem to mark them off from all the others as a separate genus, for which I propose the name Eupolyodontes, calling the

[1] "Prostomial appendages" = 1 median and 2 paired "prostomial tentacles" + 2 "palps."

[2] Compare Ehler's figure of head of Euarche tubifex (7).

[3] Not wishing to spoil the specimen I was unable to examine microscopically the structure of the different pairs of prostomial appendages.

new species, after the name of its discoverer, E. Cornishii. I would give the following as a definition of the genus and of its contained species:

1. Genus Eupolyodontes.

Acoëtidæ with peduncles of eyes arising laterally from the base of the prostomium, and fused with it on either side; short antennæ or paired prostomial tentacles arising from the anterior margin of the prostomium or slightly ventral to it; median prostomial tentacle rudimentary or absent, arising from the posterior part of the prostomium when present; palps small, no longer than the antennæ, situated very close to or on the bases of the eye-stalks. Dorsal surface of body very finely rugate transversely and segment boundaries thus obliterated. Parapodia with papillæ on the dorsal surface, which may be filamentous or even arborescent. Parapodia of buccal segment not chætiferous.[1]

Sp. 1.—E. gulo, Gr. [Polyodontes gulo, Grube (8), 'Arch. f. Naturg.,' xxi].

Eupolyodontes with antennæ arising from the anterior edge of the prostomium, but with no median prostomial tentacle. Parapodia with minute papillæ, sometimes elongated, two to five in number on the elytra-bearing segments, six or seven on the others. Only one acicle to each parapodium; chætæ of three kinds:—a comb of short stiff chætæ slightly curved at apex, a fine bundle of bipinnate ventral chætæ, and a dorsal bundle of long delicate capillary chætæ, forming the thick silky thread of the "spinning gland." Hab. Red Sea. Living in tubes.

Sp. 2.—E. Cornishii, n. sp.

Eupolyodontes with a minute median prostomial tentacle situated on the posterior part of the prostomium, and just in front of a slightly raised part of the back which forms a kind

[1] This may turn out hereafter, when new species are discovered, to be only of specific value.

of "caruncle." Prostomium slightly bilobed, paired prostomial tentacles or antennæ arising one from each lobe just below the anterior edge; palps smaller than the antennæ, each with a minute swollen basal piece. Parapodia, both those bearing elytræ and those bearing dorsal cirri, with a very large number of filamentous and arborescent branchia-like looking structures along the anterior and posterior border of each, beginning on the posterior border of the 6th chætiferous parapodium, where there is only a single bifurcate filament; three segments further back they are present on both anterior and posterior border of the parapodium, and are already numerous; they increase in number and size and amount of branching for the next few segments, and are best developed on the parapodia of the 15th to the 50th segments; they then decrease in number and size, and become more papilliform. In structure each filament is hollow, its cavity being probably an extension of cœlom; the cells of the epidermis are laden with yellow granules, which look like excretory products, and there is a very thick cuticle. Only one acicle to each parapodium. Chætæ as in E. gulo (see figs. 8 B and 8 C), with in addition certain ones with double-brush shaped tips[1] (fig. 8 A), scattered amongst the comb of stiff chætæ dorsally. The "spinning gland" is well developed in every segment after the first few, being long and coiled and occupying the cavity of the parapodium, and opening on its dorsal surface; the long fine capillary chætæ produced by it are of a silky golden colour, generally retracted but readily drawn out an inch or two (fig. 4, *cap*). Elytra smooth, anteriorly flat, those of the 2nd parapodium (the first pair) overlapping one another, but the rest well to the side, leaving the whole of the dorsal surface except for the parapodia exposed, small (relatively to the size of the animal) and scarcely imbricate; posteriorly they are swollen and pear-shaped, each being attached to the parapodium by a stalk (fig. 5). Dorsal cirri short (no longer than the branchiæ where these are well developed), outside the elytra. Ventral

[1] Resembling those of Eupompe Grubei, Kinberg (12), more than those of any other of the species of which the chætæ have been figured.

cirri rather shorter, those of the 2nd pair of parapodia being larger than the rest.

Pharynx and jaws not present in the specimen.

Colour (in spirit): of the eye-stalks dark blue-black; of the prostomium itself dark, but not quite so dark; of the dorsal and ventral surface of the body dark brownish, the parapodia somewhat lighter, and the ventral ridge below the nerve-cord also of a lighter colour; "branchiæ" darker in colour than the rest of the parapodium.

Length of single specimen, consisting of ninety-two segments, but incomplete posteriorly, 32·5 cm.; breadth, including parapodia, 4·2 cm.; of the dorsal surface of the body alone 2 cm.

Hab.—Single specimen, obtained off the mouth of the river Congo, about thirty-five miles from land, at a depth of from forty-three to fifty-seven fathoms, from a bottom of mud and weed. The colour of the water where it was taken was of a uniform reddish orange.

It is probably tube-forming, although no tube was found with it. The various points are illustrated in the figures (1—8).

With regard to the other fourteen species of the sub-family (or rather thirteen, as the Acoëtes lupina of Stimpson is probably the same as A. Pleei), reference to the list given on p. 435 will show that six genera have been formed for them. Grube (8) has long ago disposed of one of these by placing Acoëtes Pleei in the genus Polyodontes. Beddard (3) has recently proposed to throw the genera Eupompe and Panthalis into one. I agree with him, but would go further, and place provisionally both these genera in one genus with Polyodontes, bearing in mind that closer acquaintance with the different species and the discovery of new ones will probably lead hereafter to a new subdivision into genera, but probably not—it seems to me, at least—coinciding with what we now know as the genera Polyodontes, Eupompe, and Panthalis. The number of genera needed in any group of animals depends entirely on which different forms and how many of them happen to be known at the time. When only three

species[1] of this sub-family were known, all about equally distant from one another, it was quite enough to have only one genus for them all, as Grube proposed. But the greater the number of species made known the less likely are they to remain equally distant from one another, and they then fall naturally into groups, only to be reunited when all the intermediate forms are known. In my opinion the sub-family of the Acoëtidæ falls now, in the present state of our knowledge, or rather of our ignorance, into three groups, which we may call genera. One of these, the genus Eupolyodontes, I have already defined. The other stalk-eyed forms (Polyodontes maxillosus and Blainvillei; Acoëtes Pleei; Eupompe Grubei, australiensis, and indica; Panthalis Oerstedi, gracilis, melanotus, and [in part] bicolor) I would propose to put together in the genus Polyodontes, defining this genus then in its widest sense as follows:

2. Genus Polyodontes.

Acoëtidæ with peduncles of eyes arising from the front of the prostomium, and meeting, or nearly meeting, one another in the middle line in front; median prostomial tentacle well developed, paired ones present in all except P. (E.) indica and P. (P.) melanotus, and arising from the ventral surface of the prostomium at the base of the eye-stalks; palps large and well developed, arising close behind the paired prostomial tentacles; papillæ sometimes present on the parapodia, but not developed to any great extent (represented in P. (A.) Pleei, P. (E.) australiensis, P. (E.) Grubei [on elytra-bearing feet only], and P. (P.) bicolor?[2]). Parapodia of buccal segment sometimes chætiferous (at least in P. maxillosus, P. (A.) Pleei, P. (E.) Grubei, and P. (P.) Oerstedi, but not in P. (E.) indica, P. (E.) australiensis, or P. (P.) bicolor; in the other species the fact is not mentioned either way).

I have the less hesitation in placing these species of different

[1] At present, in this sub-family, it is easy to speak of these "different forms" as "species."

[2] If I understand Grube's description aright they would be here on the ventral surface of the parapodia, and not on the dorsal.

genera together into one genus, as I have been able to compare one species of Eupompe (E. australiensis) which was in the British Museum with a type specimen of Polyodontes maxillosus which Professor Bell kindly procured for me from Naples. Although the last-mentioned worm has been several times described, none of the figures of its head show very well the relations of the tentacles to the prostomium, and I have therefore figured the head from above and below (figs. 9 and 10).

The remaining species of the Acoëtidæ (Eupanthalis Kinbergi, Euarche tubifex, and (?) Panthalis nigromaculata, and (?) part of P. bicolor) I would place provisionally, but only provisionally, in a third genus, which would bear the name Eupanthalis, defining it as follows:

3. Genus Eupanthalis.

Acoëtidæ with sessile eyes, four in number; three prostomial tentacles, except (?) in E. tubifex;[1] otherwise like Polyodontes.

Although it seems simplest to make one genus for all the sessile-eyed forms, I have a good deal of hesitation in doing so on account of Grube's description (9) of what he calls two forms of Panthalis bicolor, coming, by the way, from the same locality as the specimen sent by Mr. Cornish. Grube's two specimens agree in colour, and the parapodia are alike; moreover he found them in the same bottle, which he seems to think important. But while in the one the eyes are pedunculate and apparently anterior, the palps very large, the paired tentacles beneath the median one and the elytra large, in the other the eyes are sessile, the palps shorter, the paired tentacles on the front margin of the prostomium and the elytra much smaller. Grube has already remarked that it would be very strange and quite unheard of in this family of Polychætes to find such very different forms of a single species, and he is not quite convinced of it himself. If it were so it

[1] McIntosh's remark that there is "no" median "tentacle in the specimen" seems rather to imply that there may once have been one which has been lost by accident.

would be exceedingly interesting, as it would suggest that other sessile-eyed forms might be but second forms of other species with pedunculate eyes; but I think evidence is wanting of the fact that the two specimens described by Grube as P. bicolor do really belong to the same species. Unfortunately neither of them is figured at all. Panthalis nigromaculata, which I have also placed with a (?) in this genus, would appear from Grube's figure of its head to have quite sessile eyes. In his description of it, however, he speaks of them as on protuberances.

Besides throwing light on the intrinsic relationships of the sub-family, the new worm also, it seems to me, increases the probability of the existence of a relationship between the whole family of the Polynoidæ and the family Amphinomidæ. The Acoëtidæ, in common with certain other sub-families of the Polynoidæ, resemble the Amphinomidæ in the forward movement of the first pair of parapodia. The new Acoëtid resembles them further in another peculiarity of the head which I have already mentioned, namely, the ridging of the dorsal surface of the head behind the median tentacle. The resemblance may be only superficial, but one is certainly reminded by it at once of the "caruncle" of the Amphinomidæ, which is sometimes little more than a raised part of the dorsal surface of the head. Another and more striking point of resemblance, at first sight at least, is the presence of the arborescent or filamentous, branchia-like looking structures on the parapodia, and this brings me to what I consider the most interesting point about the new worm. I have already mentioned the position of these filaments and referred briefly to their structure in diagnosing the species. Their relation to the parapodium is shown in figs. 4 and 5, a single tuft of them in fig. 6, and a transverse section of one of them in fig. 7. The state of preservation they were in makes their minute structure difficult to interpret, and I cannot be at all certain whether the central cavity is really an extension of cœlom or a large blood-vessel,—that is to say, whether there is a true space between the epidermis and wall of this central cavity or not. I am inclined to think that there is no cavity

in the filament besides the central cavity, and that there is connective tissue between this and the epidermis which has not been preserved, except for a few nuclei (fig. 7 *c. t.*). The space would then be extension of cœlom, and I believe it to be lined by a definite epithelium, although the nuclei indicating this are few and far between. (One is shown at *n.* in fig. 7.) The clot inside the cavity is more like a cœlomic clot than a blood clot. If this central cavity be cœlom, I cannot be certain of there being blood-vessels going to the filaments at all (unless certain small structures, seeming to lie in the wall of the central cavity and marked "*bl.* (?)" in fig. 7, represent them), and the filaments cannot be termed "branchiæ" in the ordinary sense of the word. The extreme thickness of the cuticle would also seem to indicate that their function is other than respiratory, and the peculiar character of the epidermis helps to show what this function is. Although, owing to the method of preservation, it is scarcely possible to distinguish cell outlines, nuclei of the epidermis cells are here and there visible, and grouped around them and apparently densely loading all the epidermis cells are numerous yellow concretions, some of them refringent, others with a somewhat darker appearance, and often massed three or four together. These resemble so closely the concretions of nephridial cells and of the cells of other renal organs described by Eisig in the Capitellidæ, and behave in the same way towards chemical reagents in as far as I have been able to test them, that I think there can be little doubt of their excretory significance. Eisig has shown how in the genus Capitella, where the nephridia appear not to open to the exterior at all, the excretory products are stored in the epidermis cells, only to be got rid of when the animal changes its skin, and, as is well known, numerous Arthropods normally store their excretory products. The filaments, then, on the parapodia of Eupolyodontes Cornishii would seem to be special organs for storing the excretory products, and perhaps also for forming them.[1]

[1] As far as I am aware nothing is known about nephridia or excretory organs of any sort in the sub-family Acoëtidæ.

In spite, however, of their being so unlike respiratory organs in structure, their outward resemblance to the "branchiæ" of other Polychætes, and especially to those of the Amphinomidæ, struck me so forcibly that I was led also to examine microscopically the structure of these for the sake of comparison; and the results are, I believe, sufficiently interesting to warrant a mention of them here, although I must defer a more detailed description and more numerous figures to a future publication.

I have examined by means of sections the branchiæ of a Euphrosyne, of two or three Amphinomes, of Chloeia flava, of Eunice gigantea, Diopatra neapolitana, Arenicola marina, and a few others. The thickness of the cuticle, although most marked in the Amphinomids, is remarkable in all. In none of them nor on any part of them is the epidermis ciliated. Very minute concretions, nothing like so large as in Eupolyodontes Cornishii, are present in the branchiæ of the Euphrosyne, one of the Amphinomes, in Arenicola marina, and, although here they are present in other parts of the epidermis as well, in Eunice gigantea. Claparède (4, p. 110) has already remarked on the thickness of the cuticle, and the absence of blood-vessels and of axial cavity in the branchiæ of Euphrosyne Audouini, and speaks of them throughout as "prétendues branchies." Schmarda shows, however, that in E. polybranchia there is a vascular network penetrating into the final ramifications, and in the Euphrosyne of which I cut sections, and which I believe to be E. borealis, there were certainly two vessels traversing the main stem of each branchia, breaking up into a capillary network in the filaments. We have, then, within the same genus forms with vascular and with non-vascular "branchiæ." In most of the Amphinomes of whose branchiæ I cut sections the filaments appeared also to be solid. There was, however, in one of them at least (fig. 12) a central part very little blocked up by connective tissue. Between this and the epidermis is retiform connective tissue (*c. t.*), and in this on either side of each filament is a blood-vessel (*bl.*), giving off

numerous branches all lying in the connective tissue. Only quite at the extremity of the filaments the connective tissue and vessels seem to have disappeared altogether. In Chloeia (fig. 13) the two vessels have increased enormously in size, and, except for being connected with one another at intervals at the tip of each filament, give off no branches. No central cavity is distinguishable, all the space which is not blood-vessel underneath the epidermis being occupied by retiform connective tissue (*c. t.*). In the other Polychætes examined the branchiæ were more normal in structure, containing an afferent and efferent vessel lying close under the epidermis, but in a well-developed extension of cœlom.[1]

From the above facts I conclude that the so-called "branchiæ" of polychætes do not necessarily serve only as respiratory organs, and indeed may even have no respiratory function at all (some species of Euphrosyne); and in the sense that we call them "branchiæ," on account of their representing the respiratory organs of allied forms, I claim to be able to apply the same term to the branching processes on the parapodia of Eupolyodontes Cornishii. When they are not respiratory, or at least not mainly respiratory in function, they may have to do with excretion, serving to store the excretory products, and probably, in the case of Amphinome at least, and those forms with blood-vessels immediately underlying the epidermis and with concretions in the epidermis cells, to form them from the blood.

In conclusion, I should like to draw attention to the minute structure of the filaments on the parapodia of the only other Acoëtid possessing them which I have been able to examine, namely, Polyodontes (Eupompe) australiensis. Although I think there can be no doubt, from their position in relation to the parapodium, as to their representing the more numerous filaments on the parapodium of Eupolyodontes Cornishii,

[1] Only in the Diopatra it was difficult to be certain of the blood-vessels, as the blood did not clot at all, and the two vessels in each of the filaments, each of them subdivided by connective-tissue partitions, would not be taken for blood-vessels, were it not for Claparède's statement that there is an afferent and efferent vessel in each filament.

their structure is very different, as will be seen by comparing the figures of two sections through one of them (figs. 11 A and B) with fig. 7. There is apparently no central cavity, nor is there anything looking at all like blood-vessel; the epidermis cells are flattened and contain no concretions: the only point of resemblance is the thickness of the cuticle. The substance of the filament near the tip seems to consist of concentrically arranged connective-tissue fibres, in which lie a few large clear cells with large distinct nuclei. Five of these (fig. 11A) are arranged radially round a common centre, and their appearance is extremely suggestive of the so-called "gill-glands" recently described in a Crustacean by Mr. Allen (1) (where, by the way, we have also an instance as shown by Kowalewsky of a branchia exercising some excretory function besides its normal function). It is true there is nothing to be seen here representing the duct described by him; but one could perhaps scarcely expect to find it, even if present, in material not preserved with a view to histological work, and also the plane of the sections might not be favorable for showing it. Nearer the base of the filament (fig. 11A) the whole space beneath the epidermis seems to be occupied by retiform connective tissue, except for a curious mass of what seem to be concentrically arranged connective-tissue fibres near the centre. The structure of these filaments bears most resemblance to that of the branchiæ of Euphrosyne—in as far as I have been able to examine them—amongst Polychætes. Here also, in the "E. borealis (?)" at least, we have similar large cells embedded in connective tissue near the apex of the filament, although not radially arranged round a common centre as in the Eupompe filament, and the large cells in a special swelling at the apex of the branchial filaments of so many Euphrosynes (including E. Audouini) are well known, although I do not know that sections of them have ever been described. The rest of the substance of the branchial filament is occupied by connective tissue, in which, however, in E. borealis (?) there are wall-less blood-vessels, though apparently there are not even these in E. Audouini.

It would be interesting if some one within reach of the other specimens of the sub-family Acoëtidæ with "branchial" filaments would examine and report on their structure.

List of Memoirs referred to.

(1) Allen.—"On the Minute Structure of the Gills of Palæmonetes varians," 'Quart. Journ. Micr. Sci.,' vol. xxxiv, pp. 75—84.

(2) Audouin and Edwards.—"Classification des Annélides," 'Annales des Sciences naturelles,' 1e sér., xxvii, 1832, pp. 435—438, pl. x, figs. 7—14.

(3) Beddard.—"Report on Annelids from the Mergui Archipelago," 'Journ. Linn. Soc.,' xxi, 1887 (1889), pp. 256—258, pl. xxi, figs. 1 and 3.

(4) Claparède.—'Les Annélides Chétopodes du Golfe de Naples,' 1868, pp. 392—396, pl. iii, fig. 2.

(5) Costa.—"Description de quelques Annelides nouvelles du golfe de Naples," 'Ann. Sci. Nat.,' 2e sér., xvi, 1841, p. 269, pl. xi, fig. 1.

(6) Eisig.—'Monographie der Capitelliden.'

(7) Ehlers.—"Results of Dredging of the U.S. Coast-Survey Steamer 'Blake,'" 'Report on Annelids,' 1887, pp. 54—56, pls. xii and xiii.

(8) Grube.—"Beschreibung neuer oder wenigbekannter Anneliden," 'Arch. f. Naturg.,' 21ter Jhrg., 1855, pp. 83—90, pl. iii, fig. 2.

(9) Grube.—"Anneliden Ausbeute S.M.S. 'Gazelle,' Monatsber.," 'Berliner Akad.,' 1877, pp. 517—519.

(10) Grube.—"Annulata Semperiana," 'Mém. Acad. St. Pétersbourg,' 7e sér., xxv, 1878, pp. 48—52, pl. iv, figs. 1 and 2.

(11) Kinberg.—'Öfvers. af. k. Vet. Akad. Fish,' 1855, pp. 386, 387.

(12) Kinberg.—"Fregate Eugenies Resa," 'Zool. Annulater,' pp. 24—26, pl. vii, figs. 34 and 35 (published part); pl. x, figs. 59—61 (unpublished part).

(13) McIntosh.—"Annelida of the 'Porcupine' Expedition," 'Trans. Zool. Soc.,' ix, 1876, pp. 404, 405, pl. lxxii, figs. 12—15.

(14) McIntosh.—"'Challenger' Reports," 'Zoology,' xii, pp. 135—139, pl. xxi, figs. 4 and 5.

(15) Quatrefages.—'Histoire naturelles des Annélés,' i, p. 216.

(16) Stimpson.—"Synopsis of Marine Invertebrates of Grand Manon," 'Smithsonian Contributions to Knowledge,' Washington, 1853, p. 36.

EXPLANATION OF PLATE 27,

Illustrating Miss Florence Buchanan's paper, "A Polynoid with Branchiæ (Eupolyodontes Cornishii)."

Fig. 1.—Dorsal view of head[1] and first ten segments of Eupolyodontes Cornishii. The elytra are represented as turned aside from their normal position to show the parapodia. Very slightly enlarged only. *t.* One of the paired prostomial tentacles.

Fig. 2.—Ventral view of head and first five segments.

Fig. 3.—Dorsal view of three segments some five or six inches from the anterior end. The elytra left in their normal position on the left side only. Turned aside to show the "branchiæ" on the right.

Fig. 4.—View from behind of a non-elytriferous parapodium from about the 50th segment. *d. c.* Dorsal cirrus. *ac.* Acicle, seen projecting behind the cut end of *sp. gl.*, the spin-gland. *d. s.* Dorsal chæta. *sp.* Spines or short stiff chætæ of comb. *v. s.* Ventral chætæ bundle. *cap.* Silky thread formed of capillary chætæ of spin-gland, drawn out from the aperture of the gland.

Fig. 5.—Similar view of an elytriferous parapodium taken from a more posterior segment, showing the swollen elytron. The branchial filaments have become much fewer in number.

Fig. 6.—A single "branchial" tuft from a segment where they are well developed.

Fig. 7.—Transverse section of one of the "branchial" filaments. *c.* Cuticle. *ep.* Epidermis laden with concretions. *c. t.* Nucleus of connective tissue (?). *c. cav.* Central cavity. *n.* Nucleus in its wall. *bl.* (?) A blood-vessel (?).

Fig. 7A.—A portion of the epidermis of the same section, enlarged to show the nucleus (*n.*) of an epidermis cell.

Fig. 8A.—Tip of a dorsal chæta.

Fig. 8B.—Tip of one of the spines.

Fig. 8C.—Tip of one of the chætæ of ventral bundle. There is not always such a marked difference at the apex as there is in the one here figured.

Fig. 9.—Dorsal view of head and first segment of Polyodontes maxillosus, much enlarged.

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[1] "Head" here is used to include the prostomium and first or buccal segment which is fused with it.

Fig. 10.—Ventral view of head and first four segments of the same. Palps turned aside to show the underlying paired prostomial tentacles.

Figs. 11A and 11B.—Two transverse sections of a "branchial" filament of Eupompe australiensis.

Fig. 12.—Transverse section of a branchial filament of an Amphinome (Eurythoë). *bl.* Blood-vessels. Other letters as in Fig. 7.

Fig. 13.—Transverse section of one of the filaments of a branchia of Chloeia flava.

On some Bipinnariæ from the English Channel.

By

Walter Garstang, M.A.,
Fellow of Lincoln College, Oxford, Naturalist to the Marine Biological Association.

With Plate 28.

The genus Bipinnaria was defined by the elder Sars (3) in the following terms:—"Corpus gelatinosum longum cylindrico-depressum, pinnis duabus, una postice terminali cordiformi, altera triangulari in medio corpore. Os appendiculis seu brachiis lanceolatis circumdatum." To this genus he referred the single species asterigera, "appendicibus seu brachiis 12 circa os."

Few zoologists meeting with this passage for the first time would, I imagine, recognise in it the description of a starfish larva; for the Bipinnaria of the text-books is invariably of that simpler, commoner, and smaller type which formed the basis of Johannes Müller's classical researches. The original Bipinnaria of Sars was a remarkably elongated creature, fully one inch in length, with a crown of polyp-like tentacles at the oral end of the body, a bilobed fin at the other extremity, and a median ventral fin placed transversely; attached to the tentaculate end of the body was a small five-rayed starfish. Sars himself was so puzzled by the animal that he referred it, not without some natural hesitation, to a special group of Acalephæ.

In October, 1846, a swarm of these Bipinnariæ visited Bergen harbour, and formed the material for some researches by Drs. Koren and Danielssen (1). These naturalists pointed out the real nature of the connection between the starfish and the Bipinnaria. Two preserved specimens of these Bipinnariæ subsequently came into the hands of Johannes Müller, and an account of them is included in the second of Müller's memoirs (2).

So far as I am aware,[1] no specimens of this interesting larva have been captured since 1846. During the month of August this year, however, I have taken near Plymouth a number of Bipinnaria larvæ which resemble Sars's larva in many points. At the same time they show some differences which seem to be important. It may eventually be proved that these dissimilarities are due to a different stage of development; or, on the other hand, the Plymouth larvæ may be shown to belong to a type of Asterid distinct from that to which Sars's larva belongs. Up to the present time, however, I have not succeeded in finding specimens in which there is any trace of approaching metamorphosis, so that the identification of the larva has not been possible.

1. Structure.

A glance at the figures on Plate 28 reveals at once the fact that the Plymouth larvæ are constructed upon a plan which is intermediate between that of Sars's Bipinnaria asterigera and the more common type of starfish larva. They agree with the former in exhibiting a great development of the præ-oral lobe; while they resemble the commoner type, and at the same time differ from Sars's larva, in the less concentrated arrangement of the paired ciliated arms.

