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The Habits and Structure of Arenicola marina.

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With Plates 1—5.

Contents.

1. Distribution: Varieties: Habits.

The common lugworm and its coiled castings of sand are familiar objects on almost all the sandy and muddy shores of Western Europe, but the exact geographical range of the species is doubtful. It has been recorded from the shores of North Siberia, Spitzbergen, Iceland, and Greenland (Wirén, 1883; Levinsen, 1883). On the north-east coast of America it has been found from the Bay of Fundy to Long Island

(Verrill, 1881). On both sides of the Atlantic, latitude 40° N. marks approximately the southern limit of Arenicola marina. South of this it is replaced in the Mediterranean by A. Claparèdii, Lev., and by A. cristata, Stimps., the latter also ranging on the west side of the Atlantic from Cape May (N. J.) to the Caribbean Sea. Its reputed occurrence on the north coast of Alaska (Murdoch[1]), at Vancouver Island (Marenzeller, 1887), Coquimbo, and South Africa requires confirmation.

An abundant, widely ranging, and undoubtedly old form such as Arenicola, might be expected to vary considerably in its habits and structure, though it has not hitherto been ascertained how far this is the case. Having paid special attention to this point, we have found that there are (at least on the Lancashire coast) two varieties of A. marina, differing in habits, structure, and times of maturity, and that there is, in addition, considerable individual variability.

(1) From high-water mark down to the beginning of the Laminarian zone, the common shore lugworms (or "lugs," as fishermen call them, in contradistinction to the second variety, or "worms") sink their U-shaped burrows to a depth of from one to two feet below the surface. One end of the burrow is marked by a casting, the other by a "countersunk" hole, through which the head of the lugworm is protruded when the tide comes in. The size and colour of the animal vary with the amount of muddy organic matter in the sand. Where there is comparatively little mud, the Arenicola average about seven inches in length and are somewhat transparent, so that the superficial blood-vessels can be clearly seen through the thin body-wall. The gills, which are not very strongly developed, are composed of nine to eleven branches, each provided with three to five pairs of short lateral twigs (Pl. 1, fig. 3). The proboscis and prostomium are only slightly pigmented, and being very vascular, appear red in colour.

Where, however, the amount of organic matter is considerable, the worms are usually about ten inches long, and their

[1] 'Proc. U. S. Nat. Museum,' Washington, vol. vii, 1884, p. 522.

prostomium, proboscis, gills, and epidermis are black. The gills are better developed than those of worms living in purer sand. These differences are probably due to more abundant nutrition. The time of maturity of both these forms of the littoral variety on the Lancashire coast is the summer, while at St. Andrews they are found mature from February to September.

(2) The second variety occurs on the Lancashire coast at the upper part of the Laminarian zone. Almost all the Arenicola from this zone (which can accordingly be obtained only at low spring-tides) are of this kind, which when fully mature, as it is from February to May, is probably one of the largest Polychæts of our shores, measuring as much as fifteen inches in length and three in girth. It is almost black, the prostomium, proboscis, and the base of the gills being markedly so. The tail is shorter in proportion to the length of the body than in the littoral variety. The burrows are of considerable length, three feet or more, and are not U-shaped, but simply vertical. Like those of the littoral variety, they are lined by a greenish coating of mucus. The dark "worms" appear to keep nearer the surface of the sand in cold weather than in summer,—at least, during the winter of 1893–4 large numbers were thrown up on the beach at Blackpool.

The most distinctive character, however, of this "Laminarian" variety is the gill (Pl. 1, fig. 2), which presents a structure hitherto only known in Arenicola cristata, Stimps. Instead of the somewhat simple gill seen in the shore lugworms, there is in the "Laminarian" variety a highly developed pinnate structure, consisting of about twelve branches united by a connecting membrane at their bases, and bearing ten or more pinnules on each side of the main axis. Such a gill is undoubtedly a much more efficient respiratory organ than the gill of a shore lugworm, though it does not appear to possess the same power of contractility as the latter, and hence probably does not contribute so much to the movement of the blood. In some old specimens the gills lose many of their finer branches, perhaps owing to friction or to the

attacks of enemies,[1] and in such cases there is an approximation to the type of gill seen in the littoral variety, though a certain amount of difference is always observable.

Thus there appear to be two varieties of the common lugworm on the Lancashire coast, distinguished by their habits, external features, and periods of maturity, but there are no important structural points of difference.

The habits of Arenicola marina at the breeding season are still to a large extent unknown, and developing eggs have not hitherto been obtained. It has been stated that, when mature, the animal is in the habit of swimming freely (Ehlers, 1892, *a*), but we are unable to confirm this. The post-larval stage, however, appears to be, for a short time, pelagic (Benham, 1893).

The curved burrow of the shore lugworm is formed by the combined action of the proboscis, the swollen anterior region of the body, and the waves of muscular contraction which pass along the body from behind forwards. When the proboscis is everted and pressed into the sand, the prostomium is slightly retracted into the body. The proboscis is withdrawn full of sand, again everted, and the body is thrust forward, partly by contraction of the longitudinal muscles, partly by a peristaltic wave produced by the circular ones. The anterior end is in this way rendered swollen and tense, and is able to enlarge the burrow, and thus a passage is gradually eaten through the sand, smoothened by contact with the skin, and lined by the mucous secretion of the epidermis. The gill region being narrower than that which precedes it, is thus, to a certain extent, protected from friction, while, as if to ensure this, the notopodial pencils of bristles are directed so as to protect the gills. After burrowing vertically downwards for a depth of from one to two feet, the worm forms a horizontal or oblique gallery, and then a second vertical one which ends at the "countersunk" hole, through which the anterior part of the worm may protrude, and so bathe the gills in fresh sea water.

[1] See the curious account of the ravages of Corophium longicorne, by d'Orbigny, 'Journal de Physique,' 1821.

The amount and value of the work done by lugworms has been estimated on the shore of Holy Island by Mr. Davison (1891), and has also been adverted to by Mr. Hornell under the name of "cleansing of the littoral." Mr. Davison finds that the castings are larger and more numerous above than below half-tide; and as the result of several estimates and measurements he calculates that on the Holy Island Sands, the entire layer of sand, to a depth of two feet, passes through the bodies of the lugworms which live in it, once in twenty-two months, and that in a year the average volume of sand per acre, which is brought to the surface in the form of castings, is 1911 tons, representing, when spread out, a layer of thirteen inches in thickness over the surface of the sands.

2. External Features.

Segmentation.—The body is divided into an anterior chætigerous portion, a middle branchial one, and a posterior caudal region or tail. The first region begins with the prostomium, and is followed by a short achætous portion (fig. 1, *MET*), which in many specimens appears to be composed of four annuli, divided, however, by secondary circular markings. The first chætigerous annulus is produced into a strongly marked ridge, just behind which the notopodial setæ (*Chn.*[1]) are inserted, the corresponding neuropodia (*Nm.*[1]) being very short and containing only a few setæ. The intervals between the chætigerous annuli are subdivided into rings, of which there are, in the "Laminarian" variety, 2 2 4 4 4 . . . , and in the littoral variety 2 3 4 4 4 . . . respectively.

The chætigerous annuli do not mark the true somites into which the body is divided. From a consideration of the internal anatomy (see p. 10) we have reasons for believing that, in the middle region of the body, the second groove behind each chætigerous annulus marks the boundary between the somites. A somite is, therefore, composed of a chætigerous annulus together with three annuli in front of, and one behind, it. The parapodia are not situated at the beginning, but slightly behind, the middle of the somites to which they

belong, thus confirming Benham's observations on the post-larval stage (1893).

The anterior region of the body is thus composed of the prostomium, six chætigerous somites, and a region between these, made up probably of two somites, but the exact number is somewhat doubtful. (See Plate 1, fig. 1, and explanation, p. 39.)

The second or branchial region of the body is composed of thirteen somites, and is distinguished by the presence of gills, a pair of which are attached to a slight fold of the skin just behind the notopodia. The first gill is variable, usually fairly well developed, but always smaller than the rest and sometimes absent. The gills about the middle of the branchial region are frequently, but not always, the largest. Both the gills and notopodia are very sensitive, and are retracted from time to time on the application of stimuli, such as a strong light. This contraction of the gills proceeds sometimes as a wave down the body, and as Milne Edwards (1838) pointed out in his classical paper, considerably assists the circulation of the blood. The neuropodia in the branchial region extend towards the mid-ventral line, so as almost to meet, and are only separated by a groove which marks the line of the nerve-cord. This groove is continued on to the prostomium by a pair of diverging arms ("Metastomial grooves") underlying the circum-œsophageal nerve connectives (Pl. 4, fig. 19, *C. Mt.*).

The tail, which is devoid of setæ and gills, is marked by a large number of secondary annuli, crowded together at first, but arranged in distinct somites of about five each, towards the hinder end. The caudal region varies much in length; some specimens have about thirty somites, but the number is not constant, possibly owing to the tendency of the worm to throw off the last few segments when irritated.

There is no change in the internal organs to mark the somite which bears the first gill, but the transition from the branchial to the caudal region is accompanied by the loss of parapodia, oblique muscles, and branchial vessels.

External apertures.—The mouth (Pl. 4, fig. 19, *C. MO.*),

when the proboscis is withdrawn, is a slightly crescentic transverse slit, bordered by papillæ and somewhat overhung by an upper lip. The anus, which is terminal, is often protruded, and the thin vascular swollen lips of the aperture project behind the last caudal segment.

The opening of the "nuchal organ" is a fairly wide slit on the upper and hinder border of the prostomium (Pl. 4, fig. 19, A and B, *NV.*). Through this aperture, sea water (or a mixture of sea water and the secretion of the surrounding glandular cells) is probably introduced.

The openings of the otocysts are difficult to see. They lie behind the prostomium on each side of the anterior end in the position marked *OT.* (Pl. 4, fig. 19, A and B). Each is placed at the point of intersection of the first transverse groove following the prostomium, with the oblique "metastomial" groove which marks the position of the nerve commissure.

The nephridial openings (fig. 1, *NO*), six in number on each side, though not so distinct as in some species (e. g. A. Claparèdii), are not difficult to find. The first is placed behind and at the upper edge of the fourth neuropodium, and the other five in corresponding positions on the succeeding somites. They are minute slightly oblique slits, sometimes exhibiting tumid lips.

Skin.—The skin is subdivided into raised polygonal areas separated by corresponding shallow grooves, and is noteworthy in being devoid of special glands. Wirén (1887) has shown that the grooves are composed of columnar cells containing pigment granules, the raised areas being made up partly of larger cells containing still greater quantities of pigment granules and partly of clavate mucus-forming cells, which produce the slimy covering of the animal with which the burrow is lined.

The 5 per cent. formalin solution of the epidermal pigment is fluorescent, but does not yield any absorption bands, merely cutting off the rays at the blue end of the spectrum. In successively thicker layers of this solution, first the violet, then

the blue, and lastly the green portions of the spectrum were cut off.

MacMunn (1889), however, has shown that the alcoholic extract of the integumental pigment shows a band in the blue and green (λ 503—468); that the residue of this solution if dissolved in ether or chloroform yields two bands, λ 503—474, and λ 465—446; and that the residue of this solution again being dissolved in nitric acid gives two bands, λ 500—468, and λ 472—443, so that a chlorophan-like lipochrome is present. It is probable that the pigment (melanin) of the skin is derived from the lipochrome of the yellow "glandular" tissue of the stomach, since the alcoholic extract of the latter yields a similar absorption spectrum.

Further investigation will be required to show in what way the transference of the pigment from the yellow peritoneal cells to the epidermis is brought about, and whether the dark-coloured, hairy-looking investment of the ventral vessel and its branches (Pl. 2, fig. 5) contributes to the melanin of the skin. In this connection the intermuscular extension of the cœlom, bringing it almost into contact with the epidermis at certain points, must be borne in mind (see p. 29).

Setæ.—The notopodial setæ are long capillary structures averaging 6 mm. in length, and bearing several rows of minute free and pointed hair-like processes (Pl. 3, fig. 10). The neuropodia in the anterior somites, which at first contain few setæ, gradually extend by addition of new ones at their ventral edge, so as to almost reach the mid-ventral line (Pl. 1, fig. 1). By isolating the entire band of the setæ the different stages in their development may be seen. The youngest setæ are always at the lower end of the series; the point of each seta is formed first, then the toothed ridge, and lastly the shaft. The fully-developed ventral seta is frequently almost smooth, owing to the wearing down of the teeth behind the apex. The middle of the shaft is straight, the inner end bent ventrally, and the outer end bent slightly dorsally, ending with a finger-shaped process bordered on the convex side by a toothed ridge, while on the concave side it is slightly produced at one

point into a minute process (Pl. 3, fig. 12, *proc.*). This process is more constant in the Laminarian than in the littoral variety. It appears to correspond, in position, to the characteristic tuft of hairs on the ventral setæ of the Maldanidæ.

According to the age of the specimen the ventral setæ differ in shape, and in the development of the toothed ridge. In setæ from a small specimen (17 mm. long) the apex was bent more sharply on the shaft than in old examples, and the teeth were very prominent (Pl. 3, fig. 9). Apparently the production of fresh ventral setæ goes on slowly throughout life, and the form which they assume before being cast out of the body, varies at different ages. Their size of course varies with the age of the worm to which they belong (see Pl. 3), but in a worm of average size their length is about ·5 to ·8 mm.

3. General Anatomy of the Internal Organs (Pl. 2).

In opening the body-cavity by a dorsal incision, the middle part of the alimentary canal is usually forced out through the cut by the pressure of the somewhat viscous cœlomic fluid. Normally this portion of the canal, being longer than the section of the cœlom in which it lies, is swung to and fro by the movements of the body. This freedom of motion is ensured by the absence of mesenteries, by the absence of any vessels running from the body-wall into the dorsal vessel, and by the length and flexibility of the branchial and nephridial vessels, which are the only connection between the stomach and the body-wall.

The cœlom is exceedingly spacious, and continuous from one end of the body to the other. In front it is divided transversely by the origins of the buccal retractors (*B. Sh.*), which form a sheath round the proboscis, and by three septa or diaphragms (Pls. 2 and 3, figs. 5 and 6). The first of these septa (*Dphm.*) is placed obliquely, arising below behind the level of the first neuropodium, and being inserted dorsally in front of the first notopodial sacs. The result of this arrangement is that between the first and second diaphragms two pairs

of setal sacs occur, caused by the forward shifting of the upper edge of the first diaphragm (fig. 5). The second and third are inserted both above and below, opposite the second groove behind the second and third chætigerous annuli. Between the first and second diaphragms, dorsal and ventral mesenteries occur, supporting the corresponding vessels; and it will be noticed that the dorsal mesentery ends in front, exactly where the first diaphragm would be inserted if it corresponded with the other two. The third diaphragm is perforated by the funnels of the first nephridia. There are, then, three diaphragms and not, as so often stated, four, and, while affording valuable evidence of the extent of the first and second chætigerous somites, they do not help in determining the number of segments which compose the achætous portion following the prostomium.

Behind the last diaphragm the body-cavity is unsegmented up to the base of the tail. The segmental arrangement of the organs, however, can be recognised by taking the funnels of the nephridia as marking the anterior ends of the somites. The slight amount of connective tissue supporting the long afferent and efferent vessels (segmental vessels) (Pl. 2, fig. 5) of the nephridia and gills, may be regarded as the remains of the septa. Allied species of Arenicola fully confirm this view.

At the level of the thirteenth pair of notopodial sacs, the segmental afferent and efferent blood-vessels, which have hitherto run nearly parallel across the cœlom, diverge. At the base of the tail, the connective tissue between them increases slightly in amount, septa forming which are continued down to the end of the body (fig. 5, *C. Sp.*).

4. Musculature.

The muscles of the body-wall are arranged in (1) an outer circular sheath, subdivided in the anterior and middle regions of the body into hoops, which cause the annulation of the skin; and (2) an inner longitudinal sheath of considerable strength and thickness divided by the nerve-cord and lines of

insertion of the notopodial sacs into three parts, two ventro-lateral and one dorsal (Pl. 4, fig. 23). The intermuscular spaces are filled by cœlomic fluid, and are probably lined by a delicate peritoneum.

In the anterior region of the body there are a few circular muscle-bands which are stronger and more obvious than the rest (fig. 5, *M. Circ.*).

The oblique muscles, which divide the cœlom longitudinally into three compartments, commence behind the third diaphragm, and disappear at the base of the tail. These muscles are arranged in thin broad bands, arising at the sides of the nerve-cord, and are inserted right and left into the body-wall at the level of the notopodial sacs. They partly cover the nephridia, and in some specimens a muscle-band is attached to each nephrostome.

The musculature of the buccal mass consists of a strong sheath of fibres derived from the longitudinal layer just behind the first diaphragm. This sheath, which is loosely attached to the proboscis by slips which run through the cœlomic space between the two structures (Pl. 3, fig. 6, *B. Sh.*), is inserted into the anterior part of the proboscis. Pressure of the cœlomic fluid at this point causes eversion of the buccal mass, which is withdrawn by the contraction of its muscular sheath.

The prostomium is retracted by a small sheet of muscle which arises partly from the longitudinal layer dorsally, and partly from the muscular covering of the circumœsophageal connectives ventrally, and it is inserted into the ventral surface of the brain, and the ventral and hinder edge of the nuchal organ (Pl. 3, fig. 6, *Nu. Tr.*).

The parapodial muscles are modifications of the longitudinal layer. One, the retractor of each notopodium, is remarkably long, reaching to the side of the nerve-cord (Pl. 3, fig. 13, *Rn.*). The protractors (*Pn.*) of the notopodia are six to eight in number, three to four being placed in front of, and three to four behind, the setigerous sac. They arise from the body-wall just below the dorsal longitudinal vessel, and are inserted into the base of each sac.

The position and relations of the three anterior septa or diaphragms, of the dorsal and ventral mesenteries between the first two of these, and the presence of regularly arranged septa in the tail region, have already been noted. It may be added that a pair of outgrowths from the first diaphragm lie under the œsophagus, opening anteriorly into the cœlomic space in front of the first septum. They are very vascular, and contract rhythmically every three or four seconds during life, and are doubtless of use in everting the proboscis (Pl. 2 and 3, figs. 5 and 6, *Dph. Ph.*).

In the caudal region the intestine is attached both above and below to the body-wall by mesenteries, in which the dorsal and ventral vessels lie.

5. Alimentary Canal (Pl. 2).

This consists (1) of an eversible buccal mass (*Bucc. M.*), of a pinkish or greenish-brown colour, which lies in front of the first septum; (2) of an œsophagus, of a light brown colour, provided with a pair of glandular pouches behind the third diaphragm; (3) of a gastric region, with yellow glandular walls, extending from the level of the heart to about that of the twelfth or thirteenth notopodium; and (4) of an intestine, of a dark brown or almost black colour, folded in a concertina-like manner by the caudal septa, and opening at the terminal anus.

During life the buccal mass (or "proboscis") is constantly being everted and withdrawn, carrying sand into the œsophagus. During eversion several rows of curved, pointed, vascular papillæ (*B. Pap.*) are first extruded. These papillæ (Pl. 3, fig. 7) in old specimens are tipped with chitin, and recall the armature of the proboscis in certain Sipunculids (e. g. Phascolion collare[1]). Then the more globular portion of the buccal mass, covered with minute rounded processes, is protruded. Finally, when fully everted, the buccal aperture is surrounded by a few pointed pigmented papillæ, which are continuous with the lining of the first part of the œsophagus.

[1] Selenka, 'Die Sipunculiden,' 1883, pl. vi, fig. 74.

The œsophagus[1] itself is slightly looped behind the second diaphragm. It is a thin-walled distensible tube, the first part of which is lined by non-ciliated mucus-forming cells. The middle portion is lined by a cuticle, and the posterior part by cells resembling those of the stomach in bearing cilia. The œsophageal pouches (*Oe. Gl.*) are somewhat flask-shaped, and open into the cavity of the œsophagus by a short tubular stalk. They are usually greenish in colour, but have a slight reddish tinge on account of their very large blood-supply. Their blood-vessels are connected with the lateral œsophageal and dorsal vessels. The cavity of the pouch is subdivided by twenty-five to thirty incomplete partitions, produced by infolding of the wall of the pouch, and therefore covered on each side by the epithelial lining of the pouch (Pl. 4, fig. 22). Between the epithelial lamellæ is a blood-sinus, which is slightly enlarged at the inner end and slightly thickened at the edge of each partition. The œsophageal pouches are lined by ciliated epithelium, covered with a fairly stout cuticle, and contain glandular cells. The walls of the œsophagus are marked by longitudinal and circular muscular impressions.

The stomach, marked out by the patches of yellow tissue on its walls, extends from the level of the heart to about the twelfth notopodial setæ. As we have already stated (p. 9), the stomach is bent upon itself and loosely attached to the body-wall. The patches of "chlorogogenous" tissue are at first arranged in symmetrical oval areas right and left of the dorsal blood-vessel, while more ventrally they are placed in two or three less regular series, and are separated from one another by a network of blood-vessels.[2] About the level of the tenth setæ these yellow areas all become subequal and arranged in a spiral manner, ending at the level of the fourteenth setæ.

Stomach and Intestine.—The muscular wall of the

[1] The histology of the alimentary canal has been carefully investigated by Wirén (1887, p. 31). Our results agree very closely with his.

[2] This network is considered by Wirén and others to be parts of a continuous sinus. We are not convinced, however, that this is really the case, and our reasons will be found on p. 17 infra.

gastric region is exceedingly thin, and composed purely of circular fibres, which appear to confer very slight powers of peristalsis upon the stomach.

The mucous lining is strongly folded, and is composed of several kinds of cells. Some of the cells in all parts of the stomach are ciliated, others are apparently digestive, and a large number appear to secrete a mucus similar to that of the œsophagus, the cells themselves being discharged into the mucus which they help to form.

Commencing about the middle of the stomach (that is between the ninth and tenth segments) is a ventral groove formed by a couple of folds of its inner and lower surface. This groove[1] (Pl. 4, fig. 23, *Gv.*) is provided with specially long cilia, which produce a current of mucus from before backwards. There are other smaller grooves on the side walls of the stomach and the anterior part of the intestine, whose general direction is downwards and backwards, and which open into the median ventral groove. The direction of the current in all these is from before backwards. The ventral groove is continued back to the anus. The intestine is dark brown or nearly black in colour externally. Its mucous lining is somewhat similar to that of the stomach, but is covered by a thin cuticle, and is not ciliated.

The process of digestion in the lugworm has not been at all fully investigated, but the series of events appear to be somewhat as follows. The sand or mud is mixed with the mucous secretion of the œsophagus, and is slowly carried backwards by peristaltic contraction. At the junction of the stomach and œsophagus the secretion of the œsophageal pouches is poured upon the sand. Wirén regards the contents of these pouches as acid and digestive. In several cases we have found the fluid neutral. In the stomach several changes occur. The secretion of the gastric cells proper is probably digestive, and this, together with a further amount of mucus, is mixed with the sand, and shaken together by the swing of the loose gastric loop. In this way the food, which apparently consists of the

[1] This groove has only hitherto been noticed by Wirén (1887).

organic substances[1] in the sand, is brought into contact with the digestive secretion. The ciliary action of the lateral and ventral grooves probably separates the digested substances from the sand and carries them slowly downwards and backwards. The lining of the stomach is very thin, and the lateral and ventral grooves are in specially close contact with the blood-plexus, in which the flow is, probably, slowly forwards, more rapidly in the sub-intestinal vessels. It seems probable, therefore, that the blood in the visceral plexus conveys the nutritive material to the hearts, which pump it along the ventral vessel to the various parts of the body.

The action of the chlorogogenous tissue round the stomach, and particularly of that in the neighbourhood of the ventral vessel and its branches, is uncertain.

6. Vascular System (Pl. 2, fig. 5).

The blood-vascular system of Arenicola attains a high degree of perfection. The large size of the chief vessels, the great development of the capillary system (especially on the walls of the alimentary canal), and the mechanism for promoting the flow of the blood, are features that distinguish it.