The primitive circumoral ciliated ring is divided as usual into two distinct portions, one of which borders the ventral side of the præ-oral lobe (figs. 1 and 2, *a. c. o. b.*); while the other (*p. c. o. b.*) not only borders the dorsal side of the præ-

[1] Metschnikoff, I find, made use of this larva in his researches on intracellular digestion. Vide 'Quart. Journ. Mic. Sci.,' xxiv, 1884, p. 99.

oral lobe, but is continued backwards down each side of the body to the posterior end, where it bends first ventrally, then anteriorly, to pass between mouth and anus across the mid-ventral region of the body. The mouth is encircled by its own adoral band (fig. 2, *a. b.*). The anterior or præ-oral ciliated ridge (cephalotroch of Lankester) is produced into three processes—a median arm anteriorly (*p. v. a.*) and a pair of lateral arms posteriorly (*r. a. v. a.* and counterpart). The posterior or post-oral ciliated ridge (branchiotroch of Lankester) is produced into eleven processes—a median arm anteriorly (*p. d. a.*), and five pairs of lateral processes. Of the lateral processes three pairs are dorsal, one pair is posterior (*r. p. l. a.* and *l. p. l. a.*), and one pair is ventral in position (*r. p. v. a.* and counterpart).

Each of the anterior unpaired arms arises from the apex of a prolongation of the præ-oral lobe, which may be termed the præ-oral appendage (cf. fig. 1). This appendage possesses a very characteristic form. It is broad from side to side and compressed dorso-ventrally, the degree of compression increasing with the age of the larva. Down each side of the appendage runs a groove which is continuous with the lateral depressions of the body of the larva, and separates the anterior prolongations of the præ-oral and post-oral ciliated bands from each other. The præ-oral appendage bifurcates after a certain distance into a dorsal and a ventral part, the two anterior unpaired ciliated arms, of which the dorsal is the larger. The point of bifurcation (fig. 1, *x.*), where the præ-oral and post-oral ciliated bands also diverge from each other, represents the morphological apex of the præ-oral lobe. This is clearly shown by a comparison of the larva with Tornaria; it is at this spot —between the anterior extremities of the two ciliated bands— that the apical plate with its tuft of cilia and pair of pigmented sensory pits is situated in the larva of Balanoglossus. The dorsal and ventral præ-oral arms (*p. d. a., p. v. a.*) are broad and flattened, and have lancet-like or tongue-shaped terminations. The dorsal arm bends backwards over the dorsal side of the body, while the ventral arm has an equally pronounced

curve ventrally. The edges of the dorsal and ventral arms are bordered by the prolongations of the post-oral and præ-oral ciliated bands respectively. The length of the præ-oral appendage and of the unpaired arms which arise from it increases with the age of the larva, and increases relatively to the general growth of the body. At the stage represented in fig. 1 the body of the larva has almost attained its full development; but the præ-oral appendage, on the other hand, is not fully grown. The increase in length of this appendage is most marked in the case of the stalk and of the dorsal arm. I am not in a position to say whether the præ-oral lobe ever reaches the extraordinary dimensions it possesses in the case of Sars's Bipinnaria, but such a development is quite within the bounds of possibility.

The larva drawn in fig. 1 was not quite my largest specimen. In the preserved condition its dimensions were as follows:

Length from apex of prostomium (x) to tips of posterior arms	1·8 mm.
Length of anterior dorsal arm from apex of prostomium (x) to tip	0·9 „
Maximum length, therefore	2·7 „

The thickness (dorso-ventral) of the præ-oral appendage was 0·2 mm.

The internal structure of the larva may be gathered from an examination of fig. 1. The alimentary canal is perfectly normal, and needs no special description. On each side of it lies one of the lateral enterocœles ($r.\ e.$), which fuse together immediately in front of the œsophagus to form the unpaired præ-oral enterocœle ($p.\ e.$). The latter is attached to the ventral wall of the præ-oral appendage for the greater part of its course; but at the base of the unpaired ventral arm it breaks away from the wall and sends a short process into the dorsal arm. The left enterocœle communicates with the exterior by means of a "water-tube" and pore, which occupy their usual position on the left side of the stomach. The restricted area occupied by the lateral enterocœles posteriorly points to the conclusion that these larvæ have by no means yet reached the final stage in their development.

The dorsal wall of the præ-oral appendage is lined by a distinct longitudinal sheet of elongated mesenchymatous muscle-cells, which are especially well developed in the region of the bend formed by the origin of the anterior dorsal arm from the stalk of the præ-oral appendage.

2. Habits.

The larvæ were captured a few miles south of the Mewstone at Plymouth. They were taken in tow-nets worked, as a rule, just above the sea bottom; once or twice they were found in the surface layers of the sea. Many of the forms taken with the larvæ were Atlantic animals which rarely visit our shores, but have been unusually abundant this year, probably owing to the prolonged calm and higher temperature of the Channel waters. This fact leads me to imagine that the adult of this form is a starfish living in deep water off the entrance to the English Channel, between the Bay of Biscay and the south coast of Ireland.[1] I invariably found the specimens of this Bipinnaria lazily swimming in the upper layers of water in the tall clear glass jars into which I have tow-netted material transferred on its arrival at the laboratory. The larvæ are almost perfectly transparent and colourless, with the exception of the tips of the posterior lateral arms, which are yellow, and the alimentary tract, which is tinged with yellow and pale brown; but the slight coloration and opacity which the larvæ exhibit are quite sufficient, when associated with their relatively large size and their remarkable mode of swimming, to render them conspicuous objects amid the various forms of life associated with them.

The mode of swimming is quite unique so far as my experience goes. During locomotion, which is usually in an upward direction, the præ-oral lobe is anterior, and the body itself is held quite rigid. Movement is effected by seemingly indolent

[1] G. C. Bourne records having taken "several very large Bipinnaria larvæ and several later stages in Asterid development" during his cruise off the south-west coast of Ireland, July, 1890. 'Journ. Mar. Biol. Assoc.,' 1890, p. 320.

but regularly repeated strokes of the dorsal arm of the præ-oral lobe in an antero-posterior direction over the back of the larva. Fig. 1 represents the position of the dorsal arm shortly before the completion of a stroke. It is obvious that movements of the broad flat dorsal arm in the direction mentioned must propel the larva forward. The strokes of the arm occur with wonderful constancy and regularity, except during short intervals of rest; I counted the number of strokes during three consecutive minutes, and found them to be 80, 81, 81. The dorsal arm of the præ-oral lobe is the only part of the body engaged in this swimming movement. It might be imagined that the ventral arm of the præ-oral appendage would also be used for swimming, but I observed nothing to indicate that such was in reality the case. The ventral arm and all the paired processes of the body were entirely inert during locomotion. The ventral arm may perhaps be called into play at a later stage of development, or its function may possibly be simply that of counteracting any tendency to rotation; in other words, it may take the part of an anterior rudder.

3. Relations.

It has been already mentioned that the Plymouth larva exhibits points of resemblance to the common type of starfish larva on the one hand, and to Sars's Bipinnaria asterigera on the other; it may, indeed, be regarded as a type intermediate between these two forms.

The body of the larva is quite normal, and the arrangement of the ciliated bands differs from that found in common Bipinnariæ only on account of the special development of the præ-oral lobe. There is, however, a difference between the two types of larvæ in the form of the dorsal arms. The common type possesses two pairs of dorsal processes, known in the nomenclature of Agassiz as the dorsal oral and the dorsal anal pairs of arms; in the Plymouth larva three pairs of dorsal processes are present. It will be seen, however, in fig. 1 that the two anterior pairs of dorsal processes of the Plymouth larva arise from a single base on each side, and that this common

base has the same position as the base of the dorsal oral pair of arms in the more familiar type of Bipinnaria. The two anterior pairs of dorsal processes in the former represent, therefore, the single pair of anterior arms of the latter. I have accordingly made a distinction in this case between the terms ciliated processes and ciliated arms; in the larva under consideration the two anterior pairs of dorsal processes clearly represent one pair of bifid arms (figs. 1 and 2, *r. a. d. a.* and *l. a. d. a.*). The significance of this distinction will be seen shortly.

Before proceeding to compare the Plymouth larva with Bipinnaria asterigera I must point out a discrepancy which has been revealed by a study of the literature upon the latter form. Müller's account of the Norwegian larva differs from those of Sars, Koren, and Danielssen with regard to the number of lateral processes. It has already been stated that Sars characterised his Bipinnaria by the possession of twelve arms round the mouth. It is clear from the later descriptions that these appendages correspond with the whole series of paired arms in other Bipinnariæ, although in Sars's larva they are crowded together in a group at the posterior end of the body. Koren and Danielssen agree with Sars in saying that there are six pairs of appendages, and they give a figure showing the arrangement of the arms. The most anterior pair corresponds exactly with the pair of anterior ventral arms of other Bipinnariæ, as it arises as a pair of processes from the præ-oral ciliated ridge. The remaining five pairs arise from the dorsal and posterior sides of the larva, and are clearly processes from the post-oral ciliated ridge; they are therefore comparable to the five pairs of processes from the same ridge in the Plymouth larva. Müller, however, not only mentions that there are six pairs of processes from the post-oral ridge, but he also gives a figure of them. He does not contrast this number with that given by the Norwegian naturalists, so that it is quite possible that he was mistaken. It is certainly not likely that any difference in this respect really existed; for the two specimens examined by Müller were

taken at the same time and from the same place as those described by Koren and Danielssen. If I am right in this supposition there is an exact correspondence between the Plymouth and the Norwegian larvæ in the number of ciliated processes. The similarity between the two forms may be traced even to details. Koren and Danielssen state that there are three pairs of processes from the back of their larva, and this is also the case in the Plymouth larva; further, one of Müller's figures (l. c., Taf. ii, fig. 1, Nos. 6 and 7) shows that the two anterior dorsal processes arise from a common base on each side, and they therefore represent a single bifid dorsal arm similar to that described in the Plymouth larva. The paired ciliated arms of the Plymouth form differ from those of the Norwegian simply in their size and arrangement, being smaller and less densely crowded together in the former than in the latter.

But in the case of the unpaired præ-oral arms there is a marked difference between the two forms. In the Norwegian larva the dorsal arm is bifid at its end, and expanded into a broad bilobed fin; in the Plymouth larva it is broadly lanceolate, and tapers to a point at its extremity. This is the only structural difference between the two larvæ, but its significance cannot be definitely determined until it is possible to trace the later development of the Plymouth larva. The much greater elongation of the præ-oral lobe and the greater length of all the paired ciliated appendages in the Norwegian form are correlated with its mode of locomotion; this has not been described with much exactness, but it has been stated that during life all the ciliated arms, both præ-oral and post-oral, are in a state of incessant agitation. In the Plymouth larva, as already mentioned, locomotion is carried on by means of the præ-oral lobe alone.

It is in this fact that the chief interest of the Plymouth larva seems to me to reside. A great development and specialisation of the præ-oral lobe for locomotive purposes is found again in Balanoglossus, although all traces of ciliated bands are lost in the course of its development from Tornaria.

This loss has obviously been brought about by the assumption of burrowing habits after the ancestor of Balanoglossus ceased to lead a pelagic life. The existence of Asterid larvæ in which the præ-oral lobe is specially developed for the purpose of swimming in the open sea seems to me, when the close similarity of Tornaria with a simple Asterid larva is taken into account, to point to the idea that the pelagic ancestor of Balanoglossus was also provided with a muscular and flexible præ-oral lobe bounded by the two ciliated bands, and used for swimming. In Balanoglossus, after the adoption of life upon the sea bottom, the præ-oral lobe, developed under pelagic influences, was used for moving about in the mud, and rapidly lost its primitive ciliated bands under the new conditions.

Literature.

1. Koren et Danielssen.—"Sur la Bipinnaria asterigera," 'Ann. des Sciences Naturelles,' 1847 (3), vii, pp. 347—352, pl. vii.

2. Müller, Johannes.—"Ueber die Larven u. die Metamorphosen der Echinodermen," 'Abh. d. k. Akad. Wiss.,' Berlin, 1850, pp. 81—94, pl. ii; 1851, pp. 61—65, pl. vii.

3. Sars, M.—'Beskrivelse og Iagttagelser over nogle mærkelige eller nye i Havet ved den Bergenske Kyst levende Dyr.,' Bergen, 1835, pp. 37, 38, pl. xv.

EXPLANATION OF PLATE 28,

Illustrating Mr. Garstang's paper on "Some Bipinnariæ from the English Channel."

Fig. 1.—Bipinnaria from Plymouth, viewed from right side, enlarged. The specimen was preserved, but retained its natural form. All outlines determined by the camera lucida. Ciliated bands.—*a. b.* Adoral band. *a. c. o. b.* Anterior (præ-oral) section of primitive circumoral band (= cephalotroch of Lankester). *p. c. o. b.* Posterior (post-oral) section of same (= branchiotroch of Lankester). Ciliated arms on the anterior (præ-oral) band.—*p. v. a.* Præ-oral ventral arm. *r. a. v. a.* Right anterior ventral arm. Ciliated arms on the posterior (post-oral) band.—*p. d. a.* Præ-oral dorsal arm. *r. a. d. a.* and *l. a. d. a.* Right and left anterior dorsal arms, each bifid. *r. p. d. a.* and *l. p. d. a.* Right and left posterior dorsal arms. *r. p. l. a.* and *l. p. l. a.* Right and left posterior lateral arms. *r. p. v. a.* Right posterior ventral arm. *a.* Anus. *m.* Mouth. *p. e.* Præ-oral enterocœle. *r. e.* Right lateral enterocœle. *x.* Morphological apex of præ-oral lobe.

Fig. 2.—Diagram of same from ventral side, to show the course of the ciliated bands. Letters as above.

Octineon Lindahli (W. B. Carpenter): an Undescribed Anthozoon of Novel Structure.

By

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With Plates 29 and 30.

This interesting form, dredged during the cruise of H.M.S. "Porcupine" in 1870, was originally studied by Dr. W. B. Carpenter, from whose drawings two plates were lithographed for publication in the 'Philosophical Transactions' of the Royal Society; but unfortunately no account of the investigation was found among his papers. The specimens were then entrusted for description to my honoured teacher, Professor H. N. Moseley; and on the last occasion that I saw him he talked to me with his customary infectious enthusiasm about this Anthozoon, on which he was at the moment engaged. He, however, was unable to finish the work, and the late Dr. Philip Carpenter then asked me to undertake it. Professor Moseley left only a description of Dr. Carpenter's figures and an introduction to his own paper; the latter, although only a rough draft, is printed here without alteration of any kind:

"In their 'Report on Deep Sea Researches,' carried on during the months of July, August, and September, 1870, in H.M. Surveying Ship 'Porcupine,' Dr. Carpenter and Mr. Gwyn Jeffreys ('Proc. Roy. Soc.,' 1871, p. 159), in their narrative of their first cruise, state as follows:—'The most remarkable novelty here [off the south coast of Spain, not far from Cape St. Vincent, in 364 fathoms] obtained was a large

collection of thin sandy discs, from 0·3 to 0·4 of an inch in diameter, with a slight central prominence; for these proved, on subsequent examination, to contain an entirely new type of Actinozoon, extraordinarily flattened in form and entirely destitute of tentacles. Dr. Carpenter, by whom this curious organism will be described, has assigned to it the name of Ammodiscus Lindahli. Further on, off Cadiz, the same curious form was obtained in depths of 227 and 386 fathoms; and on the next page of the report it is again stated that the Ammodiscus will be worked out by Dr. Carpenter."

"Apparently nothing further has been published concerning this undescribed Actiniarian. Although Dr. Carpenter devoted much time to the investigation of its structure, and made a large set of microscopical preparations of its various parts, and although he frequently mentioned it and dwelt on its importance to friends, and to myself amongst the number, he never found time to write an account of his results for publication. He, however, did prepare two quarto plates in his scrupulously careful manner of excellent figures of the animal, both entire and dissected in various ways, so as to display its most important internal structure and arrangement. Unfortunately he left no manuscript of any kind—not even a description of the plates; and the only clue to his views as to the component structures is to be derived from the series of microscopical preparations,[1] which is fully labelled throughout. The two plates, the whole series of preparations, and a considerable supply of specimens of the animal in spirits and in the dry condition were placed in my hands by Dr. Carpenter's family, with a request that I should work out a memoir from these materials embodying his results for publication, adding from my own observations anything that might be required."

"Difficulties of manipulation: sand."

"It is singular that Dr. Carpenter applied the term Ammodiscus to this new form, as this name had been conferred on an arenaceous Foraminifer in 1861. It is necessary that a new generic title should be adopted, and I propose to call the form

[1] These have not come into my possession.—G. H. F.

Octineon, from the characteristic octameral disposition of its large retractor muscles."

The animal is, therefore, Octineon (Moseley, MS.) Lindahli (W. B. Carp.).

As, in addition to the foregoing introduction, Professor Moseley left only a description in general terms of Dr. Carpenter's figures (utilised for those of the figures which have been redrawn for the present paper), it will be obvious that the whole investigation had to be begun afresh for a third time. The difficulties of manipulation to which Professor Moseley refers proved to be considerable, the animal being too small and too brittle to allow of much dissection, while the sand particles, which not only cover the external surface thickly, but are carried deep into the body of the animal by the invagination of part of the body-wall, ruin alike razor and section.

No specimens of Octineon have been recorded besides those of the "Porcupine" expedition; but Professor Lankester informs me that he has dredged an organism of the same appearance on the Terebratula ground beyond Capri. I forwarded one of the "Porcupine" specimens to my friend Dr. Paul Mayer at Naples, with the request for others in an expanded condition; but this ground is seldom visited, and the search for Octineon has not as yet been successful.

The normal form of the animal in contraction (fig. 1) appears to be that of a flat disc from which a truncated cone projects centrally upwards. All the specimens were in this state of extreme contraction. In diameter the disc varies between three and seven sixteenths of an inch, and the total thickness, including the cone, varies between one and three sixteenths of an inch.

Whether from damage to the animal, or from some other cause, the outline of the disc is often most irregular, and the position of the cone becomes thereby excentric (figs. 3, 4, 5). A few specimens are rather plano-convex and less flattened; so far as I have seen, these are the specimens in which generative organs are being developed.

The whole exterior of the animal is densely covered by particles of micaceous sand, Foraminifera, and sponge spicules, which are firmly embedded in the mesoglœa of the body-wall, after the manner characteristic of the Zoantheæ. This exterior is divisible into two surfaces, of which the lower plane surface appears to correspond to the limbus or pedal disc of ordinary Actiniaria, but is obviously never attached in adult life to stones or other large objects; the upper and more convex surface, and the cone, correspond to the lower part of the column.

At the truncated apex of the cone (fig. 1) is the opening through which oral disc, tentacles, &c., have been withdrawn. It exhibits in many specimens eight grooves which project slightly towards its centre, and which are doubtless due to the contraction of the eight retractor muscles. This opening leads into a tube which passes vertically downwards, and which has a sandy coating like that of the exterior (cf. figs. 10, 23); this appears to be the upper part of the column pulled inwards and downwards in retraction, and passes into the oral disc (fig. 23) from which spring the tentacles; below this, again, is the stomodæum. The relative position of the various regions in retraction is, therefore, that of an ordinary Actinia, and is effected in much the same manner, viz. by retractor and sphincter muscles, although these are markedly different from the corresponding structures in Actinia.

It is probably attributable to the tough character of the body-wall that the preserving fluid (apparently spirit) has been unable to penetrate, and that the cells of the interior have consequently been for the most part reduced to a pulp in which their length and distribution are alone recognisable.

In scraping off the sandy particles from the exterior surface of the organism, a necessary preliminary to section-cutting, but frequently detrimental to the anatomy, it is noticeable that the sand tends to come away in little sheets, in which the particles are cemented together by a gelatinoid substance, staining uniformly both with carmine and with hæmatoxylin, and formed apparently of an outer layer of mesoglœa. No ectoderm-cells were traceable anywhere on the exterior, a

similar condition holding also in the Zoantheæ; presumably, therefore, the secretion of (?) mesoglœa for the adhesion of the sand is effected by wandering cells from the endoderm, but I have not been able to detect their presence.

When stripped of the incrusting sand the animal has the appearance represented in fig. 2; the surface of the mesoglœa is pitted by impressions of the sandy particles, and lines indicative of the mesenteries radiate towards the central cone. The external body-wall thus exposed consists of a thick mesoglœa, lined internally by a cubical endoderm, and is provided with a single layer of endodermal circular muscle-fibres. This description applies also to that part of the column which is turned inwards and downwards in retraction, but must be extended by the fact that there is a profusion of "mesodermal" circular fibres in this region, intermingled with a few longitudinal fibres which are doubtless used in expansion; the mesoglœa is extremely thick. Just before this inturned part passes into the oral disc the sand particles are no longer adherent, the ectoderm is met with for the first time, and, like the mesoglœa below it, is thrown into six folds or ridges (fig. 13).

The oral disc is, in complete retraction, pulled outwards and downwards into pockets which contain the tentacles, as is so frequently the case in Actiniaria and Madreporaria, this withdrawal being effected by the mesenteries. I have not been able to assure myself of the exact number of the tentacles either by sections or dissections. In addition to the difficulties of investigation already mentioned, the exact anatomical relations are often very hard to make out, because, owing to the great vertical length of inturned body-wall plus oral disc plus stomodæum, the two latter are generally bent round in a J-shape in most cases (fig. 7), rendering the interpretation of sections troublesome. So far as I can make out (and I infer that Carpenter and Moseley were of the same opinion) there are twelve tentacles, arranged as is shown in fig. 9, with the addition of a twelfth tentacle on the left of the upper directive mesenteries. This would amount to an entocœlic tentacle

over each of the six chief pairs of mesenteries, and six additional (? entocœlic) tentacles between them. In the histological condition of my specimens no difference is apparent between the stomodæum and the oral disc. The contortion of the specimen was so considerable that I am unable to speak to the presence of siphonoglyphs.

It is in the arrangement of the mesenteries and their muscles that the chief interest of Octineon consists. Their exact number appears to vary considerably in different specimens: generally there are about thirty-five to forty-five; but as they are almost all devoid of filaments and of generative organs, their exact number probably has no particular significance. They are generally, but not always, in pairs of approximately equal breadth.

There are, however, always twelve larger mesenteries, which we may safely term "primary," which appear to reach the stomodæum, and to extend downwards to be inserted on the pedal disc. In spite of its Zoanthid habit of forming a sandy incrustation of foreign bodies embedded in the mesoglœa, Octineon is therefore a Hexactinian, as possessing twelve primary mesenteries; but the next point that I wish to bring out is, that of these twelve Hexactinian mesenteries only eight carry the extraordinarily powerful retractor muscles, and that these muscles are arranged in the manner characteristic of a third group, the Edwardsiæ. We have therefore, in Octineon, an Actiniarian with the characteristic habit of a Zoanthid, with the twelve mesenteries of a Hexactinian, and the eight muscles of an Edwardsid.

The section, fig. 11, is taken near the apex of the cone, and exhibits the twelve primary mesenteries. From the central tube, which is the inturned body-wall, they are cut off by the large stoma represented in fig. 23. In these mesenteries at this height, as in all the inferior mesenteries, the fact that a single layer of weak muscle-fibres occurs on the extremely thin mesoglœa lamina is recognisable. (An obvious exception is noticeable in the mesenteries 3, 3, and will be referred to below.)

There are in all four types of mesenteries present (fig. 12):—

(i) The majority have very thin laminæ of mesoglœa; very few of them ever reach the stomodæum, and then only as minute ridges on its surface which do not stretch across the cœlenteron. They carry neither filaments nor generative organs, and their musculature is so slightly developed that it is impossible to make out whether they form "pairs" according to the ordinary standard or not. (ii) Of the twelve primary mesenteries, two (5, 5) are only distinguishable from the former set by their greater length, by the longer ridge which they make on the stomodæum, and by their position with regard to the other primary mesenteries. They are devoid of filaments, generative organs, and well-developed musculature. (iii) Two others (3, 3), of similar length to those last mentioned, and, like them, represented on the stomodæum only by a central ridge, have at the free edge of the peripheral part a considerable swelling produced by plication of the mesoglœa for the attachment of muscle-fibres. They therefore carry a special pair of muscles, but are devoid of filaments (and generative organs?). Judged by the direction of the fibres, which run nearly vertically in retraction, and are at right angles to the retractors, these muscles appear to be "depressors," tending to flatten the animal when retracted. They have much the same appearance as in fig. 11 for their whole course throughout a transverse series of sections. (iv) The remaining eight mesenteries are of a remarkable type, the key to which appears to lie in the fact that the retractor muscle-fibres are shifting off from the mesenteries, and becoming separate strong muscles with belly and "tendon."

The sections schematised in figs. 14 to 18 are made from camera drawings of the same mesentery and muscle at different heights, and are from a "transverse" series, that is, in a plane parallel to the pedal disc. They should be compared with the restoration in fig. 23, it being borne in mind that the latter for clearness' sake represents the animal in a slightly less contracted condition. The mesentery consists, not of a single lamina of mesoglœa with pleatings for the increased adhesion-surface of muscle-fibres, the usual Hexactinian condition,

but of a peripheral plate (*a*) at the side of which is fastened a lateral plate (*b*); the latter unites below with a central plate (*c*) which projects radially outwards from the oral disc and stomodæum, and is of course merely that part of the mesentery which is on the central side of the stoma or perforation represented in fig. 23. The peripheral and central parts are alone shown in fig. 11, a section taken above the level at which the lateral plates grow out. In the thickened and slightly muscular free edge of the peripheral plates it is easy to recognise the homologue of the muscular thickenings of the mesenteries numbered.

Continuous centrally with (*c*), laterally with (*a*) and (*b*), and inferiorly and peripherally by a sort of tendon (*d*) with the pedal disc, is the huge retractor muscle, the general relations of which certainly suggest that it is shifting off from the mesentery. This interpretation is even more strongly suggested by figs. 19 to 22, which are taken from a "vertical" series,—that is, parallel to the plane of section in fig. 23, and read from without inwards. They are, therefore, approximately transverse to the muscle itself, and exhibit its relations to the pedal disc. The whole structure is of considerable interest, and, if the Anthozoan origin of cœlomate animals be accepted, may even be taken to indicate the method of evolution of primitive muscles (aggregates of muscle-fibres) at the sides and from the walls of the archenteric cœlomic pouches.

The mesenteries which exhibit this curious shifting of the muscles are eight in number, and, as already mentioned, they are grouped in the same manner as in the Edwardsiæ (fig. 12); a pair of "dorsal" directives is followed on each side by two (not a "pair" of) mesenteries, with their muscles on the "ventral" sides; and these face towards a pair of "ventral" directives. They carry what appears to be a trilobed filament; the ova lie in the usual position, embedded in the mesoglœa lamina (fig. 22).

Phylogenetic Considerations.

The anatomy of Octineon having been described, its systematic position demands consideration. That it is a Hexac-

tinian, with twelve primary mesenteries, of which two opposite pairs are directives, is fairly certain, in spite of the simple character of the remaining mesenteries. But the structure of its retractor muscles is so unlike that known in any other Anthozoan, that it must stand as the type of a new family, the Octineonidæ, characterised by a specialisation of the retractor muscle-fibres into a muscle separate, or nearly so, from the mesentery; probably also by other of its characters.

The next question for discussion is—Does the structure of Octineon yield any indication of its phylogenetic history, or of that of other Anthozoa? Two important papers[1] have appeared of late on the interrelations of the various groups of Anthozoa, both possessed by a central idea; namely, that the Anthozoa having a larva with eight mesenteries, the muscles of which are in many cases arranged as in Edwardsia, the Edwardsiæ are the modern representatives of the starting-point from which all our present groups originally diverged. The premiss of the argument, however, has been contradicted by van Beneden[2] so far as relates to the Ceriantheæ; it is true of about half of the recorded cases of Hexactinian development, and is an inference only in the Zoantheæ.

While admiring the brilliant ingenuity with which McMurrich and Boveri have worked out their suggestive theory, I must say that its weakness seems to lie in the absence of evidence as to what constitutes homology among mesenteries and siphonoglyphs, on which it is mainly based.

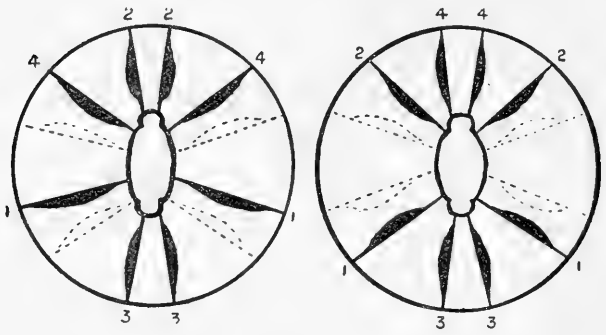
In the case of the Hexactiniæ, with which we are alone concerned at the moment, there may be an homology among the first six pairs of mesenteries when two of these are directives; at any rate, there exists no present reason to deny it to them. But the stage with twelve mesenteries is preceded by a stage with eight, a resting stage in the development of some dura-

[1] Boveri, "Ueber Entwicklung und Verwandschaftsbeziehungen der Aktinien," 'Zeit. wiss. Zool.,' xlix, 461. McMurrich, "Contributions on the Morphology of the Actinozoa: iii, The Phylogeny of the Actinozoa," 'Journ. Morph.,' v, 125.

[2] Van Beneden, "Recherches sur le Développement des Arachnactis," 'Arch. Biol.,' xi, 115.

tion, generally admitted to indicate an eight-mesenteried ancestor. Now this eight-mesenteried stage is unfortunately reached in the Hexactiniæ by various methods.[1] The moment that we begin to apply homologies to these octomeral larvæ we

Fig. A. Fig. B.



The chief eight-rayed types of Hexactinian and Madreporarian larvæ. The numbers indicate the order of appearance of the mesenteries; the dotted outlines, the position of the future mesenteries which make up the total twelve primaries.

i. Fig. A represents Sagartia, Actinia, Bunodes (Lacaze-Duthiers, corroborated by F. Dixon), an unknown larva, ? Bunodes (Boveri), and Cereactis (Boveri).

ii. Fig. B holds for Adamsia (O. and R. Hertwig), and an unknown larva, ? Tealia (Boveri).

iii. A third type differs from that of Fig. A in transposing the order of appearance of 2 and 4, and of 5 and 6 (not numbered in the figure); this occurs in Rhodactis (McMurrich), Manicina (H. V. Wilson), and Cereactis (Cerfontaine).

iv. A fourth variation is described in Halcampa (Faurot), which transposes 5 and 6 of type iii.

The four types therefore read—i. 3, 6, 1, 5, 4, 2; ii. 3, 1, 5 + 5, 2, 4; iii. 3, 5, 1, 6, 2, 4; iv. 3, 6, 1, 5, 2, 4.

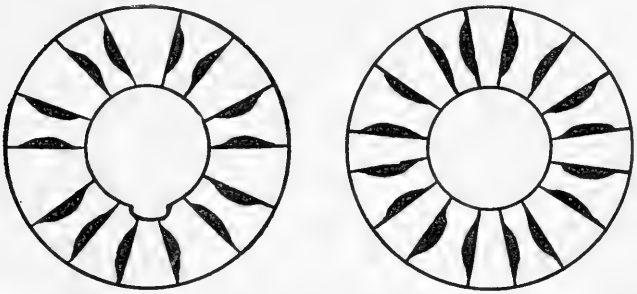
are met by a ring of difficult choices. If the twelve primary mesenteries are homologous throughout the various genera and species, there are at least three lines of descent in the group,

[1] In the Cerianthea, according to van Beneden, the octomeral stage is apparently attained by yet another path.

three octomeral larvæ of phylogenetic value with different muscular arrangements, which at first sight is unlikely. That the eight mesenteries of one type are all homologous with those of another is contradicted by a glance at the diagrams given above; they have different muscle-relations, and hold different positions in the twelve-rayed and adult stages. An objection of less weight is that if there exist that detailed homology which would follow from the value assigned to them by McMurrich and Boveri, mesenteries holding similar positions should appear in the same order in both cases, but obviously do not. To refer the differences between these two types to a "Vereinfachung," or to an "abbreviation" of development, is no explanation, and may be made to cut equally well in two directions.

Again, there arise the cases of the Monauleæ (Hertwig, 'Report on the Actiniaria,' Supplement; Chall. Rep. Zool., xxvi) and of the Holactiniæ (Boveri, "Das Genus Gyractis, eine radial-symmetrische Actinien-form," 'Zool. Jahrbücher,' Abth. Syst., vii, 241).

Fig. C. Fig. D.



Diagrams of the mesenterial relations of the Monauleæ (C) and of the Holactiniæ (D).

The essential difference between these and the Hexactiniæ lies in the fact that one pair only of 'directive' mesenteries is present in the first group, none (according to Boveri's view) in the second. Where in these can we look for homology with the Hexactiniæ? Is the single pair of directives of the

Monauleæ homologous with the pair marked 3, 3 in Fig. A, p. 470, or with the other? Are the two mesenteries opposite to this pair to be regarded as a "pair" of directives, or do they form two halves of separate pairs? This latter query applies at any point to the Holactiniæ.[1]

To crown the confusion, G. Y. and A. F. Dixon ('Proc. Roy. Dublin Soc.' [n. s.], vi) have described an abnormal Bunodes with three pairs of mesenteries and three siphonoglyphs.

It seems almost impossible in the present state of our knowledge to deny that an eight-rayed ancestor is common to the several groups of the Anthozoa: the Alcyonaria and Edwardsiæ are permanently eight-rayed; the Madreporaria, Hexactiniæ, and (without expressing an opinion on the points in dispute between Boveri and van Beneden, we may add) the Cerianthеæ have all yielded an eight-rayed resting stage in their ontogeny. Nor is it perhaps a wanton use of the evidence to say that this stage is the natural outcome of the earlier four-rayed condition, by further interradial specialisation in the first formed four chambers. The evolution of a scyphistomoid ancestor into the Lucernariæ in one direction, the Anthozoa in a second, the Scyphomedusæ in a third, is very generally accepted. The four mesenteries of the Lucernariæ and Scyphomedusæ, like the more numerous mesenteries of the Anthozoa, appear to have a threefold function: they carry (*a*) digestive cells, (*b*) reproductive cells, (*c*) muscle cells; and it is easy to conceive that, in a simple hydriform ancestor, a

[1] I have cited these two groups merely to call attention to the absence of evidence as to what constitutes homology between mesenteries, not because I can accept them as groups of equal value with the Zoantheæ, Cerianthеæ, Edwardsiæ, and Hexactiniæ. In a discussion of these points we may fairly utilise evidence drawn from the Madreporaria, which anatomically agree so closely with the Hexactiniæ. Is Lophohelia, which is devoid of directives (Fowler, 'Quart. Journ. Micr. Sci.,' xxviii, 1), to be placed with Mussa and Euphyllia (Bourne, 'Quart. Journ. Micr. Sci.,' xxviii, 21) in a group of Monaulic Madreporaria, while Amphihelia (Fowler, 'Quart. Journ. Micr. Sci.,' xxviii, 413), the very next genus, of which the corallum, the mode of growth, and budding agree almost exactly with those of Lophohelia, remains with the Hexactinic corals?

special localisation of digestive cells on ridges (which may be compared to a typhlosole) would lead to a concentration of reproductive cells in their neighbourhood for a better nutrition; and that the general musculature of the body-wall, probably originally both circular and longitudinal, like that of Hydra, might, when carried out along the ridge, become specialised on one side into retractor (longitudinal), and on the other side into protractor (circular) muscles.

The alternative hypothesis—that the ridges or mesenteries grew out for support like buttresses, and that the concentration of digestive and reproductive cells occurred on them secondarily—does not seem so probable, and has neither analogy nor observation in its favour; but the identical concentration of the three functions in Cœlenterate groups which have evidently been long independent of one another (e. g. Madreporaria and Alcyonaria), and the analogy of a typhlosolar increase of absorptive and secretive surface, occurring in many divisions of the animal kingdom, are in favour of the idea first suggested. The way in which one function may be dropped and another retained is well seen in the Ceriantheæ, where the contractile function has been taken up (? permanently retained) by the body-wall, and digestive alternate with reproductive mesenteries. Any mesentery may become specially utilised for a particular function; in Seriatopora and Pocillopora (Fowler, 'Quart. Journ. Micr. Sci.,' xxviii, 1) the six mesenteries specialised for digestion and reproduction are precisely those six which in Madrepora pocillifera and tubigera are arrested, are sterile and devoid of filaments[1] (Fowler, 'Quart. Journ. Micr. Sci.,' xxvii, 1).

[1] These two species of Madrepora were originally identified for me as belonging to the species M. aspera and Durvillei. In the recent 'Catalogue of the Madreporarian Corals in the British Museum,' vol. i, 'The Genus Madrepora' (Lond., 1893, 4to), the author, Mr. George Brook, whose premature death has recently deprived zoology of a careful student of the Anthozoa, has assigned them to the species quoted in the text.

In this case (Pocilloporidæ and Madreporidæ) I think we may safely compare the twelve mesenteries of the polyps concerned, because of the orientation afforded by their axes when compared with the axes of the colony and

Granting, then, the evolution of a four-rayed stage from a hydriform ancestor, and its further advance into an eight-rayed organism, from which branched the five great groups of Anthozoa already mentioned, we may conclude, at any rate until a good explanation is forthcoming of the apparent discrepancy between the larval types shown in Figs. A, B, and C (pp. 470–71), either (i) that, if the first eight mesenteries of the various larvæ are homologous structures, and indicative of an eight-rayed ancestor, this homology does not extend to their musculature, and that mesenteries formed after the first eight are not homologous with mesenteries of similar position in adults whose larvæ are of different types; or (ii) that, if the first twelve mesenteries are homologous structures, they may arise in any order, and this order is not of homologous or of phylogenetic significance; or (iii) that slightly diverging types have reconverged in our present group of Hexactiniæ. All three conclusions appear to me to be almost equally difficult of acceptance, although the third becomes less improbable when the facts are considered (1) that this group, at first sight so homogeneous, is being constantly shown to include Actiniaria which do not conform to the hexameral type; (2) that the order of development of the mesenteries has been efficiently studied in only about eight genera. The improbable conclusion drawn above may therefore be expressed in this way, that both from embryology and from morphology comes evidence to show that the group Hexactiniæ includes two or more groups, not clearly distinguishable in the present state of our knowledge.

When dealing with questions of this kind, in which the evidence is obviously incomplete, a writer can but express his personal beliefs; but, while he cannot claim for them the weight of admitted truths, he is allowed to apply them provisionally in that capacity. On this score the beliefs which I have stated—(1) that mesenteries are to be regarded as having arisen primarily in connection with digestive cells, secondarily

its branches; both families have axial (superior) and abaxial (inferior) directives at the ends of the long axis of the stomodæum, an orientation similar to that of the polyps of Alcyonium.

with reproductive cells and concentrations of muscle-fibres; (2) that the eight-rayed larval stage is of phylogenetic value; (3) that any of these eight or of subsequently formed mesenteries may be specialised for the performance of one function, digestive, reproductive, or muscular (whether protractor, retractor, or depressor), and may drop one or both of the other functions—may now be applied to Octineon.

At first sight this Actiniarian fits perfectly into Professor McMurrich's scheme, taking a place immediately above the Edwardsiæ, at the point where (inter alias) the lines of descent of Hexactiniæ and Zoantheæ diverge—a stage at which mesenteries 5 and 6 of Fig. A (p. 470) are throughout life less developed than the remaining eight primary mesenteries (cf. McMurrich's table, 'Journ. Morph.,' v, 150). Against this view I would urge that the numerous mesenteries of lower orders in Octineon are not incipient mesenteries, 'prophetic germs' of greater efficiency to come, but rather retrograde or arrested mesenteries, which have (phylogenetically speaking) lost their filaments, reproductive organs, and (most of their) muscle-fibres, in correlation with the extraordinary muscular development of ten primaries. The fact that the "pairs" are apparently not always of equal breadth (age) or length, and that some of them die out to reappear at a lower level—that they are, in fact, very irregularly developed—is an additional argument in favour of this view. It can hardly be denied that this degradation is true of mesenteries 5 (fig. 12) if this figure be compared with an ordinary Hexactinian diagram, and these are structurally identical with those of the lower orders. Further, if the shifting (p. 467) of the eight retractor muscles were to be carried a stage beyond its present condition, the eight mesenteries which are at present connected with them (1, 2, 4, 6, fig. 12) would be reduced to the same degraded type. Lastly, if the suggestion made above as to the origin of mesenteries be true, and they are all in the first instance physiologically equivalent, the mere fact of enormously increased efficiency in a few will surely lead either to the gradual obliteration of the rest, or to their adaptation to new

functions. This second alternative has evidently affected mesenteries 3 (fig. 12), but no others.

It is possible that the exaggerated development of retractors has occurred in mesenteries 1, 2, 4, 6, in consequence of the larva of Octineon being of the type figured as A (p. 470). Very interesting are the special muscles of mesenteries 3, in which the direction of the fibres is approximately at right angles to those of the retractors; as stated above, they are probably to be ranked as depressors,—muscles which, if present in other Actiniaria, have not yet been distinguished, and therefore are only slightly developed: their occurrence here, like the shifting of the retractors, indicates the very great specialisation which Octineon has undergone.

To the Zoanthid habit of incrustation of the external surface of the mesoglœa we can hardly attribute systematic importance, although it has not yet been described as occurring outside the group.

The evidence seems, therefore, in favour of the view that Octineon is the type of a new and highly specialised family, descended from true Hexactinian ancestors.

EXPLANATION OF PLATES 29 and 30,

Illustrating Dr. G. H. Fowler's paper on "Octineon Lindahli" (W. B. Carpenter).

PLATE 29.

These figures have been reproduced from the two quarto plates prepared by Dr. W. B. Carpenter, but never published. Professor H. N. Moseley wrote out an unrevised description of these plates, of which such part as relates to the figures selected for reproduction is here printed. My own additions are enclosed in square brackets.

I have been reluctantly compelled to differ from Professor Moseley in interpreting his "stomodæum" as the upper part of the column, his "tentacular chamber" as the oral disc, and his "stomach" as the stomodæum. Continuous series of microscopic sections (so far as I am aware, he was not at work long enough upon the animal to have these prepared) and a comparison with an ordinary Actinia in complete retraction, have left no doubt in my own mind as to the correctness of these interpretations, but I have thought it only just to him to present his actual words, and to make my own alterations in brackets.

Fig. 1.—A typical specimen, enlarged about [seven] diameters, viewed from the oral face. The entire surface is thickly set with fine sand grains, fragments of foraminiferous shells, spicules, &c. At the summit of the central visceral prominence is seen the opening of the invagination of the surface leading to the mouth.

Fig. 2.—A closely similar specimen viewed from the aboral face, with the adherent sand particles completely removed. The finer radial striæ mark the courses of the mesenteries; some of the broader radiating ridges correspond with some of the eight large retractor muscles [and their associated mesenteries. Judging by the shading of the drawing, I have no doubt that "aboral" was only a slip of the pen for "oral;" the aboral surface is very nearly flat, but in the centre of this drawing is a cone with a central pit.]

Figs. 3, 4, and 5.—Examples, with the adherent sand removed, showing irregular varieties in [the] form assumed, produced by the indentation of the margin and the outgrowth of lobes.

Fig. 6.—The invaginated stomodæum [oral disc, and inturned column] removed from the body-cavity. At its base are seen [ten of] the evaginated tentacles [lying in pouches of the oral disc] which there [surround?] the mouth, the entrance to the œsophagus.

Fig. 7.—View of the opposite side of the specimen shown in Fig. 6. The inferior aperture of the digestive tract, the opening of the stomach, is here seen [owing to the J-shaped curvature mentioned in the text]. From the eight rings [ridges?] of tissue surrounding it radiate eight mesenterial fringes; from the margin [of] the stomach, here inverted, six tentacles [in their pouches] are seen to proceed. On the peripheral extremities of [those] mesenterial filaments which lie uppermost in the figure, ovaries are seen to be present. Two large retractor muscles, in a completely retracted condition, lie just above the tentacles which are lowermost in the figure.

Fig. 8.—Base of the stomodæum [or rather, of the inturned part of the column], showing its expansion, and the mode in which the tentacles [lying in their pouches of the oral disc] proceed from its margin. The stomach [stomodæum], lying beneath it, is hidden; but the eight [mesenteries of the] retractor muscles [which were torn away in the preceding figure] are seen radiating from its margins, and some of the ovaries and mesenterial filaments are seen in situ.

Fig. 9.—Specimen viewed from the aboral surface, with a disc of the inferior body-wall removed from the central region, to expose the viscera. In the centre is seen the slit-like pleated inferior opening of the stomach [stomodæum] surrounded by the eight mesenterial filaments which radiate from it. Beyond these, and from beneath their bases, pass outwards the eight long and large retractor muscles [and their mesenteries]. The dorsal and ventral pairs of these [are the directives, and] are placed exactly opposite and in line with each other, and enclose each a single intermesenterial chamber [formed by a?] pair of mesenteries only [while the spaces between the other pairs of primary mesenteries contain a large number of mesenteries of the lower orders]. Between the bases of the muscles are seen the tentacles, eleven of which only appear. [A twelfth is probably present in the sector to the left of the uppermost directives.] The eight mesenterial filaments correspond in position with the eight retractors.

Fig. 10.—Much enlarged view of a but little compressed specimen laid open so as to exhibit the essential internal structures (probably a combined, more or less diagrammatic representation). The stomodæal tube [or rather, the inturned part of the column,] is laid open, and it is seen that the cuticle of the outer surface of the body, with its dense coating of sand and shell particles, is continued to its base. Here it opens into a discoid chamber, which may be called the tentacular chamber [formed by a folding of the oral disc], since [the pouches for] the invaginated tentacles communicate with it all round its periphery by open mouths. [In one of the pouches pointing directly towards the observer, which has been cut across, the tentacle can be seen lying as in a sheath.] In the middle of this chamber below, lies the mouth, leading into the stomach cavity [stomodæum] below. The latter is surrounded by the radiating retractor muscles, with the ovaries showing

beneath them, and by the [sheaths of the] tentacles between the muscles. Six retractor muscles are shown, and eight [sheaths for] tentacles. The directive muscles are apparently the pair on the extreme right and those on the extreme left of the figure [as there is only one entocœlic tentacle sheath between them. It will be noticed, however, that in order to tally exactly with Fig. 9 only one tentacle sheath should be pointing directly towards the observer instead of two. The right-handmost of these is drawn as a tube, but is perhaps the thick "depressor" muscle of mesentery 3 in Figs. 11 and 12. There are in this case only seven tentacle sheaths shown, the same number as indicated by Fig. 9. None of the specimens in my possession were nearly so spacious as the one here figured, but no doubt Dr. Carpenter selected the least flattened and contracted specimens for dissection].

PLATE 30.

These figures are all from my own drawings, and, with the exceptions of Figs. 12 and 23, have been outlined by the camera lucida. The histological condition was so bad that I have represented the body layers diagrammatically (except in Figs. 12 and 23):—ectoderm blocked, mesoglœa black, endoderm a grey line.

a. Peripheral plate of mesentery.
b. Lateral plate of mesentery.
c. Central plate of mesentery.
d. Tendon.
dir. Directive mesentery.
D. "Dorsal" aspect, in the Edwardsia nomenclature.
ext. col. External part of the column.
int. col. Inturned part of the column.
or. d. Oral disc.
ov. Ovary.
pe. d. Pedal disc.
r. m. Retractor muscle.
st. Stomodæum.
te. Tentacle.
te. p. Tentacular pouch of oral disc.
V. "Ventral" aspect, in the Edwardsia nomenclature.

Fig. 11.—Transverse section near the apex of the cone. Internally lies the (broken) inturned part of the column; towards this radiate the peripheral plates of the twelve primary mesenteries and a few of the lower orders; from it radiate the short central plates. The section has cut some of the tentacular pouches, exhibiting in two cases the contained tentacles, and has also shaved the upper part of two of the retractor muscles.

Fig. 12.—Diagram showing the general arrangement of the mesenteries, a description of which will be found on p. 466, et seq.

Fig. 13.—Part of a section vertical to the animal, and at right angles to the plane of the pedal disc. The inturned part of the column, distinguished by its enormously thick mesoglœa, is cut nearly at a right angle, owing to its flexure below; in this region its sandy lining is replaced by ectoderm, thrown by ridges of the mesoglœa into six folds. It is seen to open into the tentacu-

lar chamber (H. N. M.) formed by the oral disc, from which radiate the tentacular pouches. A few tentacles are cut at various angles. At the bottom of the tentacular chamber is the opening into the stomodæum, and through it into the cœlenteron. A few central plates of mesenteries project from the upper surface of the inturned column.

Figs. 14—18 are taken from a series transverse to the animal, and represent longitudinal sections of the same retractor muscle, &c., at various heights. Compare p. 468 and Fig. 23.

Figs. 19—22 are taken from a series vertical to the plane of the pedal disc, and are therefore transverse to the retractor muscle. They exhibit the way in which it appears to be shifting in position away from the mesentery.

Fig. 23.—Diagrammatic vertical section of the animal, slightly less contracted than Fig. 10; it shows both sides of a large mesentery with retractor muscles. The position of the circular "mesodermal" muscle-fibres, which in combination with the retractors achieve the inturning of the upper part of the column, is indicated by dots.

Studies in Mammalian Embryology.

III.—The Placentation of the Shrew (Sorex vulgaris, L.).

By

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With Plates 31 to 39.

A. Introduction.

As a sequel to my investigations on the placentation of the hedgehog ('Quart. Journ. of Microscop. Science,' vol. xxx, part 3, 1889) I was very anxious to collect a similar series of data with respect to different genera of Insectivora, in order to determine to what extent the development of the placenta might be said to agree in various representatives of this archaic order of Mammalia. It will be seen from the following pages—as also from what I hope to publish ere long about Indian Insectivora—that this agreement is very small indeed.

The shrew was in the first place selected[1] as a type of comparison because the mole was then already being studied by Professor Strahl, of Marburg, whose results with respect to these questions have since been published in the 'Anatomische Hefte' von F. Merkel und R. Bonnet, ii, 1892.

[1] A preliminary communication was made by me to the Academy of Sciences at Amsterdam, at its meeting of 27th September, 1890, in which the chief results, which are fully described at this paper, have already been briefly noticed (vide vol. viii, p. 79, of the 'Verslagen en Mededeelingen van de Koninkl. Akademie van Wetenschappen te Amsterdam').

Another paper on the placentation of the mole by one of my pupils is in preparation, and will be published in a few months hence. We shall then be in the possession of sufficient data concerning three European genera of Insectivora.

Although the collecting of a sufficient number of pregnant shrews appeared at the outset to be rather an arduous undertaking, still an organised search, continued through three consecutive summers, furnished me with the material required. Kleinenberg's fluid, in which the freshly extirpated uterus was immersed in toto, proved to be in this case again—after repeated comparative trials with Flemming's mixture and other reagents—the best and surest means of preservation.

The sections were cut after embedding in paraffin by means of Caldwell's or De Groot's microtome; the series were numbered and catalogued in the way indicated on p. 393 of vol. xxx of this Journal.

In the explanation of the plates which belong to this article the catalogue number of the respective section-series from which the figures were taken is everywhere mentioned, and thus a future comparison of these figures with the original sections will always be possible. To such comparison I may be allowed to invite any investigator, who, being occupied in a similar line of research, desires to become acquainted by personal observation rather than by perusal of this paper with the facts as they present themselves in Sorex. I must here mention that not all the shrews whose uteri have served for these investigations have been submitted to a separate specific determination. Moreover for most of them such determination is at present no longer possible, as only a comparatively small number ($\pm$ 100) of the total series of specimens was preserved. Nevertheless the possibility cannot be denied that in some few rare instances the uterus may have been taken not from Sorex vulgaris, L., but from Sorex (Crossopus) fodiens, Pall., which latter species is provided with one tooth less in each upper jaw, but corresponds externally very closely with the common shrew.