There are two chief vessels running, one above, and the other below, the alimentary tract from end to end,—the dorsal vessel, which contracts fairly rhythmically from behind forwards; and the ventral vessel, which is feebly, if at all, contractile. The walls of the gastric and intestinal portions of the gut are enclosed in a blood-plexus, and the œsophageal region is supplied by lateral vessels. The gastric vessels are connected with the ventral vessel by a pair of "hearts" placed a short distance behind the œsophageal pouches (fig. 5, *V.*). These hearts drive the blood from the gastric vessels into the ventral vessel.

The dorsal vessel (*D V*) arises near the anus, and as it runs along the intestine gives off in each somite a pair of branches

[1] Saint Joseph found in an Arenicola a whole Nereis almost digested. 'Ann. Sci. Nat.,' series vii, t. xvii, 1894, p. 127.

which are attached to the anterior face of the caudal septa, and which run downwards and forwards to open into the ventral vessel (Pl. 2, fig. 5). Of these there may be twenty-seven to thirty pairs. In front of the caudal region each of the last seven pairs of gills returns an efferent branch to the dorsal vessel, and between these there are three or two pairs of smaller branches which run round the alimentary canal from the ventral vessel to open into the dorsal one. From the level of the twelfth setæ to the œsophageal pouches the dorsal vessel does not receive any segmental vessels from the gills or nephridia, nor does it open directly into the heart (fig. 5). It merely receives numerous branches from the gastric plexus. In front of the heart it receives on each side a branch from the third nephridium and the fifth setigerous sac; a branch from the œsophageal pouches; and one from the second nephridium and fourth setigerous sac. It then runs on and, piercing the third diaphragm, receives a branch running on the anterior face of the diaphragm from the first nephridium and third setigerous sac. On reaching the second diaphragm it receives a branch from the second setigerous sac, and after piercing the first diaphragm receives a branch from the muscles forming the buccal sheath. Thence the dorsal vessel breaks up into capillaries around the buccal musculature, prostomium, and otocysts. From these capillaries the ventral vessel takes its origin. It gives off a small unpaired branch running in the first diaphragm and to its pouches; a paired branch arising about midway between the first and second diaphragms to the neural vessels and second setigerous sac; a single small vessel supplying the second diaphragm and the neural vessels; an unpaired vessel to the third diaphragm, to the neural vessels in that region, and to the first nephridia; a pair of branches to the neural vessels and second nephridia; and lastly, a pair to the neural vessels and third nephridia. From this point onwards the ventral vessel supplies the setigerous sacs, body-wall, nephridia (if present), and gills by large segmental vessels. The ventral vessel is very large and turgid in the gastric region, and is surrounded by tufts of dark brown chlorogogenous tissue,

which are also found in older specimens on the vessels running to the body-wall. This chlorogogenous tissue is first seen on the ventral vessel about the level of the eighth pair of setæ. In the tail the ventral vessel ends in the obliquely placed intestinal vessels which encircle the intestine, and which form, along with the capillaries from its median terminal portion, the commencement of the dorsal vessel.

Visceral Plexus.—Wirén (1887) maintains that the intestine and stomach are enclosed in a blood-sinus, thickened along certain lines which have been called the dorsal, gastric, and subintestinal "vessels." We are, however, of the opinion that the so-called sinus is a close plexus of vessels, some of which appear to have a distinct cellular lining. The dorsal vessel is, at any rate, a perfectly distinct structure with proper walls.

The subintestinal vessels (fig. 5, *S. V.*), which commence just behind the heart and run backwards, are moderately large up to the level of the thirteenth setæ, but then taper rapidly and gradually disappear. They each receive seven segmental vessels. The first of these comes from the fourth nephridium, the second from the fifth nephridium and the first gill, the third from the sixth nephridium and second gill, and the other four from the third, fourth, fifth, and sixth gills. The subintestinal vessels open through the plexus into the lateral gastric ones, and so into the heart. The flow in these vessels is probably slowly forwards.

The gastric vessels give off from the "auricle," into which they expand, a lateral œsophageal vessel (*Oe. Lat.*), which, after giving off a stout branch to the œsophageal pouches, runs forwards to the buccal mass, supplying the wall of the œsophagus, as it does so, with numerous small branches.

Neural Vessels.—These are a pair of small vessels lying one on each side of the ventral nerve-cord, and accompanying it from one end of the body to the other. They arise round the nerve-connectives from the brain from capillaries of the dorsal vessel, and receive several branches from the ventral vessel (1) midway between the first and second diaphragms, (2) from

the vessel running in the second diaphragm, (3) from a vessel just behind the third diaphragm, (4 and 5) from the vessels to the second and third nephridia. Near the middle of each somite the two neural vessels are united by cross connections, which also supply the nerve-cord (Pls. 2, 3, fig. 13, *N. V.*, *N. C. V.*).

Behind the third diaphragm the neural vessels supply the oblique muscles by branches which run the whole length of the bands, and are connected with the outer longitudinal parietal vessel (fig. 13).

Vessels of the Body-wall.—This parietal system of true vessels is highly developed in Arenicola marina. It consists of two longitudinal vessels, (1) the nephridial longitudinal vessel (fig. 22, *N. L. V.*) running just below the level of the nephridiopores, and (2) the more important dorsal longitudinal vessel (fig. 13, *D. L. V.*), which runs just above the level of the insertion of the notopodial setal sacs. Both arise just behind the first setæ, and increase in size as they pass backwards. The former receive vessels from the nephridia, just behind which they taper and disappear. The latter, which may be traced to the anus, and are largest in the branchial region, receive branches in each somite: (1) from the segmental vessels; (2) from its fellow of the opposite side. The body-wall in the dorsal and lateral regions derives its blood-supply from the nephridial and dorsal longitudinal vessels, and in the ventral region from the neural vessels. These parietal vessels (*Par. V.*) run just within the layer of circular muscles in almost every groove between adjacent longitudinal muscle-bands of the body-wall, are chiefly longitudinal in direction, but at frequent intervals there are cross connections. Branches from these vessels ramify between the bases of the epidermal cells, and are accompanied by extensions of the cœlom.

Hearts.—The hearts are a pair of muscular bulbous swellings connecting the visceral plexus with the ventral vessel on each side. Each commences with the thin-walled expansion of the gastric vessel ("auricle," fig. 5, *A. v.*) which, after giving off the lateral œsophageal branch, opens into the ventricle

(*V.*). The cavity of the ventricle is small and broken up by a spongy mass of cells. The ventricular walls are muscular, and contract from above downwards, forcing the blood into the ventral vessel. (We have sometimes seen an apparent reversal of the heart's action.) The spongy cardiac body arises by ingrowths from the wall of the ventricle, chiefly in the middle and ventral regions. It gradually encroaches on the blood space, so as to reduce it considerably (Pl. 5, fig. 36, *Card. B.*) in an old specimen. The cardiac body in a young specimen (fig. 38) is much smaller, and extends obliquely across the heart, its general direction being downwards and backwards. The cells of the cardiac body in an old specimen which we have examined are loosely arranged, so as to cause the formation of a large number of intercellular spaces, some of which are of considerable size, and which are in life filled with blood (Figs. 36—38, *B. S.*). Between the cells there are numerous fibres, which are probably muscular. The cells are apparently of two kinds, which, however, merge into each other: (1) cells whose protoplasm has a very vacuolated appearance, and which contain few or no granules (*Vac. C.*); (2) cells which contain a large number of yellowish granules in the protoplasm (*G. C.*). These latter cells are possibly glandular, and correspond to those found in the cardiac body of other Polychæts. The function of the cardiac body may be, as Schaeppi (1894) suggests, to prevent regurgitation of the blood from the ventral vessel into the heart when the diastole commences. The "cardiac body" of Polychæts, as hitherto described, is an unpaired structure lying in the dorsal vessel. That of Arenicola, however, is paired and in no way connected with the dorsal vessel. Hence a strict homology is scarcely probable.

Blood.—As Professor Lankester was the first to point out, the blood of Arenicola is strongly impregnated with hæmoglobin, but there has been no thorough investigation of the constituents of the plasma. Krukenberg (1882), it is true, made some experiments which led him to believe that there were no coagulable albumens in the blood of his specimens; but as they

were in a starving condition, a fresh examination is very desirable. A large quantity of albumen is certainly present, which when the specimens are fixed becomes very hard and brittle.

We have seen small cells (4 μ in diameter) in the blood-vessels of the nephridia, but it is doubtful if these are the blood-corpuscles, which we have not been able to demonstrate.[1]

General Remarks on the Circulatory System.—No other system of organs shows the true segmentation of the body of Arenicola so well as this. The lines of demarcation between the somites from one end of the body to the other are marked by the segmental vessels passing from the ventral to the dorsal vessel and breaking up on their way in the body-wall, nephridia, or gills. Throughout the gastric region, however, this arrangement is somewhat disguised, owing to the loss of the connection with the dorsal vessel, an alteration caused probably by the necessity for leaving this part of the alimentary canal freely moveable.

Wirén evidently believes that there is no capillary system except in the gills and the alimentary canal. He suggests that the assimilation of food and oxygen by the tissues is effected chiefly through the mediation of the cœlom, which he points out is parcelled off in the intermuscular spaces, by a channelling out of the subepidermic connective tissue, into "perihæmal canals." Though this suggestion is a valuable and correct one, we have found a very perfect system of capillaries in the skin in all parts of the body, and in the nephridia and septa the same is the case. The extension of the cœlom into the intermuscular and subdermal spaces has, however, all the appearance of acting as the equivalent of lymph-spaces of higher forms. The transformation of the constituents of the blood into cœlomic fluid takes place in all probability with especial rapidity in the neighbourhood of the dark chlorogogenous processes of the ventral vessel (cf. Cuénot, 1891).

[1] Since writing this we have discovered that these small cells are the blood-corpuscles.

7. The Gills (Pl. 1, figs. 2—4).

The general characters of these organs have been mentioned in the introductory part of this paper, and little remains to be added.

There are thirteen pairs of gills from the seventh to the nineteenth chætigerous somites inclusive. The shape varies from the short dendritic type of the littoral form to the delicate, richly-branched gill of the Laminarian variety. The gills are hollow, being outgrowths of the body-wall enclosing an extension of the cœlom, and what little evidence we have of their development (see Benham, 1893) points to their being independent structures, and not modified dorsal cirri.

The walls of the gills, though thin, are muscular, and there are also muscular bands stretching across the cavity of the gill (fig. 23); and Milne Edwards has pointed out that the contraction of the gills, which often proceeds like a wave from before backwards down the sides of the body, must exert a powerful influence in propelling the blood partly into the efferent vessels, and partly to the parietal capillaries.

The ventral vessel supplies all the gills with their afferent branches. The first seven pairs return the blood to the subintestinal vessels, and so to the heart; while the efferent branches of the remainder open into the dorsal vessel.

8. Nervous System and Sense-organs.

This system is composed of the brain, the œsophageal connectives, the ventral nerve-cord, and the nerves arising from these. We have not been able to demonstrate a visceral nervous system.

The brain (Pl. 5, figs. 25, 26) is placed in the prostomium, of which it forms the chief part, being only separated from the epidermis by blood-vessels lying in extensions of the cœlom. It is a small elongated structure, measuring ·75 mm. in length in ordinary shore lugs, and 1 mm. in the large "Laminarian" variety. At its anterior end the brain is divided into two stout cornua (*A. Cr.*), separated by a cleft containing blood-vessels. About the middle of the brain the cornua unite, but only for a

very short distance, a second connective-tissue partition dividing the smaller posterior cornua (*P. Cr.*), which gradually taper off and end at the hinder edge of the nuchal organ (Pl. 5, fig. 25).

Sections of the prostomium of the littoral variety of Arenicola (immature specimens, 4″ long) exhibit a thick covering of ganglion- and glia-cells, forming the dorsal surface of the brain (fig. 24); a central fibrous portion; and a strong ventral membrane, into which the greater part of the prostomial muscles are inserted, though a few fibres are attached in front of and between the anterior cornua (Pl. 5, fig. 25). In older specimens, and particularly in mature examples of the "Laminarian" variety, the ganglion-cells are more scattered, and in other ways the brain shows greater differentiation. The anterior cornua, for example, are not only deep and thick, but give off from their dorsal surface short stout branches, along which the ganglion-cells are scattered, and which supply the prostomium. The central fibrous part of the brain also grows out ventrally in these large examples, separating the hitherto compact layer of cells and carrying them outwards or leaving them in clumps, and not evenly arranged as in young Arenicola.

From the anterior cornua a large nerve arises on each side, in front of the origin of the œsophageal connectives. It passes out to the under surface of the epidermis, and supplies the papillæ on the upper surface and the sides of the mouth. The epidermis of the prostomium itself is in close contact throughout its whole length with the ganglionic covering of the processes arising from the dorsal and lateral surfaces of the brain. The posterior cornua seem to be specially connected with the nuchal organ, against which they lie and terminate (Pl. 4, fig. 21).

The most remarkable histological feature of the brain is the close contact between the large ganglion-cells of its upper surface and the sensory epithelium of the prostomium (figs. 20 and 24). Racovitza (1896) has figured (Pl. 5, figs. 48 and 49) a similar condition in Clymene. It is only at this point

that the nervous system of the adult Arenicola marina can be said to have an epidermal position. Elsewhere it is separated from the epidermis by the circular musculature.

The circum-œsophageal nerve-connectives arise from the large anterior cornua in the form of two thick cords, covered on their outer surfaces by ganglion-cells (figs. 20, 21, 25, *Oe. Comm.*). From them a pair of short nerves (fig. 26, *OT. N.*) arise supplying the otocysts, and several longer ones are distributed to the oral papillæ of the ventral region of the mouth. The line of the connectives is marked externally by the "metastomial groove" (Pl. 4, fig. 19, *C.*), and the commencement of the ventral cord by the junction of these grooves, which occurs on the ventral surface just in front of the first chætigerous annulus. The nerve-cord is protected by a delicate connective-tissue sheath, a thin sheath of circular muscle, and a thin layer of epidermis. Though nearly circular in section it is somewhat flattened from above downwards, but exhibits scarcely a trace of segmentation externally or internally. The ganglion-cells are arranged in two ventral groups, while the fibrous portion of the cord is dorsal. In the tail the ganglionic masses increase in size, and are separated from the skin by a thicker layer of circular muscle-fibres. Two "giant-fibres" are present in the branchial region, a single one only in the anterior and tail region.

From the cord a paired series of nerves is given off with great regularity, one opposite each groove separating the annuli of the somites, so that there are five nerves on each side of the body in each somite. These lie in the body-wall just beneath the circular layer of muscle, and, in some places where this layer becomes obsolete, they lie just under the epidermis. Dorsally these nerves thin out and become very difficult to trace.

Sense-organs.—There is no doubt that the prostomial lobes, the nuchal organ, and the otocysts are sense-organs; but there are, in addition, certain other structures, such as the setæ[1] of the notopodia and some of the buccal papillæ, which,

[1] Retzius has described free nerve-endings on these setæ. 'Biologiska Föreningens Förhandlingar,' Bd. iii, Hefte 4—6, 1891, p. 85.

on account of their position, movements, and the nerves ending in them, may be considered as probably belonging to this category.

Professor Ehlers' (1892) account of the nuchal organ and otocysts is an almost exhaustive description of these organs in Arenicola. We have worked over the whole subject again, however, and are able to add a few points to this important paper.

The nuchal organ belongs to the prostomium, whereas the otocysts belong to the metastomium. The prostomium and the nuchal organ are found, in varying degrees of complexity, in nearly all Polychæts; the otocysts, however, occur in few and widely separated families.

The general appearance of the prostomial lobes and the opening of the nuchal organs have already been described. Seen from the dorsal surface the former consists of a small median papilla and two larger lateral prominences (Pl. 4, fig. 19), which together correspond with the single prostomial papilla of allied forms (cf. Racovitza's figure of Leiocephalus, 1896, pl. v, fig. 5). In young Arenicola these lobes are transparent, and therefore red from the underlying blood-vessels. In old specimens they become dark-coloured and opaque from the deposition of pigment in them. In no species of Arenicola have eyes been discovered, although they are known to occur on these lobes in many related genera.

The prostomial epithelium is a complex of several distinct kinds of cells,—unaltered columnar elements, fusiform sense-cells, each ending in a conical prominence, glandular cells, and apparently also "wandering cells" from the body-cavity. Underneath the epithelium is a connective tissue continuous with the supporting tissue, the neuroglia of the brain, which binds together the large ganglion-cells of the cornua of the brain. The prostomial sensory structure thus formed is very sensitive to light, but what function it subserves has not been determined with accuracy.

Nuchal Organ.—To the outer side of the lateral prostomial lobes is a depression guarded externally by a fold (just

above *Nu.*, Pl. 4, fig. 19, *B.*). These two pits form the beginning of the nuchal organ and indicate its paired origin. Further back they unite to form a transverse groove (bordered by the hinder edge of the prostomium), which is continued inwards as a deep pit to the hinder margin of the brain (Pl. 5, fig. 25). From the posterior cornua of the latter the nuchal organ is innervated.

In its paired form and under the names "Wimperorgane," "Wimpergrübschen," the nuchal organ is well known in almost all families of Polychæts, and a similarly placed organ is found in Sipunculids,[1] not to mention other more distantly related groups. It is always associated with the posterior lobe of the brain, and arises as a pair of pits from the surface of the prostomium. Of its development in Arenicola, however, we have no evidence, but the two depressions in front of the main part of the organ, together with the paired nerve-supply, point to its double nature.

The epithelium of this deep, pigmented pit (Pl. 4, fig. 21, *Nu.*) is composed of long columnar ciliated cells, glandular cells which secrete the mucus in which the cilia work, and slender sense-cells. It seems probable that the whole organ is olfactory in function.

Otocysts.—The otocysts of Arenicola marina are a pair of flask-shaped structures projecting into the body-cavity close to the outer edge of the œsophageal nerve-commissures. They open externally by a couple of apertures (Pl. 4, figs. 19, A and B, *OT.*), at that point on the "metastomial groove" where the latter is crossed by the first groove of the body following the prostomium. The body of the flask is placed at an angle with the "neck," and contains the otoliths. It is lined by non-ciliated columnar sense-cells and supporting cells, which are surrounded by the nerve-fibres and connective-tissue fibrils, figured by Ehlers (1892, pl. xii). The neck of the otocyst is made up of a columnar epithelium covered with a thick cuticle, which gradually merges into the epidermis of the external surface, and ciliated cells only occur in its lower portion. A

[1] Ward, 'Bull. Mus. Harvard,' vol. xxi, 1891, p. 143.

short nerve from the œsophageal commissure supplies the otocyst.

If the otocyst of a fresh shore lugworm be rapidly dissected out under sea water and mounted, the sand-grains will be seen to execute a most extraordinary movement. Each one is rotating slowly and jostling its fellows, so that the whole contents of the flask are in a state of commotion. The fluid in which the otoliths move is slightly viscous, and is a secretion of the walls of the otocyst, mixed with a little sea water. The sand-grains are covered with a distinct layer of some chitinoid substance soluble in boiling potash. Acids have no appreciable effect upon these grains, and under the polariscope they react as quartz does. Hence it seems clear that the otoliths of Arenicola marina (the other species of the genus differ most remarkably in this respect, as well as amongst themselves) are quartz grains covered by an organic film, and surrounded by a fluid which is not merely sea water.

Large specimens of the "Laminarian" variety were examined without being opened under sea water, and the otocysts were mounted by us in cœlomic fluid. No movement of the otoliths was observed even in specimens which were perfectly healthy in all respects. The otoliths sometimes filled the expanded part of the organ, and it is possible that they had no room to turn round. But it appears to us more likely that if we assume the cause of the rotation to be the diffusion caused by liquids so different as sea water, in which the preparation was first mounted, and the somewhat viscous, perhaps albuminous fluid inside the otocyst; then if we mount the otocysts in the same kind of fluid which they contain, no movement should occur; and the experiment showed that in these cases no movement did occur. The whole matter is one of very great interest, especially in view of the probable functions of such an organ as the otocyst. Ehlers has suggested that the movement is due to the cilia at the bottom of the neck of the otocyst; but the same extraordinary movements are seen in the otocyst of A. Grubii, which is closed and has no cilia. We quite agree with Ehlers that there are no cilia in the expanded part

of the otocyst where the movement has been noticed, but we are of the opinion that the quivering motion of the otoliths is not a normal phenomenon, but is due to diffusion currents.

9. Nephridia.

There are six pairs of nephridia, belonging to somites 4 to 9. Of these the first pair seems to be unrepresented in any other species of Arenicola, and its variation in A. marina points clearly to a gradual degeneration which it appears to be undergoing at the present time. It is not only the smallest of the series, but is sometimes represented merely by a funnel or by the secretory and terminal portions. Very rarely both the first nephridia are mere funnels, and again one may be fully developed and the other rudimentary, but they are never absolutely wanting. Their small funnels, which are of a bright pink colour, are placed on the anterior face of the third diaphragm with the long axes vertical (Pl. 2, figs. 13 and 14). One lip (the outer) is produced into processes corresponding to the dorsal lip of the other nephridia. The secretory portion is elongated, narrow, and usually brownish in colour, and the terminal portion opens just above the fourth neuropodium (Pl. 1, fig. 1) at a decidedly lower level than is the case in the succeeding nephridiopores.

The remaining five pairs are always in adults fully developed. They are attached to the body-wall partly by connective tissue, partly by the broad bands of oblique muscle which obscure them at first sight (Pl. 2, fig. 5). The nephrostomes are very long, and bent upon the rest of the organ. The narrow slit-like aperture has a dorsal vascular lip bearing finger-shaped or spatulate ciliated processes, and an entire ventral one. The cilia just within the mouth of the funnel are exceedingly long, and produce a current tending to carry cœlomic fluid and corpuscles into the cavity of the organ. The middle or secreting portion is brownish (in old worms almost black), owing to the excretory granules which are formed in its cells. The terminal rosette-shaped bladder, which is slightly lighter in colour, opens by a minute slit-like aper-

ture through the body-wall, which thins out at this point (Pls. 1 and 4, figs. 1 and 22, *NO.*).

The blood-supply to the nephridia (Pl. 4, fig. 18) is derived from the ventral segmented vessels, which divide, one branch going to the funnel of the nephridium and the other to the body-wall. The former traverses the funnel, sending a vessel into each of the ciliated processes, and giving off numerous small branches to the lips of the funnel. After traversing the funnel the vessel runs over the secreting portions of the nephridium, supplying the genital strand in its course, and finally ramifies on the terminal portion. The blood is collected again into small vessels, which open into the dorsal longitudinal or nephridial longitudinal vessels of the body-wall, from which it is returned largely to the dorsal or subintestinal vessels, but in part passes into the parietal vessels.

In young specimens the funnels are naturally simpler, but have similar positions and relations, as may be seen in figs. 16—18, which show nephridia from worms 29·5 and 44 mm. long, in which the processes on the dorsal lip are being formed. In the post-larval stage (Benham, 1893) the nephridia have no funnels, the development of which has still to be investigated.

10. Cœlom.

The cœlom of Arenicola is well developed, and continuous in all its parts. Not only does it form the space between the alimentary tract and body-wall from one end of the body to the other, but it is carried along with the blood-vessels into the intermuscular spaces. Thus the blood-vessels of the prostomium, of the buccal sheath, and of the body-wall generally, are accompanied by cœlomic canals which very probably serve as lymphatic spaces from which nutritive matters can be absorbed by the surrounding tissue, and into which waste nitrogenous substances may be excreted.

The segmentation of the body-cavity is very faintly marked. Anteriorly three diaphragms, perforated just above the nerve-cord, are present, whose position and relations are indicated

on fig. 5, and Pl. 3, fig. 6. The whole middle region of the body is devoid of septa, which, however, reappear on the last two somites of the branchial region, and are present throughout the tail in a complete form, though they are perforated to allow of the more thorough circulation of the cœlomic fluid.

Arenicola fresh from the sand exhibits a series of peristaltic waves of the body-wall from behind forwards, which can be easily seen if the gonads are sufficiently developed to cause slight swellings, which each wave carries forwards. These waves of fluid are probably of considerable physiological value. They assist the circulation of the fluid, the cœlomic cells, and the developing reproductive cells. They inflate the anterior digging part of the worm, and thus assist in burrowing. By their action the contents of the gut will tend to travel slowly backwards, the weak visceral musculature being probably insufficient by itself to cause the requisite amount of movement of the sticky sand: while in defæcation the main agent is doubtless the pressure of the cœlomic fluid on the intestine, brought about by violent contractions of the body-wall.

The cœlom is lined by a very thin layer of flattened cells, which undergo remarkable changes in certain parts of the body, resulting in the formation of (1) chlorogogenous tissue, (2) ova or spermatozoa, (3) cœlomic corpuscles.

The cœlomic fluid is a mixture of sea-water and globulins, among which only paraglobulin has hitherto been detected (Krukenberg, 1882, p. 87). We find that the specific gravity of the fresh fluid (including corpuscles) varies slightly, but is on the average 1·0288.[1]

On exposure to air this fluid coagulates, and a delicate fibrous network is formed, binding the corpuscles together. If carmine is injected into the cœlom, it is removed by the cœlomic corpuscles, by the cells lining the cœlom and by the

[1] It was found to be least (1·0270) in specimens which had been kept for some time in sea water, and greatest (1·0311) in those which had been kept for thirty-six hours in moist seaweed only. The specific gravity of the sea water used was 1·0264.

nephridia, and there is no trace of carmine in the cœlom after forty-eight hours.[1]

Cœlomic Corpuscles.—These abundant cells occur in two chief forms, which probably pass into one another. The first varies from 8 to 20 μ in length, is amœboid, and usually contains yellow or brown granules of a very highly refractive character. The pseudopodia are often grouped at the two ends of the cell (Pl. 5, fig. 24). The longer forms of this kind of corpuscle pass into the second or spindle-shaped cells of the cœlom, which measure as much as 50 μ in length, and contain no coloured granules. These fusiform elements are most abundant, and constitute the most characteristic features of the cœlomic contents.

The chlorogogenous tissue of the ventral vessel and its branches in the body-wall consist of groups of cells about 20 μ in length, full of large slightly yellow or deep brown granules, which are not highly refractive. The tissue in old black worms is immensely developed, so as to completely cover the vessel by the masses of hair-like threads, each thread consisting of a small blind diverticulum of the vessel surrounded by the chlorogogenous cells.

11. Reproductive Organs.

Thanks to the researches of Cosmovici (1880), Cunningham (1887), Kyle (1896), and others, the true ovaries and testes of Arenicola marina are now known to arise by proliferation of the peritoneal covering of an extension of the blood-vessel supplying the funnels of the nephridia. It is not certain that there is a corresponding gonad on the first pair of nephridia, but on each of the following five pairs the gonads are present during the breeding season. In both sexes the organ is a mass of cells, from which the ova or spermatoblasts break away at a very early stage, to ripen in the cœlom. The rachis is continuous with the posterior angle of the nephrostome, and is developed around a backwardly projecting process of the

[1] Schneider, 'Arbeit. Naturf. Gesellschaft,' St. Petersburg, Bd. xxvii, Heft 1, 1890.

nephridial vessel which comes off segmentally from the ventral vessel (Pl. 4, fig. 18, *G. V.*).

In large Arenicola, at certain seasons, the vascular process has no gonad, and it is possible, as Cuénot (1891) suggests, that a formation of the amœboid corpuscles of the cœlom takes place at this point when the animal is not breeding.

After passing through the earliest stages of their development in the genital rachis, the young reproductive cells may be found at the breeding season in all stages of development in the cœlom. The ova do not exhibit any considerable changes except in size in attaining maturity. They are nourished either directly from the cœlomic fluid, or possibly (Cuénot, 1891) by the amœboid cells acting as follicle-cells, though we have seen nothing to support this view. Extrusion of a polar cell (?) has been observed by us in an ovum only about half the definitive size (Pl. 5, fig. 35, A and B). In the spherical ripe ova (which measure ·16 mm. in diameter) a distinct but very thin vitelline membrane is present, and a small quantity of food-yolk in the form of very small granules in the protoplasm. The production of ova by the fertile vascular processes of the nephrostomes must be extraordinarily great, since the spacious body-cavity of a large worm is eventually filled to bursting with them by the end of February.

We have not followed the development of the spermatozoa in great detail. The youngest stage which we have found in the cœlom contained eight spermatoblasts arranged round a vesicular-looking blastophore (Pl. 5, fig. 30). Further division and elongation of the outer ends of the cells to form the tails of the spermatozoa produces the stages seen in figs. 31 to 34. The masses of spermatids are not spherical, but disc-shaped, their thickness being only about one quarter of their long diameter. They contain a cavity, the remains of the blastophore, together with a small quantity of a slightly fibrous coagulum in the centre of the cavity. Curiously enough, perfectly ripe males were comparatively rare in March and May of this year, when mature females were abundant. In most cases the body-cavity was full of spermatids in great bundles,

as in fig. 34. The ripe spermatozoa closely resemble those of A. Grubii, which have been accurately figured by Claparède.[1] They measure ·058 mm. in length, and possess a curiously shaped head, ·004 mm. in length, and an extremely long slender tail (·054 mm. long). The head (figs. 28 and 29) is divisible into three regions,—a rounded disc-like cap (*S.*) at the anterior end, which is partially divided by a median groove; the nucleus (*N.*), which is large and oval in shape; and the "middle piece" (*M.*), which bears posteriorly a depression into which the tail is inserted. This depression is formed only at the time when the spermatozoa are fully ripe. The tail (*T.*) in the specimens which we have been able to obtain appeared to be a somewhat stiff filament, which could only be bent to a comparatively small extent.

The breeding season of the "Laminarian" variety of Arenicola marina lasts from February to May on the Lancashire coast. The large black "worms" which may be dug out during the great spring tides of these months are then distended with ova or spermatozoa. Males and females are not distinguished by external characters, but owing to the slight discharge of gonads from the nephridiopores consequent on the tense condition of the body, it is often possible to distinguish the sex of an example without dissection. It is at present impossible to state how long these Arenicola live and how many times they breed.

The ordinary littoral lugworms of the Lancashire coast and of the Isle of Man are not mature in the spring, and contain at most a few very small eggs. In the summer (August) of 1896 we found mature specimens, and we believe that this variety breeds through the summer, commencing at about the time when the deeper water form has ceased.

Relation of the Nephridia to the Reproductive System.—As is well known, the ova and spermatozoa escape by the nephridiopores, but it does not seem to have been noticed before, that in both males and females the bladders of the last five pairs of nephridia are specially enlarged (Pl. 3, fig. 15, *Bl.*),

[1] 'Annélides de Naples,' 1868, pl. xix, fig. 2, *C.*

and contain mature ova or spermatozoa, so that upon irritation a simultaneous discharge through all these apertures may occur. In one worm only eight inches in length the bladder of the nephridium was swollen with ova so as to measure 14 mm. in length and 6 mm. in width. During the discharge of ova from the female the eggs are caught by the slimy mucus covering of the body, and, owing to the movements of the animal, collect in strings round the body. We have not observed the formation of gelatinous capsules in which the eggs may be laid, since we have not worked at the oviposition of this species, about which nothing is at present known. At certain times of the year, chiefly in the spring, the nets used by shrimpers on the sandy coast near Lytham are almost choked by the balls of eggs, each moored by two "cables" to the sand. Whether these eggs belong to Arenicola remains to be seen, but their form differs from that of Phyllodoce found so commonly in early spring.

It has generally been assumed that the number of nephridia and gonads occurring in Arenicola marina is typical or fairly typical of the genus, and it is usually stated that the number of both these organs is a small one (five or six). An investigation of several other species of Arenicola, the results of which we hope shortly to publish in full, have shown that A. Grubii and A. Claparèdii have five pairs of nephridia, and apparently the same number of gonads, whereas A. ecaudata has no less than thirteen pairs of nephridia, twelve of which bear large and complicated gonads of a size and complexity which is scarcely equalled by any other Polychæt. What relations exist between A. marina and the other species of the genus cannot be discussed here, but it may be stated generally that the genus exhibits greater variety in the development of several systems of organs than has been hitherto suspected, and that it is no longer possible to exemplify the characters of Arenicola as a genus by using their particular grade of development in A. marina as a type.

12. General Summary.

The following is a recapitulation of the new points which we have found in Arenicola marina.

1. On the Lancashire coast, and probably elsewhere, two well-marked varieties of Arenicola marina occur, differing, as the following table shows, in general appearance, in their habits, in the structure of their gills, and periods of maturity.

Name.	Habitat.	Colour.		Gills.	Breeding Season.
		Adult.	Young.		
"Shore lugs," or littoral variety, 6—8″ long, exceptionally 10″	The sandy and muddy shores of bays, estuaries, and harbours, extending from high water mark to and sometimes beyond low tide level Burrow U-shaped	Greenish brown or reddish black	Semi-transparent, yellowish or brown	Moderately developed. Branches with 3—5 pairs of gill-plumes	July, August.
"Worms," or Laminarian variety, 8—15″ in length	The sandy shore exposed at extreme low spring tides, occasionally extending above this. Burrow a vertical shaft	Black or very dark brown	Dark red, opaque	Very well developed. Branches with usually about 12 pairs of dichotomously arranged plumes	January to May.

2. The cilia lining the central or gastric region of the alimentary canal are specially arranged (1) on the sides of a ventral groove which is continued to the anus, and (2) on curved shallow grooves running downwards and backwards into the former. The current caused by the action of these cilia carries a stream of mucus and of digested food slowly backwards and away from contact with the mass of sand in the gut. As these grooves are in close connection with parts of the visceral plexus, absorption may take place from them.

While the ventral groove is morphologically equivalent to the similar structure of Oligognathus (described by Spengel[1]),

[1] "Oligonathus Bonelliæ," 'Mitt. Zool. Stat. Neapel,' iii, 1882.

and probably to the "siphon" of Capitellids, we have seen no reason for regarding it or any other part of the alimentary canal as "respiratory" in function.

3. In the circulatory system the two hearts each contain a cardiac body. This structure is composed of masses of granular and vacuolated cells, projecting into the cavity of each ventricle. Functionally they may be regarded as glandular valves preventing the reflux of blood into the gastric sinuses. While previously unknown in Arenicola, the "cardiac body" has been long known in allied genera (Ophelia, Trophonia, Chlorhæma), but as an unpaired structure in the dorsal vessel (Schaeppi, 1894). Hence, though histologically similar, it is very doubtful whether the paired structure of Arenicola, which has no connection with the dorsal vessel directly, is homologous with the unpaired organ of other Polychæts.

Contrary to Wirén (1896), we regard the dorsal vessel as a distinct structure, the gastric blood-system as a plexus, and we find that the nephridia and body-wall, as well as the gills, are well supplied with capillaries.

4. Both the large pinnately-branching, and the smaller dendritic, types of gill occur in A. marina. The usual statement that the latter type of gill characterises this species, and that the former type is characteristic of A. cristata, must therefore be modified.

5. The brain is divided by a narrow cleft throughout the greater part of its length. The anterior cornua supply the prostomium, the buccal papillæ, and give off the œsophageal nerve-connectives. The middle region of the brain supplies the upper part of the prostomium, and the posterior cornua innervate the nuchal organ.

In young specimens the almost uniform covering of ganglion-cells of the brain is in close contact with the peculiar and complex sensory epithelium of the prostomium, but in old specimens of the "Laminarian" variety fibrous outgrowths from the dorsal and lateral surfaces of the brain scatter this ganglionated covering.

6. The nuchal organ, though apparently single, shows traces

of a double origin. It is probably an olfactory organ, and is developed from the posterior region of the prostomium.

7. The otoliths consist of quartz grains surrounded by a delicate chitinoid film, as Ehlers stated. The peculiar commotion observed in otocysts mounted in sea water was not noticed in others examined in cœlomic fluid. Hence the motion is probably a result of diffusion currents.

8. The first pair of nephridia are in process of reduction. In the others the form of the funnel at an early stage is described and figured. In adult examples the terminal portions of the nephridia act as receptacles for the ripe ova or spermatozoa.

9. The specific gravity of the cœlomic fluid varies slightly, but is on the average (including the corpuscles) 1·0288, thus being only very slightly denser than sea water (1·0264).

10. The general analogies of Arenicola with certain other limnivorous Chætopods are very striking. With the Sipunculids the Arenicolidæ agree in the chitinous spines tipping the proboscis papillæ, the buccal papillæ, the strong retractors of the "proboscis," the capacious and largely unsegmented cœlom, the general character of the musculature, the thin-walled looped alimentary canal with its ciliated ventral groove, the action of the body-wall in producing waves of cœlomic fluid auxiliary to the process of burrowing and defæcation, and lastly, the pigmented nuchal organ. If we acknowledge the many points of agreement, which have for the most part arisen independently, between these two distantly related families under similar conditions of life, the true relationship between Arenicola and other genera of Polychæts can only be ascertained by exercising the greatest caution in not confusing convergent adaptational characters with true genetic resemblances.

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EXPLANATION OF PLATES 1—5,

Illustrating Mr. F. W. Gamble's and Mr. J. H. Ashworth's paper on "The Habits and Structure of Arenicola marina."

List of Reference Letters.

A. Cr. Anterior cornua of the brain. *An.* Anus. *Au.* "Auricle" of the heart. *Bl.* Bladder or terminal part of the nephridia. *Blph.* Blastophore of spermatoblast. *B. Pap.* Papillæ of the buccal mass. *Br.* Gills. *Br. Aff.* Branchial afferent vessels. *Br. Eff.* Branchial efferent vessels. *BR.* Brain. *B. S.* Blood spaces in the heart. *B. Sh.* Sheath of retractor muscle enclosing the buccal mass. *Bucc. M.* Buccal mass. *Card. B.* Cardiac body. *Chl. Tiss.* Chlorogogenous tissue on the stomach and ventral vessel. *Chn.* Notopodial chætæ. *C. F.* Cardiac fibres. *C. Sp.* Caudal septa. *D. L. V.* Dorsal longitudinal vessel. *D. Nph.* Dorsal lip of the nephrostome. *Dph. Ph.* Diaphragmatic pouch. *Dphm.* $^{1-3}$ Diaphragms or anterior septa. *Ep.* Epidermis. *G.* Refringent granules in cœlomic cells. *Ga.* Ganglion-cells of the brain. *Gast. Lat.* Lateral gastric vessel. *Gast. V.* Gastric vessels. *G. F.* "Giant fibres." *Gl. Op.* Opening of the œsophageal glands into the œsophagus. *G. S.* Granular cells of the heart. *Gv.* Ventral groove of alimentary canal. *G. V.* Gonidial vessel. *Int. V.* Intestinal vessels. *M.* "Middle piece" of spermatozoon. *M. Circ.* Circular muscles. *Mes. D.* and *Mes. V.* Mesenteries supporting the dorsal and ventral vessels between the first and second diaphragms. *MET.* "Metastomium," or achætous portion of the body immediately following the prostomium. *M. Long.* Longitudinal muscles. *MO.* Mouth. *M. Ob.* Oblique muscles. *M. Par.* Parapodial muscles. *M. Pr.* Retractors of the prostomium. *N.* Nucleus. *N. Aff.* Afferent vessel to the nephridia. *N. C.* Ventral nerve-cord. *N. Cap.* Nephridial capillaries. *N. Eff.* Efferent vessel from the nephridia. *NLV.* Nephridial longitudinal vessel. *Nm.* $^{1-10}$ Neuropodia. *No.* $^{1-6}$ Nephridioporcs. *NPH.* $^{1-6}$ Nephridia. *NPHM.* Nephrostomes. *NS.* Nervous elements and connective tissue round otocyst. *Nu.* Nuchal organ. *Nu. Tr.* Retractor muscle of nuchal organ. *N. V.* Neural vessels. *Oe.* Œsophagus. *Oe. Comm.* Circumœsophageal nerve-connectives. *Oe. Gl.* Œsophageal glands. *Oe. Gl. V.* Vessel of œsophageal glands. *Oe. Lat.* Lateral œsophageal vessel. *O. Ot.* External opening of otocyst. *OT.* Otocysts. *OT*^{1}. Neck of otocyst. *Oth.* Otolith. *OT. N.* Nerve to otocyst. *Par. V.* Parietal vessels. *P. Cr.* Posterior cornua of brain. *Pn.* Protractor of notopodium. *Pr.* Prostomial lobes. *Rn.* Retractor of notopodium. *S.* Cap of spermatozoon. *S. V.* Subintestinal vessels. *T.* Tail of spermatozoon. *V.* Ventricle of the heart.

Vac. Vacuole. *Vac. C.* Vacuolated cells of the heart. *V. Nph.* Ventral lip of nephrostome. *V. V.* Ventral vessel. *I. II. III. IV.* &c. Somites beginning with the first chætigerous.

PLATE 1.

Fig. 1.—The anterior end of a large specimen of the "Laminarian" variety seen from the left side, to show the external features, the segmentation of the body-wall in relation to the internal metamerism, the nephridial apertures, and the commencement of the branchial region. The achætous region following the fully everted buccal mass (*Bucc. M.*) extends forwards as far as the groove indicating the insertion of the first diaphragm dorsally (*Dphm.*[1]). We have considered the first chætigerous annulus and the annulus behind this, as composing the first chætigerous somite (*I*), although we are fully aware that, owing to the obliquity of the first diaphragm, and the absence of landmarks in the achætous region in front of this septum, it is somewhat hazardous to delimit this first chætigerous somite. × $\frac{5}{3}$.

Fig. 2.—View from the right side of two somites from the anterior part of the branchial region of a specimen of the "Laminarian" variety 7 inches long. The fourth gill is shown in detail, while the third and fifth are cut down to the base of the main branches. The large size of the spreading branches and the somewhat pinnate arrangement of the lateral twigs distinguish the gill of this variety of A. marina from that of the ordinary shore lugworm seen in figs. 3 and 4. The webbing at the bases of the branches is generally much more marked in old black examples than in immature dark red specimens such as the present. × 14.

Fig. 3.—Fifth gill of the right side of a shore lugworm 8 inches long, to show the features characteristic of the littoral variety of Arenicola marina. The branches are united by extensive connecting membranes, between which the blood-vessels of the gill are faintly visible. × 14.

Fig. 4.—The first gill of the right side from the same specimen as Fig. 3. The ventral branches are apparently the last to develop, and are only just budding off the secondary leaflets. × 14.

PLATE 2.

Fig. 5.—Dissection of a large "Laminarian" variety, to show the general characters of the internal anatomy (conf. pp. 9 to 10). The body-wall has been cut along the mid-dorsal line, the flaps pinned back, and the alimentary canal turned over to the left side. The special features shown are the vascular system, the nephridia, the septa, and muscles. × 2.

PLATE 3.

Fig. 6.—View of a vertical longitudinal section of Arenicola marina taken somewhat to the left of the middle line. The thickness of the body-wall is exaggerated. The stomach has been cut away behind the heart, to show the oblique muscles and the second nephridium. The main blood-vessels only are indicated, the object of the figure being to show the exact position of the three diaphragms (*Dphm.* $^{1-3}$), of the buccal or proboscidal sheath (*B. Sh.*), and the relations of these to the external segmentation. × 3.

Fig. 7.—Chitinoid spines covering the buccal papillæ of that part of the proboscis which is first protruded during eversion. They may be compared with the figures of "hooks" from the proboscis of Sipunculids (e.g. Phascolion) shown in Selenka, 'Die Sipunculiden.' Caustic potash preparation. × 50.

Fig. 8.—Papillæ in situ on the base of the proboscis of young worm. × 6.

Fig. 9.—A group of neuropodial setæ from a very young Arenicola marina 16 mm. long. The shape and strongly-toothed ridge distinguish these setæ from those of the adult (figs. 11 and 12). The youngest setæ are on the left side of the figure. × 300.

Fig. 10.—Notopodial seta 6 mm. long. × 16.

Fig. 10A.—The tip magnified. × 50.

Fig. 10B.—The toothing on the notopodial seta highly magnified. × 450.

Fig. 11.—Neuropodial seta (× 20), and enlarged (× 120).

Fig. 12.—A group of developing neuropodial setæ in situ in the neuropodium (*Nm.*) of a "Laminarian" specimen. × 70. *Proc.* is referred to on p. 9.

Fig. 13.—The fourth and fifth chætigerous segments of the left side of a large mature "Laminarian" specimen. The first two nephridia are shown. The figure is a study of the blood-vessels of the nerve-cord, of the oblique muscles, and of the connection between the nephrostomial and the dorsal longitudinal vessels (*D. L. V.*). × $3\frac{1}{3}$.

Fig. 14.—The first nephridium from the specimen shown in fig. 13, seen from the dorsal surface, to show the gonidial vessel (*G. V*^{1}.) bearing blind, vascular processes. The gonidial vessel on this nephridium is sterile. × 4.

Fig. 15.—Fifth right nephridium of an adult male, to show the bladder distended with spermatozoa. The nephrostome is widely open. Seen on February 24th, 1897. × 4.

Fig. 16.—The second nephridium of the right side of a specimen 29·5 mm.

long, seen from the dorsal surface, to show the gonidial vessel (*G. V.*), the commencing processes of the dorsal lip, and the position of the external opening (*No.*[2]) with regard to the neuropodium (*Nm*[5].). The ventral lip of the nephrostome (*V. Nph*[2].) is seen through the dorsal one. × 60.

Fig. 17.—Funnel of the first left nephridium from the same specimen as fig. 16, seen from the right side, to show the vertical position of the nephrostome and the commencing processes on the anterior lip. × 90.

PLATE 4.

Fig. 18.—The second right nephridium from a specimen 44 mm. long. Dorsal view, to show the remarkably complete capillary circulation and the extension of the vessel of the dorsal lip to form the gonidial vessel (*G. V.*). The ventral lip (*V. Nph*[2].) is seen by transparency. × 65.

Fig. 19.—Three views of the anterior end of a specimen 8 inches long (littoral variety), to show the prostomium, nuchal organ, openings of the otocysts, and the secondary annulation of the skin. A. From the left side. B. From above. C. From below. × 12.

Fig. 20.—Transverse section across the middle of the prostomium to show the brain, the three prostomial lobes, and the rich blood-supply of this region. The brain lies in the central prostomial lobe, and its covering of ganglion-cells is closely applied to the overlying sensory epithelium. The section is cut across in the region of the posterior cerebral cornua (*P. Cr.*). × 65.

Fig. 21.—Transverse section of the same series as Fig. 20, across the nuchal organ, *Nu.*, the hinder cornua of the brain, and one otocyst, with its contained otoliths. × 65.

Fig. 22.—Transverse section of the body a short distance behind the third diaphragm at the level of the openings of the œsophageal pouches (*GL. Op.*). The external aperture of the second nephridium is shown on the right side. The subdivision of the body-cavity into three longitudinal portions, and the structure of the œsophageal pouches, are well seen. × 38.

Fig. 23.—Transverse section of the body in the branchial region at the level of a parapodium. The neuropodium is cut through its entire length on the left side. On one side of the nerve-cord a retractor muscle from the notopodium arises, on the other an oblique muscle. The vascular supply of the body-wall, setæ, and gills is well seen. × 38.

PLATE 5.

Fig. 24.—Amœboid and spindle-shaped cells of the cœlom. × 1000.

Fig. 25.—Sagittal section of the brain slightly to the left of the middle line, from a young littoral form about 3 inches long. The mass of ganglion-

and glia-cells underlying the epithelium of the prostomium is distinct; some of the cells of the latter are shown bearing sensory processes. The nuchal organ, *Nu.*, is cut at its full depth. × 85.

Fig. 26.—View of a dissection of the brain, œsophageal connectives, otocysts, and the buccal sheath. The commencement of the neural vessels from capillaries of the organs just mentioned, is shown. Seen from the dorsal surface. The buccal mass, cut transversely, lies in the centre of the figure. × 6.

Fig. 27.—An otocyst with the otoliths composed of quartz grains. The sensory epithelium and the surrounding nervous and supporting cells are seen. × 160.

Fig. 28.—Otoliths to show the chitinoid covering of the quartz grains. × 500.

Fig. 29.—Ripe spermatozoon seen on March 10th, 1897. Length of head 4 μ, length of tail 54 μ. × 3000.

Fig. 29A.—Head and portion of tail of an immature spermatozoon seen on February 22nd, 1897. × 3000.

Figs. 30—34.—Stages in the development of the spermatozoa.