As no deviation from the normal succession of develop-

mental phenomena has been noticed in my preparations which would require a similar explanation, we may safely conclude that either the phenomena of placentation are identical in Sorex vulgaris and Sorex fodiens, or that no uteri of the latter species are among those that have been investigated and figured by me.

In the illustrations of this paper I have endeavoured to give the drawings on each plate, as far as possible, on scales of enlargement that will allow of a more direct comparison of the different figures among themselves. In figs. 1 to 16 the general outlines (drawn with the camera) of the process of placentation in the shrew are diagrammatically delineated. The maternal tissues are there represented by red, the embryonic tissues by black lines. The red numbers indicate the catalogue number of the specimens; the small numbers (red or black) have reference to the number of the figure in which the region thus indicated is drawn with more histological detail under higher power.

Leaving the histological questions to be fully discussed further on, I will first, by the aid of these figs. 1 to 15, give a general and succinct description of the principal facts that present themselves concerning the growth of the shrew's blastocyst, and its more intimate connection with the maternal tissues and the maternal circulation.

All these figures have been drawn with the camera from the actual preparations with Zeiss's apochromatic obj. 16 mm., oc. 1, tub. 160, distance between ocular and paper 24 cm. They are thus strictly comparable also in regard to size.

To this paper no chapter is added in which the points of agreement and of difference between the results here obtained and those to which recent investigators (Duval, Strahl, Fleischmann, Marius, Minot, Heinricius, Lüsebrink, &c.) have arrived with respect to other species of Mammalia, are discussed.

Nor are any general and comparative considerations with respect to the theory of placentation here advanced. This was done on purpose, because the material is already at hand

which will enable me to study the placentation process of yet five other genera of Mammalia hitherto not examined. It is preferable to reserve the discussion on points of criticism and on general conclusions, and to describe for the moment only the facts and phenomena as they present themselves in every one of the genera separately.

B. The Process of Placentation in Outlines.

The following paragraph, preceding those in which the detailed observations are recorded, is meant to be a recapitulation of the general features by which the shrew's placentation is characterised. It precedes the full record of the details instead of closing it, because the former will be better understood and more easily grasped if certain general notions have first been discussed.

The placentation of the shrew is brought about by processes that take place in the maternal tissue, and by processes that affect the blastocyst. At the outset these two sets of processes are quite independent of each other; later on, when the blastocyst has come to adhere against the maternal tissue, they are closely related; still later the participation of the mother towards the constitution of the ripe placenta is again reduced to the maternal blood by which this organ is permeated.

The maternal processes are—

1°. Unexpected and somewhat peculiarly shaped local distensions of the uterine wall, accompanied by changes in the distribution of glandular tissue, &c., in this wall.

2°. Considerable local proliferations of maternal uterine epithelium.

The embryonic processes are—

1. Local changes in the outer wall of the blastocyst.

2. Special development of certain portions of the trophoblast which finally constitute a syncytium, in which the allantois-villi and the embryonic blood are in the closest contact with maternal blood, the latter circulating in spaces of embryonic issue without any endothelial lining.

Beginning with the processes in the maternal tissue, we notice that the distensions above referred to change the aspect of the uterine horns, which were originally cylindrical ducts, most considerably.

Not only in this sense, that spherical swellings indicate the advance of pregnancy, as in other mammals, but the distensions take a definite pear shape very soon after the embryo has wandered from the oviduct into the body of the uterus. These swellings are first connected by portions of great tenuity, which only widen in the later stages of pregnancy.

This distension is undoubtedly independent of any direct action of the blastocyst. It is a maternal phenomenon preparatory to the adhesion of the blastocyst. Simultaneously with it the epithelial lining of the lumen of the distended portion undergoes important changes.

These changes consist in a rapid increase of the epithelium cells, which become more than one layer thick, and between which vascular channels are enclosed, the final result being—

1°. A concave bell-shaped surface opposite the mesometrium, on which numerous newly formed epithelial crypts open out, gland openings being here and there interspersed between the much more numerous mouths of the crypts.

2°. Lateral cushion-shaped surfaces, where no similar crypts are present, and against which the blastocyst first adheres by means of a zonary strip.

The embryonic processes are the following:—The blood-vessels of the area vasculosa on the yolk-sac spread out against this zonary strip, those of the allantois (soon ramified in digitate villi) against the concave surface referred to sub. 1°; not, however, before most important changes have taken place in these two regions—changes that consist in the development of a syncytial tissue of embryonic origin out of the outermost layer of the blastocyst.

In this embryonic syncytium two regions—the counterparts of the maternal proliferations above referred to—may be distinguished:

1. A zonary syncytium in the region of the area vasculosa.

This syncytium owes its origin to that portion of the surface of the blastocyst which we will call the omphaloidean trophoblast.

2. A bell-shaped syncytium opposite the mesometrium. This syncytium owes its origin to that part of the outer wall of the blastocyst which expands simultaneously with the formation of the amnion (being, in fact, the epiblast of the outer amnion fold), and which we will call the allantoidean trophoblast.

Both syncytia enclose numerous cavities, into which maternal blood penetrates.

The omphaloidean syncytium with the area vasculosa applied against it is not indented by villi of the yolk-sac, such as are found in the hedgehog. The area vasculosa is after some time removed, the regions against which it has been applied (proliferation and syncytium) being gradually but entirely resorbed. A new layer of maternal uterus-epithelium applied against the muscularis arises directly below the resorbed portions.

When this has come about the bell-shaped syncytium opposite the mesometrium remains alone in the field, and undergoes a series of further transformations and complications, which change it into the full-grown discoid placenta of the shrew.

These transformations can be summarised as follows:

(*a*) The allantoidean trophoblast is applied against the concave maternal surface, and sends knob-like projections into the mouths of the maternal crypts, the maternal epithelium being destroyed wherever the trophoblast adheres. The projections serve to fix the trophoblast very firmly against the maternal proliferation. They do not enter the mouths of uterine glands; these are simply blocked by the trophoblast.

(*b*) The trophoblast undergoes a differentiation into an outer layer, which assumes the syncytial character more fully, and contains paler nuclei (plasmodiblast—van Beneden), and an inner layer of which the nuclei stain more strongly (cytoblast—v. Beneden).

(*c*) Internuclear blood-spaces are developed in the plasmodi-

blast, and enter into communication with the maternal spaces that have been laid bare after the disappearance of the maternal epithelium.

(*d*) The trophoblastic protuberances that have penetrated into the crypts are hollowed out. Allantoidean villi enter into these cavities.

(*e*) The allantois sends new villi against the cytoblast, which, however, do not continue to grow centrifugally. The cytoblast itself grows centripetally, the peripheral portions being gradually transformed into plasmodiblast, the central portions at the same time ensheathing the newly formed villi. The latter are thus, while gaining in length, enclosed by an identical trophoblastic matrix (in which maternal blood circulates), as are their earliest predecessors.

(*f*) As the placentary region increases in breadth, space is gained for the free development of these secondary villi. The maternal proliferation at the same time flattens out to a superficial covering of the growing placenta, and is finally reduced to isolated nuclear remnants.

(*g*) In the final stage of the placenta the allantoidean villi are no longer recognisable as such, and the intervening trophoblast is stretched to the utmost; consequently there is only the thinnest layer of plasmodiblast tissue to separate the maternal blood fluid from the embryonic.

Trophoblastic lacunæ containing maternal blood can be very easily distinguished from those spaces in which embryonic blood circulates by the fact of the much smaller size of the maternal blood-corpuscles.

In the ripe placenta the most intricate intermixture of these two sets of spaces is thus detected with great facility, whereas the details of the genetic history of the placenta that are here brought forward can by this important detail be easily traced and tested.

The recapitulation here given shows that the placenta is essentially an embryonic neo-formation, which is permeated by maternal blood that circulates in spaces devoid of endothelium. This embryonic neo-formation is preceded by a

considerable proliferation of maternal epithelium, which, however, does not enter into the constitution of the ripe placenta, but affords facilities of fixation and nutrition for the embryonic neo-formation in its earliest stages.

The discoid placenta is, in the later stages of pregnancy, the only connection between fœtus and mother. The zonary connection in the omphaloidean region is only temporary. Below this another ring-shaped modification of trophoblast appears at an early stage, and persists till the end as a thickened circular membrane, forming an annular constriction round the allantoidean vessels that connect the fœtus with the placenta. There is reason to suppose that both this subdivision of the trophoblast and that which is applied against the mesometrical surface of the uterus-lumen play a part in the transport of nutritive material to the inner cavity of the yolk-sac, where it is being absorbed by specially modified hypoblast-cells.

C. Details of the Process of Placentation.

1. The Changes in the Uterine Tissues.

The various and successive changes by which the uterine tissues are affected during pregnancy visibly influence the outward aspect of the uterus. The position of the embryo and placenta is not only marked by a swelling which increases in size as pregnancy advances, but this swelling undergoes certain unexpected changes of shape which we shall have to notice in some detail. Several figures of the uterus in early stages of pregnancy were already given in my paper on the development of the germinal layers of Sorex (this Journal, vol. xxxi, 1890); of these I will here refer to figs. 13, 14, 15, 42, 65, and 83.

The swellings are knob-like projections separated by the more tubular portions (fig. 47). With advancing pregnancy the rounded knob becomes more ovoid, the tubular connecting portions being, if possible, yet narrower (fig. 39). Of the figures here alluded to, the following correspond to the figures of transverse sections given in this paper:

Fig. 83 (1890) is the uterus of which a section is figured in fig. 7 (1893).
„ 65 (1890) corresponds to . . . . „ 6 (1893).
„ 42 (1890) „ „ . . . . . „ 5 (1893).
„ 15 (1890) „ „ . . . . . „ 4 (1893).
„ 13 (1890) „ „ . . . . . „ 3 (1893).

On Pl. 34 of this paper further stages of pregnant uteri in external view are figured. The numbers between crotchets have reference to the catalogue number of the specimen; and as these numbers are also attached in red type to the transverse sections of Pls. 31 and 32, it will be easy to compare these two sets. The egg shape of the swelling (figs. 4—10) is seen to be soon exchanged for a more pear-shaped one with a faint constriction in the middle (figs. 11—13). The furthermost projecting surface is the incipient placenta; the swelling closer to the mesometrium is that of the inverted yolk-sac and of the growing embryo itself. This latter swelling very soon is seen to increase at a more rapid rate than the former; in fig. 38 it has become somewhat triangular, the placental area projecting in knob-like fashion. The connecting portions are yet very narrow tubular ducts. As pregnancy advances further these portions are finally also widened out, and in the fully ripe uterus the embryos are no longer separated by such formidable constrictions as in the early stages, the placental area having at the same time become much more flattened out (figs. 44, 45).

We now turn to the transverse sections, and the various histological changes which accompany this transformation in the outward shape of the uterus.

In the stage of figs. 1 and 2, where the embryos are yet contained in the oviduct, and where any outwardly visible local swelling is not detectable, we see the uterine muscularis still in its full thickness, the outer layer of fibres being longitudinal (fig. 16), the inner circular. Inside of the muscularis the mucosa is of very varying thickness, as can be gathered at a glance from the figs. 1, 2, and 16. Close to the mesometrium a folded epithelium without any uterine glands opening out into the lumen is present, and separated by an inconsiderable

layer of connective tissue from the muscularis. The glands are concentrated along the opposite surface, even more to the right and to the left than exactly opposite the mesometrium, where the mucosa is again somewhat thinner (fig. 16). In this glandular portion the glands are very closely packed together, and very tortuous; they open out in that portion of the lumen which in fig. 1 and fig. 16 forms a longitudinal groove opposite the mesometrium. This narrow groove is thus flanked by two cushion-shaped swellings of the mucosa. In these thickenings more considerable blood-vessels are present right and left (fig. 16, *bl.*); smaller blood-vessels are seen between the muscularis and mucosa, capillary ducts between the glands.

It is very remarkable how in the next stage the transverse section of this same region has undergone very considerable changes, independently of any direct or active co-operation of the blastocyst, which is as yet not adherent to the uterine wall.

If we take the mesometrium as our starting-point, we notice that in fig. 3 there are yet traces close to this mesometrium of the two lateral recesses of the uterine lumen which were visible in fig. 1, below the cushion-shaped swellings, carrying glands and blood-vessels. Instead of the groove-shaped portion of the uterus lumen that was found opposite the mesometrium in fig. 1, and that formed a ⊥ shape with those two lateral recesses, we now find a wide bell-shaped space, between which and the different parts of the uterine wall the relations, more especially with respect to comparative thickness, have become very different. Better than a detailed description, a comparative glance at figs. 3 and 1 will explain this process. A most considerable amount of stretching has taken place, the antimesometrical part of the uterine wall has been reduced to one half and even less of the thickness it had in the preceding stage, and only the cushion-shaped regions yet fairly recognisable as such have increased in thickness. The distribution of the glands has assumed a very different aspect; they are no longer close together, but stretched over a wider area, and

more numerous to the right and left than opposite to the mesometrium. Their openings are in the same place as before, but instead of the perpendicular groove of figs. 1 and 16, the region where they open out has become the upper part of the concave upper wall of the bell-shaped lumen.

The blood-vessels are found in corresponding situations as before. The lateral swellings have chiefly increased by connective-tissue proliferation, the muscularis having here also become thinner (compare figs. 16 and 18), and the epithelium being as yet only one cell-layer thick. The histological details are indicated in figs. 17, 18, and 66. In the last figure it is seen that the epithelial lining also of the antimesometrical concave surface is in this stage not more than one cell thick.

It is this point which is the first to be modified. And this modification is at the same time the most important change that takes place in the maternal tissues preparatory to the reception, fixation, and nutrition of the blastocyst. Moreover it is a process which in other mammals has up to now not been noticed, rather the contrary. The most recent trustworthy observations on the placentation of mammals have brought to light numerous instances amongst Insectivora, Rodentia, Carnivora, and Cheiroptera, where the maternal epithelium of the uterine lumen disappears in early stages of pregnancy. I have myself described and figured this phenomenon in the hedgehog. And thus it is certainly both an unexpected and an important fact that in the shrew proliferation of this same uterine epithelium takes place to a very considerable extent, and that the cell material resulting from this proliferation is of such high importance for the further attachment of the embryo. Still we shall afterwards have to notice that the complicated epithelial arrangement resulting from this proliferation is not permanent, but that it disappears and is destroyed either simultaneously or some time after the embryonic trophoblast becomes attached to it, thus bringing about a final stage in which comparison with the other mammals (where the uterine

epithelium disappears without any special antecedent proliferation) is again possible.

This epithelial proliferation must now be described in detail. When we compare fig. 4 with fig. 3 it is seen that the diameter of the uterus and of its lumen have not undergone any appreciable increase. Yet the epithelial proliferation has commenced in fig. 4, and is already three to five cells thick in what will afterwards be the placental region ; twelve to eighteen cells on the two lateral cushion-shaped surfaces, where by this time (cf. fig. 4) the blastocyst has commenced to adhere to the maternal tissues. The difference between this and the foregoing stage is still better seen if we compare fig. 19 with fig. 17, fig. 20 with fig. 18, whereas figs. 67—69 will allow us to discuss the histological detail. The preparation figured in fig. 67 leaves no doubt as to the proliferation being epithelial.

Here—and also in very many sections that were not figured —the karyokinetic processes (well preserved and sharply defined after staining with picro-carmine) leave no doubt about the origin of the proliferated cell matter. It is the epithelial cells lining the lumen that throw off new cells, which become mixed up with connective-tissue elements that belong to the layer situated between the epithelium and the muscularis. However, the epithelial elements are much more numerous than the connective-tissue ones. The proliferating process goes on more rapidly on the lateral cushion-shaped surfaces; the difference between the epithelial layer and the cells that have originated out of it is soon effaced; these cells are themselves rapidly multiplying, and thus the first distinction is about this time created between the lateral maternal tissues against which the yolk-sac adheres, and the bell-shaped maternal surface, against which the allantois is going to be applied. Although there is no sharp boundary line, still in fig. 20 and fig. 4 it is easy enough to distinguish them in a general way.

Another distinguishing feature between these two portions of the uterine surface is of great importance, viz. that in the placental region the proliferated epithelial elements are very soon seen to arrange themselves in a particular manner below

the epithelial layer from which they have sprung. At comparatively regular distances the cells of the proliferation arrange themselves in a peculiar radiating fashion, leaving a central part without nuclei surrounded by an overcapping layer of nuclei (fig. 69). In transverse sections this arrangement of the proliferated cells could be termed fan-shaped, the centre of the fan's radii being situated somewhere in the uterine epithelium.

In the following stages this arrangement becomes converted into a functionally more important one. The centre of the fan-shaped structure becomes an open crypt, the protoplasm breaking up, and the peripheral nuclei forming the epithelial lining of the crypt. The uterine epithelium breaks away from under the crypt, and the inner lining of the crypt solders with the surrounding epithelial surface at the lower border. All this is figured in detail in figs. 70 and 71; whereas figs. 22 and 23 elucidate the same processes, and at the same time the comparative thickness of the proliferation as compared with the connective tissue and muscularis of the uterine wall. These figures, compared with figs. 17 and 19, undeniably show that the connective tissue has also increased in bulk. The open crypts, secondary derivates of the epithelial proliferation, are now spread over the concave surface where the placenta is going to develop. Between the openings of these crypts the mouths of the uterine glands are situated. It is not as difficult as it would perhaps seem to be to recognise a gland from the newly formed crypts. Figs. 21 and 68 make this clear; especially when the latter is compared to fig. 71, the well-defined glandular epithelium is ever so much more distinct than the epithelium of the crypt, which as yet is only sharply circumscribed where it passes into the surface layer (fig. 71) and encloses a distinct lumen.

This lumen of the crypt becomes clearly circumscribed—and with it the boundaries of the cells lining the crypts—in the now following stages of figs. 24, 25, and 74.

The proliferation process has here reached its maximum development; its products, the epithelial crypts, are now ready

to receive the processes of the trophoblast in the cavities of its crypts. Between the epithelial proliferation here more fully described maternal blood-vessels, supported by connective tissue, have from the first taken their course. In figs. 24 and 25, but more especially in fig. 74, the relation of this connective and vascular stroma to the epithelial tissue of the crypts can be clearly seen. There can be no doubt but that the proliferated epithelial tissue is more massive than the sanguiniferous strands penetrating between these epithelial crypts.

In comparison to the thickness of the wall of the uterus the epithelial proliferation in figs. 24 and 25 is also seen to have considerably increased in significance, and if we now compare the outline figs. 3 and 4 with figs. 5—7, all of them corresponding to the stages hitherto considered, we see that matters have assumed a very different aspect, and that the lateral cushion-shaped parts where the first omphaloidean attachment of the blastocyst is brought about are no longer much thicker than the antimesometrical concavity, but rather the contrary.

The epithelial proliferation, which at first took place at a slower rate in this placentary region, has very rapidly overtaken in its growth the lateral portions. The uterine glands, although they are considerably flattened when the proliferation and crypts have grown to the size of figs. 6 and 7, are always in the possession of their duct, which takes its course towards the surface parallel to the long axis of the crypts. In the lateral regions of epithelial proliferation against which the blastocyst has become attached there is no semblance, as we have already observed (cf. fig. 7), of the formation of crypts ; here too, however, delicate vascular tracts can be noticed between the proliferated epithelial cells (fig. 20), these vascular spaces remaining in communication with the vessels of the deeper connective tissue. We have now obtained detailed information concerning various changes which the maternal tissues undergo previous to and simultaneously with the definite fixation of the blastocyst against these tissues. When this fixation has come about a new phase is entered upon, which is characterised by important modifications. And whereas up to here we have noticed a

marked progressive development in the maternal tissues, the phase we now enter upon is one in which embryonic proliferations play the by far more considerable part. Proliferations of the outer layer of trophoblast, more fully to be described in the next paragraph, are henceforth seconded by vascular development, first in the omphaloidean region (the vessels of the area vasculosa), later on in the tissues of the allantois, which make use of the roads that have been opened up by the trophoblast in the future placental regions.

We may summarise the characteristic features of this new phase as follows:

1. In the uterine tissue—

(*a*) The newly formed epithelial crypts are slowly but gradually invaded by trophoblastic protuberances and excrescences, which exercise both histolytical and vasifactive functions.

(*b*) The lateral cushion-shaped proliferations of maternal epithelium against which the trophoblast of the area vasculosa is applied undergo a decided histolytical resorption, and finally disappear.

2. In the blastocyst—

(*a*) The amnion is formed.

(*b*) The area vasculosa on the yolk-sac is completed.

(*c*) The allantois originates.

(*d*) The trophoblastic annulus and the trophoblastic protuberances noticed sub 1, *a*, make their appearance.

It will be seen from the above that the chief feature that is novel to and characteristic of this phase is the intimate fusion over a very extensive surface of embryonic and maternal histological elements, coupled with interesting and as yet only imperfectly understood histolytical and histogenetical processes. This makes it impossible to describe any further what was set down for this paragraph, viz. "the changes in the uterine tissues," without making continual references to the growth of the embryonic trophoblast. It is for that reason

preferable to postpone the detailed description of the further stages to the next paragraph.

In this one I will only indicate in a few sentences the general outlines of the further changes that affect the maternal uterine tissues down to the period of parturition.

The lateral cushion-shaped thickenings disappear with comparative rapidity, a new epithelial coating being rapidly formed behind them, out of the confluence of what are in the beginning (fig. 83) defects or fissures in the deeper regions of these thickenings. The first appearance of this process of dehiscence in the deeper layers of the lateral portion is given in outline in fig. 7, and figs. 8—12 furnish us with different aspects of its further progress. It thus comes about that a superficial portion of the maternal tissue against which the area vasculosa is applied becomes more and more loosely attached to the deeper lying portions, that strands of cells (figs. 49 and 50, *s.*) connect them in some, but open spaces separate them in more places, and that finally also the last strands of tissue disappearing (cf. left half of fig. 12) a tongue-shaped projection of tissue remains, which is only connected with the rest of the uterine tissue superiorly, i. e. along the margin of the placental region. From this moment onwards the disappearance is even more rapid, and in fig. 13 these tongue-shaped projections are no more present; the portion of the trophoblast adhering to them (omphaloidean trophoblast) has become modified in a way which will be discussed in the next paragraph, and at the same time the placentary region has considerably increased. The important process of the formation of a new epithelial layer in the deeper portions, simultaneously with the process here referred to, can be better gathered from those figures in which not only the outline, but also the histological detail is given, viz. figs. 84 and 88. The latter figure shows at the same time that this new epithelium is thus disposed (with numerous folds) that it can allow of an extraordinary amount of stretching, as will be necessary in the subsequent stages of further advanced pregnancy.

From the moment the stage has been reached which is re-

presented in fig. 13, the uterine tissue enveloping the embryo is stretched and thinned out more considerably as far as the non-placental region is concerned. But even in the placental region the considerable local thickening brought about by the formation of the placenta is, as we shall see in subsequent paragraphs, not due to proliferation of maternal uterine, but of embryonic trophoblastic tissue. Here, too, the actual uterine tissue is flattened and stretched, its inner boundary line being recognisable by the presence of deeply stained nuclear remains of the blind ends of the epithelial crypts, of which the origin and further development was noticed above, further blood-vessels, and finally a few hardly recognisable gland remnants. It deserves observation that the maximum of the decrease of the true uterine tissue in the placental region is brought about in stages long before parturition comes about (cf. fig. 14).

In the later stages of pregnancy the deeper regions again somewhat increase in thickness in comparison to the bulk of the placenta, preparatory to the severing of this organ and to the restoration of the uterine surface after parturition.

As a detailed discussion of all these changes would fall outside of the scope of this paper, I will now only complete this rapid sketch by special reference to some of the figures.

Figs. 18 and 20, when compared to fig. 16, bear testimony to a very considerable increase of the uterine connective tissue between the epithelium and the muscularis, partly preceding, partly simultaneous with the epithelial proliferation. This growth of connective tissue begins in the lateral cushion-shaped regions; somewhat later it is also noticed in the future placentary region. In figs. 21—23 this is represented under lower, in figs. 66, 72, and 73 under higher powers. A network of blood-vessels is evidently being spun out in these regions for the purpose of supplying the placenta in the later stages of pregnancy. The comparative thickness of the connective-tissue layer, which reaches its maximum in the stage of uterus No. 73, is soon encroached upon by the epithelial proliferation from which it is separated by the fibrillar layer, which is very clearly indicated in fig. 74, and which in the earlier stages (cf.

figs. 68, 72, and 73) is seen to consist of an intermediate layer of cells which are much closer together than the connective-tissue cells further outside, and which are much more decidedly fusiform than the proliferating epithelium cells situated below them.

The reduction of this connective tissue has commenced in figs. 24, 25, and 74; in the following stages (figs. 26—30) it has reached its maximum, and if we compare one of them under stronger powers (fig. 89, same stage as fig. 29) we see that the maternal blood-vessels, which are situated outside the proliferated region, and which supply the latter and the placentary lacunæ with blood, are directly enclosed by the muscularis. The same can be noticed for the lateral regions in figs. 56, 57, 83, and 88 *a*. Of compressed glands distinct traces can yet be found, sometimes even (cf. fig. 30) with an unexpected distension among the proliferated epithelial cells.

The later increase in thickness of connective tissue between the muscularis and the proliferated region (or rather the remnants of it), which follows on the phases of compression or suppression just described, can be noticed in figs. 31 and 32, fig. 54 being a view of part of the latter figure under yet stronger power. In fig. 54 we see between the muscular and the connective-tissue elements peculiar corpuscles (*c.*) having the aspect of dark granules round a lighter centre. In earlier stages they are noticed in the blood-vessels, but as later on they are not inside but outside those vessels, I have no suggestion to offer as to their significance.