Fig. 30.—The 8-celled stage, in which the spermatoblasts leave the testis. × 500.

Figs. 31 and 32. Later stages. × 500.

Fig. 33.—Two cells from a stage much later than the preceding, showing the commencement of the tail. × 2000.

Fig. 34.—Discoidal mass of almost ripe spermatozoa. × 500.

Fig. 35.—Developing ova. (*a* and *b*) show a polar body (?) × 250. (*c*) is a ripe ovum enlarged 125 times.

Fig. 36.—Longitudinal section of the heart of an Arenicola 250 mm. in length, to show the cardiac body. × 32.

Fig. 37.—Histology of a portion of the cardiac body of fig. 36. × 500.

Fig. 38.—Longitudinal section of the heart of a young Arenicola 65 mm. in length, to show the cardiac body at an early stage of development. ×50.

The Aseptic Cultivation of Mycetozoa.

By

Casper O. Miller, M.D.

With Plates 6 and 7.

Observations on the Cultivation of Mycetozoa.

Until the work of de Bary nothing was known about the development of Mycetozoa further than that they appeared as a slimy mass from which the sporangia were formed. He made a short report (1) on the development of the zoospores from the spores at the "Naturforscherversammlung," in Göttingen, in 1854, which was followed by his other publications (2, 3). He speaks (8) of keeping portions of plasmodia in glass dishes containing water, or on slides, but they died in a few days without forming sporangia. Spores of Æthalium septicum, planted on moistened tan on the 2nd of May, showed at the beginning of July colourless plasmodia, which continued through July without further development. Another culture of spores of the same plasmodium, planted the 13th of August, developed many zoospores, and on the 8th of October plasmodia were seen. Spores of Lycogala, planted in a dish containing water and decaying pine-wood, developed zoospores within twenty-four hours; about the fourteenth day there were plasmodia present, which at the end of a fortnight had died without forming sporangia. He also planted spores of Stemonitis obtusata on decaying pine-wood, and found plasmodia on the fourteenth day, but they did not develop further. De Bary was unable to determine whether the plasmodia de-

veloped from a single zoospore or by the fusion of a number of zoospores.

Cienkowski (6 and 7) planted spores of Licea pannorum, Wallr., on decomposing carrots, and obtained plasmodia. He also planted the spores in water placed on slides, and saw the zoospores fuse to form plasmodia. Spores of Physarum album=Chondrioderma difforme, planted on microscopically small portions of vegetable fibre, developed plasmodia on the fourth day, and twenty-four hours later they fructified, so that under good conditions they completed their cycle of development in five days.

Lieberkühn (9) described a plasmodium which he found in the bottom of a glass vessel in which spongillia were being cultivated.

Cienkowski (16) cultivated Didymium libertianum in water. In one to two weeks plasmodia appeared in the water or creeping on the wall of the vessel.

He also found a plasmodium in fresh water containing algæ. He studied it in hanging drop-cultures and on the slide. He thought it probably was the same species which Lieberkühn had studied. Sporangia did not form in any of his cultures.

Stahl (22) cultivated Æthalium septicum on moist tan, and saw a species of Physarum form small-stalked sporangia on a filter-paper culture. He did not use any aseptic precautions, and does not state how long it took the sporangia to form after planting the spores.

Ward (25) found a plasmodium which formed sporangia on the roots of hyacinths which he was cultivating in water containing a small percentage of salts of lime, magnesia, potash, and soda. He then made a decoction of hyacinth roots, which he boiled and used to make drop-cultures. By planting the spores he succeeded in getting the zoospores and plasmodia in drop-cultures and on slides without other forms than bacteria. The cover-glasses were heated, and the cardboard used in making the moist chambers was boiled.

Strasburger (26) obtained Chondrioderma diff. by placing macerated stalks of Vicia faba on moistened filter-

paper under a bell-jar ; the sporangia developed after a few days. He also made drop-cultures of the spores of Chond. diff. in a decoction of cabbage-leaves or bean-stalks, leaving fragments of vegetable fibre in the fluid. He heated the cover-glasses and needle used in making the inoculations, but added algæ and bacteria to the cultures. In many of the cultures the development did not go further than the formation of microcysts, but in more favorable cultures plasmodia developed which fused with each other, and on the fourth or fifth day they crawled out from beneath the cover-glass and formed sporangia.

Wingate (32), in describing Enteridium rozeanum, says that Roze (12) cultivated plasmodia in earthenware dishes filled with sphagnum and water, into which he thrust dead branches of trees, pieces of decayed stumps, &c., which were taken from the neighbourhood of Paris to America. He obtained various plasmodia, and studied them until they formed sporangia. I have unfortunately not been able to procure the original work by Roze.

Lister (34) cultivated Chond. diff., and obtained the sporangia in from ten to fourteen days after planting the spores. The writer has only seen a short report of the paper in Just's 'Jahresbericht,' so that he does not know what methods were employed.

Celakovski (38) used the method which Pfeffer (35) found useful for obtaining plasmodia. He placed dried stalks of Vicia faba, or the leaves and stalks of other plants, particularly of Typha latiflora, in broad crystallising dishes, poured enough water in the dishes to cover the greater portion of the nutrient material, covering the dishes with suitable lids, and sterilised them at a boiling temperature. He then planted spores of Chondrioderma diff. and Didymium macrocarpon. In from six to fourteen days plasmodia of the former were found in the cultures. He frequently obtained the two plasmodia together by simply moistening the stalks of Vicia faba, and placing them in a covered dish. By repeatedly transplanting he obtained the Didymium alone, without the

Chond. diff. He fails to mention how long the interval was between the planting of the spores and the formation of the sporangia. He also planted the spores of Arcyria punicea, Pers., Trichia nutans, Libert, and Stemonitis dictyospora, Rostaf., on sterilised decayed beech-wood in flat crystallising dishes, containing water to the depth of ·5 cm. He did not see the plasmodia of the first two in the water; they developed in the interior of the wood, and only appeared on the surface when the sporangia were formed. The Stemonitis developed plasmodia in the water, and fourteen days after their first appearance the sporangia were formed. He fails to state how long it took for the plasmodia to develop after the spores were planted.

Although Celakovski sterilised his nutrient media and the vessels, no observations were made as to the presence or absence of contamination. It is very difficult to prevent the contamination of cultures in a large flat dish, when the lid is removed or lifted for the purpose of examining the culture.

My cultures were first made as controls for another series of experiments, but the results seem of sufficient interest to publish as a separate paper.

The Methods employed.

In the summer of 1890, while making some experiments at the pathological laboratory of the Johns Hopkins Hospital, to determine what Protozoa one finds in the air, a number of flasks, containing sterilised water with 2 per cent. of milk added, were left uncorked for a number of days. Of the flasks one showed zoospores of Mycetozoa, which were transplanted a number of times. The zoospores and plasmodia developed, but no sporangia appeared.

The first systematic attempt of the writer to cultivate Mycetozoa was made at the Zoological laboratory at Heidelberg in 1893.

A culture was prepared in the laboratory for the study of Infusoria, by simply placing unsterilised hay in a glass jar with

unsterilised hydrant water, the jar being covered by a glass plate. On examining the culture ten or twelve days later zoospores of plasmodia were found in the water, and sporangia of plasmodia developed on the hay a few days later.

A number of similar cultures without aseptic precaution were then made of hay gotten from different sources, and they all showed the presence of plasmodia.

Next a series of cultures were made in tall narrow beakers, they being first closed with a large plug of cotton and sterilised in a hot-air steriliser. The beakers were then filled about half full with unsterilised hay. Care was taken to first wash the hands and sterilise the scissors, so as to be moderately certain that no spores of plasmodia were introduced from the hands or instruments. Water which had been sterilised in flasks was then poured into the beakers, until most of the hay was submerged, care being taken not to cover it completely.

In a few days the hay projecting from the surface of the water was covered with mould fungi. A pair of sterilised forceps was then used to remove the stalks of hay covered by the fungi, care being used to loosen up the hay so as to have some of it projecting above the water. If the hay is entirely submerged plasmodia may not develop, but when prepared as above, all of the cultures prepared with hay, whether gotten in Heidelberg or Baltimore, developed plasmodia. It would appear that plasmodia are constantly present on hay in one form or another.

Cultures prepared in the same way with the stalks of wild carrot picked out from the hay did not develop plasmodia.

A series of cultures were made by putting dried chestnut and oak leaves in sterilised Erlenmeyer flasks with sterilised hydrant water. In a number of these cultures plasmodia developed.

Elsewhere (45) the writer has described the aseptic methods employed in the cultivation of Protozoa, but for Mycetozoa some modifications are necessary. They will grow in sterilised dilute hay infusion, or 2 per cent. of milk in water, but for the formation of sporangia it is in general advantageous, and for

some forms essential, to furnish them a mechanical support as a means of getting out of the water.

The medium which has proven the most generally useful is prepared as follows. A handful of hay is placed in a jar and washed repeatedly until the water remains colourless. It is then covered with fresh water and allowed to soak overnight. The following day the water is poured off, filtered, diluted with fresh water until it is of a white-wine colour, and 2 per cent. of milk is added to the infusion. It is then filtered, put into a flask, and sterilised for future use. The macerated hay is cut and placed in Erlenmeyer flasks; the first portion is cut short enough so as to form a tolerably compact layer in the bottom of the flask to the depth of 1 cm.; the rest is cut sufficiently long to form a very loose layer reaching about two thirds the way up the sides of the flask, care being taken not to allow any of the stems to reach the cotton. Sufficient water is placed in the flasks to cover the hay, and they are sterilised for fifteen minutes. On the following day fresh water is substituted, and they are again sterilised. The water is once more poured off, and enough of the hay infusion and milk previously prepared is added until it is about 1 cm. deep. The flasks are then sterilised in a steam steriliser for ten minutes on three successive days. They are then ready for use.

After soaking the hay for twenty-four hours in water, and boiling it several times in fresh water, about all of the soluble substance has been extracted, and the diluted hay infusion with 2 per cent. of milk is added; we thus have a medium of tolerably uniform composition.

Of the cultures gotten from the air several contained mould fungi, which were eliminated by putting the cultures in the oven at a temperature of 37° C.

One culture contained chroococci, and these were eliminated by keeping a series of cultures in a dark closet. It is not possible in every case to eliminate other protozoic forms that may be present, but one may at times succeed by taking advantage of the fact that the encysted forms withstand drying. In this way one may sometimes succeed in separating Myce-

tozoa from the Infusoria, Amœbæ, and other protozoic forms found in hay infusions.

The cultures are usually transplanted by means of a sterilised pipette.

Bacteria are found in all the cultures, and studies have been made with the view of finding out what effect bacteria have on the growth of Mycetozoa, and what bacteria, if any, are more favorable to their growth.

It is not the writer's purpose to discuss the influence of the bacteria in this connection, but he will leave it for a future communication.

The Mycetozoa cultivated.

Physarum cinereum.

This was the first plasmodium from the air which was cultivated. It will grow and form plasmodia in water with 2 per cent. milk or in dilute hay infusion. The best cultures are obtained when the hay also is present as described above.

In all the cultures where sporangia are formed, the plasmodia grew in the fluid and crawled on the side of the flask above the fluid preparatory to the formation of the sporangia. Although the largest plasmodia form in cultures containing hay, yet the sporangia only form on the glass.

The plasmodia spread out on the glass in the form of a yellowish-white network, consisting of primary trunks from which run branches anastomosing with each other, the network becoming finer as the periphery is approached. At the periphery there is a more or less flattened perforated protoplasmic plate with a scalloped border. In the cultures not containing hay the principal trunks extend to the water; in cultures containing hay the plasmodia spread out from stems of hay leaning against the side of the flask (fig. 2), and it cannot be determined whether branches extend to the water.

In the more vigorous cultures the plasmodia are large enough to cover the whole inner surface of the flask above the water, but do not pass to the cotton plug.

After remaining on the glass above the water for from two to twelve days, the protoplasm collects at one or a number of points at the periphery of the network, and forms sporangia, leaving behind a so-called hypothallus, retaining the shape and outlines of the original network, but much paler in appearance. The sporangia vary in number according to the size and vigour of the plasmodia. In one culture there were only two sporangia; in other cultures the sporangia form groups, the larger of which may contain from seventy to eighty sporangia. In the first stage of the formation of the sporangia the protoplasm is of a more yellow colour than that of the network. As the sporangia assume their completed shape the colour becomes a brownish red, which changes to a greyish white when the development is completed.

The sporangia are sessile, resting on a broad base. When isolated they are round, oval, or kidney-shaped. At times they are united, forming a long drawn-out sporangium with constrictions at irregular intervals. The small oval or round sporangia may measure as little as 0·5 mm. in diameter, the long drawn-out ones may measure as much as 7 mm. On examination with the low power by reflected light the surface shows irregularly shaped small white elevations, between which are darker areas. Under the high power these white areas are seen to consist of aggregations of coarse granules, which dissolve on the addition of hydrochloric acid with the formation of gas bubbles. The sporangia have no columella, and the sporangium wall is colourless. The capillitium is made up of a network of thin, colourless fibres attached to the wall of the sporangium. At the point of communication of the fibres there is a more or less flattened triangular or polygonal thickening, containing granules of lime. The spores are smooth and of a brownish-violet colour, measuring 8·5—13·5 μ in diameter. The majority of the spores are spherical, but occasionally there are oval or irregular forms. From a study of the structure and the arrangement of the sporangia of this plasmodium, it would appear that it is identical with Physarum cinereum, Pers.

Stemonitis.

In July, 1892, another series of flasks containing sterilised milk, 2 per cent. in water, was exposed for a month to the air; they were then closed with cotton and examined. In three of the flasks were flagellate bodies which the writer thought corresponded to the description given by Bütschli (29) of Mastigamœba. From these flasks cultures were made, and in one of the transplantations plasmodia developed. At that time the writer had not studied plasmodia sufficiently to recognise the relationship between the zoospores and the plasmodia, and inasmuch as there were similar flagellates in all three flasks, he concluded that the plasmodia and the flagellates were independent forms. Nothing further was done with the cultures until July, 1893, when they were again transplanted, and plasmodia developed in all three cultures. At that time they were cultivated with 2 per cent. of milk in water, and in hay infusion. The zoospores and the plasmodia grew, but there was no formation of sporangia. The cultures were then made in flasks containing hay, with the idea that the plasmodia would be enabled to get out of the water to form the sporangia. Upon placing hay in the flasks a number of cultures formed sporangia.

All three plasmodia belong to the genus Stemonitis.

From a study of the sporangia there is no difficulty in deciding that two of them are distinct, while it is not so evident that the third one differs from one of the others, but the writer is inclined to the opinion that it is also a distinct species. The writer is not familiar with the American Mycetozoa, and has not been able to get all of the literature on the subject; it is possible that they agree with species already named. It will be necessary to refer to each of these cultures, and it will be more convenient simply to designate them as Stemonitis A, B, and C.

At a variable period after the inoculation of the cultures there appears, rather suddenly, a large yellowish-white plasmodium lying on the hay at the surface of the water, which

may cover an area measuring about 1 by 2 cm. They are composed of a network of short, thick, anastomosing branches, from the periphery of which extend branching, sausage-, horn-, or club-shaped prolongations. There is not much change in the appearance of the plasmodium for forty-eight hours; during this time it may change its location on the hay, but the motion is a slow one. At the end of this time the motion becomes more rapid. The plasmodium moves some distance from the surface of the water, and settles upon the hay or on the glass. In one of the cultures the whole plasmodium had moved 6 cm. in four hours. When it has found a suitable place the peripheral prolongations are drawn in, there is no longer any evidence of the presence of a network; it then appears as an oval or rounded, conical or flat, yellowish-white mass, the surface of which is covered by a number of closely crowded small hemispherical prominences. From each of these prominences is formed a cylindrical sporangium. Soon after the sporangia assume their permanent form, the yellowish-white colour begins at the base to change to a reddish colour, which gradually ascends to the apex, and finally becomes a reddish or dark brown colour.

It takes from twelve to eighteen hours from the time the plasmodium leaves the water until the sporangia are fully developed.

The well-developed sporangia are cylindrical, closely crowded, and placed more or less perpendicular to the membranous hypothallus, from which extends a branch going to the surface of the water, indicating the route which the plasmodium took. When the sporangia are formed on the glass the plasmodia take an oblique course up the side of the glass.

There are slight differences in the appearance of the plasmodia of the three cultures, but the difference is not alone sufficient to enable one to say that they are distinct species. The peripheral prolongations of Stemonitis B are usually longer and thicker than Stemonitis A. The network of Stemonitis C is a more open one than that of the other two.

In some cultures the sporangia are imperfectly developed.

A typical sporangium of Stemonitis A measures about 3 mm. in height, including the stalk, which is 0·167 mm. The diameter of the sporangium is about 0·3 mm., and is usually uniform throughout. The sporangium may be thicker toward the apex or base. The apex is usually rounded, but at times is more acute; the base may or may not be symmetrical. The measurement of the stalk given above is about the average, and applies to fig. 9. In a few instances the capillitium extends to the hypothallus; in other instances the stalk may be 0·5 mm. long. The columella tapers gradually from the base to near the apex, where it divides into several branches, becoming continuous with the capillitium. Occasionally one finds a spindle-shaped thickening of the columella. The primary branches of the capillitium usually come off at an acute angle from the columella, forming one series of anastomoses, and then divide into smaller branches, which go obliquely to the surface network. The surface network usually extends over the entire sporangium. The meshes of the network average from 8 to 33 μ. On the surface network are distributed small wart-like thickenings. The colour of the capillitium is a brownish violet. The spores measure 7—13 μ, and are of a violet-brown colour; the membrane is finely warted.

The sporangia of Stemonitis B (fig. 10) measures 3·5—3·83 mm. in height, not including the stalk, which is about 1·37 mm. long. They are tolerably uniform in thickness, measuring about 0·27 mm. in diameter. The columella tapers gradually from the base to near the apex, dividing into branches which become continuous with the capillitium. The capillitium fibres come off at right angles to the columella, forming one series of anastomosing arches from which pass out secondary fibres placed perpendicular to the surface; they break up into branches which become continuous with the surface network. The capillitium is of a dark violet-brown colour. At the point where the primary fibres anastomose one frequently finds membranous expansions which are more marked than in the sporangia of Stemonitis A, but these

membranous expansions vary a good deal in sporangia from the same culture. The spores are of a light violet colour; they have a smooth membrane, and are tolerably uniform in diameter, measuring 7·9—8·5 μ.

The sporangia of Stemonitis C resemble those of Stemonitis B. The sporangia of Stemonitis C measure 3·3—3·7 mm. in length, and 0·37 mm. in thickness. The columella is not infrequently bent on itself at about the upper four fifths. The secondary fibres of the capillitium are longer than in the sporangia of Stem. B. The stalk measures 0·68—1·16 mm. in length. The spores are smooth, of a brownish-violet colour, measuring 7·4—11 μ in diameter.

It would therefore appear that the differences between the sporangia of Stemonitis B and C are not less than those which separate some of the forms which are described in works on the subject under different names. It is possible that further cultivation may show that they are the same.

Hay and Leaf Cultures.

In cultures made with unsterilised hay in jars without aseptic precautions, or in flasks with aseptic precautions, one finds bacteria, fungi, monadina, infusoria, and plasmodia developing with uniform regularity. Chondrioderma difforme and some species of Didymium, usually microcarpon, appear together or singly, the Chondrioderma being most frequently present. As has been stated before, some plasmodium appears in every culture made with unsterilised hay.

By the drying method the Chondrioderma diff. and Didymium microcarpon have been separated and cultivated aseptically in flasks. They both form sporangia on the hay, and on the glass above the hay.

In a culture of Chond. diff. made in dilute hay infusion with 2 per cent. milk added, which had been kept in the dark for several weeks and then placed in the light, sporangia formed under the surface of the water. The sporangia were small, round, or pear-shaped, and did not show the presence of

any granules of lime in the sporangium wall. In all the other cultures observed the sporangia formed on the hay or on the sides of the flask above the level of the fluid.

In speaking of the classification of his plasmodium, Ward (25) says, "It is, indeed, not improbable that we have here an aquatic form of Didymium difforme, one of the commonest of our Myxomycetes; and if so, we have another proof of the all but uselessness of attempting to classify the lower organisms until we know more of their habits under varying conditions." From the writer's experience, he questions whether Ward did not really have the ordinary form of Didymium diff. = Chondrioderma diff. in his cultures, and whether the character of the fluid in which they grew, and the other conditions surrounding them, did not cause the sporangia to form only on the roots under the water or on the moist roots above the water.

Didymium farinaceum was obtained from a culture made with unsterilised leaves taken from the forest.

In one flask containing leaves, and in two containing pine-needles, plasmodia developed and formed sclerotia above the water on the side of the flask, but no sporangia appeared, so it was not possible to determine what species they were.

Spores of Æthalium septicum obtained from a tan-pile were planted in flasks, and yellowish plasmodia developed, but no sporangia formed. Spores from several varieties of Stemonitis collected at Heidelberg were planted in flasks. The zoospores and plasmodia developed, but only one of them formed sporangia.

Spores of Ceratium porioides, gotten from a pine stump, dried and planted aseptically, developed zoospores which have been cultivated for about four years, and as yet the writer has failed to find any plasmodia or sporangia. So far as I have been able to discover, no one has succeeded in cultivating plasmodia of any of the Ceratiomyxa.

The Time of the Appearance of the Large Plasmodia, and of the Formation of the Sporangia.

Plasmodia, as we usually find them in nature, appear rather suddenly on decaying wood, tan, or leaves, and within a short time they form sporangia. We know little about their previous growth.

Some Mycetozoa may form sporangia during any of the warm months, while, according to de Bary (21), others are characterised by forming sporangia only during a short time in the year. As has already been mentioned, Cienkowski and Strasburger obtained sporangia on the fifth day after planting the spores of Chond. diff., and Lister obtained them in from ten to fourteen days. Celakovski (38) mentions that the sporangia of Stemonitis dictyospora, Rostaf., developed fourteen days after the appearance of the plasmodia, but does not state how long it took the plasmodia to develop.

Rex (36) mentions having seen Stemonitis Bauerlinii form sporangia on a decayed log in the autumn, and the next summer the same species formed sporangia three times on the same log at intervals of a month. One cannot say that the spores fell back on the log, developed zoospores, and from these new plasmodia grew and formed sporangia.

In the cultures made with unsterilised hay in water the conditions are practically the same. The forms of the Mycetozoa, whether microcyst, sclerotia, encysted zoospores, or spores, have been dried for months. The hay is placed in the water and kept at the room temperature. The sporangia of Chond. diff. appeared on the hay from the twenty-fourth to the twenty-ninth day. Crops of sporangia continue to be formed on the hay every few days for from two to four weeks.

Didymium microcarpon first show sporangia on the hay from the twenty-first to the twenty-fourth day, and continue to form sporangia for several weeks.

When there were only a few stalks of hay projecting above the surface of the water the sporangia appeared, but were less

numerous than when a good many of the stalks projected. The time from the planting of the cultures until the sporangia form varies considerably.

Cultures of Stemonitis A, B, and C formed sporangia as early as the thirtieth, and as late as the seventy-sixth day. Two cultures made from the same parent culture in the same media developed sporangia on the thirty-second and seventy-sixth day respectively.

As a rule but one set of sporangia developed in the same culture. Sporangia do not develop in all the cultures; at times large plasmodia form on the hay and degenerate without forming sporangia.

Physarum cinereum formed sporangia from the twenty-second to the sixty-fourth day.

Didymium farinaceum formed sporangia on the dried leaves on the fifty-seventh day.

Æthalium septicum formed large plasmodia about the fifty-fifth day, remained on the side of the flask for about ten days, and then degenerated without forming sporangia.

Plasmodia under natural conditions leave their moist or wet habitat, crawl to the surface when it is dry, and they are exposed to the light. In some of my cultures the formation of the sporangia seems to have been delayed by keeping the culture in the dark; some of the cultures were kept in the dark six weeks, and after being in the light for several weeks formed sporangia.

The zoospores develop readily in the oven at 37° C., but no sporangia formed in any of the cultures. The absence of light may have had something to do with the result.

Time of the Day at which the Sporangia develop.

De Bary (8) studied the formation of the sporangia of Physarum sulphureum, Didymium serpula, Æthalium septicum, and Stemonitis ferruginea, and found that usually the sporangia began to form in the afternoon or late evening, and the development was completed in some cases by

the next morning; in others not until near the middle of the day.

In one observation by the writer, the plasmodium lying on the hay at the surface of the water began about noon to crawl up the side of the flask. By 6 p.m. the plasmodium had collected at the point where the sporangia formed; by 7 p.m. the branches were drawn in, and the surface was covered by a number of hemispherical projections; and by 6 a.m. the following day the sporangia were fully formed. In other cultures observed the plasmodia were resting at the surface of the water at 6 p.m.; by 9 o'clock the next morning they were out of the water, and the sporangia had begun to assume a cylindrical shape. By 11 a.m. the shape of the sporangia was fully developed; the colour appeared first in the base of the columella, gradually going to the apex. By 2 p.m. the sporangia were of a brownish-red colour except at the apex, which was yet a yellowish-white on the surface. By 5 p.m. the colour was fully developed and the sporangia were completed.

The sporangia of Phys. cinereum, so far as observed, began to be developed at 3—6 p.m., and were completed by the next morning. The sporangia of Chond. diff., Didym. microcarpon, and Didym. farinaceum also developed for the most part at night.

Observations and Speculations concerning the Formation and Growth of the Plasmodia.

In his first studies De Bary failed to show how the plasmodia develop, whether by growth from a single zoospore or by the fusion of a number of zoospores.

Cienkowski (6, 7) described and pictured the fusion of the zoospores to form small plasmodia, and he saw plasmodia which had later taken in foreign particles, spores, and microcysts.

De Bary (8, 21) accepted Cienkowski's results, although he never saw the zoospores fuse.

Ward (25), in speaking of the fusion of the zoospores to

form plasmodia, says, "The inference becomes almost a certainty after watching the specimens under cultivation;" but he did not actually see them fuse.

Strasburger (**26**) also describes the fusion of the zoospores to form Myxamœba.

The writer has not been fortunate enough to observe the fusion of the zoospores, but the accuracy of the observations of such competent observers as Cienkowski, Strasburger, Lister, and others can hardly be doubted. In the cultures, as the writer has studied them, however, he does question whether the fusion of the zoospores is the chief mode by which the plasmodia grow.

If a few drops of a culture containing microcysts of Stemonitis, with suitable bacteria, be inoculated in a flask containing sterilised water, with milk 2 per cent., the bacteria multiply at the expense of the milk. Within two or three days the fluid loses the slight opalescent appearance which it had, and on microscopic examination there are no longer milk globules present. I think, from our knowledge of bacteria, we can conclude that at least a portion of the milk has been consumed by them. During this time the zoospores have multiplied by division; they feed on the bacteria, and possibly some elements of the milk which the bacteria may not have appropriated. In a few days the zoospores begin to encyst, and by the end of the second week the majority of the zoospores are encysted, while a smaller number remain active. If control cultures are made from the flask, it will be found that there are not near so many bacteria present as there would be in a flask containing a similar medium inoculated with the bacteria alone which grow with the zoospores. In from ten to fourteen days small plasmodia may appear; they increase in numbers and in size, and later large plasmodia are present.

In cultures made in flasks containing hay, with milk 2 per cent. in hay infusion, essentially the same changes take place, but the hay interferes somewhat with the examination. If examined about the end of the second week one finds bacteria, encysted zoospores, active zoospores, and a few small plasmodia. The

plasmodia increase in number and in size, but they are not seen macroscopically. If the culture be one which forms sporangia on the thirtieth day, and it is examined about the twenty-sixth day, one finds more small plasmodia and a smaller number of microcysts present in the fluid than at the previous examinations. A large plasmodium appears, rather suddenly, on the twenty-eighth day, lying on the hay at the surface of the fluid. It does not increase noticeably in size for two days, and then passes up the side of the flask to form sporangia. If the fluid is examined the small plasmodia have disappeared for the most part from the fluid.

One may have examined the culture the previous day without having macroscopically observed the presence of a plasmodium. It must have originated by the fusion of a number of small plasmodia, or have grown as a large plasmodium in the interior of the stalks of hay. One stalk of hay is not large enough to accommodate the plasmodium, and no branches of plasmodia are seen connecting the various stalks. The writer is of the opinion that it originated by the fusion of a number of small plasmodia. Plasmodia large enough to be seen macroscopically have been observed by the writer to fuse on the slide.

What takes place in the culture seems to be as follows:— the bacteria multiply at the expense of a portion of the nutrient material: the zoospores multiply at the expense of the bacteria, and possibly some nutrient material which was not consumed by the bacteria; the majority of the zoospores encyst; small plasmodia develop from a single zoospore or by the fusion of several zoopores; the plasmodia take in and digest active and excysted zoospores and bacteria; finally, the small plasmodia fuse to form the large plasmodium.

Celakovski (38) studied the action of the plasmodia Chond. diff., Didymium microcarpon, and Æthalium septicum on various substances placed in the fluid with them. He saw them take in microcysts which, after ingestion, were not found in vacuoles, but were simply surrounded by protoplasm. After two days the microcysts were expelled unchanged; if dried

and again moistened they gave origin to active zoospores. He thus reached the conclusion that the plasmodia did not digest the microcysts.

It is well to consider the condition under which he placed the plasmodia. He removed them from the fluid to which they were accustomed, washed them in fresh water, and placed the microcysts, spores, &c., on or near the plasmodia. In some instances he washed them several times. To the writer this seems harsh treatment. It cannot be wondered at that they were not in a condition to digest foreign substances, and that under normal conditions he got peculiar results.

The writer has observed living plasmodia which had taken in microcysts and rounded off zoospores which had not yet formed a cyst wall. In these instances the zoospores were lying in vacuoles. Plasmodia placed on slides under a cover-glass (with active and encysted zoospores in the same fluid in which they grew) and allowed to spread out were killed with picric and acetic acid, and stained with piero-carmine. One finds in such specimens microcysts and rounded-off zoospores lying in vacuoles. They are in various stages of degeneration, and stained with varying degrees of intensity. From a study of the specimens the writer does not see how one can reach any other conclusion but that the microcysts and zoospores are digested. If one examines a culture after having developed sporangia, there are a smaller number of microcysts present than there was some time previously.

If one places a few drops of a culture containing zoospores of Stemonitis on a slide under a cover-glass, placing it in a moist chamber for some hours, on examination he will find many of the zoospores creeping around on the slide, feeding on the bacteria. If, now, a point is examined at one side of the cover-glass, and a drop of sterilised water be added to the culture at the opposite side of the cover-glass, it will be observed that the zoospores instantly draw themselves together, many of the vacuoles will disappear, and the bacteria or undigested granules which were in the vacuoles will appear as granular particles enclosed in the protoplasm. It will be

some minutes before the vacuoles reappear and the zoospores begin to feed again.

It is not an infrequent experience that some protozoic forms are killed by simply placing them in fresh water. The plasmodia may not be as sensitive as the zoospores. They may have sufficient vitality not to be killed by the treatment to which Celakovski subjected them, but the writer questions whether the results obtained under the conditions in his experiment can be used as a basis for conclusions as to what plasmodia do in the fluids in which they thrive. His studies also showed that the plasmodia did not digest the encysted or active Colpoda which they had ingested. By the study of plasmodia taken from hay cultures and placed on a slide with a few drops of fluid containing Colpoda, the writer obtained results which showed that they do digest Colpoda.

The observations of Lister (44) also show that plasmodia do take in microcysts, enclose them in vacuoles, and digest them.

Observations on the Ingestion of Foreign Substances and the Multiplication of the Zoospores.

Lister (30) described the taking up of bacteria by the zoospores of Stemonitis fusca and Trichia fallax. The bacteria were drawn in by pseudopodial prolongations, which always came off from the posterior extremity, and were carried to vacuoles near the nucleus, where they remained until digested. He also gave them particles of carmine, which were ingested but not digested.

The zoospores of Stemonitis A, B, or C have not been observed to put out pseudopodial prolongations and draw in food particles, but the writer has seen the zoospores of Stemonitis A, B, C, and Ceratium porioides take up bacteria and particles of carmine by means of a kind of vacuole which forms at the surface of the body (fig. 12). It is situated most frequently in the anterior half, but may form at any portion of the surface. The formation of these vacuoles can best be studied in a culture which has been on a slide for some hours. If a drop is taken

from a flask culture, placed on the slide, and immediately examined, the vacuoles are not always found. In favorable cultures the zoospores are found creeping on the slide and changing their shape; the flagellum is in active motion. At the posterior portion of the body a pseudopodial prolongation is put out, by means of which they adhere to the slide. Not far from the attachment of the flagellum there arises a projection, which is placed more or less perpendicular to the surface of the body. At first sight it appears to be simply a conical or papillary projection from the surface layer of protoplasm. On closer examination a thin fold of protoplasm is seen to arise from the whole length of the projection, and extends forward toward the flagellum, where a second similar projection arises. At this stage the two projections, with the thin fold-like connections, form a funnel-shaped depression. The apices of the two projections approach each other and fuse, converting the funnel-shaped depression into a closed vacuole, which passes backward, and is lost among the vacuoles near the centre of the body. The flagellum is in active motion, and throws the bacteria or particles of carmine into the funnel-shaped depression, which then closes and forms the vacuole. The writer has seen bacteria and particles of carmine taken up in this way. When these vacuoles are located in the posterior region of the body, the flagellum has not been observed to throw the bacteria into them. Fig. 12, *a*—*j*, shows the different stages in the formation of these vacuoles. The zoospores of Phys. cinereum have not been observed to form these vacuoles.

The amœboid stage of the zoospores of Phys. cinereum is much more pronounced than that of Stemonitis A, B, C, and Cerat. porioides. A large part of the active existence of the zoospores of Phys. cinereum is passed without the presence of flagella. They put out pseudopodia which are more or less angular, and their change of shape resembles more that of an amœba. They may ingest their food after the manner of amœbæ.

The zoospores of Stemonitis A, B, C, and of Ceratium porioides are characterised by rarely being without a fla-

gellum, and when they do change their shape the pseudopodia are more rounded.

The zoospores of plasmodia usually have a single flagellum, but they may have two or four. The writer has not seen zoospores of the Endosporia with more than one nucleus, whereas large zoospores of Cerat. porioides have occasionally been found which have two distinct nuclei (fig. 12, *l.*).

In all species of plasmodia which the writer has examined, one can distinguish two forms of microcysts in the cultures. In the simple form the cyst wall is made up of a single homogeneous membrane, closely applied to the protoplasm, as indicated in fig. 12, *m.* In the second form the cyst wall is made up of an outer thick membrane, irregular or scalloped in outline, and an inner, thinner membrane, which is closely applied to the protoplasm (see fig. 12, *n.*, *o.*). One finds cysts intermediate between these two varieties. The simple microcysts of Stemonitis A measure 5—7 μ in diameter, the thick-walled cysts measure 10—14 μ in diameter.

In cultures of Stemonitis A, B, and C, and Phys. cinereum made in hay infusion, the thin-walled microcysts remain unstained, whereas the membrane of the thick-walled cysts may be stained a brownish colour.

In old cultures made in hay infusion at times one sees microcysts with dark brownish, almost black pigmented granules in their interior.

Lister (**44**) speaks of the spores of Ceratiomyxa as having four "nucleus-like" bodies, and pictures them indistinctly in his plates. The writer's observations show, from staining with picro-carmine, that these bodies are true nuclei.

Famintzin and Woronin (**10**) showed that after the protoplasm escaped from the spores of Ceratium it remains at one place for some time, undergoing amœboid changes. It then divides into four round segments, each of which divides and gives origin to two zoospores. Lister (**44**) describes the naked spore dividing into eight spherical segments which remain attached to each other. These develop flagella, and then separate.

The writer has seen them divide after the manner described by Famintzin and Woronin (see fig. 13).

Microscopical Appearance of the Plasmodia and the Structure of the Nuclei.

Cultures made in fluids without the presence of hay offer the best facilities for studying the plasmodia. The smaller plasmodia are usually found lying in or upon a clump of microcysts and bacteria. The larger plasmodia can be seen spread out on the bottom or sides of the flask. The closeness of the network, the size of the branches, and the peripheral arrangement of the network can be studied.

For microscopical study, the plasmodia, with a few drops of the fluid in which they grow, are placed on a slide by means of a pipette. A cover-glass is carefully laid on, and is supported by small bits of wax at each corner to prevent injuring the plasmodia by pressure. The specimen can be immediately examined, and then placed in a moist chamber for twelve to twenty-four hours, after which it may again be examined.

If it is desired to preserve the specimen, the plasmodium can be fixed and hardened on the slide and stained by any of the usual methods. Hardening in picric and acetic acids, and then staining in picro-carmine, give good results.

Fig. 6 represents a segment of a plasmodium taken from a culture of Stemonitis A. Some of the clumps containing microcysts, bacteria, and plasmodia were placed on a slide. The specimen was examined at the expiration of an hour, and a number of rather long, finger-like, blunt, unbranched protoplasmic processes were seen radiating from the periphery of the clumps. At the expiration of twenty-four hours the clumps were surrounded by a network of protoplasmic branches. The primary trunks are large, and extend from the clump toward the periphery of the network. They anastomose with each other, and are also connected by means of a finer network of secondary fibres. At the periphery of the network are irregular, angular, flattened, protoplasmic expansions, which at times unite and form irregular plates, as shown in the

figure. Occasionally one gets specimens where the fusion of these expansions is more extensive.

When the plasmodia spread out as in the figure, one may see microcysts which have been taken up from the clump by the plasmodium and carried along the branches toward the periphery.

One frequently finds small plasmodia composed of only a few branches which do not form as close a network as in the figure, and the ends of the branches are not so angular.

The writer has not, as yet, sufficiently studied plasmodia from Stemonitis A, B, and C to be able to point out any constantly marked characteristics which distinguish them. They are all more transparent and not so granular as the other plasmodia which have been studied.

Fig. 7 represents a portion of the periphery of a small plasmodium of Phys. cinereum taken from a culture and allowed to spread out in the same way. It has more the appearance of a spread-out, perforated, protoplasmic plate than a network of branches, and one cannot distinguish between primary and secondary branches.

Fig. 8 represents the peripheral expansion of a plasmodium taken from a hay culture in which were Chon. diff. and Didym. microcarpon. Here there are several large trunks going to a broad peripheral expansion, less perforated than Phys. cin.

The nuclei of the plasmodia of Stem. A are distributed irregularly along the branches of the network and in the peripheral expansions. In some of the larger branches they may be collected in groups, where, as in other branches, they are situated at irregular intervals. In the larger branches the most of the nuclei lie in the peripheral layer of protoplasma, often immediately under the surface. As a rule one does not see the nuclei in the living plasmodia, but occasionally, if the nucleus lies at the side of the branch, a nucleus can be seen as a spindle-shaped body just beneath the surface, and may cause a bulging at that point.

The shape of the nuclei is usually that of a flattened oval

disc. When seen on the edge they appear spindle-shaped. They contain from one to six or seven small bodies, which may provisionally be called nucleoli. One finds nuclei containing two nucleoli. These nuclei are at times constricted, and suggest stages of division.

Strasburger (23) studied the division of the nuclei of Trichia fallax during the formation of the sporangia. He succeeded in finding stages which showed karyokinesis. Rosen (39) studied the division of the nuclei in the forming æthalia of Æthalium septicum, but found the division of the nuclei simpler than that described by Strasburger.

Lister (44) describes the division of the nuclei of zoospores and of the nuclei of the plasmodia by karyokinesis, but also concludes that they divide by direct division. The writer has not been so fortunate as to find nuclei dividing by karyokinesis, although frequent search was made for them.

The zoospores can frequently be seen to divide and form two zoospores, and to the writer there seems to be evidence that at times the protoplasm of the microcysts breaks up into a number of small segments, and these segments enlarge and develop zoospores.

The nuclei of Phys. cinereum are smaller than those of Stemonitis A. They are spherical, containing one or more nucleoli, and are distributed in every part of the protoplasm, but are more abundant in some portions than in others.

Before concluding the writer wishes to acknowledge the invaluable assistance which he received from Professor Bütschli while pursuing these studies in Heidelberg, also to Professor Wladimir Schewiakoff, then assistant at the laboratory of Heidelberg, for his assistance in the preparation of the plates.

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EXPLANATION OF PLATES 6 and 7,

Illustrating Mr. Casper O. Miller's paper on "The Aseptic Cultivation of Mycetozoa."

Fig. 1.—Sporangia of Chondrioderma diff. on the glass above the fluid, from a culture made with unsterilised hay.

Fig. 2.—Physarum cinereum, showing sporangia at the periphery of the plasmodial network which radiated from the end of a stalk of hay leaning against the glass.

Fig. 3.—Stemonitis A, with the sporangia in process of formation on the side of the flask at a point where a stalk of hay touched the glass. The colour had not fully developed. The course which the plasmodium took is shown by the thread which descends obliquely to the water.

Fig. 4.—Sporangia of Stemonitis B, fully developed on stalks of hay.

Fig. 5.—A plasmodium of Stemonitis lying on the hay at the surface of the water before it ascends to form sporangia.

Fig. 6.—A portion of plasmodium of Stemonitis A., which had spread out under a cover-glass. The trunks radiate from a clump of encysted zoospores, some of which were enclosed in the protoplasm. Drawn with a camera lucida. × 130.

Fig. 7.—A portion of a plasmodium of Physarum cinereum spread out under a cover-glass. × 130.

Fig. 8.—A portion of a plasmodium gotten from hay. × 130.

FIG. 9.—A sporangium of Stemonitis A, drawn with a camera lucida × 66.

FIG. 10.—A sporangium of Stemonitis C. × 66.

FIG. 11.—A sporangium gotten on a stump at Heidelberg. It has not been identified with any of the species described in works on Mycetozoa.

FIG. 12.—Zoospores of Stemonitis. 12 *a*—*h*. A zoospore in the act of forming a nutrient vacuole, and taking in a granule of carmine. 12 *i*, *j*, showing the location of the nutrient vacuoli posteriorly. 12 *k*. A zoospore with four flagella. 12 *l*. A zoospore of Ceratium porioides, with two flagella and two nuclei. 12 *m*. An encysted zoospore, with a thin wall. 12 *n*, *o*. Encysted zoospores, with thick walls.

FIG. 13 shows different forms of spores of Ceratium porioides, with the four nuclei and four zoospores developing from one spore.

On the Development of Tubulipora, and on some British and Northern Species of this Genus.

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With Plates 8—10.

Introduction.

The principal object of the observations described in the present paper was to test the conclusion, at which I formerly arrived, that a process of embryonic fission is of normal occurrence in Cyclostomatous Polyzoa. The process had already been demonstrated in Crisia (15) and in Lichenopora (16), and I am now able to show that the development of Tubulipora[1] takes place on essentially the same lines. In the course of these observations it became apparent that the discrimination of the British species of Tubulipora had not been characterised with sufficient precision.

The satisfactory determination of the species of Cyclostomatous Polyzoa is not an easy task. There are perhaps few cases, even in this group, in which the synonymy is in a more involved state than in the genus Tubulipora. Many of the specific diagnoses which have hitherto been given are based on immature specimens, or are for other reasons insufficient for purposes of identification, and the compilation of unduly long lists of synonyms is for this reason undesirable.

[1] A preliminary note (17) on this subject was published, in which the genus appears as Idmonea.

The question is further complicated by difficulties in deciding which names have the greatest claim to be assigned to particular species.

Before proceeding to the accounts of the species the scope of the paper may be stated. I have given diagnoses of two Northern species, one of which I believe to be new, and of all the forms ordinarily recognised as British species of Tubulipora, with the exception of T. lobulata, Hassall.

I have not had a sufficient supply of this supposed species to enable me to come to any clear conclusion about it. Many of the colonies which I have found on shells in the deeper Plymouth dredgings (20—30 fathoms) agree well enough with Hincks's description of this form. I have also seen similar specimens from the Liverpool district, kindly lent to me by Professor Herdman; but I have not been able to examine uninjured mature colonies. In the absence of perfect ovicells, it is possible that some of the specimens supposed to be T. lobulata may be worn examples of other species of Tubulipora. I prefer, therefore, to express no opinion with regard to this form.

The paper is divided in the following way:

I. Structure of the colony and of the ovicell. (The terms "oœciostome" and "oœciopore" are here proposed.)

II. History of the species and genus.

III. Synonymy, diagnoses, and accounts of the species. (New species, T. aperta.)

IV. The nature of certain vesicles found in the tentacles and other parts, with a few statements relating to the budding and the structure of the adult zoœcium.

V. Description of the development. (The terms "axial lobe" and "lateral lobes" are here proposed.)

VI. The morphology of the internal parts of the ovicell.

I. Structure of the Colony and of the Ovicell.

A colony of Tubulipora, as of other Cyclostomes, takes its origin in the well-known circular primitive disc (Pl. 8,

fig. 2) formed by the calcification of the body-wall of the metamorphosed larva. The early zoarial development is well described by Barrois (1, p. 70, pl. iv, fig.), whose account perhaps refers partly to T. phalangea and partly to T. plumosa; and it results in all species in the formation of a colony which is at first pyriform or fan-shaped. The terms "proximal" and "distal" will be used with relation to the primitive disc. The distal curved margin of the colony is bounded by the "terminal membrane" (16, p. 93), which consists partly of an uncalcified cuticle (ectocyst) and partly of underlying protoplasmic structures. The terminal membrane does not remain as a continuous sheet, since portions of it are continually cut off by the upgrowth of the calcareous septa which form the lateral walls of the zoœcia or ovicells. Each of these structures is thus closed by a derivative of the original terminal membrane, and this name may consequently be applied also to the uncalcified distal wall of each unit of the colony (fig. 24). Both the zoœcia and the ovicells are added to, after their first formation at the growing edge, only by the prolongation of parts of the calcareous tubes which have already been developed, though this is not altogether true of the Lichenoporidæ, in which the character of the colony is altered by the subsequent development of "cancelli." It thus follows that a young Tubulipora colony is identical, so far as its calcareous structures are concerned, with the proximal part of the same colony at a later stage of its existence; and that in order to understand what a particular colony or ovicell was like at an early period of its development, it is only necessary to imagine the distal parts of the colony suppressed.

The form of a particular colony is due to the behaviour of its terminal membrane, which is identical in its extent with the growing margin. Should this remain undivided, and continue to grow symmetrically, a pyriform or flabelliform colony will result, according to the relative activity of growth in the longitudinal and transverse directions. Under certain circumstances, probably due to unfavourable conditions, the colony becomes mature by developing an ovicell without losing its

pyriform shape. I have found mature colonies of this type commonly in at least three of the species described in the present paper, namely in T. phalangea, T. flabellaris (fig. 4), and T. aperta (fig. 2).

In other cases the transverse growth of the terminal membrane is more active, and the flabelliform character becomes more marked (figs. 1 and 5), its lateral edges commonly growing proximally so as more or less to encircle the primitive disc. This flabelliform shape typically occurs in well-grown colonies of T. flabellaris and T. aperta. In other species the terminal membrane commonly divides at an early stage, so that there is a cessation of growth between the two parts of the divided membrane. The colony then grows into two lobes, which usually diverge from one another. This form of growth is well indicated by Pallas (33, p. 248) in his original account of T. liliacea (= T. serpens, auctt.). Colonies of this species, as well as those of T. phalangea and T. plumosa, often become mature in this condition; but in all three species further divisions of the terminal membrane[1] usually take place, the colony thus becoming variously lobed. In T. liliacea alone of the forms described in this paper, there is in many cases a marked tendency for the lobes to become free and erect; and an Idmonea-like form thus results.

The common basal part of a colony consists of a mass of pyramidal tubes with pointed proximal ends, the arrangement being comparable with that of a honeycomb, all the "cells" of which look in one direction, represented in Tubulipora by the growing margin of the lobe. The base of the colony, or basal lamina, is the sum of the lower walls of the more proximal parts of the zoœcia. It is completely adherent to the substratum in many species of Tubulipora, but it may grow out freely from it, and the colony thus becomes erect. Each zoœcium is developed at the growing edge by the formation of a new radial septum in connection with the basal

[1] A commencing division of the terminal membrane is indicated on the right side of fig. 1.

lamina, and grows at first in a more or less horizontal, centrifugal direction. As its length is added to at the growing edge, its upper wall gradually rises above the general level of the colony, and some of the zoœcia thus form projecting ridges (fig. 1). As the zoœcium rises up in this way, its basal wall splits off from the basal lamina, between which and itself a cavity thus originates. This cavity is the beginning of a new zoœcium, which continues to grow in the same manner.