2. The Further Changes of these same Tissues in connection with the Attachment of the Blastocyst, and with Different Phases of its Later Development.

In the foregoing paragraph changes in the maternal tissue have been described that occurred independently of any simultaneous embryonic growth that was in direct contact with the maternal surface.

In this paragraph we shall have further to develop the history

of these changes, and at the same time to trace the detail of the very important interaction which henceforth becomes established between maternal and embryonic growths—

(*a*) In the region of the yolk-sac.

(*b*) In the region where the allantois completes its growth.

As far as the embryo is concerned, it is obvious that these two regions are always very distinct. In the maternal tissue, on the contrary, there is direct continuity between that portion of it which is going to be in contact with the yolk-sac and that which is preparing for the adhesion of the allantois, although certain important differences have been noticed in the preceding paragraph (formation of crypts, &c.).

The continuity ceases in the later stages of pregnancy, when the yolk-sac is again removed from any contact with the maternal tissue, because then the corresponding portion of the maternal tissue is gradually resorbed and disappears. But even before this final disappearance certain landmarks can be noticed, by which it is possible to distinguish an allantoidean from an omphaloidean region in the uterine wall. As early as the stage of fig. 10, connective tissue is seen to penetrate in wedge shape between the proliferated epithelium cells that shall contribute towards the placenta and those that are in contact with the yolk-sac. This is no full separation of the two regions, but all the same a valuable indication of their extension.

The actual contact between the outer layer of the blastocyst and the maternal tissue is the first step of a new series of transformations. It is a zonary region of the blastocyst that first becomes attached to the maternal proliferation. This zonary region is equatorially situated with respect to the embryonic area; the latter is, moreover, always facing the concavity of the uterus lumen opposite the mesometrium. There is great probability, as far as I can see, that the zonary region here alluded to is already present before the attachment has yet come about. The thickened trophoblast cells that were figured in vol. xxxi, Pl. XXXVII, fig. 27, of this Journal, in my paper on the "Ger-

minal Layers of Sorex," can hardly be anything else than the predisposed zonary region of attachment. It is, however, worthy of note that when the attachment comes about the trophoblast cells that are applied against the maternal surface are by no means more bulky, but rather excessively flattened. This may be consequent upon a very rapid increase in surface of the blastocyst.

Figs. 4 and 5 give us the two earliest phases of the attachment of the blastocyst.

The fact that the two uteri, No. 52 and No. 73, contained together no less than fifteen blastocysts, which have all been sectionised, enables us to follow all the phases of this attachment in detail. Three of the fifteen are not yet adherent to the maternal surface; and of the remaining twelve, those that may be said to represent the very earliest stage are as yet only adherent by a small portion of the belt-shaped region; others are already attached on one side; the majority, however, are fixed all round, so that the fixation certainly comes about in a very short space of time. In a few of the cases here mentioned there was no visible change either in the maternal proliferated cells or in the flattened embryonic trophoblast cells, against the inner surface of which the still more flattened hypoblast can be easily detected. These cases, no doubt, represent the earliest incipient stages.

In the next stage there is a change both in certain of the trophoblast and in certain of the maternal cells, in some cases the maternal, in other the embryonic cells taking the lead. The changes in the embryonic cells affect both the aspect of the protoplasm and of the nucleus.

When examined in the preserved sections the cells are seen to contain fine filaments which testify both to a small increase in bulk of these flattened cells and to a more frothy arrangement of their protoplasm. In some cases the nucleus of these modified trophoblast cells is readily distinguishable, in others it has undergone a transformation into smaller, deeply stained granules, that (figs. 51 and 49) sometimes even become extremely minute.

The simultaneous change in the maternal proliferated epithelium is this, that the sharply defined surface demarcation of the layer tends to disappear, that the cell boundaries between the cells of the superficial layers become less distinct, that the nuclei stain more deeply, lose their sharp outline, and take the aspect of spherical drops of nuclear matter in which the minute details (nucleolus, granula, &c.) of ordinary nuclei can no longer be detected. These changes, both in protoplasm and nuclei, cause this part of the maternal tissue to be so exceedingly like the adherent embryonic cell matter, that very soon a distinction between the two becomes more difficult and even impossible.

A most effectual adhesion of the two tissues has now come about; they may be said to be fused together. Especially in the stage of fig. 5 (uterus, No. 73) the cementing process between trophoblast and mucosa is very perfect, and in many of the blastocysts secondary changes in the tissues of the belt-shaped region of attachment have not yet commenced. Where these are initiated they have the character of a granular degeneration, as indicated in the lower part of fig. 51. In the upper portion the fused parts have a yet more uniform aspect.

A more detailed and at the same time comparative inspection shows that this granular degeneration affects both the maternal and the trophoblast cells. In how far it is preparatory to vasifactive processes in this region cannot exactly be made out, maternal blood penetrating into capillary spaces in this zone of fusion independently of any preceding granular transformation.

Amongst the six blastocysts of uterus No. 73 there was one yet partially protected by its zona pellucida; this is undoubtedly abnormal for this stage, and a few other peculiarities in its development seem to confirm this view. As such I may mention partial and local proliferations and thickenings in the trophoblast instead of the flat application and parallel fusion against the mucosa.

The stages represented by the uteri 45, 42, and 51 will be discussed together. In all of them the fusion has become yet

more intimate, and so has the confluence of protoplasm by which a syncytial layer of embryonic derivation is created, in which intercommunicating spaces for the maternal blood are gradually evolved. The possibility of distinguishing maternal from embryonic derivates in this layer is yet further diminished ; in the stage of figs. 7 and 49 (uterus No. 42) there are, however, indications that secondarily a deeper layer of the trophoblast becomes more sharply marked off against the syncytial layer, and may thus be compared to what Ed. van Beneden[1] has called for the bat the cytoblast, i. e. a deeper layer of the trophoblastic tissue in which the cellular character as distinct from the syncytial is more prominent. Here in Sorex it is only just indicated, and in many sections very imperfectly visible, but certainly somewhat more distinct in later stages.

Altogether the phenomena here described in the omphaloidean region of Sorex do not testify of a very intense physiological significance of this region. They have only an evanescent existence for the short period that the area vasculosa comes to extend along the belt-shaped zone of the original attachment of the blastocyst. No omphaloidean villi (as in Erinaceus) contribute to the increase of the surface or to a more intimate contact between maternal and embryonic circulation. As such the arrangement is perhaps more the hereditarily transmitted reminiscence of former higher perfection, which has been gradually overruled by the allantoidean arrangement.

The phase in which the interchange of nutritive materials and of oxygen between the maternal blood and that of the area vasculosa may be said to have reached its maximum is represented in fig. 10 (uterus No. 106).

In all the sections of this phase I find very marked self-injections of numerous capillary blood-spaces that are immediately contiguous with the above-mentioned cytoblastic layer, and only separated by this from the now fully developed circulation of the yolk-sac. These blood-spaces originated in the syncytial layer, as was noticed above, and their lumen was

[1] 'Bulletin de l'Académie Royale de Belgique' (3), vol. xv, p. 351.

brought into communication with the maternal capillaries during the histolytic processes there described (cf. fig. 83).

As pregnancy advances, and as spaces that have originated in these lateral proliferations by dehiscence (fig. 83) increase in number and in size, the communication between the capillary lacunæ in the syncytium and the afferent uterine blood-vessels is brought about by vessels that take their course through persisting strands of proliferated epithelial tissue that keep up the connection between the deeper permanent and the superficial epithelial tissue (*s, s,* figs. 9, 10, 50, 84).

The latter is, so to say, scaled off from the rest of the mucosa. Even the connecting strands just noticed disappear one by one, and in the stage of figs. 11 and 12 the maternal blood-flow through this region, which reached its maximum in the stage of fig. 10, has again become diminished, histological and degenerative phenomena of the cells and the nuclei at the same time increasing. There is a very marked difference between the cells of this and the placental region. The component cells of the latter are seen by the number of karyokinetic figures to be in rapid cell division; those of the lateral omphaloidean region show no signs of such activity; growth has here come to a standstill after the phase of fig. 10. In many sections a sort of pseudo-karyokinesis was observed in some of the component cells. I have figured this phenomenon in fig. 84 *a*. It may be gathered from these figures that the nuclei rather testify towards a degenerative than towards a reproductive process.

There is every reason to believe that in the process of final resorption of the omphaloidean region of the mucosa an active part is played by a special modification of the trophoblast, to which we shall henceforth give the name of the trophoblastic annulus, and which is seen in what is evidently its most active phase in figs. 10 and 84, i. e. simultaneously with the maximum development of the omphaloidean circulation and congestion. It is then seen to be a ring of trophoblastic tissue of increased thickness as compared with the other portions of the trophoblast. The component trophoblast cells are

high and columnar, the nuclei situated in the inner half. There is a very gradual passage of these modified cells to the flattened ones which form the non-placental trophoblast below the annulus. At the upper rim of the annulus the passage is more abrupt. The ring, which is nowhere adherent to the uterine surface but distinctly curved away from it, is there attached to the region of fused maternal and embryonic elements, against which the area vasculosa is spread out. There is, in fact, no interval between the cells of the omphaloidean trophoblast and those of the trophoblastic annulus. The terminal sinus of the area vasculosa is very close to the upper rim of the trophoblastic annulus (fig. 88). From the point where the annulus meets the uterine wall there has been from the first moment of its appearance (figs. 6—8, and 48—50) an indication that cells properly belonging to the annulus proliferate in a downward direction (*an'* in these figs. and in fig. 84), and are then applied against the tongue-shaped maternal shred which has already been more than once alluded to. This becomes more and more evident in later stages, and then forms, as will be seen below, the permanent, also ring-shaped, membranous connection between the trophoblastic annulus and the outer circumference of the placenta.

In order to form an opinion as to the physiological significance of the trophoblastic annulus we shall first have to describe a very peculiar phenomenon, of which the first traces appear about the time of the completion of the amnion, during the congestion of the tissues consequent upon the gradual completion of the omphaloidean circulation. The histolytical processes which at the time of this congestion are in full activity, and which have partly contributed to prepare this circulation of maternal blood outside the area vasculosa, are undoubtedly connected with this phenomenon. It is an actual hæmorrhage which is invariably found to a lesser or greater extent just outside the trophoblastic annulus, between it and the permanent and the proliferated epithelium. As early as the stage of uterus No. 51 (figs. 8, 9, and 50) there are traces of it. In the last-named figure the extravasate is as yet insignificant; in

the stages of figs. 10—12 it has become much more important; there is a distinct blood-clot in which the separate blood-corpuscles can generally yet be traced in the sections, pressed between the trophoblastic annulus and the uterine wall (fig. 84). This blood-clot marks the lower end of what will speedily become a tongue-shaped projection in the sections (fig. 88), but which, in fact, is a thin cylindrical shred of degenerating tissue, and which represents the remnants of the proliferated omphaloidean region of the mucosa. Simultaneously with the resorption of these parts the placenta increases in circumference. The trophoblastic annulus thus apparently comes to be situated higher and higher up (figs. 11 and 12). The area vasculosa is lifted off from the surface with which it has entertained relations of exchange, but which is now no longer available for that purpose.

Finally (fig. 13, uterus No. 130) the circumference of the placentary region has so considerably increased that the trophoblastic annulus, as has already been indicated, is now connected with the inner rim of the placenta by the same strip of trophoblast, and that could be noticed in its earliest stages on figs. 10—12, 48, 50, and 84, and that has now grown out to a membrane. As such it has more and more asserted itself, its first phases being figured as noticed above, its final stages being indicated in figs. 13—15, and more considerably enlarged in figs. 33 and 34.

The last remnants of the omphaloidean trophoblast are visible in the stage of fig. 13, though not indicated in that figure. We find it in this stage as a mass of semi-resorbed cell-remains mixed up with blood and nuclear detritus, and pressed in between the outer surface of the trophoblastic annulus and the outer rim of the placenta. In the stage of figs. 14 and 15 no further remains were present. The resorption has here become final, and the allantoidean regions of mucosa and trophoblast are now not only predominant, but entirely monopolise the nutrition of the blastocyst.

We are now enabled more clearly to picture to ourselves what part the trophoblastic annulus has to play in all this.

And we can definitely affirm that this part is an active one after inspection of such preparations as those of fig. 84. They show us that the blood extravasate above alluded to, which is such a constant and characteristic feature of the phase of development that is here under discussion, is bodily absorbed by the cells of the trophoblastic annulus. The blood-corpuscles that have been set free between the uterine wall and the trophoblastic annulus are seen to enter the cells of the latter to a very considerable extent, and I have no doubt that during life this process takes place on an extensive scale. Whether the cells of the trophoblastic annulus which thus absorb maternal blood-corpuscles in a phagocytical way produce other matter out of them, thanks to a special activity of their protoplasm, must be left an open question. Still it is an undoubted fact that about this time the cavity of the yolk-sac gradually comes to contain matter which forms a characteristic coagulum in this and the next phases.

This coagulum grows and increases, and is spread out against the hypoblast that lines the mesometrical concavity of the yolk-sac (fig. 56). It has a yellowish-green, glassy, and yet partly granular appearance, sometimes with different shades of colour disposed in parallel layers (fig. 57). It appears to be densest in the immediate proximity of the hypoblast; towards the lumen of the yolk-sac it grows less dense, and in the phases of fig. 13 and following it fills the yolk-sac more or less completely, the area vasculosa and its modified hypoblast, of which we shall come to speak by-and-by, being bathed by it.

In the early phases of development the cavity of the yolk-sac does not contain similar coagulable matter. The first appearance coincides (*a*) with the increased resorption and final disappearance of the congested omphaloidean mucosa; (*b*) with the increase in size of the yolk-sac, which is then applied with its whole surface against the mesometrical wall of the uterus.

These particulars should be borne in mind when we are going to speculate upon the origin and significance of the coagulum. And if we then see that the trophoblastic annulus

stands from the first in a particular relation to that omphaloidean mucosa, and to the disintegrated products into which it gradually dissolves, and that the cells of the annulus actively absorb the blood-corpuscles that are set free during this disintegrating process, the hypothesis does not seem a strained one which assumes the annulus to participate in the production of the coagulable matter that is gradually accumulating inside the yolk-sac.

Only one layer of hypoblast-cells coating the trophoblastic annulus on the inside separates the protoplasm of the latter from the yolk-cavity (figs. 50, 84, and 88). And the slight increase in size of these hypoblast cells where they are applied against the annulus (figs. 50 and 84) rather favours than contradicts the supposition that they too aid in transporting matter that is originally outside the yolk-sac towards the inside of it, albeit with changed chemical and physical properties.

The cells of the annulus merge very gradually, as was already noticed, into those of the non-placental trophoblast. As this is applied against the uterine epithelium, immediately behind which numerous maternal blood-vessels convey the blood from the mesometrium to the placenta (figs. 15 *a*, 15 *b*, 56, 57, 11—13), it is neither a strained hypothesis to suppose that here, too, certain substances transudate from the blood-vessels through the uterine epithelium, are absorbed by the cells of the non-placental trophoblast, and transported through this and through the underlying hypoblast into the cavity of the yolk-sac. Such a regular passage directed inwards through these layers would go far to explain the very regular growth and increase of the coagulable material all round the surface where the non-placental trophoblast is in contact with the uterine epithelium. Trophoblastic annulus and non-placental trophoblast, continuous anatomically, would then also physiologically play a part to a certain extent analogous.

We must not forget that if the hypothesis of the derivation of the coagulable matter here enunciated is not accepted, another source must be indicated from whence this matter can have been derived. And if we consider the other tissues by

which the yolk-sac is bounded we find that only the area vasculosa remains.

Now it is undeniable that the area vasculosa increases in surface and its cells in bulk even after it has been delaminated from the surface of the omphaloidean mucosa (figs. 11—13). This considerable increase goes on in later stages of pregnancy. Arborescent excrescences (figs. 15, 15 *a*, 65) contribute to extend its surface yet further, and we would thus be able to argue that all this enlargement was at the same time the expression of a heightened activity, by which the coagulable matter filling the yolk-sac is produced.

Such a conclusion would, however, to my idea, confuse cause and effect. The unexpected ulterior development of the vascular area on the yolk-sac, and the increase in bulk of the hypoblast cells can hardly be explained as meant to contribute to the production of special contents of the yolk-sac, which would then be available for the growth of embryonic tissues only on condition that the processes were reversed, and that by the same channels that have first deposited the coagulable matter it was next absorbed and conveyed to the embryo.

The explanation is ever so much more natural that it is the presence of this coagulable matter inside the yolk-sac which has brought about the increased ulterior growth of the vessels of the yolk-sac.

The upper surface of the latter, in consequence of the peculiar growth of embryo and placenta already noticed, has come to be inverted into its own cavity, and over a considerable surface the hypoblast cells beneath the area vasculosa can thus be directly bathed by any fluid that is contained in the yolk-sac. If this fluid has nutritive properties, as in the case of Sorex it may very reasonably be expected to have, then the increased growth of the area vasculosa and the increased efficiency of the apparatus (in casu of trophoblastic annulus and non-placental trophoblast) by which this nutritive matter is prepared and conveyed, is at the same time understood.

And so the disintegration of the omphaloidean portion of the mucosa may be said to lead indirectly to the production in a

second instance of food material for the growth of the embryo, this material being at the same time partially derived from other sources.

We have now to give a somewhat fuller account of the changes in the area vasculosa that have been so often referred to. The most evident change relates to the hypoblast cells of this region, which become more bulky and which take a most vivid green colouring, visible through the distended tissue of the unopened living uterus. This colouring matter is extracted by alcohol. It was not further chemically analysed, but after the ordinary treatment of the specimens with hardening, staining, and embedding reagents, it can yet be distinguished as a greenish-brown or reddish-brown colour. The increase in the size of the hypoblast cells can already be detected when the stage of fig. 12 is reached. From that of fig. 14 onwards it is, however, most clearly marked, and at the same time another process comes in the foreground in this region, viz. the formation of embryonic blood-corpuscles. Different stages in the formation of those corpuscles are represented in figs. 58—63, and I have no doubt that the preparations there figured will well repay a conscientious study of this process. As the dimensions of the vascular surface of the yolk-sac increase so considerably in the period from fig. 13 onwards, new vessels are being formed in all directions, and become visible as proliferations that rise above the level of the surface (figs. 58 and 59) and gradually form a raised network with the pre-existing larger vessels. The whole of this network is bathed, as was noticed above, by the contents of the yolk-sac. In these vessels the future lumen is filled with cells which in the first instance are fixed and immoveable (fig. 58, extreme right), each of them being granular and only gradually changing their aspect—transition stages being present—in that of the ordinary embryonic blood-corpuscles. At the same time the latter are seen to become looser (fig. 58, middle), and are gradually attracted into the general embryonic circulation.

As to the first origin of these cells, it can be noticed that they arise out of larger polynuclear ones (figs. 59—63), of

which in their turn the first origin will have to be settled. For the present I will refrain from giving my own opinion on the point whether these mother-cells are of direct hypoblastic origin, or whether they are proliferations of the ever so much thinner mesoblastic tissue which lines the massive hypoblast of the area vasculosa.

Lately new researches on the origin of the blood and the blood-vessels (C. K. Hoffmann, *a. o.*) have brought this question again very much into the foreground, and rather than here treating it incidentally I will limit myself to the pointing out of the shrew's yolk circulation as a favorable object for the study of these problems.

Figs. 64 and 65, taken from the same section, show two blood-vessels of the yolk-sac in a much later stage. The upper one is a very much flattened space in a stretched portion of the area vasculosa; the lower one is in the region close to the free border of the placenta (cf. fig. 15 *a*), where the surface of the area vasculosa is thrown into very numerous folds, the free space in the yolk-sac being in these later phases of pregnancy more and more reduced. The coagulum is all the same present in these later stages. The hypoblast cells are here seen to have yet further increased in size.

An important peculiarity that should here be mentioned, now that the blood-corpuscles of the embryo and their neoformation are being noticed, is this, that they are of such a different size from those of the mother. Such is the case, not only in the early (figs. 80—82, 85, 86, 89), but also in the later stages of pregnancy, and offers a most valuable advantage for recognising maternal from embryonic circulatory spaces. This is especially important in the placental region, where the relative intermixture of these spaces is so extremely complicated, and where quite normal self-injections are thus available, showing the finest blood-spaces with the utmost clearness (figs. 52 and 53).

We have now finished the description of the later phenomena in the omphaloidean region and the area vasculosa, in that of the trophoblastic annulus, and of the non-placental

trophoblast, as also of the participation of these different regions in the processes of attachment and partly of nutrition of the blastocyst. There now remains for our consideration that yet more important portion of the trophoblast which takes an active part in the formation of the placenta—the allantoidean trophoblast.

Its first appearance as an independent layer is coincident with the formation and completion of the amnion. From the very first (cf. fig. 7) the amnion fold (that can primarily be observed behind the embryo) has an ever so much thicker outer than inner fold. This difference in thickness must be set down to the account of one of the component cell-layers—the epiblast, whereas the somatic mesoblast is and remains exceedingly thin. I have reason to believe (though I will reserve the consideration of this hypothesis to a later publication) that the growth of the amnion fold is not really a slow turning over of a spread-out layer, but that from the very first—even as long as they are spread out flat—the inner and the outer curve can be said to be distinct.

In the earliest phase the thickened shield of epiblast is sharply set off against the continuation of the epiblast external to it. It seems to me very probable that from cells derived from the first-named shield the epiblast entering into the inner fold of the amnion is derived. The point of meeting between the inner and the outer fold would then correspond to the rim of the shield in the earliest phases. Inner and outer fold increase simultaneously and at an equal rate. In Sorex they always meet at a sharp angle; they do not pass into each other along a smooth curve. Be this as it may, the fact remains patent that the outer fold of the amnion is composed of the somatic mesoblast above referred to, and of a thickened layer of epiblast cells. This layer, as will be seen by reference to figs. 26, 27, and 75—79, is in many places two and sometimes more cells thick: it is the allantoidean trophoblast. It cannot be strictly defined topographically, as the allantoidean trophoblast passes into the omphaloidean trophoblast quite gradually. Still, the name is convenient for designating that portion of the

trophoblast which forms a hemispherical overcapping of the embryo, which is situated opposite the non-placental (equally hemispherical) trophoblast, and which is separated from the latter by two ring-shaped zones—the omphaloidean trophoblast and the trophoblastic annulus.

The allantoidean trophoblast is a massive layer, and though the cells may be less high individually than those of the trophoblastic annulus, still they seem to be yet more active physiologically speaking, and stain more deeply with picro-carmine. Another peculiarity to be noticed on the allantoidean trophoblast even before the completion of the amnion (cf. figs. 8 and 9) is the presence of warts or projections of proliferating cells. They arise in the first instance independently of corresponding cavities on the maternal surface against which the allantoidean trophoblast is going to be applied. But when this application has come about, a trophoblastic projection is present at whatever point we find the maternal surface indented, i. e. at the mouth of every epithelial crypt.

The numerous crypt openings are thus in the stages of uteri 51 and 106 (figs. 8—10 and 75) already partially blocked by trophoblastic cell material, and the trophoblast cells continue to proliferate in the first instance there where they have penetrated into the crypts. The trophoblast applied against the concave surface between the crypt openings is also in proliferation; later on (figs. 13 and 94) this becomes yet more marked.

When once the crypt openings are filled by massive knobs of proliferated trophoblast, the latter are seen to become hollowed out very rapidly, a phenomenon which goes apace with the further development of the allantois. The latter makes its first appearance while the amnion is being completed (fig. 8). After this has come about, and while the omphaloidean circulation reigns as yet supreme (fig. 10), the allantois rapidly extends against the concave surface when the trophoblast has just become adherent against the mouths of the epithelial crypts that have arisen in this region in preparation of the processes which are now going to follow. In these processes both this region of the trophoblast and the allantois play the principal parts.

Before describing those processes in detail it will be well once more to summarise them. Superficially it would seem as if the phenomena could be thus characterised:—the trophoblastic knobs become inserted in the crypts and vascularised by the allantois, and by further growth and division of all these parts the full-grown placenta comes into existence. Nothing is, however, further from the truth. It can be easily understood that the number of crypts is limited when once the trophoblast has come to be applied against the latter. Thus new crypts and knobs cannot possibly arise in the same way as the original ones after the adhesion of the trophoblast against the maternal surface.

Neither do I find traces, when once the trophoblastic protuberances have become firmly fastened in the crypts and have received allantoidean villi in their subsequent cavities, of any penetration of trophoblastic tissue into the proliferated maternal tissue between the already existent maternal crypts.

The positive interpretation of the facts which I desire to substitute for the insufficient hypothetical suggestions just brought forward is the following:—After the maternal crypts have received the plugs of trophoblast in their cavities a destruction of the maternal epithelium as far as it is in contact with the trophoblastic plugs follows.

Trophoblastic proliferation and histolysis in the surrounding maternal tissue going hand in hand, the firm adhesion of the blastocyst in this placental region is very soon brought about.

And it is to obtain this degree of very firm connection that the crypts and protuberances have to a great extent served. The trophoblast has, so to say, become anchored by its wart-like protuberances in the maternal proliferation, and it is now going to prepare the placenta by its own activity. For this the firm adhesion between trophoblast and mucosa which has now been established is of the highest importance. It is henceforth possible for the trophoblastic tissue to expand into a syncytium of considerable size, and to tap the maternal circulation without any danger of unservice-

able extravasates. The trophoblastic knobs may yet penetrate somewhat further into the crypts, but it is not in this penetration that the chief feature of the placental development is found. That chief feature is the increase in thickness of the trophoblast, and the decrease in thickness of the maternal crypt region. From this latter blood passes into the former; no further maternal contribution, nor indeed any penetration of maternal growths between the trophoblast (or the allantois villi that are enclosed therein), can anywhere be noticed.