It follows from this description that each zoœcium proximally reaches the basal lamina, as in Lichenopora (16, woodcut, p. 84); a younger zoœcium always originating from the basal side of an older one, and making its appearance distally to it after a certain time.

As the zoœcium continues to grow longer, its distal part becomes free from the common basal mass of the colony. It may become completely free on all sides, and it then, by the activity of its own terminal membrane, develops into a curved tube, which is generally cylindrical, though in some cases with an oval transverse section and orifice. Very commonly in Tubulipora the zoœcium does not become free on its basal side, where it remains connected with one or more of its younger neighbours. In this way it forms the beginning of a row, usually uniserial, of connate zoœcia, as show in figs. 1, 5, and 9. The adjacent zoœcia of a series are separated by a flat septum, corresponding with the intersecting plane of two cylinders. Any one of the middle zoœcia of a simple series will thus have four flat walls (fig. 1).

The shape of the orifice corresponds with that of the zoœcium, being at first angular in the case of serial zoœcia. If, however, growth of these goes on actively, the series may be resolved above into its constituent units, each of which then becomes cylindrical with a round orifice.

The ovicell of Tubulipora is an enlarged zoœcium. This is shown by several facts, the most striking of which is that when young it has a polypide, and then differs in no way from an ordinary zoœcium. The proximal end of the ovicell thus takes part in the formation of the basal lamina. On looking

down into the mouth of the open calcareous funnel (fig. 4.7), which is the condition of the young ovicell at the time when it commences to expand distally, a tubular cavity can be seen, which passes down to the basal lamina and represents the ovicell in its zoœcium stage.

The upper wall of the expanded distal part of the ovicell is thickly perforated by pores (fig. 1). These pass through the calcareous layer; they are wider internally and constricted externally, where they are closed by a cuticle, the pore being entirely filled with a few cells. The pores are always much more numerous in the upper wall of the ovicell than in the walls of the zoœcia, as Waters has pointed out (45, p. 277, and in other papers). It is perhaps probable that the function of these pores is mainly respiratory, to provide for the gas-exchange which must be necessary for the development of the great mass of larvæ which are formed inside the ovicell. The definitive assumption of the ovicell-character by a zoœcium is thus marked by a great increase of the porosity of its upper wall.

In some species, and notably in T. flabellaris (fig. 4, ovicell 2) and T. aperta, the more porous part can in some cases be seen to be separated from the less porous part by a sharp line. The proximal part has the appearance of a zoœcium; and we thus have an external indication of the time at which the fertile zoœcium became definitely an ovicell.

An ordinary zoœcium was seen to become, sooner or later, free from the common basal part of the colony, unless it remained connected with it by forming one of a connate series or of a fasciculus of zoœcia. The corresponding process in the ovicell is the formation of its tubular portion ending in the orifice. This part, in all respects comparable with the upstanding free part of an ordinary zoœcium, is, however, developed late, and can only be seen in ovicells which are nearly mature. The ovicell, in fact, remains a part of the common basal mass of the colony for a long period, during which its distal end is expanding and increasing the space available for the growth of the embryos. Before the distal

expansion begins the ovicell has become separated by younger zoœcia from the basal lamina, and its floor is thus formed by their proximal parts. The upper part or roof of the ovicell spreads out horizontally as the conspicuous, porous, calcareous film usually described as the ovicell. If no fresh zoœcia were formed at the growing edge, the roof of the ovicell would be a simple fan-shaped film. But new zoœcia continue to grow in the same way as if no ovicell were present (Pl. 10, fig. 32). The floor of the ovicell, formed by the upper walls of the younger zoœcia, or really by the septa dividing its own body-cavity from that of these zoœcia, thus rises into radial ridges which encroach on its cavity. As these become more vertical they meet the growing horizontal roof of the ovicell, and with further prolongation stand up from it at a right or obtuse angle.

In most of the species investigated the zoœcia which are younger than the ovicell are arranged in connate series. But whereas the proximal parts of the fertile lobes have their zoœcia in obliquely transverse, alternate rows (fig. 5), these series usually become radial in the region of the ovicell. In other words, a young zoœcium which has reached the roof of the ovicell in the manner just indicated does not become free, but remains connected with the expanding growing edge by a still younger zoœcium. A radial series of connate zoœcia thus results, and the roof of the ovicell is divided into two lobes, one on each side of the zoœcial series. After a time the series may become completely free from the growing edge, and the two adjacent lobes of the roof of the ovicell may then unite distally to the series.

In my preliminary note (17, p. 212) I have stated that the zoœcia may thus be left as columns passing freely through the cavity of the ovicell. I believe that this statement is not correct for Tubulipora. The cavity of the ovicell may indeed surround the zoœcium, or series of zoœcia, on the proximal, lateral, and to some extent on the distal sides; but I have found no evidence that two lobes which have become contiguous on the distal side of a zoœcium ever really unite, as

they certainly do in Lichenopora. In transparent preparations it can be seen that the cavities of the two lobes remain separated by a vertical radial septum, the upper edge of which is generally indicated as a line on the roof of the ovicell even in dried colonies (fig. 5.s.).

As the radial series of zoœcia are formed all round the periphery, the ovicell itself consists of a cavity which branches dichotomously at the proximal end of each series. In large, actively growing colonies (fig. 5) the ovicell usually branches in a palmate manner, and the primary lobes may undergo further dichotomy once or more. The extent to which the ovicell branches is not a specific character, but is probably dependent partly on nutrition and partly on temperature. Figs. 2 and 4 represent mature fertile colonies of T. aperta and T. flabellaris respectively, and fig. 1 a fertile lobe of T. plumosa. The ovicell shown in the last figure is much more complicated than that shown in the former two; but it must be distinctly understood that the ovicell of T. flabellaris and T. aperta may be large and much branched in vigorous colonies.

After the ovicell has reached a certain size it develops its tubular portion, ending in the orifice through which the larvæ make their escape. Here, however, there is some modification in the course of the development as compared with an ordinary zoœcium. While the orifice of the latter is merely the part which, for the time being, forms the distal end of the zoœcium, the lobes of the ovicell undergo a considerable amount of growth, in most cases, after the orifice is fully formed. Sooner or later, however, the peripheral ends of the lobes, closed merely by a soft terminal membrane during growth, become completely calcified, except in the abnormal specimens of T. aperta (fig. 2), described under the heading of that species, in which accessory openings may be formed by the outgrowth of the lobes into tubular passages which more or less resemble the real orifice of the ovicell. The complete calcification of the peripheral ends of the lobes of the ovicell takes place, as in other Cyclostomes, by the encroachment of the calcareous

roof on the terminal membrane, which is, so to speak, gradually constricted until it completely disappears.

My study of Tubulipora has fully confirmed the conclusion stated in my paper on Crisia (**13**, p. 128), that the form of the orifice of the ovicell is of great importance for the discrimination of the species. As I am convinced that this structure will become increasingly important in the systematic study of the Cyclostomata, I venture to think that a special terminology may be convenient for descriptive purposes; and I therefore propose to term the passage by which the larvæ escape from the ovicell the "oœciostome," and its actual external orifice the "oœciopore." The oœciostome is usually a tubular or funnel-shaped structure, as in the species of Tubulipora here described, and in many other Cyclostomes. The part connecting the cavity of the ovicell with the oœciopore may be termed the "tube" of the oœciostome, whatever its shape. The tube is not a necessary part of the oœciostome, since it is absent in Crisia aculeata, Hassall, in which the oœciopore represents the entire oœciostome.

The structure of the wall of the tube is commonly different at its two ends. The proximal portion is often pierced by pores identical with those in the roof of the ovicell, of which it is a direct continuation (fig. 1), while the distal portion is imperforate. Valuable specific characters are afforded by the shape, size, and relations of the oœciostome, and by the size and position of its tube and oœciopore.

The constancy of the characters of the oœciostome has been verified by the examination of numerous specimens of several species from various localities. While the shape and size of the entire colony, and therefore of the ovicells, has been shown to be highly variable, the oœciostome retains its character, whatever the condition of the colony. I do not, of course, mean to assert that there is no variation in the oœciostome. Variation does occur, sometimes within fairly wide limits; but it is usually possible to decide the species at once by an inspection of its oœciostome. In order to do this it may be necessary to have a fully developed oœciostome, since the

specific characters are not completely shown in the immature condition of this structure. There are occasional cases in which the oœciostome of a given colony may appear intermediate between two species. In most of these cases comparison with other specimens will, however, usually leave no doubt of the existence of a definite character for the oœciostome of each species, in spite of the occasional occurrence of difficult cases.

The value of the characters of the oœciostome in diagnosing a species is strikingly illustrated by the specimens of T. flabellaris, Fabr., which have come into my hands. Fig. 4 is an Arctic specimen of this species, dredged by Colonel H. W. Feilden on July 1st, 1895, in Barents Sea, and presented by him to the Cambridge Museum of Zoology. Although the colony is very much smaller than the form of this species figured by Smitt (40, pl. ix, fig. 6), it possesses no less than six complete ovicells, each with a fully formed oœciostome (1—6), five of which are visible in the position in which the colony lies, besides one immature ovicell (7). The ovicells are crowded and are very small, exhibiting hardly any of the lobing which may occur in this species as in others. The principal characteristic of the species, the flattened shape of the oœciostome, is nevertheless as well marked in each ovicell as it is in the lobed ovicells of a larger colony, sent to me by Dr. Nordgaard from Hammerfest.

The above case can hardly be regarded as a mere individual abnormality. I have examined four specimens (including the above) from Colonel Feilden's collection, and all of them showed the same peculiarities. Two other colonies (about the same size as the above) had each four small, contiguous ovicells with fully formed oœciostomes, and an immature fifth ovicell. The last consisted (unlike the others) of two diverging lobes, which may, however, have been formed from two larvæ. One lobe had two ovicells with oœciostomes, and at least one immature ovicell, and the other lobe had one ovicell with a fully developed oœciostome. In every ovicell the long flattened oœciostome, which I regard as the distinguishing

feature of this species, was developed on precisely the same type.

A case of the abnormal development of four contiguous ovicells has been described by me (**13**, p. 166, pl. xii, fig. 13) in Crisia ramosa. These cases are interesting as showing the essential similarity of the ovicell and the zoœcium. The production of an ovicell is probably induced by the development of an embryo from one of the eggs which so commonly occur in young zoœcia, most of which do not ordinarily become fertile.

The simple ovicells of the specimens of T. flabellaris from Barents Sea are similar to those which were figured by Savigny (**37**, pl. vi, figs. 4.2 and 5.2), and described by Audouin under the names of Proboscina Boryi, and P. Lamourouxii. Ovicells of a similar type are known in Diastopora suborbicularis, Hincks, and in many fossil forms. They are figured, for example, by Gregory (**12**, pl. i, fig. 6; pl. iii, fig. 3), and this type of ovicell is given by Walford (**41**, p. 73) as a characteristic of his genus Pergensia, although I cannot agree with him in thinking that the character shows any approach to the Cheilostome genera Lekythopora and Pœcilopora. It appears to me probable that the stunted specimens of T. flabellaris above described have reverted to a more primitive condition (a conclusion borne out by the evidence of fossils) in developing a less complicated form of ovicell than is usually produced in other recent species, and even in T. flabellaris itself. This reversion may be regarded as due to the small size of the colony, in conjunction with the successful development of primary embryos in several contiguous zoœcia. It is probable that this points to a time when the ovicells were ordinary zoœcia, and that the restriction in the number of fertile zoœcia now so characteristic of Cyclostomes is not a primitive feature of the group.

References to the oœciostome in any form hitherto described as Tubulipora or Idmonea are curiously rare; and most authors do not seem to have considered the possibility of the occurrence of this essential part of the ovicell. Smitt (**40**)

has described it in Idmonea atlantica (p. 443), in I. serpens [= T. liliacea of the present paper] (p. 446), in T. fimbria [= T. aperta] (p. 455), in T. flabellaris (pp. 456, 457), and in T. lobulata (p. 457). The descriptions there given are not, however, sufficiently precise to be used in discriminating the species, nor are they illustrated by figures which really show their character. Waters (**44**, pp. 256—259, and **43**, p. 339) has described the oœciostome in one or two species of Tubulipora or Idmonea; and Kirkpatrick (**22**, p. 22, pl. iv, figs. 6 *a*, 6 *b*) has described and figured the oœciostome of I. pulcherrima. These are almost the only references I have been able to find to the oœciostome of recent species of Tubulipora. It appears to me that a Tubulipora colony reaches its fully adult condition when the ovicell, with its oœciostome, is fully formed. If this view be correct, the importance of the oœciostome as a specific character is intelligible. Colonies in which this structure is not yet developed are in a state of immaturity. It is perhaps as reasonable to characterise a species of Tubulipora without taking account of its oœciostome as it would be to describe a new species of Cervus without describing the form of the antlers. It is unfortunately impossible to describe the oœciostome of a Cyclostome in the majority of cases, and particularly in fossil specimens; but I think the description of this structure should be an essential part of a diagnosis wherever it can be given.

The absence of the oœciostome in a given case may depend on the season of the year at which the specimen was obtained. It is, for instance, somewhat difficult to find the oœciostomes of the British species in colonies collected in the spring, in which the formation of the ovicell is beginning; but it is easy to find them in uninjured healthy colonies collected in the summer.

The following table illustrates the use which can be made of the oœciostome in distinguishing the species treated of in this paper. The "lower" surface of the colony is that by which it is fixed, so that "upwards" means away from the basal surface:

1 { Zoœcia more or less obviously arranged in connate series, at least in well-developed colonies . . 2
Zoœcia not in connate series, even in the fertile lobes. Oœciostome with a well-developed tube, usually more or less free, the oœciopore being larger than an orifice and looking upwards T. aperta (Pl. 8, fig. 2)

2 { Oœciopore much larger than an orifice, opening upwards or obliquely horizontally T. plumosa, W. Thomps. (fig. 1)
Oœciostome or oœciopore not larger or only slightly larger than an orifice . . . 3

3 { Tube of the oœciostome recumbent on a zoœcium or series of zoœcia, the oœciopore opening horizontally or downwards 4
Tube of the oœciostome free, greatly compressed, the oœciopore being slit-like T. flabellaris, Fabr. (fig. 4)

4 { Oœciopore larger than an orifice, opening horizontally T. liliacea, Pall. (=T. serpens, auctt.) (figs. 7, 8)
Oœciopore concealed, smaller than an orifice, looking downwards T. phalangea, Couch (figs. 5, 6)

The average size (in thousandths of a millimetre = μ) of the oœciopores and of the orifices is stated in the following table, the greater diameter of the oœciostome being added in T. phalangea, the only species in which the oœciopore is narrower than the oœciostome:

	Oœciopores.	Orifices.
T. phalangea . .	120 (oœciostome, 150)	160
T. flabellaris . .	165	175
T. liliacea . . .	260	165
T. aperta . . .	280	170
T. plumosa . . .	355	185

It should be expressly noted that the diameter of the oldest zoœcia of a colony may be much smaller than that of the younger zoœcia. The above averages are taken from zoœcia which have reached their full size.

II. History of the Species and Genus.

The tenth edition of the 'Systema Naturæ' gives the diagnosis of certain species referred to the genus Tubulipora, of which the first or type species is T. musica, the organ-pipe coral. Amongst these (p. 790) occurs T. serpens.

The twelfth edition contains (p. 1271) a diagnosis of T. serpens, as "T. tubulis cylindricis erectis brevissimis distantibus axillaribus, basi repente dichotoma divaricata." This is practically identical with that given in the tenth edition, to which a reference is given, with the further reference 'Amœn. Acad.,' i, p. 105, t. 4, f. 26.

The species is thrown up on the shores of the Baltic, while a similar but smaller form occurs in the Mediterranean, the only locality mentioned in the former edition.

A reference to the first figure which is quoted by Linnæus, namely, to that contained in his 'Amœnitates Academicæ' (vol. i, 1749), leads to the conclusion that the original description did not refer to the species which is now usually known as Idmonea serpens. The figure is on a plate headed "p. 312," and the description is on p. 209 [not 105]. The figure, with which the description agrees, represents a stone bearing a closely adherent species, consisting of an open network of tubes with single pores at considerable intervals, usually at the angles of the meshes. It is hardly possible to recognise any similarity to any species of Tubulipora or Idmonea; but, on the contrary, the figure is strikingly suggestive of the Alcyonarian Sarcodictyon catenatum, Forbes, and closely resembles the figure of that species given by Herdman in the 'Proc. Liverpool Biol. Soc.,' vol. ix, 1895, pl. viii, fig. 2.

Whether the description of Linnæus referred to Sarcodictyon or to an Alecto-like form must be left an open question,[1] but I think that it can have had no connection with any species of Tubulipora or Idmonea.

In 1755 (**9**, p. 74) Ellis described, under the name of the

[1] Milne Edwards (**28**, p. 331) believed that it referred to an "Aulopore."

"small purple Eschara," the form which is now commonly known as Idmonea serpens. The purple colour and the parallel arrangement of the zoœcia are expressly mentioned. The figures *e* and E on pl. xxvii represent the species as growing on a substance which is doubtless the stem of the "sickle coralline" (Hydrallmania falcata), as appears from the description, on p. 75, of the Cellepora shown on the same stem. The occurrence on this species of Hydroid is eminently characteristic of the "small purple Eschara."

In 1766 (**33**, pp. 248, 249) Pallas described the same species under the name of Millepora liliacea, referring to Ellis's description and figures; and it is given as Millepora tubulosa in the well-known work of Ellis and Solander (**10**, p. 136).

In the enlarged thirteenth edition of the 'Systema Naturæ' (1788) Gmelin complicates the question by describing the species under no less than three different names.[1] The first of these (tom. i, part 6, p. 3754) is Tubulipora serpens. Linnæus's diagnosis is repeated, but a reference to former editions of the 'Systema' is omitted, while the small purple Eschara of Ellis, and Millepora liliacea of Pallas are given as synonyms. The second (p. 3790) is Millepora tubulosa, Ell. and Sol., the small purple Eschara of Ellis appearing a second time as a synonym. The third (p. 3790) is Millepora liliacea, Pall., and Tubulipora serpens, Linn., is given as a synonym.

It appears to me that the Linnæan name must be rejected, and, following the ordinary laws of priority, that the choice must lie between Tubulipora liliacea, Pall. (1766), and T. tubulosa, Ell. and Sol. (1788). If the tenth edition of the 'Systema Naturæ' is adopted as the commencement of the binomial system, Pallas's name has the right to be accepted; while the adoption of the twelfth edition as the starting-point would necessitate the employment of T. tubulosa, Ell. and Sol. I shall follow the example of Mr. Hincks[2] in regarding as valid Pallas's

[1] As has already been pointed out by Lamouroux (**25**), p. 66.

[2] See his remarks on Flustra securifrons, Pall., on p. 122 of the 'British Marine Polyzoa' (1880).

names, published in the year before the part of the twelfth edition of the 'Systema Naturæ' which referred to Zoophytes.

I have come to the conclusion, from my study of the development, that it is not possible to separate "Idmonea" liliacea generically from the British forms recognised as "Tubulipora," although I claim no novelty in that conclusion. It thus becomes necessary to consider whether the genus which includes the two species should be called Idmonea or Tubulipora.

The genus Tubulipora was founded by Lamarck (**23**) in 1816, while Idmonea is due to Lamouroux (**25**), and dates from 1821. Lamarck's type-species is T. transversa, said to be found on Fucus in the Mediterranean. The small purple Eschara of Ellis, and Millepora tubulosa of Ellis and Solander, are given as synonyms, and from this, with the diagnosis, it might be concluded that T. liliacea, Pall., is the type-species of the genus Tubulipora. H. Milne Edwards has, however, figured (**30**, pl. ix, figs. 3 and 3*a*) a specimen from the Paris Museum with the statement (p. 218, note) that it is the one from which Lamarck's description was taken. He regarded the species as an Idmonea (**29**, p. 332), a course which is hardly justifiable considering that it was the type-species of the earlier genus Tubulipora. If, then, we are to accept Milne Edwards' figures as a correct representation of Lamarck's species, Idmonea becomes, on his showing, a synonym of Tubulipora.

If a generic distinction between Tubulipora and Idmonea, in the ordinarily understood sense, can really be maintained, this is a regrettable conclusion, since it results in the substitution of Tubulipora for Idmonea, and would necessitate the use of some other generic name for the species usually understood to belong to Tubulipora. If Lamarck's type-specimen is still in existence, and the evidence that it is the type-specimen is satisfactory, I suppose there is no option but to regard the synonyms which he himself gave for T. transversa as erroneous. But as the evidence is perhaps not quite certain, and as, moreover, it is not clear that any

generic difference between Tubulipora and Idmonea can be maintained, I shall regard the species described in this paper as members of the genus Tubulipora.

The type-species of Lamouroux' genus is Idmonea triquetra, a fossil form from the "terrain à polypiers" (Bathonian) of Caen. The description might lead to the inference that the species is erect, but Gregory (**12**, p. 134) states that it is always an encrusting form, and has justly remarked that it is therefore impossible to define Idmonea as consisting only of erect species. If this is so, it becomes very difficult to draw any line between Tubulipora and Idmonea. Dr. Gregory's catalogue includes no species of Tubulipora, but he distinguishes (p. 134) a family Idmoniidæ from the Tubuliporidæ mainly by the existence of regular transverse rows of zoœcia in the former. I do not think that this distinction can be maintained, either as the character of a family or even of a genus. Lamarck's type-species of Tubulipora was defined as having its zoœcia in transverse series, and this feature is strongly marked in other recent species which are ordinarily included in that genus. The only character in Dr. Gregory's diagnosis of Idmonea (p. 134) which is not applicable to many species of Tubulipora is the ridged or triangular cross-section of the branches, and it is very doubtful if this is really a valid generic difference.

From a superficial examination of the ovicells of Idmonea atlantica, and from a consideration of Smitt's description and figures (**40**, p. 443, pl. iv, figs. 5, 7), it appears to me that this form at least is closely allied to T. liliacea.

III. Synonymy, Diagnoses, and Accounts of the Species.

The material on which the following account was based was collected mainly in the Salcombe Estuary, in South Devon, in March and April. I have to thank Dr. A. M. Norman for having recommended me to choose that place as a base of operations. Other specimens were collected by myself while working at the Plymouth Laboratory and on other parts of

the English coast, and in Norway. I have to express my great indebtedness to my friends who have kindly given or lent me specimens from other localities; and particularly to Professor Herdman and Miss Thornely for specimens from the Liverpool district; to Professor M'Intosh for material from Scotland; to Dr. O. Nordgaard for specimens from Norway; and to Dr. F. M. Turner for material from Guernsey. I must also express my obligation to Mr. A. H. Church for determining one or two seaweeds on which my specimens were found, and for some observations on the amount of annual growth of the colony; and to Mr. S. D. Scott for some observations on the excretory vesicles.

Tubulipora, Lamarck.

Zoarium with a distinct basal lamina, adnate or erect, beginning as a pyriform or flabelliform colony, which may become lobed by the division of the terminal membrane. Lobes short and adherent, or longer and dichotomously divided once or more often, sometimes becoming erect. Zoœcia with a free, cylindrical, terminal portion; or connate in obliquely transverse series, in which they are separated by flat septa corresponding with the intersection of two cylindrical zoœcia. The series are arranged alternately on opposite sides of the axial line of the lobe, but the transverse arrangement usually becomes radial in the distal part of the fertile lobes. Ovicell an enlarged zoœcium, which extends into the intervals between the parallel or radial series.

The number of the tentacles is usually eleven or twelve in the three species I have studied by means of sections. Of these, T. phalangea and T. plumosa seem to have eleven tentacles in most cases, Milne Edwards (**30**, p. 195, note), giving the number as twelve for the former. In T. liliacea I have counted twelve tentacles in most cases, a number agreeing with Dalyell's statement (**6**, p. 86); but one polypide had thirteen, and several had eleven.

T. liliacea, Pallas (figs. 7—9).

Tubulipora and Idmonea serpens, auctt. (not Tubipora serpens, Linn. [**27**, p. 1271], nor Fabr. [**11**, p. 428]).

Small purple Eschara, Ellis (**9**, p. 74, pl. xxvii, figs. *c*, E).

Millepora liliacea, Pallas (**33**, p. 248).

Millepora tubulosa, Ell. and Sol. (**10**, p. 136).

Millepora tubulosa and M. liliacea, Linn. Gmel. (**28**, p. 3790).

Tubipora serpens, Dalyell (**6**, p. 85, pl. xviii, figs. 11—15).

Tubulipora serpens, Johnst. (**19**, pl. xxxi, figs. 4—6; and **20**, pl. xlvii, figs. 4—6). Couch (**5**, pp. 105 [part], 106). Smitt (**40**, pp. 399, 444, [part], pl. iii, figs. 4*a*—4*c*, 5*a*, 5*b*; pl. ix, figs. 1, 2*a*, 2*b*). Busk (**2**, pp. 25, 26 [part], pl. xxii, figs. 1—3).

Idmonea serpens, Hincks (**18**, p. 453, pl. lxi, figs. 2, 3). Levinsen (**26**, p. 76, pl. vii, figs. 6—10).

Zoarium adnate or erect, its form being greatly influenced by the substance on which it is growing; commonly dividing several times dichotomously. Zoœcia curved for the most part in one plane, with the serially connate, alternate arrangement strongly marked, though sometimes obscured in small or irregular colonies. In well-branched colonies the inner zoœcia are much longer than the outer ones, so that the height of the transverse series diminishes greatly in passing from the inner to the outer side. Ectocyst usually vitreous and hyaline. Oœciostome about 260 μ in diameter, slightly larger than the orifice of a zoœcium, opening horizontally.

Common on Hydroids (especially Hydrallmania falcata), from 20 to 40 fathoms; but also found on Cellaria from the same depth, and on red seaweeds from shallower water.

This is the "small purple Eschara" of Ellis, and the Tubulipora or Idmonea serpens of most writers. I have explained on p. 86 the reasons for rejecting the familiar specific name.

The distinctive feature of this species is the form of the oœciostome (figs. 7 and 8). In size it is intermediate between the corresponding structure of T. plumosa and that of T. phalangea, and is somewhat larger than the orifice of an ordinary zoœcium. The difference between the oœciostome of T. liliacea and that of T. phalangea (figs. 5 and 6) is not always apparent at first sight, the oœciopore being often concealed in both cases. But whereas in the latter species it is seldom possible to see the oœciopore in any position in which the uninjured colony may be placed, it is nearly always

possible in T. liliacea to see it by inclining the colony in a suitable direction. The oœciopore typically opens horizontally; or, in other words, its plane is vertical to the upper or exposed surface of the ovicell. The edge of the oœciopore may be slightly everted, so as to form a narrow brim, or there may be no eversion; and the upper lip may be horizontal and more prolonged than the lower lip.

In all cases the oœciopore is relatively large, varying from 230 μ to 270 μ, that of an ordinary zoœcium being 145 μ to 190 μ. When the structure is placed in a suitable position it is possible to see some way down the tube of the oœciostome (fig. 7). This cannot be done in T. phalangea.

The tube is moderately long, and is recumbent on a zoœcium or series of zoœcia. It may occur on the proximal side of the series, and look towards the oldest part of the colony, or it may be placed on the distal side and look towards the growing edge.

T. liliacea is very common on certain Hydroids, particularly on Hydrallmania falcata, a fact familiar to many of the older naturalists, and on Sertularella. It is easily obtained from the masses of Hydroids brought up by trawlers in water of twenty fathoms or more. The form of the entire colony varies a good deal. It may remain closely attached to the narrow stem of the Hydroid, its basal lamina curving round the stem and thus giving rise to very irregular colonies; or it may remain attached merely by a small central area, and grow out into free, erect branches, as in the var. radiata of Hincks. In this condition it assumes a typical Idmonea-form, having a very strongly marked alternate arrangement of connate plates of zoœcia.

In a particularly fine specimen of this form, from the Trondhjem Fjord, which I owe to the kindness of Dr. Nordgaard, a single series consists in some cases of as many as eight zoœcia, the inner ones being very much taller than the outer ones. The tip of the longest branch is about 11 mm. from the centre of the colony. The oœciostome belonging to this branch is on the distal side of the seventh series (of one

side of the branch) from the bifurcation preceding the ovicell; whereas in another branch the oœciostome is on the proximal side of the eighth series of one side, the ovicell itself beginning immediately after the fifth series.

The ovicells of this colony extend through a region of four or five transverse series of zoœcia on each side of the fertile lobe. The shape of the ovicell is of course affected by the strongly marked alternate arrangement of the series, and its roof is thus a comparatively narrow, curvedly zigzag band, running along the middle of the lobe, and giving off an interserial lobe on the convex side of each bend. The ovicell may thus be described as consisting of a regularly undulating axis, with an alternately pinnate arrangement of simple lobes extending between the series of zoœcia. The ends of the branches are bifurcated, but the same ovicell extends into both halves of the fork by division of its main axis. The same arrangement is figured by Smitt (**40**, pl. iv, figs. 5 and 7) in Idmonea atlantica.

A great contrast to this Idmonea-like colony was afforded by a fine specimen from the Liverpool district kindly lent to me by Professor Herdman. A narrow branch suddenly expanded into a nearly semicircular fertile lobe, 6 mm. in transverse diameter. The zoœcia in this lobe had a Tubulipora-like arrangement, consisting of radial series, which showed a distinct tendency to become frayed out into separate zoœcia at their upper borders. The oœciostome had the typical form, and other colonies from the same locality were in no way different from the more ordinary type of T. liliacea.

I think there can be no doubt that the form of oœciostome which I have described is quite characteristic of this species. Although I first noticed it in a number of specimens from the Plymouth district, it is not a local peculiarity, since I have found precisely the same form in the specimens which have just been alluded to from Trondhjem and Liverpool, as well as in a series of colonies from Hydroids dredged in St. Andrews Bay (20 to 27 fathoms), kindly given to me by Professor M'Intosh. The variations in the size of the oœciopore

are indicated by the following list of measurements of the transverse diameter:

Plymouth (on red seaweed)	220 μ.
St. Andrews (on Hydroid)	230 μ.
Plymouth (on Hydroid)	260 μ.
Trondhjem	265 μ.
Plymouth (on Hydroid)	280 μ
Liverpool (probably on Hydroid)	300 μ.

The average of this series of measurements is 260 μ.

T. phalangea, Couch (figs. 5, 6).

Tubulipora phalangea, Couch (**5**, p. 106, pl. xix, fig. 7 [figure bad]). Johnston (**20**, p. 273 [part], pl. xlvi, figs. 1—4). Busk (**2**, p. 25, pl. xxiii, fig. 2).

Tubulipora flabellaris, Hincks (**18**, p. 446, pl. lxiv, figs. 1—3).

Tubulipora verrucaria and T. verrucosa, Milne Edwards (**29**, pp. 337, 328, 323, pl. xii, fig. 1).

Zoarium entirely adnate, variously lobed, sometimes consisting of a series of divaricated lobes, sometimes almost circular in outline, and then reaching a maximum diameter of at least 15 mm. In stunted specimens the terminal membrane may not divide, but gives rise to a single small fertile lobe, the whole colony being pear-shaped. Zoœcia serially connate, the series alternate near the base of elongated branches, but becoming radial in fertile lobes. The series are commonly resolved above into their component elements, the zoœcia having a longer or shorter free cylindrical portion; but they may remain entirely connate to their ends. Zoœcia narrow and long compared with those of most other species. Oœciostome (fig. 5) about as large, at its widest point, as an orifice, averaging 150 μ in diameter, the tube bent completely round, so that the oœciopore (fig. 6), which averages only 120 μ in its longer diameter, looks down on to the roof of the ovicell, and can rarely be seen without dissection of the colony. The tube of the oœciostome is adnate to a series of zoœcia, and its upper exposed surface is convex. The primary zoœcium diverges from the plane of attachment to a greater extent than in most species, and the proximal part of the colony is usually rather deep and narrow.

Common (in Devonshire) on red seaweeds, shells, and stones from about three fathoms to moderately deep water. I have seen one specimen from the Outer Hebrides.

The reasons for maintaining that this species is distinct

from T. flabellaris, Fabr., are given below, under the account of that species.

The oœciostome of T. phalangea is shown in figs. 5, 6. The convex upper surface is really the outer part of the wall of the tube, which has the form of a ∩, one limb of which rises vertically from the roof of the ovicell, the other being shortened and opening downwards. It results from this arrangement that it is quite impossible to see the adult oœciopore in the great majority of cases without breaking off the series of zoœcia which bears the tube, and turning it over until the oœciopore becomes visible. It is then seen to have the form shown in fig. 6, being conspicuously smaller than that of T. liliacea. The diameter of the oœciopore varied from 110 to 135 μ in five specimens measured (average 118 μ), the widest part of the entire tube varying from 110 to 180 μ (average 139 μ) in the same specimens. The oœciostome may be quite symmetrical, or it may be distorted so that the oœciopore looks obliquely downwards. When the oœciostome is typically developed (as in the great majority of cases), it differs to a striking extent from that of any other species described in this paper.

In its typically developed form this species is distinguishable by its very long and slender zoœcia, the ends of which are more commonly dissociated from their neighbours (and are therefore completely cylindrical) than in T. plumosa, with which it commonly occurs. The character of the oldest zoœcia is of some value in distinguishing the species. While T. plumosa is usually depressed in the oldest part of the colony, this species has rather the opposite tendency, and the primary zoœcium usually grows upwards at a considerable angle from the plane of support, the interval being occupied by the proximal ends of the next zoœcia.

The general characters are well described by Couch (**5**, pp. 106, 107), though his fig. 7, pl. xix (wrongly given as fig. 8 in the text), is excessively bad. H. Milne Edwards (**29**, pl. xii, figs. 1, 1*b*, &c.) gives excellent figures of this species, accompanied by some anatomical details (pp. 323, &c.), under the

name of T. verrucaria, Fabr., accidentally given as T. verrucosa in one place on p. 328. The name employed by Milne Edwards cannot be retained, since the Madrepora verrucaria of Fabricius is a Lichenopora. From an inspection of the original description and figures I can see no sufficient reason for believing that the fossil Diastopora plumula, Reuss, is identical with the present species or with T. flabellaris, Fabr., although Pergens (**34**, p. 9) considers the specific name given by Reuss to be the correct name of one of the forms to which the name T. flabellaris has been ascribed.

T. phalangea is common in the Salcombe Estuary, at a depth of 3 to 5 fathoms, on red seaweeds, where it occurs in company with T. plumosa, and on dead shells. It is equally common at Plymouth from 3 to 15 fathoms; and I believe that the greater number of specimens of Tubulipora found on shells in the shallower parts of the Plymouth district belong to this species. In the deeper water (20 to 30 fathoms) a considerable proportion of the specimens may belong to the form identified by Mr. Hincks as T. lobulata, Hassall; but I am at present unable to express any positive opinion with regard to Hassall's species.

I have seen a typical specimen of T. phalangea, kindly lent to me by Professor M'Intosh, from the Outer Hebrides; but the rest of my material has been obtained from Devonshire. T. phalangea is very variable in the form assumed by the colony. It may consist merely of a single small, fertile lobe, the whole colony being then pear-shaped, and closely resembling the Obelia tubulifera of Lamouroux (**25**, p. 81, pl. lxxx, figs. 7, 8), a Mediterranean form with which it may be identical. It may consist of a small number of well-separated lobes, or it may have an almost completely circular outline. Colonies of the last type may reach a diameter of nearly an inch. The complexity of the ovicell varies greatly with the size of the colony, the large colonies having very complicated ovicells with numerous palmately arranged lobes extending between the radial series of zoœcia; fig. 5 being by no means

an extreme case of this kind. The smaller colonies have simpler ovicells, but this is also the case in other species.

My Salcombe specimens were dredged in March and April; and an examination of this material gives some hints with regard to the meaning of the differences in size. A very large number of colonies found on shells were small and pyriform, although in many cases possessing a mature ovicell; while other small colonies consisted of only two or three lobes. These colonies were nearly all brown, and more or less encrusted with foreign matter. Here and there an old lobe had recommenced to grow, and had given rise to a fresh and clean lobe, whose brilliant white colour (in spirit specimens) forms the most striking contrast with the older lobes. The colonies of Tubulipora have in fact the power, which is probably common to all Cyclostomes, of regenerating new zoœcia from various parts of the old colony (cf. **13**, p. 141). In the species now under consideration a small part of the edge of the old colony here and there becomes active, so that a series of fresh lobes, with narrow bases, may be seen growing out from various parts. These lobes have in many cases acquired a considerable size by the beginning of April, and have developed mature ovicells. A few quite young, healthy colonies were found amongst these specimens. It is probable that these last were colonies which had recently commenced their existence, that the brown specimens belonged to a previous year, and that the fresh lobes proceeding from them were entirely recent growths. If this is a correct inference, it may be suggested that the small brown colonies were produced late in the year when the temperature was becoming low, so that although they became mature so far as the external characters of their ovicells were concerned, they were unable to grow large. There was of course no doubt that the finely developed specimens were actively growing when they were dredged.

I think it probable, therefore, that the difference in the development of the entire colony may, in some cases at least, be of a seasonal nature.

I may here refer to some interesting remarks which have

been made to me by Mr. A. H. Church, whom I had consulted on the growth of Tubulipora. Mr. Church informs me that Rhodymenia ciliata, a red seaweed on which I obtained nearly all the material (T. phalangea and T. plumosa) which I have used for sections, is an annual. It follows, therefore, that even the largest colonies (some 12—15 mm.) found on this seaweed must represent the growth of one year. Mr. Church informs me that R. ciliata usually dies in the winter, the middle of which period may be regarded (for Algæ) as February, but that the specimens I dredged (in a sheltered estuary) at the beginning of April must have grown in the preceding year. I have not noticed processes of regeneration in colonies growing on seaweeds, the evidence from which is not entirely concordant with that from shells. The specimens growing on Rhodymenia (and the same is true of specimens of T. plumosa collected at the same season on Saccorhiza bulbosa) show no apparent discontinuity of growth, nor were any stunted mature colonies observed in this situation. The colonies were probably far too large to have grown in the year in which they were collected; and although the growth may have been less active or dormant during the winter, there was no interruption sufficient to give rise to a marked discontinuity, as in the specimens growing on shells. It may, however, be noticed that there is no reason for assuming that these latter were the growth of the year immediately preceding. They may be evidence of unfavorable conditions more than one year before, in which case the absence of similar colonies on the Rhodymenia growing in the same locality would be due to the fact that this plant is an annual. I think it follows from the observed facts that growth may start at any time when the species is breeding, and that a colony which begins existence in the summer continues to live through the winter, and produces ovicells in the spring.

Mr. Church informs me that in some cases of regeneration which he has observed (on a glass bottle) the central part of the colony had decayed, leaving the new growths with a vacant

space in the centre. I have had no opportunity of examining cases of this kind.

Hincks (**18**, p. 447) has referred to a curious lobed Tubulipora, about an inch in diameter, which he has met with in Salcombe Bay. I have obtained some specimens which appear to correspond with Mr. Hincks's description. One or two of these colonies were from the Salcombe Estuary, and had the oœciostomes of T. phalangea. Two others were sent to me from Plymouth by Mr. Church, and had the oœciostomes of T. plumosa. The variety is a very curious one, and is characterised by its nearly circular outline and by the great crowding of the zoœcia, the series being placed very close together, and the lobes of the well-branched ovicells being correspondingly narrow. On closer examination a considerable difference (no doubt specific) between the Plymouth and the Salcombe specimens becomes apparent. A perfect colony of the former measures about 15 mm. in diameter; it is composed of twelve well-marked lobes, and closely resembles the form of T. plumosa which is found on Saccorhiza. These lobes can be readily made out without any magnification, whereas the Salcombe specimens do not appear obviously lobed when examined with the naked eye.

I regard this variety as due to an excessive growth of the edge of the colony, resulting, by the mutual pressure of the lobes, in a crowding of the zoœcia, and in the acquirement of a circular form. This appears to me to be a further instance of the tendency of different species of Tubulipora to assume the same general form as the result of some unknown factors in the environment.

T. flabellaris, Fabricius (fig. 4, described on p. 82).

Tubipora flabellaris, Fabr. (**11**, p. 430).

Tubulipora flabellaris, Smitt (**40**, p. 401, pl. ix, figs. 6—8).

? T. flabellaris, Levinsen (**26**, p. 76, pl. vii, figs. 1—3).

Zoarium entirely adnate, more or less fan-shaped in form in well-developed specimens. Stunted colonies may occur, as in the preceding species. Some of the zoœcia are free, others are in connate series, which are more or less

developed, and become radial in fertile lobes. Oœciostome consisting of a greatly compressed tube, whose oœciopore is slit-like. The longer diameter of the slit averages about 165 μ (130—180 μ), being equal to or somewhat less than the diameter of an orifice. The tube is not recumbent on a series of zoœcia, but stands up freely from the roof of the ovicell, its two narrow edges being placed in a radius of the colony.

This seems to be an essentially Northern species, and I have no evidence of its occurrence in British waters. I have examined specimens from Greenland (the locality from which the type-specimens came), Barents Sea (50 fathoms, growing on Cellularia peachii), and Hammerfest; and I have also obtained what I believe to be a young form of this species from Godösund, Björne Fjord, Norway. The specimen figured (fig. 4) is a stunted, somewhat abnormal form of this species from Barents Sea (see the description on p. 82), but it well illustrates its characteristics by showing five perfectly typical oœciostomes.

Professor Smitt (**40**, pp. 400—402, 454, 458), who is followed in this respect by Mr. Hincks, regards T. phalangea of Johnston as identical with T. flabellaris, although he believes the original T. phalangea of Couch to be identical with T. lobulata, Hassall. It appears to me that the characters of the oœciostome are amply sufficient to separate T. phalangea from T. flabellaris. I first became acquainted with the oœciostome of the latter in two colonies from Hammerfest, kindly sent to me by Dr. Nordgaard. The collection of Polyzoa given to the University Museum of Zoology by Miss E. C. Jelly contained a moderate number of specimens on seaweed from Greenland. The form and size of the oœciostome were identical in these and in the Hammerfest specimens. The colonies from Greenland, although very small and stunted (one of them, with only twenty-seven zoœcia, possessing a complete ovicell), did not resemble the Barents Sea specimens from deeper water in possessing a multiplicity of ovicells.

It can hardly be doubted that the Greenland specimens, sent by Miss Jelly, belong to Fabricius' species. The flabelliform shape, the connate series of zoœcia, the occurrence on seaweed, and the small size (given by Fabricius as 1½ lin. in transverse diameter), all agree closely with the original description. The species has been more fully described by Smitt, who gives excellent figures. In two of these (**40**, pl. ix, figs. 6, 8), re-

presenting specimens from Spitzbergen, the peculiar form of the flattened oœciostomes, with their radially arranged flat sides, is indicated, while the flattened form is expressly mentioned on p. 457. I have not seen specimens so finely developed as that shown by Smitt in his fig. 6, in which the radial serial arrangement of the zoœcia is strongly marked (as many as twenty being stated to occur in one series). The difference between Smitt's specimens and those examined by me may, however, be seasonal, as suggested under the last species.

It may be remarked that Smitt's conclusion that T. phalangea is a synonym, partly of T. lobulata, Hass., and partly of T. flabellaris, Fabr., does not appear to have been based on an examination of actual specimens of the first-named species.

Tubulipora aperta, n. sp. (figs. 2, 3).

Tubulipora fimbria, Smitt (**40**, pp. 401, 452, pl. ix, fig. 5).

? Tubulipora fimbria, Levinsen (**26**, p. 75, pl. vi, figs. 45—50).

Zoarium entirely adnate, pyriform, flabelliform, or lobed. Zoœcia not serially connate, or only exceptionally united in very short series. Ectocyst with few pores. Oœciostome about 280 μ in diameter, larger than an orifice, more or less funnel-shaped; the oœciopore opens upwards, and is circular or oval. Tube of the oœciostome usually more or less free, and diverging from the zoœcium on which its base is recumbent, the edge of the oœciopore often resting on the wall of another zoœcium. Accessory openings sometimes present at the ends of the lobes of the ovicell.

Common on the fronds of Laminaria saccharina in Norway. My largest colony is 5·25 mm. in transverse diameter.

This species, which I believe to have hitherto received no distinctive specific name, has been described and figured by Smitt under the name of T. fimbria. This name, applied by Hincks to T. plumosa, Thomps., was given by Lamarck to an immature specimen of Tubulipora, of which the locality is not recorded, and I give my reasons for not accepting it on page 107. The name aperta is suggested in reference to the wide oœciopore, which is usually clearly visible from above,

and is not concealed either by the zoœcia or by other parts of the oœciostome. The specimens on which my account of this species is based were found principally at Godösund, a small island off the north of Tysnäsö, at the entrance to the Björne Fjord in Norway. They were not uncommon on the fronds of Laminaria saccharina, where they occurred in company with Lichenopora verrucaria, Fabr. Most of the specimens collected at the end of June had fully developed ovicells. Specimens collected at Lervik, in the Hardanger Fjord, at the same period in a previous year were, however, not provided with ovicells. Smitt describes the species as occurring on Laminariæ and other Algæ from the Gullmar Fjord (in the south of Sweden) to Spitzbergen, so that the species may fairly be regarded as a Northern one.

I think there can be no doubt of the specific distinctness of this very beautiful form, which is more easily recognised, at all stages of its growth, than are most of the European species of Tubulipora. It differs strikingly from the other species here described in having its zoœcia isolated, or only associated two or three together. The connate arrangement found in other forms is usually completely absent; and even in the fertile lobes the zoœcia stand up for the most part singly from the roof of the ovicell, the lobes of which may thus unite distally to the zoœcium. Even in these cases the suture or septum between the two ovicell-lobes is easily seen near the growing edge, and I have no reason to suppose that contiguous lobes really fuse at any time.

The zoœcia are more clearly marked off from one another in the basal part of the colony than is the case in most species, the proximal part of a zoœcium being very convex as far as the suture where it joins another zoœcium. In other species parts of the zoœcia may form more or less extensive flat surfaces, as is well seen in the connate series or in the basal adherent parts of the colony. The ectocyst of T. aperta has a delicate appearance, and there are noticeably fewer pores on the zoœcia than in most other species. Numerous concentric lines of growth, passing transversely across the zoœcia, are clearly

marked, as Smitt points out. The zoœcia are relatively large, averaging about 175 μ in diameter.

The oœciostome more nearly resembles that of T. plumosa than that of the other species here described, but it differs from it in the fact that the basal region of the tube, which is perforated by pores, is commonly partially free (fig. 3), whereas in T. plumosa, as in T. phalangea and T. liliacea, the porous part is wholly adnate to a zoœcium, and only the non-porous termination of the tube is free. The oœciopore typically opens upwards, or away from the basal surface of the colony. The proximal part of the tube is attached to a zoœcium, and is followed by a considerable free length of tube (part of which is porous), which dilates upwards, so as to be more or less funnel-shaped. This funnel-shaped portion commonly grows away from the first zoœcium and towards the free part of a second zoœcium, on which its edge rests. In T. plumosa the whole of the tube is recumbent on the same zoœcium or zoœcia. In some specimens of T. aperta, and especially when the tube is wedged in between two zoœcia which are near one another, the arrangement above described is not so obvious. The oœcio-pore is circular or oval, and is of considerable size, its longer diameter usually varying from 200 μ to 370 μ, and averaging about 280 μ. One abnormally small oœciopore was only 135 μ, and one unusually large one was 435 μ, but the usual range is given by the figures first stated. The edge of the oœciopore may be slightly everted, or evenly circular or oval. In some cases it is somewhat indented on one or both sides, and may then closely resemble that of T. plumosa.