The trophoblast is spun out against a maternal surface specially prepared for its firm adhesion. A glance at figs. 10—15 will further explain this. In these figures the maternal crypts represented by the red dotted lines are not represented in their real number. The fact is, they are very closely pressed together in the early stages (cf. figs. 24 and 74), and only those crypts were inserted in the drawings with the camera (figs. 6—13) which happened to possess a more or less distended lumen.

The depth of the zone occupied by the crypts is seen to remain about stationary between the stages of the figs. 11—13. On the contrary, the depth of the zone in which the trophoblastic protuberances are situated has very considerably increased.

It follows from this that when once the firm adhesion is brought about there is no very active further penetration of the trophoblast into the crypts, nor any extension of the cryptal and intercryptal proliferation downwards between the trophoblastic tissue. Great activity is, however, displayed; first, in the trophoblast between the villi, where intercommunicating spaces are being evolved, that enter into communication with the maternal circulation; second, in the trophoblastic layer that covers the inner concavity of the developing placenta.

The latter layer attains to a most considerable thickness (cf. figs. 13, 30, and 94), preparing new points of insertion for secondary and tertiary allantoic villi (figs. 13, 29, 30, 31, 91, 94), which in their turn grow out to the length of the

first formed as the placenta yet further increases in size and in thickness.

Simultaneously with this growth of secondary and tertiary allantoidean villi, the thick layer of trophoblast in which their insertion took place furnishes the material for the blood-cavities by which these extending villi are being surrounded. That same trophoblast layer has in the further stages (figs. 14 and 15) no longer such a considerable thickness, and we can now understand how there can be no doubt that all the tissues that contribute to the formation of the part of the placenta which is marked in black are of embryonic origin.

During the considerable increase in superficial extension of the placenta the cryptal region has at the outset not remained quite stationary, though its increase is in no comparison whatever to that of the trophoblastic region. Its growth is more adapted to the obvious fact that it has to spread over an ever enlarging area. This leads to a decrease in thickness that is very obvious when we compare the figs. 13, 14, and 15 with each other. And whereas in the first-named figure the crypts are yet very clearly observable, this is much less the case in figs. 14 and 15, the whole of the cryptal and intercryptal tissues being here only represented by a layer of deeply staining nuclear matter and nuclear detritus.

After having given this summary description of the important modifications that go on in the placentary region, we shall have to analyse in further details the processes that cause them, and to furnish the confirmation of the interpretation here given. We must then go back to the stage of uterus Nos. 42 and 45 (figs. 24, 25, and 74).

We here have the mucosa before us of the placental region in the phase of its highest development, anteriorly to the adhesion of the allantoidean trophoblast against it. The epithelial crypts, that have originated by the proliferation above described (p. 493), are in possession of a very distinct epithelial lining, passing continuously into that of the uterus lumen. They are closely pressed together, and some of them bifurcate towards the outer circumference, the blind ends of the crypts being

thus considerably more numerous than the mouths. Between the high and massive epithelium of two neighbouring crypts there is everywhere a core of connective tissue with capillaries, the latter with a flattened endothelium. Especially close to the surface of the mucosa many of these blood-spaces are disposed parallel to that surface, some of them immediately below the epithelium.

In fig. 74 it can be easily seen that the character of the tissue between the crypts is not that of ordinary connective tissue, nor that the endothelium referred to is as yet very much flattened. The tissue is in a state of most active proliferation, and the resemblance to the proliferating epithelium is very close. In the stages of figs. 26, 28, 29, 30, the epithelial character comes yet more into the foreground, because the tissue between the crypts is now reduced to actual capillary vessels, taking their course strictly radially. In this stage the endothelium is really flattened (fig. 82), and the radial capillaries are on all sides supported by cryptal epithelial cells.

The moment the adhesion of the trophoblast has come about the maternal epithelium disappears. Whether it partly disintegrates in its place, or whether it is actively absorbed by the outer layer of trophoblast cells, cannot be decided with absolute certainty; perhaps both processes contribute, and the phenomenon is readily comparable to what was noticed in the omphaloidean region and represented in fig. 83.

In fig. 75 the maternal epithelium is yet seen to the right of the trophoblastic knob; to the left of this it is disintegrating between the darker stained outer trophoblast layer and the sanguiniferous deeper layer. In the same way the maternal cryptal epithelium disappears wherever a trophoblastic knob penetrates into a crypt; in this case the preparations countenance the view that active destruction and absorption of the maternal cells by the trophoblastic ones takes place (fig. 80). As a rule the nuclei belonging to the trophoblast are smaller than those of the maternal proliferation, although this is of course no rigorous means of distinction (cf. figs. 82 and 89).

However this may be, a stage is soon reached—and figs. 11, 28, and 80 are good representations of it—in which the trophoblast forms a continuous layer over the maternal surface, and over the mouths of the crypts, acting as a sort of pseudo-epithelium.

This trophoblast layer has at the same time commenced another transformation, which is of the highest importance for the correct interpretation of the further phenomena of placentation. Of this transformation the earliest appearance of the allantoidean trophoblast—even before it is as yet applied against maternal surfaces—has given evidence already. We there notice in the free trophoblast an evident tendency to differentiate into two layers, the inner one of these generally staining more intensely (figs. 75—78). This duplicity is also marked in the trophoblastic knobs (figs. 27, 27 *a*, 78, and 79). We may safely infer that this subdivision of the trophoblast is the same as that which was noticed by van Beneden for the bat, and afterwards by Masius, Duval, and others for the rabbit and other rodents. Van Beneden applied the names of "cytoblast" and "plasmodiblast" to these closely contiguous subdivisions of the trophoblast.

We will follow his example, and henceforth designate in the allantoidean trophoblast of Sorex the inner layer as the cytoblastic, the outer as the plasmodiblastic one. Both of them rapidly increase in extent and thickness as the trophoblast continues to spread over the maternal surface. Superficial inspection of preparations, as those of figs. 28 and 80, would lead us to the conclusion that the real extent of the trophoblast was limited to the darkly stained layer and knobs there indicated. Still these only represent the deeper cytoblast. The fact is that the plasmodiblast, which is the outer layer, commences to be fused intimately with the maternal tissue in the stage of figs. 27, 27 *a*, and 79, and is busily engaged in developing lacunary and intercommunicating spaces just outside the limits of the darker cytoblast layer (fig. 79). These future blood-spaces are spread out in the plane of the layer, and thus come to be directly contiguous to the actual blood-

vessels that are already present in the maternal proliferation, which, as we have seen on p. 516, are also very markedly spread out horizontally below the layer of epithelium, which has come to disappear now that the trophoblast has taken its place. Out of this immediate contiguity an actual communication of the maternal blood-spaces with those in the plasmodiblast is very soon developed, as can be clearly seen in fig. 80. The plasmodiblast does not henceforth develop independently of the cytoblast; on the contrary, it is continually being added to by cells or cell sheets from the darker stained cytoblastic layer getting more detached and travelling inwards. This is clearly indicated in fig. 93, which has reference to the uterus No. 85, i. e. one stage later than (fig. 12) uterus No. 3 (fig. 11), from which fig. 80 is taken. In fig. 93 the detachment of cytoblastic elements from the more deeply stained layer that arrange themselves into parallel superposed layers of plasmodiblast, between which open spaces develop, in which blood gradually penetrates, is very distinctly visible, as it is also in numerous preparations of this and the earlier stages that have not here been figured.

As further growth goes on, these horizontal blood tracts assume a more vertical course, new horizontal ones developing below them (cf. fig. 89).

Also in fig. 87 the penetration of blood in trophoblastic spaces is indicated,—this time in a section that cuts the placenta circularly (cf. fig. 11 *b*).

Thus, through the action of the massive trophoblast, not only a firm attachment of the blastocyst in the placentary region is brought about, but between the trophoblastic knobs which have caused that firm attachment a sanguiniferous plasmodiblast is very early established. Allantoidean villi penetrating into the trophoblastic knobs that become hollowed out as they lengthen centripetally are thus bathed by maternal blood circulating in embryonic spaces.

There is thus neither necessity nor even possibility for any further penetration of proliferating maternal tissue between the villi. That space is filled by the allantoidean trophoblast,

composed of cytoblast and plasmodiblast, which are in continuous growth and in rapid increase.

Of the mother only the blood and the blood-corpuscles penetrate into the numerous and intricate lacunæ of this region. Both the external cap of maternal tissue and the internal core of trophoblastic tissue (with allantoidean villi embedded in it) out of which the placentary region is composed, show the traces of their respective growth in numerous karyokinetic figures. In the stage of fig. 11 these are yet numerous in the maternal crypt region; in that of fig. 13 they are already rare or absent, and the increase of this layer may be said to have ceased. On the contrary, the number of karyokinetic figures in the cytoblast is in this stage very striking. Of direct nuclear division, without karyokinetic intermediate stages, examples are found in the plasmodiblast.

The glands, as far as they persist in the placentary region (sometimes with unexpected local dilatations), are not invaded by trophoblastic knobs or villi; their openings towards the uterine lumen are closed by the trophoblast, and they play no part in the fixation of the blastocyst, or in the facilitation of the intercourse between the maternal and the embryonic circulation. In the latest stages of pregnancy all vestiges of the glands have disappeared in the region between the villi.

And so we see that there is in the placentary region a gradual substitution of maternal tissue by embryonic tissues, corresponding in extent to what is indicated in figs. 10—15 by the extension of the red and the black divisions.

Always with this restriction, that in the region which is indicated by black lines maternal blood penetrates into the trophoblastic tissue that fills up all the white space between the black lines.

Thus the maternal blood is transported from the maternal arteries into channels that are wholly built up of embryonic material, returning back along similar spaces to the superficial (red) covering of the placental region and to the maternal veins.

The phenomena of growth and development of the tropho-

blastic tissue that surrounds the allantoidean villi (and through the intervention of which the latter are bathed by maternal blood) are thus identical with the further phenomena of growth and development of the placenta. The latter does not contain maternal elements other than blood. Trophoblastic tissue is the material out of which the placenta is built up.

It is soaked with maternal blood, and allantoidean vessels with their ramifications have been carried into it by the villi. In the ripe placenta the villi are no longer recognisable as such, more or less in the same way as I have formerly described and figured this for the hedgehog (l. c., figs. 56 and 57).

And whereas in the growing placenta of Sorex a maternal (red) and an embryonic (black) part can be distinguished, in the full-grown one the maternal portion is no longer present as such, but forms an insignificant and interrupted sheet of nuclear remains between the main mass of the placenta and the muscularis with the afferent and efferent vessels. It is in the plane of this sheet that the severing of the placenta at birth is effected. In the shrew it is actually shed as in the hedgehog, and not resorbed in loco as in the mole.

We must now attend to a few details connected with this process of development. Starting from the figs. 81, 89, 93, 82, and 94, which have already been referred to, we must repeat that in these stages the fusion of trophoblast and maternal epithelial proliferation has become most intimate, that it would be difficult to draw any sharp line of demarcation in figs. 81, 82, and 89, but that, all the same, the difference between plasmodiblast and maternal crypt tissue and between trophoblastic blood-spaces and maternal capillaries is unmistakable. The thickness of the maternal layer in comparison to the embryonic one is about 1 : 1 in figs. 12 and 89. The active increase of the maternal crypt tissue by further proliferation has now nearly come to a standstill, and henceforth the growth of the placenta means the increase of the trophoblastic strands and of the villi between them. The increase of the plasmodiblast at the cost of new layers of cytoblast was already noticed above. But as pregnancy advances certain

other processes of growth of these parts come under observation. It is no longer in the lamellar fashion (of fig. 93) that the cytoblastic elements come to be transformed into plasmodiblastic tissue, at least not generally. A phenomenon more frequently observed in the later stages, when the uterine swellings have reached the size of fig. 38 (cf. fig. 31) and more, is represented under higher powers in figs. 90—92. The separation between cytoblast and plasmodiblast is less distinct. In the region immediately surrounding the villi this was already noticed in comparing the two stages of figs. 81 and 82 that follow so closely upon each other. But whereas in the stage of fig. 82 the distinction between cytoblast and plasmodiblast had become difficult between the villi, fig. 94 (belonging to the same stage as fig. 82) shows that it was yet very easy towards the lower surface of the placenta, where the trophoblastic proliferation is most active.

Later on, however, as the comparison of the last-named figure (94) with those just mentioned (90—92) shows, the line of demarcation becomes less marked. There is yet a certain difference in the staining, and many of the cytoblast nuclei are recognisable by their more copious absorption of picro-carminate. But one glance at the figs. 90—92 will convince us of the very gradual transition between cytoblast and plasmodiblast. Also with respect to the fact that hardly any more cell boundaries can be distinguished between the cytoblast nuclei; so that not only the plasmodiblast, but also the cytoblast, should then be considered as a syncytium.

Now the new process of transformation of cytoblastic into plasmodiblastic—i. e. into sanguiniferous—tissue consists in the appearance of nests of nuclei marked off by a comparatively very distinct line of demarcation (fig. 91). These nests gradually separate themselves from the surface, and are apparently carried inwards (fig. 90), where for some time they persist (fig. 92). After that they gradually develop cavities, into which maternal blood-corpuscles are seen to penetrate, and which thus become part of the general circulatory apparatus of the plasmodiblastic syncytium. Fig. 91 clearly shows, however,

that such blood-spaces not only arise in those nests of nuclei, but also between them. The whole of the set of blood-spaces in the syncytial tissue here referred to is thus gradually developed by processes that, though closely comparable, are not yet identical in the earlier and in the later phases of pregnancy. The final point to which they lead up, and which we find represented in the fully ripe placenta (figs. 32 and 52—54), is this, that the lower surface of the placenta where the vessels of the allantois are applied against it very much resembles the phase of fig. 90, but that the rest of the trophoblastic tissue is so exceedingly attenuated between the allantoidean villi and their ramifications that by the stretching of the syncytium only a very thin partition of trophoblast, with nuclei regularly distributed in it (figs. 52, 53, and 86), separates the maternal blood from the embryonic.

The latter, circulating in the allantoidean villi, is surrounded by the tissue of the villus, which in the earlier stages is indeed substantial (figs. 85 and 86). There is to each villus a central blood-space (figs. 85 and 30) and numerous peripheral ramifications immediately under the surface (figs. 81, 82, 85, and 86). But as the placenta increases in size, the trophoblast in tenuity, and the villi with their ramifications in length, this primarily more massive tissue is also extraordinarily stretched, and finally not more than the thickness of one cell ensheaths the embryonic corpuscles in the villi.

I have no doubt that in the fully ripe placenta even this covering disappears as far as the finer ramifications are concerned, and that there only the thin trophoblastic partition above referred to separates the maternal from the embryonic blood.

We may conclude from the foregoing that the passage of blood from the maternal vessels into the embryonic trophoblast takes place in the shrew at a later period of the development than in the hedgehog. For this passage communications must necessarily originate (p. 35) between the maternal blood-vessels, and the embryonic lacunary spaces which are intended for the

reception of that blood. The genesis of such communications is undoubtedly facilitated in the shrew by the fact that not only the trophoblast is composed of young and newly formed cells, but that the same holds good for the maternal proliferation with the crypts. The spaces which in both are destined for the transport of maternal blood fuse, so to say, in statu nascenti, and this helps to explain how in the shrew the process of fusion can only be traced with so much difficulty, and how the boundary lines between maternal and embryonic proliferations become so very soon untraceable.

The final reduction of the maternal cryptal tissue to a layer of nuclear nests interspersed between the placentary tissue and the muscularis (see figs. 32 and 54) need not be discussed in detail. The reduction is a gradual one, and partly figured in fig. 86, where the maternal tissue between this cryptal proliferation and the muscularis has not yet developed anew—rearranged itself preparatory to parturition, as it has already done in fig. 54.

The more deeply stained maternal nuclear nests just referred to are thrown off with the placenta. The way in which the maternal surface regenerates after parturition will not be here discussed, but will be reserved for another paper, in which I will then include comparative considerations with respect to other genera of Insectivora (Erinaceus, Talpa, and Tupaja).

EXPLANATION OF PLATES 31 to 39,

Illustrating Professor A. A. W. Hubrecht's paper "Studies in Mammalian Embryology. III.—The Placentation of the Shrew (Sorex vulgaris)."

List of Abbreviations.

a. T. Allantoidean trophoblast. *o. T.* Omphaloidean trophoblast. *tr. an.* Trophoblastic annulus. *an'.* Embryonic cells which grow downwards from the upper rim of the trophoblastic annulus and adhere against the maternal tissue. *np. T.* Non-placental trophoblast. *k.* Trophoblastic knobs which become hollowed out and into which allantoidean villi penetrate. *U.* Lumen of the uterus. *u. E.* Uterine epithelium. *gl.* Uterine glands. *M.* Mesometrium. *ep.* Epithelial crypts in the proliferating portion of the maternal mucosa. *S.* Strands of epithelial tissue between the superficial and deeper maternal layers in the omphaloidean region. *bl.* Blood-vessels. *lm.* Longitudinal, and *cm.* Circular fibres of the muscularis. *y.* Yolk-sac. *a. v.* Area vasculosa. *E. c.* Extra-embryonic cœloma. *am.* Amnion. *pr. am.* Proamnion. *all.* Allantois. *vi.* Allantoidean villus. *g. c.* Green coagulum in the yolk-sac. *mes.* Mesoblast. *s. m.* Somatic mesoblast. *hy.* Hypoblast. *nn.* Nuclear nests in the syncytium (incipient blood-spaces).

PLATES 31 and 32.

Outline sketches of median transverse sections through uterus and embryo in successive stages of pregnancy of Sorex vulgaris. All the figures drawn with the camera. × 27. Embryonic tissue in black, maternal tissue in red outlines. The red numbers in brackets refer to the catalogue number of the specimen. The small numerals connected with dotted lines refer to figure-numbers on the subsequent plates in which the region thus indicated is represented as seen under stronger powers. Cf. Figs. 36—47 for the outward aspect of the uterus in the stages from that of Figs. 8—15.

Figs. 1 and 2.—The blastocyst has not yet arrived in the cavity of the uterus, but is still contained in the oviduct.

Fig. 1.—Utr. Mus. Cat. n°. Sorex 124*f*, 3 *r*. 4 *s*.
Fig. 2.— ,, ,, 110 2, 2 *r*. 16 *s*.

Figs. 3 and 4.—Sudden and peculiar extension of the uterus lumen and of the wall, the latter having decreased in thickness considerably opposite the mesometrium. The blastocyst has arrived in the uterus.

Fig. 3.—Utr. Mus. Cat. n°. Sorex 2 *a*, 5 *r*. 21 *s*.
Fig. 4.— ,, ,, ,, 52 *e*, 3 *r*. 26 *s*.

FIGS. 5 and 6.—Proliferation of the maternal epithelium having extended over the surface opposite the mesometrium, the uterine wall has here again thickened. The blastocyst has become attached to the maternal surface by means of the omphaloidean trophoblast.

Fig. 5.—Utr. Mus. Cat. n°. Sorex 73 *f*, 2 *r*. 19 *s*.
Fig. 6.— „ „ 45 *c*, 3 *r*. 14 *s*.

FIG. 7.—First appearance of amnion, trophoblastic annulus and area vasculosa. Dehiscence in lateral maternal tissue, which has commenced in the stage of Fig. 5, has here made rapid progress.

Fig. 7.—Utr. Mus. Cat. n°. Sorex 42 *e*, 3 *r*. 11 *s*.

FIGS. 8 and 9.—Proamnion and amnion simultaneously in process of formation. Secondary maternal epithelial crypts at their maximum of independent development. First appearance of trophoblastic knobs on the allantoidean trophoblast. Area vasculosa does not as yet extend down to trophoblastic annulus.

Fig. 8.—Utr. Mus. Cat. n°. Sorex 51 *g*, 3 *r*. 29 *s*.
Fig. 9.— „ „ 51 *d*, 2, 4 *r*. 2 *s*.

FIG. 10.—Amnion closed and completed; allantoidean trophoblast partly applied against concavity of future placentary region; trophoblastic knobs just entering epithelial crypts. First appearance of allantois as free knob projecting in extra-embryonic cœlom. Area vasculosa completed down to trophoblastic annulus. Active participation of the latter in resorbtion of hæmorrhagic extravasates consequent on disintegration of lateral tracts of maternal tissue.

Fig. 10.—Utr. Mus. Cat. n°. Sorex 106 *e*, 3 *r*. 9 *s*., and 106 *h*, 1 *r*. 9 *s*.

FIGS. 11 and 12.—The trophoblastic excrescences have penetrated into the secondary epithelial crypts and have in their turn become hollowed out. Villi of the allantois penetrating into these trophoblastic cavities. Epithelium of maternal surface and crypts has disappeared wherever embryonic trophoblast is in contact with it. In Fig. 11 the area vasculosa commences to loosen its hold upon the lateral maternal surface; in Fig. 12 it is hardly any longer adherent. The actual distance between the trophoblastic annulus and the placentary region has at the same time diminished. The embryo is being sunk into the yolk-sac, thus pushing before it the area vasculosa, which is thereby turned inside out. The differentiation of the allantoidean trophoblast into a cytoblastic and a plasmodiblastic portion has commenced. In Fig. 12 the last traces of the ensheathing of the allantoidean villi by distinct cytoblast are yet visible as a thin black line.

Fig. 11.—Utr. Mus. Cat. n°. Sorex 3 *b*, 2, 1 *r*. 9 *s*.
Fig. 12.— „ „ 85 *g*, 3 *r*. 1 *s*.

FIGS. 11 *a* and 11 *b*.—Two sections along a plane perpendicular to all the foregoing. Same stage as that from which Fig. 11 was taken. The planes of sections accompanied by the corresponding numbers are indicated in Fig. 11 by lines and numerals. In Fig 11 *a* only secondary crypts of maternal epithe-

lium and uterine glands, *gl.* (flattened against the outer circumference), are cut, the former being indicated by dotted lines. In Fig. 11 *b* trophoblastic protuberances, which have already penetrated into the more centrally situated crypts, are indicated by thin black lines. Other dotted lines inside of these refer to allantoidean villi filling up the cavity of the hollow trophoblastic protuberances.

Fig. 11 *a*.—Utr. Mus. Cat. n°. Sorex 3 *a*, 2, 5 *r*. 19 *s*.
Fig. 11 *b*.— „ „ 3 *a*, 2, 6 *r*. 17 *s*.

Fig. 13.—The visible contrast between the maternal secondary crypts and the trophoblastic ingrowth, ensheathing allantoidean villi, has considerably diminished, making it more and more difficult to distinguish the maternal from the embryonic histological elements in the placentary region. Of the crypts, however, the peripheral blind ends are yet preserved and sufficiently distinct. In the trophoblast we notice a region of special activity on the concave placental surface where the cytoblastic thickening is particularly considerable, secondary allantoidean villi that are rapidly forming being ensheathed by it. The increase in size of the placenta is accompanied by the total disappearance of the lateral maternal tissue against which the area vasculosa has been applied. The whole of the area vasculosa (which has not stopped growing, but, on the contrary, remains on the increase) is henceforth situated below instead of above the trophoblastic annulus. The attachment between the latter and the border of the placenta has become a circular membrane. The head of the embryo is still enclosed in a proamnion, the cavity of the yolk-sac is being considerably encroached upon by the embryo, and the inverted area vasculosa is sinking down into it.

Fig. 13.—Utr. Mus. Cat. n°. Sorex 130 *a*, 3 *r*. 1 *s*.

Fig. 14.—There is a considerable increase in the size of the placenta, but the relation of the parts has remained very much the same to what it was in Fig. 13. The layer of proliferating trophoblast is no longer so exceptionally thick on the concave placental surface. The trophoblast (and the allantoic villi embedded in it) occupies seven eighths of the thickness of the uterine wall in the placental region. The remnants of the cryptal region have been still more considerably reduced. The trophoblastic annulus has undergone no further modification. The area vasculosa has extended considerably.

Fig. 14.—Utr. Mus. Cat. n°. Sorex 61 *a*, 2 *r*. 17 *s*.

Figs. 15 and 15 *a*.—The border region of the placenta just before birth. The actual thickness has hardly increased when compared to Fig. 14, but the ramification of the allantoic villi and the intervening strands of trophoblastic tissue is ever so much more complicated. The maternal epithelial proliferation has now finally become reduced to mere nuclear remnants, staining more deeply with carmine. The thickness of the maternal tissue outside these nuclear elements has somewhat increased when compared to Fig. 14. In Fig. 15 *a* peculiar foldings of the wall of the yolk-sac are indicated.

Fig. 15.—Utr. Mus. Cat. n°. Sorex 100, 3 *r*. 10 *s*.
Fig. 15 *a*.— „ „ 100, 1 *r*. 8 *s*.

Fig. 15 *b*.—The wall of the uterus (thick red line), the non-placental trophoblast and the yolk-sac in the vicinity of the mesometrium, drawn on the same scale and from a similar preparation as Figs. 15 and 15 *a*.

Fig. 15 *b*.—Utr. Mus. Cat. n°· Sorex 90 *a*, 1 *r*. 8 *s*.

PLATE 33.

(All these figures × 100.)

Fig. 16.—Part of a transverse section (cf. Fig. 1) through a uterus in which the blastocysts are as yet contained in the oviducts. The coiled uterine glands are seen to be massed together in the antimesometrical regions. The uterine lumen is more or less ⊥-shaped. Opposite the mesometrium the uterine epithelium is much higher now than in the stages of Figs. 17 and 19.

Utr. Mus. Cat. n°· Sorex 124 *f*, 3 *r*. 3 *s*.

Fig. 17.—Part of a section through the wall of the uterus opposite the mesometrium, in a later stage of pregnancy (cf. Fig. 3 and Fig. 66). The epithelium is flattened consequent upon the stretching of the wall and the distension of the lumen.

Utr. Mus. Cat. n°· Sorex 2 *a*, 5 *r*. 18 *s*.

Fig. 18.—Ibid., through the region where the uterine wall of the same specimen is thickest (cf. Fig. 3). The epithelium has as yet not entered upon any proliferating process.

Utr. Mus. Cat, n°· Sorex 2 *a*, 5 *r*. 25 *s*.

Fig. 19.—The same as Fig. 17, but of a somewhat later stage (cf. Fig. 4). Proliferation of the epithelium has commenced (cf. Figs. 67—69).

Utr. Mus. Cat. n°· Sorex 52 *e*, 3 *r*. 24 *s*.

Fig. 20.—The same as Fig. 18, but in a stage in which the proliferation of the lateral uterine epithelium is already considerable (cf. Fig. 4). This proliferation differs in character from that of Figs. 67—71, there being no formation of crypts. The omphaloidean trophoblast will very soon become adherent against it. Blood circulates in capillary spaces between these proliferated epithelium cells.

Utr. Mus. Cat. n°· Sorex 52 *e*, 3 *r*. 24 *s*.

Figs. 21—26.—Later stages of a portion of the uterine wall opposite the mesometrium (cf. Figs. 4—9). The secondary epithelial crypts which originate by the peculiar proliferation of the uterine epithelium, that commenced in Fig. 19, gradually attain their full development in Figs. 25 and 26 (cf. Fig. 74). In the latter figure many of the crypts are not indicated because they are flattened and pressed together. Maternal blood-vessels are everywhere present between the crypts. In Fig. 26 the amnion is nearly complete, and

the allantoidean trophoblast is beginning to be applied against the uterine epithelium.

Fig. 21.—Utr. Mus. Cat. n°. Sorex 52 *i*, 3 *r*. 22 *s*.
Fig. 22.— „ „ 73 *f*, 2 *r*. 20 *s*.
Fig. 23.— „ „ 73 *b*, 2, 1 *r*. 16 *s*.
Fig. 24.— „ „ 45 *c*, 3 *r*. 17 *s*.
Fig. 25.— „ „ 42 *c*, 3 *r*. 8 *s*.
Fig. 26.— „ „ 51 *f*, 4 *r*. 15 *s*.

Figs. 27 and 27 *a*.—The same, with the allantoidean trophoblast applied against the uterine wall and the trophoblastic protuberances, *k*., fitting into the secondary epithelial crypts, *ep*. The specimen from which these figures were taken having been differently preserved from all the others, and the histological details of the proliferated epithelium being less distinct, this region is indicated by a blank space in Fig. 27. There are unmistakable indications of the separation of this trophoblast into a cytoblastic and a plasmodiblastic portion (cf. Fig. 79).

Fig. 27.—Utr. Mus. Cat. n°. Sorex 106 *f*, 3 *r*. 12 *s*.
Fig. 27 *a*.— „ „ 106 *f*, 3 *r*. 4 *s*.

Fig. 28.—The same, in a yet later stage. The trophoblastic protuberances, *k*., adhere firmly in the epithelial crypts, *ep*., the protuberances being partly hollowed out and filled out by incipient allantoic villi, *vi*. Plasmodiblast extends between the darker zone of the cytoblast and the protuberances.

Utr. Mus. Cat. n°. Sorex 3 *b*, 3 *r*. 17 *s*.

PLATE 34.

Figs. 29—34 × 100. Fig. 35 × 1½. Figs. 36—47 natural size.

Fig. 29.—Fragment of a section through a yet later stage (cf. Fig. 12). The allantoidean trophoblast has already considerably developed; the cytoblastic portion of it has been more deeply stained by the picro-carminate. *P*. Tissue belonging to the maternal epithelial proliferation. *T*. Tissue belonging to the trophoblast (cytoblast + plasmodiblast). The sanguiniferous villi of the allantois that penetrate into this have on purpose been entirely omitted in this figure. White spaces in this figure thus indicate where they are situated. Only the lower boundary line of the allantois is indicated, with a few excrescences adhering against the trophoblast and representing incipient new villi (cf. Fig. 30). Comparison with Figs. 12, 81, and 89 will further elucidate this preparation. The difference should be noticed between the extent and situation of the area vasculosa in this and in the next figure (cf. also Figs. 12 and 13).

Utr. Mus. Cat. n°. Sorex 85 *g*, 2 *r*. 17 *s*.

Fig. 30.—The same, in a later stage (cf. Figs. 13, 82, and 94). The cytoblastic trophoblast is only here and there visible as a distinct layer outside the allantoic villi; generally it cannot be readily distinguished from the

intervening trophoblast (plasmodiblast) surrounding the blood-spaces (cf. Figs. 13, 82, and 94). On the concave free surface of the placenta, however, the cytoblastic trophoblast has attained to a maximum of thickness. It is here, moreover, more or less honeycombed, incipient new allantoidean villi entering into those recesses and being there attached and subsequently vascularised. In this figure the connective tissue of the allantoic villi has been indicated, but the spaces in these villi through which the embryonic blood-corpuscles circulate have been left open (white). Two full-grown double villi, both of them bifurcating, are here indicated, and three or four incipient ones. The trophoblastic layer, *an'*, being the continuation of the trophoblastic annulus (cf. Figs. 9—13, 50, 84, and Figs. 33 and 34), is closely applied against the allantois, but does not in any way fuse with it. A uterine gland, with partly distended, partly flattened lumen, is visible in this preparation. *P.* and *T.* as in Fig. 29.

Utr. Mus. Cat. n°. Sorex 130 *a*, 3 *r*. 10 *s*.

Fig. 31.—A yet further stage of placentation. The trophoblastic region in which the allantoidean villi are embedded occupies about five sixths of the thickness of the wall; the epithelial proliferation and gland-remains about one sixth. Here, too, the embryonic blood-corpuscles are not indicated, and the parts of the villi where they circulate remain white. The wide allantoidean vessel which supplies the three to four villi here represented is only indicated in outline, as is also the lower boundary line of the allantois. The maternal proliferation is yet more reduced, and of the crypts no distinct remains persist (cf. Figs. 14 and 86).

Utr. Mus. Cat. n°. Sorex 61 *a*, 2 *r*. 3 *s*.

Fig. 32.—A fragment of a section through the ripe placenta, perpendicular to the surface, with low power (cf. Figs. 15 and 15 *a*). The spaces in which embryonic and maternal blood-corpuscles circulate, although interwoven in a most complex manner, can be distinguished (*a*) by the size of the blood-corpuscles, which are very much smaller if maternal, (*b*) by the fact that in the syncytium the maternal corpuscles circulate in spaces without any trace of endothelium, whereas round the embryonic corpuscles the traces of the endothelium of the allantoidean vessels (which in this figure are again represented by white spaces) are often preserved as flattened nuclei (cf. Figs. 52—54).

Utr. Mus. Cat. n°. Sorex 100, 2 *r*. 12 *s*.

Fig. 33.—The trophoblastic annulus, terminal portion of area vasculosa, and downward membranous continuation by which the trophoblastic annulus is connected with the placental border, in the stage of the uterus No. 77 (cf. Figs. 35 and 44). To the right of the membrane, *an'*, a transversely cut allantoidean vessel is indicated (compare with Fig. 14).

Utr. Mus. Cat. n°. Sorex 77 *a*, 3 *r*. 4 *s*.

Fig. 34.—The same for a yet later stage, viz. that of uterus No. 100. The

area vasculosa is here not indicated; only its point of attachment is marked by a thin line.

Utr. Mus. Cat, n°. Sorex 100, 2 *r.* 1 *s.*

Fig. 35.—View of the inside of the uterus (in a stage corresponding to Fig. 44, which is somewhat further advanced than Fig. 14) after the mesometrical half has been dissected away. The view is thus directed towards the inner surface of the placenta. Three considerable allantoidean vessels, pressed in between the amnion (which is not indicated in the figure) and the area vasculosa turned inside out (cf. Fig. 14), are seen to take their course towards a central ring-shaped opening, through which they disappear. This ring is the upper constriction of the trophoblastic annulus. The area vasculosa is attached to it immediately below this rim. There is in the figure an irregular lighter centre, and a darker outer circumference. The latter is continued on the (here absent) inner surface of the mesometrical half. This darker colour is caused by the special green pigment of the hypoblast cells of the yolk-sac.

Prep. Sorex n°. 77.

Figs. 36—47.—Different phases of pregnant uteri of Sorex vulgaris following on the earlier stages that were represented in vol. xxxi of this Journal, Pl. XXXVI (and following) figs. 13, 15, 43, 65, and 83. The figures are natural size, drawn after the spirit specimens; the catalogue number between crotchets.

Fig. 36.—Uterus, No. 101 (one of the swellings; cf. Figs. 60, 61, 90—92).

Fig. 37.—Uterus, No. 106. *a—h.* The individual swellings, each of them containing an embryo (cf. Figs. 10, 27, 76, 79, 84).

Fig. 38.—Uterus, No. 61 (one of the swellings; cf. Figs. 14, 31, 58).

Fig. 39.—Uterus, No. 130 (cf. 13, 30, 82, 85, 94).

Fig. 40.—Uterus, No. 85 (cf. Figs. 12, 29, 81, 88, 89, 93).

Fig. 41.—Uterus, No. 90 (one of the swellings).

Fig. 42.—Uterus, No. 26 (one of the swellings; cf. Figs. 57, 59, 62).

Fig. 43.—Uterus, No. 51 (cf. Figs. 8, 9, 26, 50, 55, 75, 77, 78).

Fig. 44.—Uterus, No. 77 (one of the swellings; cf. Figs. 33, 35).

Fig. 45.—Uterus, No. 100 (one of the swellings; cf. Figs. 15, 32, 52—54).

Fig. 46.—Uterus, No. 80 (one of the swellings; cf. Fig. 86).

Fig. 47.—Uterus, No. 3 (cf. Figs. 11, 28, 56, 80, 87).

PLATE 35.

Figs. 52—54 enlarged × 200. Figs. 48—51, 55—65 enlarged × 260.

Fig. 48.—First appearance of the trophoblastic annulus in a stage corresponding to Fig. 6 (though not from the same preparation). About a dozen cells in each section take part in the formation of the annulus. These cells

are as yet only somewhat enlarged. They are not the same enlarged trophoblast cells that were figured for Sorex on Pl. XXXVII, fig. 27, vol. xxxi of this Journal. The latter have become applied against the maternal tissue, and have given origin to the omphaloidean trophoblast. The shred of omphaloidean trophoblast (*o. T.*) that is here figured gives ample indication of active histolysis going on in this portion of the trophoblast. In the preparation the shred here figured is not applied against any maternal tissue, and its trophoblastic derivation is thus all the more indubitable. It is important to determine this, as it will be seen in Figs. 49—51 how eminently difficult is the unravelling of maternal and trophoblastic tissue in the omphaloidean regions as soon as these two have become contiguous.

Utr. Mus. Cat. n°. Sorex 45 *c*, 2 *r*. 28 *s*.

Fig. 49.—In this figure the trophoblastic annulus is rather less than more distinct than in the former (cf. Fig. 7). The omphaloidean trophoblast is undergoing a granular transformation simultaneously with degenerative phenomena in the maternal tissues.

Utr. Mus. Cat. n°. Sorex 42 *e*, 2 *r*. 14 *s*.

Fig. 50.—The maternal tissue in partly degenerative resorption (cf. Fig. 8), in consequence of the fusion with trophoblastic tissue. To the right strands of epithelial tissue (*s*.) keep up the connection between the superficial and the deeper maternal layers not indicated in this figure. These latter become transformed into a new uterine epithelium (cf. Figs. 84 and 88). The fusion between omphaloidean trophoblast and maternal tissue is very complete in this preparation just above the upper rim of the trophoblastic annulus. The hypoblastic nuclei are distinct. There are traces of blood-extravasate between the trophoblastic annulus and the maternal tissue (cf. Fig. 84).

Utr. Mus. Cat. n°. Sorex 51 *b*, 2 *r*. 17 *s*.

Fig. 51.—A yet earlier phase in the attachment of omphaloidean trophoblast against the lateral maternal epithelial proliferation (cf. Fig. 5). A considerable portion of the trophoblast has undergone a granular metamorphosis. Above this there is another region where the distinction between embryonic and maternal proliferated tissue is indeed impossible. In this stage a trophoblastic annulus is not yet even indicated.

Utr. Mus. Cat. n°. Sorex 73 *c*, 3 *r*. 13 *s*.

Figs. 52—54.—Three regions of the placenta that was represented in Fig. 32, more considerably enlarged. Fig. 52 from the lower, Fig. 53 from the middle, Fig. 54 from the upper portion. The difference in size between embryonic and maternal blood-corpuscles (the former being by far the larger) renders distinction between maternal and embryonic blood-spaces very easy. The preparation may be considered as a very perfect self-injection. The maternal blood circulates in spaces delimitated on all sides by trophoblast that is spread out to the utmost degree of tenuity. The embryonic blood circulates in similar spaces, nowhere communicating with the former, and showing here

and there flattened remnants of the endothelium of the vessels of the allantoidean villi. In Fig. 52 the embryonic blood-spaces are wider than in Fig. 53. In Fig. 54 no blood-corpuscles are indicated, whereas the remnants of the secondary epithelial crypts are visible as nuclear conglomerates. *C.*, peculiar corpuscles in the deeper maternal layers.

Utr. Mus. Cat. n°. Sorex 100, 2 *r.* 12 *s.*

FIGS. 55—57.—Three figures of different phases of development of the non-placental trophoblast and of the extenuated uterine wall. Fig. 55 taken from the phase of Figs. 8 and 9. The lower wall of the blastocyst is in this phase comparatively thick, the hypoblast even much more so than the non-placental trophoblast. In Fig. 56 (corresponding to Fig. 11) the same layers have become more considerably attenuated, and so has the uterine wall, the epithelium of which is consequently no longer thrown into any folds. In Fig. 56 a thin layer of a greenish granular coagulum is applied against the inner surface of the yolk hypoblast. In Fig. 57 this has considerably increased in thickness, and can be traced in the lower half of the yolk-sac all round. The greenish coagulum has parallel surfaces. To the left of *g c.* in Fig. 57 a further granular precipitate is indicated, which in this preparation fills part of the crescentic yolk-sac. In comparing Fig. 57 with 56 we see that the further extenuation of the muscular layers of the uterus is especially marked.

Fig. 55.—Utr. Mus. Cat. n°. Sorex 51 *e*, 4 *r.* 13 *s.*
Fig. 56.— „ „ 3 *b*, 4 *r*, 4 *s.*
Fig. 57.— „ „ 26 *a*, 3 *r.* 10 *s.*

FIGS. 58—65.—Eight different regions and different stages of development of that surface of the yolk-sac, which becomes inverted from the stage of Fig. 12 onward. This is the surface, stretching between the trophoblastic annulus and the embryo, on which the area vasculosa develops. After the inversion the vascular region increases in surface, and so does the number of the vessels. The hypoblast cells increase in size, proliferate freely, and acquire a deep green tinge. Neoformation of blood-corpuscles is very actively going on in this region.

Fig. 58.—Utr. Mus. Cat. n°. Sorex 61 *a*, 2 *r.* 10 *s.*
Fig. 59.— „ „ 26 *a*, 3 *r.* 15 *s.*
Fig. 60.— „ „ 101 *a*, 3, 3 *r.* 15 *s.*
Fig. 61.— „ „ 101 *a*, 3, 2 *r.* 12 *s.*
Fig. 62.— „ „ 26 *a*, 2 *r.* 10 *s.*
Fig. 63.— „ „ 92 *a*, 2, 1 *r.* 10 *s.*
Fig. 64.— „ „ 100, 1 *r.* 8 *s.*
Fig. 65.— „ „ 100, 1 *r.* 8 *s.*

PLATE 36.

All the figures in this Plate × 480.

Fig. 66.—Part of the uterine wall opposite the mesometrium, in the phase of Fig. 3 (cf. Fig. 17). The stretching of the uterine wall is very considerable; the blastocyst has not yet become attached.

Utr. Mus. Cat. n°. Sorex 2 *a*, 5 *r*. 23 *s*.

Fig. 67.—Part of the same, one stage further (cf. Figs. 4 and 19). Proliferation of the uterine epithelium has commenced. Karyokinetic figures demonstrate the participation of the epithelium cells.

Utr. Mus. Cat. n°. Sorex 52 *b*, 4 *r*. 20 *s*.

Fig. 68.—Section through another swelling of the same uterus, showing the mouth of one of the uterine glands (cf. Figs. 4 and 21).

Utr. Mus. Cat. n°. Sorex 52 *i*, 3 *r*. 21 *s*.

Fig. 69.—Section through the same swelling from which Fig. 67 was taken, somewhat further from the mesometrium (cf. Fig. 4). The proliferated epithelium cells have commenced to arrange themselves in a radial fashion. Uterine epithelium as yet continuous. A few cells in the border region, between the epithelial proliferation and the connective tissue of the uterine wall, are seen to become spindle-shaped in Figs. 68 and 69. This is later on further accentuated into a special fibriform layer (cf. Figs. 72—74, and also Figs. 22—25).

Utr. Mus. Cat. n°. Sorex 52 *b*, 4 *r*. 15 *s*.

Fig. 70.—Section through a further stage of development. The proliferation forms already a thicker layer, the uterine epithelium breaks away from under the radially arranged groups of cells, which are going to be the secondary epithelial crypts.

Utr. Mus. Cat. n°. Sorex 73 *f*, 3 *r*. 22 s.

Fig. 71.—Another section through another swelling of the same uterus. The passage of the uterine epithelium into that of the crypt is already more complete, but quite different from the mouth of a gland.

Utr. Mus. Cat. n°. Sorex 73 *e*, 3 *r*. 14 *s*.

Fig. 72.—The boundary region between the epithelial proliferation and the connective tissue of the uterine mucosa. The blood-vessels in the latter are more spacious than the capillary ducts in the former. Of the uterine glands one is partly cut along the lumen, the other only tangentially through the wall.

Utr. Mus. Cat. n°. Sorex 73 *b*, 2, 3 *r*, 8 *s*.

Fig. 73.—The same, in a somewhat later stage. The proliferated epithelium cells are more closely packed, and between them and the connective tissue there is a layer of fusiform cells, first noticed in Fig. 69. This section was somewhat too tangential to show the secondary crypts well (cf. Fig. 25, which has reference to the same uterus).

Utr. Mus. Cat. n°. Sorex 42 *e*, 2 *r*. 13 *s*.

PLATE 37.

Fig. 80 × 200. All the other figures × 260. Both the maternal and the embryonic blood-corpuscles are coloured red on this Plate.

Fig. 74.—Part of a section through the future placentary region when the proliferation of the maternal epithelium, which precedes the adhesion of the blastocyst, has attained its maximum of development. The crypts reach through the whole thickness of the proliferation (cf. Figs. 6 and 24).

Utr. Mus. Cat. n°. Sorex 45 *c*, 3 *r*. 17 *s*.

Fig. 75.—One of the proliferating knobs of allantoidean trophoblast penetrating into the mouth of a secondary epithelial crypt in the future placentary region. Large nuclei reveal other epithelial crypts tangentially cut (cf. Figs. 8 and 9).

Utr. Mus. Cat. n°. Sorex 51 *e*, 2 *r*. 8 *s*.

Fig. 76—79.—Four different portions of the allantoidean trophoblast before its adhesion against the maternal tissue. In all—but most especially in Fig. 79—the differentiation of cytoblast and plasmodiblast has commenced.

In Fig 76 the amnion is just being completed.

In Figs. 78 and 79 distinct and massive trophoblastic knobs are represented.

Fig. 76.—Utr. Mus. Cat. n°. Sorex 106 *e*, 2 *r*. 19 *s*.
Fig. 77.— „ „ 51 *a*, 2 *r*. 24 *s*.
Fig. 78.— „ „ 51 *c*, 5 *r*. 20 *s*.
Fig. 79.— „ „ 106 *f*, 4 *r*. 15 *s*.

Fig. 80.—A similar section to that of Fig. 28 (cf. also Fig. 11), more considerably enlarged. The allantoidean trophoblast and its protuberances have become fused with the maternal tissue, and have further proliferated under partial destruction of the latter. The trophoblastic knobs are being hollowed out, allantoidean villi penetrating into the cavities thus originating. The differentiation of plasmodiblast between the protuberances has commenced.

Utr. Mus. Cat. n°. Sorex 3 *b*, 2, 1 *r*. 17 *s*.

Fig. 81.—Part of the placentary region of Fig. 12, more considerably enlarged. The upper half of the figure represents maternal tissue (secondary epithelial crypts, with blood capillaries between them), the lower half trophoblastic tissue. Below this is the allantois, with a distended blood-vessel and two villi. The trophoblast is subdivided in a more deeply stained cytoblast (which is the superficial layer, and which visibly ensheathes the villi) and an intervening mass of cells (plasmodiblast) in which the maternal blood circulates. In this figure these two have been torn asunder in the lower part of the figure; in more normal circumstances they firmly adhere together. The exact boundary line between the "plasmodiblast" and the maternal tissue cannot be distinctly indicated; it takes its course somewhere

between the tops of the villi. With Fig. 81, Figs. 29, 89, and 93 should be directly compared, as they are all preparations from the same uterus.

Utr. Mus. Cat. n°. Sorex 85*f*, 3 *r*. 13 *s*.

Fig. 82.—A section through a corresponding region in a later stage (cf. Fig. 13). The plasmodiblast between the villi is being attenuated as the number and the length of the villi increase. The more deeply staining layer of cytoblast surrounding the villi is no longer distinct; its elements have assimilated with the intervening plasmodiblast. The passage of blood from a maternal vessel, with distinct endothelium, into the trophoblastic blood-spaces can be distinctly traced in this and the neighbouring sections. The cytoblastic layer of the trophoblast, if it is no longer distinct round the villi, is all the more massive on the free concave surface of the placenta, which is, however, not represented in this figure (cf. Figs. 30 and 94, taken from the same uterus).

Utr. Mus. Cat. n°. Sorex 130 *a*, 2 *r*. 13 *s*.

PLATE 38.

Figures 83—85 × 260. Fig. 84*a* × 60. Fig. 86 × 480.

Fig. 83.—Fragment of a section through the region of the omphaloidean trophoblast and the adjoining maternal tissue, in an early stage (cf. Fig. 6). One of the first phenomena of dehiscence in the deeper proliferated maternal layers is visible as a solution of continuity in the middle of the lower half of the figure. The omphaloidean trophoblast is considerably thickened—without as yet being adherent to maternal tissue—in the upper half of the figure; in the lower half adhesion has come about, and at the same time considerable histolytical transformations have commenced. Maternal uterine epithelium is only intact in the very topmost portion of the figure. Maternal blood is seen to pass from the blood-spaces of the maternal proliferation into the syncytial tissue, in the region where maternal and trophoblastic elements have fused together. Remnants of compressed uterine glands are seen between the muscularis and the epithelial proliferation.

Utr. Mus. Cat. n°. Sorex 45 *e*, 5 *r*. 6 *s*.

Fig. 84.—Section through the uterine wall in the region of the trophoblastic annulus (cf. Fig. 10). There is a considerable blood-extravasate between the annulus and the tissues that are in process of resorption. Blood-corpuscles are being actively absorbed into the protoplasm of the cells of the trophoblastic annulus. In the region of the omphaloidean trophoblast there is a very intimate fusion between embryonic and maternal tissue. In the deeper layers of the proliferated maternal epithelium dehiscence is actively going on, and a fresh layer of uterine epithelium is developing below the tissues that are being resorbed. From the upper rim of the trophoblastic

annulus a cell-layer (*an'*.) descends, that is parallel to the annulus but adherent to the maternal tissue. The hypoblast adhering against the trophoblastic annulus is cut tangentially in the upper part of the figure. Its continuation along the omphaloidean trophoblast, missing in this section, is again present in the neighbouring sections.

Utr. Mus. Cat. n°. Sorex 106 *a*, 3 *r*. 15 *s*.

Fig. 84 *a*.—Pseudo-karyokinetic and other phases of the nuclei of Fig. 84 in the region where histolysis is most actively going on.

Fig. 85.—An allantoidean villus in an early stage; massive, with a central blood-vessel and peripheral blood-spaces communicating with that central one (cf. Figs. 30, 81, 82, and 86).

Utr. Mus. Cat. n°. Sorex 130 *a*, 2 *r*. 13 *s*.

Fig. 86.—The tops of two other allantoidean villi, in a later stage of pregnancy (the embryonic blood-corpuscles are here represented as red discs, the maternal as dots). The tissue of the villus is less compact, the blood-spaces in it are arborescent and partly intracellular. Between the two villi, as well as right and left of them, strands of trophoblastic tissue are pictured, carrying spaces with the so-much smaller maternal blood-corpuscles. It has here already become considerably more difficult to distinguish between the tissue of the villus and of the intervening trophoblast than it was in Figs. 81 and 82. On the other hand, it is here not yet quite so difficult as in the still later phases of pregnancy that are represented in Figs. 52 and 53. There is as yet hardly any other tissue between the muscularis and the maternal epithelial proliferation, as far as it is yet preserved (cf. Figs. 13, 14, 15, and 54).

Utr. Mus. Cat. n°. Sorex 80 *a*, 3 *r*. 5 *s*.

PLATE 39.

Figs. 87, 90—92 × 480. Fig. 88 × 100. Figs. 89, 93, 94 × 260.

Fig. 87.—A portion of Fig. 11 *b*, more considerably enlarged. In the free space in the middle of the figure an allantoidean villus is going to penetrate. The layer of darker stained nuclei surrounding that space is the deeper (cytoblastic) layer of the trophoblast. Blood already circulates in trophoblastic spaces.

Utr. Mus. Cat. n°. Sorex 3 *a*, 2, 5 *r*. 22 *s*.

Fig. 88.—The region of the trophoblastic annulus of Fig. 12, under higher power. Lateral maternal proliferated tissue already considerably resorbed. New epithelium that has originated behind this (cf. Fig. 84) is further developed and conspicuously folded.

Utr. Mus. Cat. n°. Sorex 85 *a*, 2 *r*. 14 *s*.

Fig. 89.—Another part of Fig. 12, more considerably enlarged, further to demonstrate the fusion between the proliferated maternal and trophoblastic

tissues. Blood circulates in the maternal proliferation in capillaries that generally have a distinct endothelium. In the trophoblast it circulates in intra- and intercellular cavities. Two allantoidean villi are here only indicated by their embryonic blood-corpuscles. Between these villi the cytoblastic and plasmodiblastic portion of the trophoblast is situated, the two having become artificially separated from each other as in Fig. 81. It is impossible to distinguish between those cells that have a maternal origin and those that are derived from the embryo, otherwise than genetically.

Utr. Mus. Cat. n°. Sorex 85 *f*, 3 *r*. 15 *s*.

Figs. 90—92.—Three portions of the allantoidean trophoblast taken from one and the same series of sections of uterus No. 101 (Fig. 36). These sections indicate how the allantoidean trophoblast proliferates and is transformed into intercommunicating blood cavities, nuclear nests breaking away from the outer layers of the syncytium, and arranging themselves into blood channels by processes of stretching and hollowing out. In principle it is this same process by which, in the earlier phases such as that of Figs. 79, 80, and 93, the trophoblast has developed and become the peculiar sanguiniferous interstitial tissue between the villi.

Fig. 90.—Utr. Mus. Cat. n°. Sorex 101 *a*, 1, 3 *r*. 5 *s*.
Fig. 91.— „ „ 101 *a*, 1, 1 *r*. 10 *s*.
Fig. 92.— „ „ 101 *a*, 1, 2 *r*. 10 *s*.

Fig. 93.—The same in an earlier stage (uterus No. 85, Fig. 40; cf. also Figs. 12, 29, 81, and 89). The darker layers of cytoblast have the somewhat more faintly stained plasmodiblast between them. The latter is in this stage seen to be split off from the former in parallel layers that develop blood-spaces between them (cf. Figs. 29, 30, and 89). The cytoblast at the top of the figure belongs to a cavity in which an allantoidean villus is situated (cf. Fig. 29, which represents the same stage).

Utr. Mus. Cat. n°. Sorex 85 *a*, 2 *r*. 23 *s*.

Fig. 94.—The same, one stage later (cf. Figs. 13, 30, and 82) at the period that the concave layer of cytoblast has attained its maximum of development. Here, too, the newly-formed blood-spaces are primarily arranged in a more lamelliform way close to the inner layer, whereas higher up between the villi this vasifactive plasmodiblast is already more like that of Figs. 90—92. Indentations on the lower surface for the reception of secondary or tertiary allantoidean villi.

Utr. Mus. Cat. n°. Sorex 130 *a*, 3 *r*. 10 *s*.

Some Further Contributions to our Knowledge of the Minute Anatomy of Limnocodium.

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With Plate 40.

While engaged in a study of the anatomy of Limnocnida tanganjicæ I was naturally rather desirous of making a comparison between the structural features of Limnocnida and those of the first-described fresh-water medusa, Limnocodium Sowerbii, in order to attempt to ascertain if the two forms possessed any similar modifications which might possibly be brought into relation with their life in fresh water.

For the furtherance of this object Professor Ray Lankester very kindly placed all such preserved material of Limnocodium as existed in the Department of Comparative Anatomy of the Oxford Museum at my disposal. I take this opportunity of thanking Professor Ray Lankester for this and all other assistance he afforded me during the progress of my work, which was carried on in the new Laboratory of Comparative Anatomy at Oxford. I also wish to express my deep sense of gratitude to the President and Fellows of Magdalen College for enabling me to continue my studies in Oxford by prolonging my Demyship at that College.

Hitherto I have, unfortunately, not had an opportunity of examining any fresh specimens of Limnocodium. The material which was best suited for histological examination had been killed in osmic acid some years previously, but was neverthe-

less well enough preserved for the elucidation of many structural points. All my observations are based on sections cut by the ordinary paraffin method and stained in various ways, but those which had been coloured with Kleinenberg's hæmatoxylin gave as satisfactory results as any. Notwithstanding the age of the material, I have been able to confirm most of the observations of Allman and Lankester (**2** and **4**), and to add some further details regarding the structure of the tentacles, the sense-organs, and the male reproductive organs.

The general proportions of the various parts of the body as seen in meridional section are shown in Pl. 40, fig. 1. In the section of the individual there figured the mouth of the bell is considerably contracted, so that the manubrium is enclosed in the subumbrellar cavity; but in the living condition, when the animal is floating in the water, the bell has a much more flattened shape, and the manubrium then projects considerably beyond the margin of the umbrella. The mesoglœa of the umbrella is fairly uniform in thickness throughout, being only very slightly thicker in the centre than near the circular canal. In the manubrium, however, the mesoglœa is not so evenly distributed, but is thickest at the distal end of that organ, the proximal end being almost completely destitute of any gelatinous middle layer whatever. This distribution of the mesoglœa may be in relation to the great extensibility of the manubrium. It is probable that the extension of that organ is chiefly effected by the elongation of its proximal end, and that a well-developed muscular layer exists between the limiting epithelia of this region.

In none of the preceding papers are the figures of the arrangement of the organs situated at the margin of the umbrella satisfactory, while that of the "diagrammatic meridional section" of Allman (**4**, p. 132) is erroneous and misleading. The relations of the organs situated round the periphery of the umbrella are exhibited in transverse section in Pl. 40, figs. 2 and 3. Fig. 2 is a section passing along a radial canal (*r. c.*) and through the base of a radial tentacle (*te.*), while fig. 3 is taken several sections further on, passing

through the base of another tentacle, not a radial one. The circular canal (*c. c.*) as seen in cross-section is roughly triangular in shape. The epithelia of the radial canals and the endoderm lamella join the epithelia of the circular canal at the apical angle, while the velum and tentacles arise near the interior and exterior basal angles of the circular canal respectively. On the basal side, i. e. the side between the attachment of the velum and that of the tentacles, the ectoderm is much thickened and modified to form the "nettle-ring" (*net.*), and between the nettle-ring and the point of attachment of the velum is the nerve-ring (*n. r.*).

Tentacles.—The tentacles are usually carried turned back over the aboral surface of the umbrella, and, like those of many of the Trachymedusæ, are adnate to the margin of the umbrella for a short distance. The tentacle roots are not entirely surrounded by mesoglœa as are those of Cunina, but only lie in a furrow on the umbrella margin. This attachment of the tentacles to the umbrella is doubtless connected with their upright carriage, and is very similar to the condition obtaining in Limnocnida; but in Limnocodium the embedded part or "root" of the tentacle consists of endoderm only, whereas in Limnocnida the embedded tentacle root is ensheathed with ectoderm.

The structure of the tentacles has been described both by Professor Allman and by Professor Lankester. With regard to the question of the presence or absence of an axial cavity and of the condition of the endoderm, the account by Professor Allman (**4**, p. 133) is as follows:—"I could find no indication of a cavity in the tentacles; but they do not present the peculiar cylindrical chorda-like endodermal axis formed by a series of large, clear, thick-walled cells which is so characteristic of the solid tentacles in the Trachomedusæ and Narcomedusæ."

Professor Ray Lankester, in an addendum to his second paper (**3**), says, "Endoderm-cells consist of a dense, highly refringent substance, which is somewhat wrinkled by the action of the reagent;" and further, "In some cases a small amount

of granular cell-substance may be seen radiating from the nucleus, but the whole cell body otherwise has been metamorphosed into a homogeneous cartilaginoid substance. There is no continuous lumen, although the cells are disposed in a single series around the axis of the tentacle, and leave, on shrinking, a small space where their adaxial surfaces should come into contact. This potential lumen appears not to be continuous even in the specimens treated with reagents, and in living specimens it has no existence."

In all the individuals which I have hitherto examined the larger and older tentacles were always hollow throughout their length (Pl. 40, figs. 2 and 5), and it is only to the younger and smaller ones that the above-quoted descriptions of Allman and Lankester can apply. Moreover, in a considerable number of sections of tentacles examined, the lumen of the tentacles was found to be directly continuous with the lumen of the ring canal, and the endodermic lining of the tentacle was directly continuous with that of the ring canal as seen in section in Pl. 40, fig. 2.

From this it appears that the tentacles of Limnocodium are, morphologically speaking, hollow tentacles, though it is quite possible that under certain circumstances they often contract to such an extent that the lumen vanishes. An indication of this contractile power is afforded by the existence of a powerful circular muscular coat at the bases of the ectodermal cells. In obliquely cut sections such as the one figured in Pl. 40, fig. 5 *a*, these circular muscles may be seen at the two ends of the section as transverse lines (*circ. m.*).

The endodermal lining of the tentacles consists of very large clearish cells very similar to those of the tentacles of Limnocnida. Their contents are very probably of a gelatinous nature, which gives the tentacles a certain amount of firmness.

Nervous System.—The nerve-ring lies on the inner or subumbrellar side of the circular canal at the attachment of the velum. It is seen in transverse section in Pl. 40, figs. 2 and 3. The nerve-fibres composing the ring, as in other medusæ, are divided into two bundles separated by the sup-

porting lamella of the velum. Of the two divisions of the nerve, the outer division on the side next the nettle-ring is the most strongly developed of the two. Its fibres are perfectly well demarcated from the surrounding cells, and are always easy of observation. They seem, moreover, to be enclosed in a sheath or neurilemma. On the other hand, the fibres which constitute the inner bundle of the nerve-ring ("unterer Nervenring" of Hertwig) are more difficult of certain demonstration, since they are not distinctly separate from the bases of the ectoderm-cells of the region.

Sense-organs.—Concerning the velar sense-organs, marginal bodies, or refringent bulbs, I have nothing to add to Professor Ray Lankester's description. I can only confirm his account of these most remarkable organs in every particular. They consist of a small almost spherical multicellular refringent body, which in the fully developed organ is attached by a thin stalk near the nerve-ring, and is suspended in an elongated sac which is embedded in the thickness of the mesoglœa of the velum. The general relations of the organ are shown in Pl. 40, fig. 3, while in fig. 6 are several views of sections of refringent bulbs carefully drawn with the camera lucida. The refringent bulbs consist of cells of two kinds. The more peripheral ones—the "cortical cells" of Lankester —are thin, and often so much stretched over the more central or "medullary cells" that they are difficult of observation. These cortical cells are of ectodermal origin, and are continuous with the lining of the sac in which the entire organ is enclosed. The medullary cells impart the highly refringent appearance to the bulbs, and are of endodermal origin, being budded off from the lining of the circular canal, and subsequently become completely enclosed by ectoderm. In the young condition these medullary cells have a granular appearance (Pl. 40, fig. 6, *a*, *med. c.*) like the cells lining the circular canal, and only become clear and refringent as the bulb approaches maturity. Even in fully developed bulbs a few of the medullary cells near the point of attachment of the bulb still retain a certain granular character (fig. 6, *d*, *e*).

In his original paper Professor Ray Lankester drew attention to the unique nature of this peculiar organ, and pointed out its relation to the endodermal sense-organs of other craspedote medusæ, such as the Trachomedusæ.

The interest attaching to this organ is now all the greater because another medusa (Limnocnida tanganjicæ) has been discovered with similar and similarly situated sense-organs, of which the axial cells are also endodermal. The new medusa, moreover, is also an inhabitant of fresh water. In Limnocnida the organ is identical in every important respect, the chief differences being that the sacs are not prolonged into the velum, and that the refringent bulbs, as a rule, consist of fewer cells, but in both Limnocodium and Limnocnida no otolithic concretion is formed.

Endoderm.—The endoderm of the gastric cavity has already been the object of a very thorough investigation by Professor Ray Lankester in his paper on the "Intra-cellular Digestion of Limnocodium" (3). Proceeding from the mouth towards the stomach, three regions have been distinguished by Professor Lankester, all differing in regard to the nature of their epithelial lining. The endoderm of the first region nearest the mouth is composed of more or less granular cubical cells, the nuclei of which are situated near the bases of the cells (Pl. 40, fig. 9). The epithelium of the second region (fig. 10) is very much higher; the cells composing it are possibly ciliated, though no cilia could be observed in the preserved material. The nucleus is situated near the middle of the cell, and separates the protoplasm of the inner half, which is fairly clear, from the very granular protoplasm of the outer half. The epithelium lining the third region (fig. 11) or stomach proper is composed of very large cells, and it is in this region that intra-cellular digestion occurs. Among the large vacuolated digestive cells are numerous goblet gland-cells, and here and there a fragment of food may be seen which is undergoing intra-cellular digestion, as in Pl. 40, fig. 11, *x*.

Fig. 12 illustrates the very abrupt transition between the large-celled digestive epithelium of the third region and the

very small and cubical cells which coat the subumbrellar wall of the stomach. Similar cells are found lining the radial canals and part of the circular canal. The genital sacs are lined with a taller epithelium, a description of which is given below.

The circular canal is lined with a low epithelium, similar to the one lining the radial canals, except on the outer side. There, between the point of attachment of the velum and the origin of the tentacles, all round the inside of the thickened patch of ectoderm which forms the nettle-ring, the cells of the circular canal become much thicker, and in some places the cell outlines are not well defined (Pl. 40, fig. 2, *end.*). The function of the modified cells is not at all clear at present; but it is noteworthy that in Limnocnida a modified mass of cells occurs in exactly the same position, but is very much more largely developed.

Reproductive Organs.—As the material at my disposal consisted solely of male individuals in various stages of maturity, I have not been able to examine any females.

The reproductive organs in the male consist of four sac-like outgrowths on the subumbrellar aspect of the four radial canals. The distal walls of these sacs are very thick, being chiefly composed of testicular tissue, as shown in section (Pl. 40, fig. 7). The lumen of each of the sacs is a ventral diverticulum of the lumen of the radial canal, and is lined by a continuation of the endoderm of the radial canal. The cells of this endodermal lining of the gonads are columnar, and rather taller than the cells of the ordinary epithelium of the radial canal. The nucleus of each cell is roundish, with a well-marked nucleolus, and is situated near the base of the cell. The protoplasm is highly granular, and near the free margin of many of the cells it contains an ovoid mass which stains deeply, and is probably the product of some secretory activity of the cell (Pl. 40, fig. 8, *end.*).

The mesoglœa is exceedingly reduced where the testicular tissue is thickest, but it is fairly well developed all round the stalk of the sac (fig. 7, *m. s.*), to which it imparts some rigidity.

Immediately to the outside of the mesoglœa the great bulk of tissue is chiefly composed of developing spermatozoa. As in the spermarium of Oceania, described by O. and R. Hertwig (1, p. 27), three different tissue zones may be distinguished. Proceeding from within outwards (Pl. 40, fig. 8), the first or basal layer of ectoderm consists chiefly of large round nuclei with but little protoplasm proportionately; secondly, there is a thick layer of spermatozoa in various stages of development; and thirdly, there is an epithelial covering over all, the cells of which send down processes in among the bundles of spermatozoa, and also seem to be in connection with long fusiform cells penetrating between the spermatozoa in the second layer.

In a single favorable section through the gonad of a male Limnocodium of a certain degree of maturity all the various stages of developing spermatozoa can be observed; consequently Limnocodium is a far more favorable object for the examination of the process of spermatogenesis than the majority of Hydroids, in which, as a rule, a complete series of stages of developing spermatozoa are not found in the same gonophore. In most Hydroids in which spermatogenesis has been studied the developing spermatozoa of one gonophore all progress at about the same rate, and so in a mature bud the younger stages do not occur, and vice versâ. In fact, it is of rare occurrence that more than two different stages of developing spermatozoa occur in the same bud. In Limnocodium, on the contrary, all stages are often present.

The following stages in the development of the spermatozoa may be distinguished:

1. The sperm mother-cells (Pl. 40, fig. 8, *a*), situated next the endoderm. These are characterised by their large nuclei, which stain but slightly. They more or less correspond to the Hertwigs' first layer in their description of the spermarium of Oceania (1). Each of the nuclei of these cells has a well-marked nucleolus. Eventually they divide by karyokinesis, and give rise to—

2. The daughter spermatoblasts (fig. 8, β). The nuclei of these cells are relatively much less than half the size of the

nuclei of the sperm mother-cells of the first layer, but their chromatin is in a more compact condition and stains more deeply. The nuclear matter of these cells now apparently undergoes a sort of condensation, as indicated both by diminution in size as compared with the size of the cell, and also by greater intensity of tingibility (fig. 8, γ). At this stage the cells apparently undergo a second division, but whether by karyokinesis or not could not be ascertained, owing to their extreme minuteness. In either case the result is—

3. A number of cells (fig. 8, δ) with very small, deeply staining nuclei, which, by the drawing out of their protoplasm into the tail, give rise to—

4. The spermatozoa themselves (fig. 8, ε). The spermatozoa are of the ordinary hydroid type, with well-marked heads and relatively short tails. They probably escape to the exterior by the dehiscence of the outer epithelium of the spermaria.

At stage 3 the cells seem to become segregated into groups.

The process of spermatogenesis, as described above, is totally at variance with the views of André de Varenne (5) regarding the condition of the nucleus of the sperm-cells. De Varenne makes the following statement:—"Dans toute la durée du développement des spermatozoides, en prenant la cellule mère dès son début, le noyau n'a pas changé." Limnocodium certainly affords us a most effective refutation of any such view, if any further objection to the view was needed after the extensive researches of Thallwitz (6) on hydroid spermatogenesis.

Conclusion.

In conclusion, I am afraid that the foregoing observations do not shed very much light upon the question of the systematic position and genetic affinities of Limnocodium. It is seemingly a case in which an increase of knowledge is correlated with an increase of difficulties. All attempts to find a resting-place for Limnocodium in the system of Haeckel have been unsatisfactory. Of the four sub-orders into which the Medusæ are divided by Haeckel, neither the Anthomedusæ nor the Narcomedusæ can receive Limnocodium on account of the position

of the gonads. Hence the Leptomedusæ and the Trachomedusæ are the only two sub-orders which can be considered.

Allman (4) enrolled Limnocodium among the Leptomedusæ on the erroneous assumption that the sense-organs were entirely derived from the ectoderm of the velum. This, however, was shown not to be the case by Ray Lankester (2), who proved their partial endodermic origin, and accordingly transferred the medusa to the ranks of the Trachomedusæ. This change he, moreover, supported by pointing out that Limnocodium resembles the Trachomedusæ in certain other respects. On the other hand, it has been urged, and I think justly, that Limnocodium has a fixed hydroid stage, a thing which is quite unknown among Trachomedusæ, and certainly absent in some of them. In my own opinion, to include Limnocodium among the Trachomedusæ in the present state of our knowledge cannot but render that group an unnatural one, or more unnatural than it is at present. On the other hand, it cannot be denied that Limnocodium has reached a Trachomedusan grade of development in the possession of sense-organs with an endodermal axis. Limnocodium, then, is a medusa descended from Leptomedusan ancestors, which has developed sense-organs with an endodermal axis independently of the Trachomedusæ.

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A complete bibliography of Limnocodium Sowerbyi up to 1890 will be found in No. 7 in the above list.

EXPLANATION OF PLATE 40,

Illustrating Mr. R. T. Günther's paper, "Some Further Contributions to our Knowledge of the Minute Anatomy of Limnocodium."

Reference Letters.

c. c. Circular canal. *circ. m.* Circular muscle. *cort. c.* Cortical cells of sense-organ. *ect.* Ectoderm. *end.* Endoderm. *m.* Mouth. *med. c.* Medullary cells of sense-organ. *mn.* Manubrium. *ms.* Mesoglœa. *net.* Nettle-ring. *n. r.* Nerve-ring. *r. c.* Radial canal. *s. o.* Velar sense-organ. *te.* Tentacle. *v.* Velum.

Fig. 1.—Meridional section through an entire Limnocodium, in which the velum is strongly contracted. The ectoderm (*ect.*) is represented by a single line; the mesoglœa (*ms.*) is shaded; the endoderm (*end.*) lining the gastric cavity is represented by a double contour. × 15.

Fig. 2.—Radial section of margin of umbrella, showing the circular canal (*c. c.*) with a radial canal (*r. c.*), cut longitudinally, opening into it. The basal portion of a radial tentacle (*te.*) and of the velum (*v.*) are also represented.

Fig. 3.—A similar section to Fig. 2, but not passing through a radial canal and tentacle. In this section a velar sense-organ (*s. o.*) in its chamber has been cut through.

Fig. 4.—Longitudinal vertical section of a portion of the velum, showing several of the chambers of the velar sense-organs cut across. Note the thin pavement epithelium lining these chambers.

Figs. 5 *a*, *b*, *c*.—Transverse sections of tentacles, showing lumen. 5 *a* is cut diagonally, and shows the circular muscles (*circ. m.*) at the two ends as transverse stripes.

Fig. 6.—Five drawings of sections of velar sense bulbs. *a* is a longitudinal section of quite a young stage which has only just become separated from the endoderm. *b* and *c* are transverse, *d* and *e* longitudinal sections of mature sense bulbs. *e* shows the stalk of attachment.

Fig. 7.—Radial section through an entire spermarium (*sp.*) and through a portion of the radial canal on which the spermarium is situated.

Fig. 8.—Section through a portion of the wall of the spermarium of another individual. α—ϵ are various stages in the development of the spermatozoa.

Fig. 9.—Endodermic lining of the oral end of the manubrium (Region I).

Fig. 10.—Endoderm of the middle or region II of the manubrium.

Fig. 11.—Section through the wall of Region III of the gastro-vascular cavity. Note the much vacuolated nature of the ordinary digestive cells of this region, and also the numerous goblet gland cells (*g. c.*), each filled with numerous granules of secreted matter. *x* is a foreign particle undergoing intra-cellular digestion.

Fig. 12.—Section through an upper corner of the gastro-vascular cavity, showing the junction between the endoderm of Region III, figured in Fig. 11, and the small-celled endoderm of the subumbrellar roof of the gastro-vascular cavity.

Note on the Mesenteries of Actinians.

By

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In a paper published in the last number of the 'Quarterly Journal of Microscopical Science' (193, January, 1894) on "Octineon Lindahli," the author, Dr. G. H. Fowler, gives a figure—woodcut Fig. A,—and as my name is given in connection with it, perhaps I may be allowed to make a statement regarding it. The figure "represents Sagartia, Actinia, Bunodes (Lacaze-Duthiers, corroborated by F. Dixon)." Some time ago Professor Haddon, who was then writing the first part of his "Revision of British Actiniæ," asked me to cut sections of young specimens belonging to the genera whose development Lacaze-Duthiers had described. He asked me to do this because the Hertwigs, from their observations on Adamsia diaphana, assumed that Lacaze-Duthiers had made a mistake in determining which were the first eight mesenteries to arise in Sagartia, Actinia, and Bunodes. They assumed that in these genera, just as in Adamsia diaphana, the most "ventral" of the dotted mesenteries in Fig. A of Dr. Fowler was developed so early as to form one of the eight mesenteries in the stage with eight mesenteries. If this were so the eight-mesentery stage in Sagartia, Actinia, and Bunodes could not correspond to the permanent condition in Edwardsia, or to the condition described first by Professor Haddon for the larva of Halcampa, because in these no "lateral" mesentery is present with its muscle plate pointing "dorsalwards." The Hertwigs' figure for Adamsia is given by Dr. Fowler in Fig. B, and this represents what they assumed to be the arrangement also in young specimens of Sagartia, Actinia, and Bunodes.

The sections which I made at this time showed that the Hertwigs' assumption was incorrect, and that Lacaze-Duthiers was right in his determination of the first eight mesenteries. They showed that the two mesenteries assumed by the Hertwigs to be formed very early—in fact, to be the first formed—were not present at all in the eight-mesentery stage, but only arise as this stage is passing into the twelve-mesentery stage. The sections confirmed Lacaze-Duthiers that the four mesenteries dotted in Dr. Fowler's Fig. A were the latest developed. Unfortunately I was not able to obtain specimens younger than these with eight mesenteries, and so made out nothing regarding the order of appearance among these eight. Professor Haddon, however, in his "Revision" ('Sci. Trans. Roy. Dub. Soc.,' vol. iv, series 2, p. 350) states, "In these representative species of three different families of Actiniæ the development of the mesenteries is similar in all, both as regards the order of their appearance and the disposition of their muscles, and they are also identical with those of the larva of Halcampa." This from my observations is too wide a statement, and it would only have been safe to say "in these three species the disposition of the first eight mesenteries is similar to that found in Edwardsia and in the larva of Halcampa, the arrangement of the muscle plates also corresponding; further, the arrangement of the next four mesenteries takes place in positions similar to those noted for Halcampa."

That this was really the point on which Professor Haddon wished to insist, and that he was not thinking of the order in formation of the mesenteries of the eight or "Edwardsia stage," is, I think, certain to anyone who reads his paper on "The newly hatched Larva of Euphyllia," read before the Royal Dublin Society in March, 1890. In this last paper, on p. 133, Professor Haddon states that the order of development among these first eight mesenteries in the forms studied by Lacaze-Duthiers requires re-investigation, and that in Euphyllia, at all events, he has a priori reason for believing that the order of Lacaze-Duthiers is not present, but that that described by Wilson for Manicina obtains. Hence it is almost certain that

the too wide statement made in the "Revision of British Actiniæ" would have been corrected by Professor Haddon if he had had an opportunity of seeing the proofs of it; unfortunately, however, he started for Torres Straits immediately after it was read.

It thus is evident that the type represented by Sagartia, Actinia, and Bunodes is separated from the type represented by Manicina (Wilson), Ariactis (McMurrich), and perhaps also Euphyllia (Haddon), merely by observations of Lacaze-Duthiers which were made without sections.

I can only add my regret that this mistake should have caused so much confusion.

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