In one or two colonies the proximal part of the exposed portion of the ovicell had the characters of an ordinary zoœ-cium, the pores being very few and widely scattered. At the level where the ovicell commences to enlarge, in these cases, the pores become suddenly numerous, and the zoœcium takes on the character of an ovicell. The line where the pores become more numerous probably corresponds with a time at which the embryonic structures reached a certain stage of development. The same peculiarity in the ovicell was also

noticed in some of the proximal ovicells of the specimens of T. flabellaris from Barents Sea described on p. 82.

I have observed one or two interesting variations in this species. In two or three colonies, zoœcia of double the normal width occur, with an orifice measuring as much as 380 μ in its major axis, but having the normal diameter in its minor axis. In one of these cases a groove down the large zoœcium indicated that its size was due to the absence of the septum which should normally have divided it into two zoœcia. A similar large zoœcium was found in a colony of T. flabellaris from Greenland. I have not seen more than one of these giant-zoœcia in a single colony, and they do not occur at all in most cases. It is not impossible that they may be zoœcia which made an abortive attempt to develop into ovicells.

In one instance an oœciostome was recumbent on a giant zoœcium (cf. the remarks given in Waters' paper, **45**, p. 277), but there was no similar relation in the other cases. Walford (**42**, p. 80) has characterised his genus Cisternifera (said to be Cheilostomatous) by the occurrence of giant-zoœcia in the colony, and has in the same place (p. 79, pl. v, figs. 14, 15) described similar giant-zoœcia or "cistern-cells" in "so-called Diastoporæ" from the Great Oolite. These latter, at any rate, appear to me to correspond with the large zoœcia of T. aperta and T. flabellaris.

A more interesting variation was noticed in two colonies in which an ovicell had developed accessory oœciostomes (fig. 2). Since the ovicell of Tubulipora is an enlarged zoœcium, its oœciopore is homologous with a normal orifice, and it follows that an ovicell should have only one oœciostome. This is actually the case in most species, and in most colonies which I have seen of T. aperta. When the growth of the ovicell is completed, each of the lobes other than that ending in the oœciostome normally becomes closed by a porous calcareous film. In the abnormal colonies of T. aperta, however, one or more of these lobes has not closed completely, but has grown at its distal end into a tube, which may completely simulate

the normal oœciostome, which can be seen in the more proximal part of the ovicell.

One of these cases is a small pyriform colony (fig. 2), beginning with the usual primitive disc, which measures 185 μ in diameter. One ovicell is present, with a normal oœciostome, its tube (fig. 3) being long and to a large extent free, and the oœciopore measuring 265 μ. To the apparent right of the oœciostome one of the lobes of the ovicell ends in an accessory oœciostome (fig. 2.1), 105 μ in diameter; while on the other side three lobes have accessory oœciostomes. Of these, No. 2 measures 165 μ; No. 3 is not yet fully formed; and No. 5 is 90 μ in diameter. A smaller tube (4), at the end of a very small ovicell-lobe, had closed except for a minute terminal pore.

A second case is that of a portion of a larger colony. The fragment is rather more than 2 mm. in transverse diameter, and has five ovicell-lobes, each of which has an accessory oœciostome, a normal one being present more proximally. One of these closely resembles the normal oœciostome, while another has about the same diameter at its base, but then becomes much constricted, finally opening by an orifice much smaller than a normal orifice.

I have not had time to examine sections of this species, but it appears to me highly probable that the accessory oœciostomes are functional in providing a means of escape for the larvæ which find their way into the lobes to which they respectively belong.

Tubulipora plumosa,[1] W. Thompson (fig. 1).

Tubulipora plumosa, W. Thompson, in Johnston (**20**, p. 274 [immature]).

Tubulipora phalangea (part), Johnston (**20**, p. 273, especially the statements given on the authority of Mr. Peach).

[1] Thompson's name first appeared in 1847. In the same year Reuss ("Foss. Polyparien d. Wiener Tertiärbeckens," in Haidinger's 'Naturwiss. Abhandl.,' Band ii, Abth. 1, 1848, p. 39) described Defrancia pluma and (p. 51) Diastopora plumula. The former, at any rate, is doubtless a Tubulipora, and is given as a member of this genus by Manzoni ('Denkschr.

Tubulipora flabellaris, Johnston (**20**, p. 274, pl. xlvi, figs. 5, 6 [immature]). Busk (**2**, p. 25 [part], pl. xxiv, figs. 1—3; pl. xxv, fig. 2).

? Tubulipora flabellaris, Busk (**3**, p. 23, pl. v, figs. 1, 1*a*—1*c*).

The locality given in the text is Station 315 [Falkland Islands], and the specimens figured presumably came from that locality. On this account I regard the determination of the species as uncertain, in the absence of any description of the oœciostome.

Tubulipora fimbria, Hincks (**18**, p. 448, pl. lx, figs. 3, 3*a* [immature]).

Zoarium completely adnate, variously lobed, reaching a diameter of an inch in the finest specimens. The serial, connate arrangement of the zoœcia is strongly marked, the series becoming radial in fertile lobes. Zoœcia large. Oœciopore much larger than an ordinary orifice, averaging about 355 μ in its greatest diameter. The tube is funnel-shaped, and the oœciopore opens directly or obliquely upwards, one of its edges being usually somewhat indented. Tentacles containing (homogeneous) excretory vesicles (see p. 113).

Common in the cavities and on the outside of the remarkable nodular rooting bulbs of Laminaria (Saccorhiza) bulbosa, where it reaches its greatest size, and on red seaweeds from shallow water.

I have examined large numbers of specimens of this species from Salcombe, Devonshire, and from Plymouth. I have also obtained typical specimens from Guernsey and from Roscoff.

This well-marked species appears frequently in the literature of the subject in its immature form, in which condition it has been supposed to be adult. Its real adult condition has been presumably mistaken for T. liliacea (T. serpens, auctt.) by most writers; but it is clearly alluded to by Johnston (**20**, p. 273), who quotes Mr. Peach as the authority for stating that it luxuriates in the bulb of Laminaria bulbosa, and that it reaches the diameter of nearly an inch. It is there given as a form of T. phalangea, while on the next page Johnston describes the species in its young state as T. flabellaris.

These specimens were sent to Johnston as T. plumosa by

K. Acad. Wien,' xxxviii, Abth. 2, 1878, p. 20, sep. copy); it has, moreover, a considerable resemblance to Thompson's species. The specific names pluma, plumula, and plumosa are similar in meaning, but they are sufficiently distinct in sound. I think there is no practical inconvenience in reviving Thompson's name, and in a group where the synonymy is so involved it is advisable to retain any name that can possibly be used when it is moderately certain what was originally meant by it.

Mr. W. Thompson, whose name I have adopted. The description and figures given by Johnston leave no doubt in my mind that Thompson's T. plumosa is the form of which I have given a diagnosis above. The flat central region of the colony, which alone is developed in Johnston's figs. 5 and 6 (pl. xlvi), is eminently characteristic of the present species. This form is indeed not invariably assumed by it, but is very distinctive when it does occur. In this condition the zoœcia are often strongly ridged transversely, in the manner described in Johnston's work, and the serial arrangement of the zoœcia is hardly apparent unless the colony is looked at from its distal edge. The shortness of the tubes alluded to by Johnston is of course due to the fact that they are immature.

Specimens with this depressed central region are usually easy to distinguish from T. phalangea, with which the species is associated on red seaweeds. The radial ridges formed by the more projecting zoœcia are the commencements of the series of zoœcia. As the colony increases in diameter, the freshly added parts of these projecting zoœcia project more and more, younger zoœcia are developed connately with them on their lower side, and the serial arrangement becomes as strongly marked as in any other species of Tubulipora.

T. plumosa is described by Hincks (**18**, p. 448, pl. lx, figs. 3 and 3*a*) from immature specimens as T. fimbria, Lamk. Fig. 3 of this author shows the beginning of an ovicell on the right side, although the septa between the ovicell and the contiguous zoœcia are not all indicated. Mr. Hincks expresses a doubt as to the identity of this species with T. fimbria, Lamk., but accepts the name with some hesitation on Smitt's authority. Smitt's T. fimbria is, however, in my opinion, a different species, and it is here described as T. aperta. The figure of T. "fimbria," given by Milne Edwards (**29**, pl. xiv, fig. 2) from a specimen labelled by Lamarck, does not specially resemble T. plumosa.

The distinctive feature of T. plumosa is the oœciostome (fig. 1), which is a wide, funnel-shaped structure, looking up-

wards or obliquely upwards, the oœciopore being conspicuously larger than an orifice. Its tube is recumbent on a series of zoœcia, and does not often become completely free, as is normally the case in T. aperta. The shape of the oœciopore is usually complicated by a slight fold or indentation of one side, as shown in fig. 1, or of both sides. The size of the oœciopore varies from 270 μ to 430 μ, the average being 355 μ.

T. plumosa reaches its largest size in the cavities of the bulb-like bases of Laminaria (Saccorhiza) bulbosa. One of the finest colonies I have seen was approximately circular, with a diameter of 21 mm., and its peripheral parts were formed of sixteen separate lobes, some of them bifurcated. These colonies are attached to the seaweed by remarkable ridges on their under side,[1] similar to those which have been figured by Waters (44, pl. vii, figs. 2, 3) in an Australian form, termed by him T. fimbria, forma pulchra, MacG., and by Busk (3, pl. v, fig. 1.*b*) in T. "flabellaris." The ridges do not project greatly, and their general arrangement is radially dichotomous. As the colony increases in diameter, the ridges increase in length, often dividing or undergoing a cessation of growth. When the ridge is re-formed it commonly grows in such a way as to prolong the direction of its more proximal portion. In one case a ridge was observed measuring 1·36 mm. without interruption, but the ridges are usually much shorter than this. If the ridge is narrow its axis may be occupied by a single row of pores, which correspond with the ordinary zoœcial pores, but are considerably larger. If the ridge is broader the number of rows of pores is correspondingly increased, and large areas of the lower side are sometimes in the form of broad, irregular, porous areas, which radiate out into narrower ridges towards the distal part of the colony. In some cases the ridges are broken up into short pieces, only large enough to contain a few pores, sometimes only a single pore. If the base of the colony is, by reason of an irregularity in the surface of the seaweed or for any other cause, separated

[1] The colonies can be conveniently removed from the seaweed by prolonged boiling with caustic potash.

from its base of support, these short ridges may grow out into short foot-like columns of support.

The lobes of this form of T. plumosa are often narrow, with nearly parallel radial sides. In well-grown colonies the parallel sides of adjacent lobes are commonly in close proximity, and they may then unite by means of these attaching processes, which grow towards one another and fuse. This may occur distally, but not proximally, so that narrow fenestræ are left between the united lobes, and these are most easily seen from the back of the colony. A colony whose lobes have thus united may have an almost completely circular edge, and may at first sight appear to have an undivided terminal membrane. That this is not the case can usually be seen by careful examination, and the original lobes are indicated not only by continuing to develop their zoœcia in alternate, connate series, but also by a slight undulation of the growing edge, due to the fact that the distal border of each lobe has a convexity which has a shorter radius than that of the entire colony. Somewhat similar colonies of this species growing on shells have been described above (p. 99).

I have not found these attaching ridges or processes on specimens of this species growing on other seaweeds, nor have I found them in any other species. It appears, therefore, possible that the ridges are, from some cause, a special adaptation connected with the occurrence of T. plumosa on this particular seaweed.

T. plumosa appears to be a shallow water form. Its fondness for Saccorhiza bulbosa has been already referred to. It is dredged at Plymouth in great numbers on Cystoseira granulata from a few fathoms depth. On this seaweed the colonies are small and irregular in their growth, owing to the small diameter of the stems round which they grow. I obtained numerous specimens, which were much more convenient for examination, growing with T. phalangea on Rhodymenia ciliata from the Salcombe Estuary. The flat surfaces of this seaweed induce a regular growth in the Tubulipora, which are thus well adapted for the preparation

of sections which it is desired to obtain in given planes. My study of the development of this form has been entirely based on material obtained from this source.

I have found T. plumosa on the empty carapace of a large crab (Cancer pagurus), but I have not obtained many specimens which I could certainly refer to this species on shells or stones. Its typical habitat may be considered to be the surface of seaweeds from shallow water, but some of the specimens of Tubulipora from shells in deeper water may also belong to the same species. Should it, in fact, prove identical with T. lobulata, Hass., this name would have priority over T. plumosa.

IV. The Nature of certain Vesicles found in the Tentacles and other Parts, with Remarks on the Structure of the Adult Zoœcium and on the Budding.

The account of the occurrence of these vesicles must be preceded by some statements with regard to the development of the polypide-buds and the structures connected with the orifice of the adult zoœcium.

a. Budding and Structure of Orifice.

There are no really detailed accounts of the budding of Cyclostomes, though some information is given by Ostroumoff (32).

I have not satisfied myself with regard to the manner in which the first rudiment of the bud is derived from the terminal membrane. The young bud (fig. 23) is bounded externally by a more or less solid mass of cells of some thickness, which may be regarded as the protoplasmic part of its terminal membrane, and is probably in the main ectodermic. Within this, two or three excretory vesicles appear at a very early stage in all the three species investigated. More internally is a deeply staining two-layered mass of cells, at first forming a hollowed plate, concave externally, but soon taking the form of the well-known vesicular polypide-bud of Ectoprocta. In correlation with the fact that the proximal ends of the zoœcia

are narrow and pointed, the younger stages of the bud are longer than is usually the case in the Gymnolæmata; and a longitudinal section of the entire mass of protoplasmic structures appears as a sharply acute-angled triangle, filling up the narrow pointed tube which is at present the only representative of the future zoœcium.

It may be assumed, on the analogy of other cases,[1] that the inner layer of the vesicular bud is ectodermic, and the outer layer mesodermic. The distal part of the bud gives rise to the tentacle-sheath, into which the tentacles project; while the proximal part develops into the remainder of the polypide, in much the same manner as in other Ectoprocta.

Immediately on the distal side of the two-layered polypide-bud, which can be distinguished by the great readiness with which it takes up colouring matters, there appears at an early stage a cavity, lined by a thin layer of cells, which ultimately gives rise to that part of the introvert which lies between the calcareous "orifice" of the zoœcium and the "diaphragm" which forms the distal end of the tentacle-sheath. This cavity is figured and described by many writers on the Ectoprocta. It is shown by Nitsche (**31**, pl. xxxv, fig. 2) and by Prouho (**36**, pl. xxiii, figs. 1 and 3), and it appears to be of general occurrence throughout the Ectoprocta. Davenport (**7**, and elsewhere) having termed the cavity of the tentacle-sheath the "atrium," this space, with its wall, will be alluded to in future as the "vestibule," without thereby implying any exact homology with the similarly named part of Polyzoon larvæ. I have previously figured the vestibule of a young Cyclostome-bud (**15**, pl. xxii, fig. 1), although the space in question was then erroneously described as the tentacle-sheath.

In some cases the young vestibule appears as an invagination of the ectoderm of the terminal membrane, but it is often difficult to obtain direct evidence on this point.

The vestibule of the adult zoœcium is usually a space of considerable length, the diaphragm being a constriction between it and the tentacle-sheath, as originally described by

[1] Cf. especially Seeliger (**38**).

Nitsche in Membranipora membranacea. The vestibule is lined with a flexible cuticle, which can be made out in longitudinal sections through an adult orifice as a collapsed tube continuous externally with the chitinous part of the terminal membrane of the zoœcium, and internally with the diaphragm, through which the tentacles are protruded.

In a living, perfectly healthy colony, each zoœcium is seen to be closed by a delicate terminal membrane, perforated by a minute hole at its centre, the membrane and its perforation being stretched across the extreme end of the calcareous zoœcium. A similar membrane closes the young ovicells, and appears to be stretched continuously over the edges of the calcareous septa which are forming the walls of new zoœcia at the growing margin of the colony. The size of the perforation in the membrane of the adult zoœcium can be altered in a way which suggests the alterations in the diameter of the pupil of the eye, though I have not succeeded in demonstrating the mechanism of the movements. Before protrusion of the tentacles the pupil, or in reality the opening of the introvert, is widely dilated, and the tentacles are pushed through it.

The perforated terminal membrane appears to be similar to what Jullien (**21**, p. 38) has described as the "irisoidea" in Cheilostomes (Microporella malusii), although Pergens (**35**, p. 509) has adversely criticised Jullien's results on this point.

In colonies which are still alive, but less healthy, the terminal membrane may no longer appear flush with the surface of the zoœcia, but may be sunk down to some little distance from the orifice (fig. 29), and be deeply concave distally or upwards. There is little doubt that this is the result of an unhealthy condition; but it is important to notice that the position of the terminal membrane can vary in the tube because in sections and in colonies which have been mounted whole the distal ends of the zoœcia are often completely empty of cellular structures for a distance equal to once or twice the diameter of the zoœcium. The same phenomenon is noticed in the ovicells, in which the growing edge of the living body-wall is usually at some distance from the edge of the cal-

careous part. I regard these as post-mortem changes due to the action of reagents, and I think it probable that in the healthy zoœcium or ovicell the distal end of the body-wall is in the immediate neighbourhood of the edge of the calcareous wall which it secretes.

The pupil-like opening seen in the terminal membrane of living colonies is doubtless the external opening of the vestibule. When the membrane is in its proper place it is probable that the body-wall of this region is of no great thickness; but in the more usual retracted condition, which is probably artificial, the irisoid (adopting Jullien's name), or in other words the terminal membrane of the zoœcium, generally appears as a thick mass of nucleated protoplasm.

The young zoœcium with its polypide-bud has been seen to consist of the following parts (cf. **15**, pl. xxii, fig. 1) :—an external terminal membrane (irisoid), a vestibule, and the two-layered vesicular bud. Further examination has shown, however, that the outer layer of the bud is reflected at its distal end into a thin membrane (fig. 23, *s. m.*), which encloses the whole bud, a cavity occurring between it and the bud. In older polypides the alimentary canal, the retractor muscles, and the reproductive organ, whether ovary or testis, lie in this space, which is the body-cavity. The reflected layer seen in the young bud may probably be regarded as the somatic mesoderm. This perhaps throws some light on the morphology of the cavity lined by the inner layer of cells of the secondary embryo (cf. **15**, p. 223, pl. xxiv, fig. 23). The inner layer applies itself closely to the dorsal ectoderm in the region which will give rise to the primary polypide bud. If that bud were developed by an invagination of the entire dorsal wall, and the shape of the cavity of the inner layer were simplified by the eversion of the sucker, the cavity in question would closely resemble what has just been described as the body-cavity of the bud.

b. Excretory Vesicles.

The most casual inspection of sections of the three species of Tubulipora which I have specially studied reveals the

presence of remarkable brown vesicles in various parts of the polypides and ovicells (see figs. 20, 22), provided that the material was prepared with certain reagents. As I have, moreover, found these vesicles also in decalcified preparations of T. aperta, it is highly probable that they are of normal occurrence in a portion of the genus at least, and perhaps throughout the whole genus, and that they play some important part in the physiology of the colonies. What that part may be is somewhat doubtful. I have hesitated whether to consider them as reserve-stores of nutritive material or as excretory bodies; but the balance of evidence appears to me to incline towards the latter view.

I first found the vesicles in sections of material which had been prepared with corrosive sublimate. The vesicles are with this preparation excessively resistent, and therefore retain their form and their yellowish-brown colour in sections of colonies which have been decalcified with nitric acid, embedded in paraffin, and stained with hæmatoxylin or borax carmine. They are found principally in one of three places: (*a*) beneath or in the terminal membranes of the zoœcia and buds; (*b*) beneath or in the terminal membrane of the ovicell; (*c*) in the tentacles. They occur in the first two situations in T. plumosa, T. liliacea, and T. phalangea; and in the first, at least, in T. aperta, of which I have not examined sections. So far as I know at present the occurrence of the vesicles in the tentacles is almost restricted to T. plumosa; and so constant have I found this character (during the spring months) that I have used it as a means of distinguishing sections of this species from those of T. phalangea. In one case, however, excretory vesicles occurred in the tentacles of a specimen of T. phalangea, determined as such by the characters of its oœciostome. The colony contained an old ovicell (early stage G), but most if not all of its polypides were degenerating, and the whole colony was unusually heavily charged with excretory vesicles. It thus appears that the vesicles may occur in the tentacles of T. phalangea under certain circumstances; but I think it is none the less true that in the early spring, on the coast of

Devonshire, the tentacles of T. plumosa normally contain these vesicles, and those of T. liliacea and T. phalangea do not normally contain them.

I spent some time at the Plymouth Laboratory during the spring, for the purpose of endeavouring to decide the nature of these vesicles. Material was unfortunately not plentiful, but some results were obtained. The most convenient way of studying the vesicles was to dissect out the fresh polypides of T. plumosa, and after separating the tentacles from one another to examine them in sea water. Although there is some difficulty in dissecting out the polypides, decalcification cannot well be employed, because the vesicles are at first readily altered by reagents.

The vesicles of T. plumosa do not occur in the lumen of the tentacles, as might at first be supposed; but they are situated in the external epithelium on the abaxial side of the lumen (fig. 26). In specimens obtained in the early spring no trace is seen, in fresh material, of the typical vesicles found in sections, but the epithelium contains a row of greenish refractive vesicles, which are commonest near the tips of the tentacles, but may also occur more proximally. These vesicles are not bounded by any distinct membrane, but appear rather as drops of a fluid substance contained in the epithelium. Other smaller granules of yellowish pigment (*p.*) occur here and there in the epithelial cells, but these have a more solid appearance, and are not affected by reagents in the way characteristic of the larger vesicles.

In sections, on the contrary, the vesicles appear to be bounded by a sharply defined membrane; and it was therefore necessary to consider whether they might be symbiotic Algæ. With the view of testing for starch and cellulose, I added a solution of iodine in potassium iodide to the fresh tentacles, with somewhat surprising results. The vesicles, which were at first more or less irregular in outline, immediately rounded themselves off and became darker in colour, an active Brownian movement at once becoming apparent in their interior (showing that they are really fluid). This lasted for a brief period,

at the end of which (fig. 27) each of the homogeneous greenish vesicles took on the appearance of the sharply contoured spherical bodies seen in sections, a certain number of brownish granules being precipitated in their interior, and usually coming to rest on the inside of the wall of the vesicle, the fluid contents of which are now colourless.[1] The subsequent addition of strong sulphuric acid gave no blue colour, and did not greatly alter the vesicles. These changes took place in the same way in vesicles which had been freed from the polypides during the dissection.

The greenish vesicles were found in the fresh material in the tentacles (in T. plumosa alone) and beneath the terminal membranes, i. e. in precisely the same places as those in which they had been previously noticed in sections.

The above-described reaction was due to the iodine and not to the potassic iodide. This was shown by the fact that a solution of potassic iodide (*a*) in distilled water, (*b*) in sea water, was added to other specimens without producing any effect. On adding a fragment of iodine to either solution the characteristic precipitation at once took place. The same result was produced by adding sea water in which a fragment of iodine had been rubbed up.

It soon became apparent, however, that iodine was not alone in precipitating the contents of the homogeneous vesicles. The same result was produced by the following reagents :— distilled water, solution of corrosive sublimate (in fresh water or sea water), strong ammonia, osmic acid.

The darker (browner) colour assumed by the vesicles in the first experiment is not merely the effect of staining by the iodine, since it occurs with other reagents, even with distilled water. In all cases the colour of the homogeneous vesicle at once becomes darker, and an appreciable interval occurs during which nothing else can be made out. The Brownian movement then commences, the granules being at first invisible individually. The vesicle soon contains numerous minute

[1] The vesicles are very similar to those which have been figured by Durham ('Quart. Journ. Mic. Sci.,' xxxiii, pl. 1, fig. 3) in Spatangids.

granules in active movement. These become larger, and on coming to rest form the granules seen even in sections in the interior of the vesicles. One fresh vesicle always gives rise to one precipitated vesicle.

The amount of the precipitate produced by various reagents is not identical. Ammonia gives rise to a specially copious precipitation, the vesicles now looking very dark, and sometimes dissolving after a prolonged action of the ammonia. The addition of strong potash now broke up the precipitated granules, with some indication of solution, during which a purplish colour appeared. Osmic acid similarly gives rise to a very dark-coloured precipitate; but when added to vesicles which have recently been precipitated by corrosive sublimate, it merely slightly darkens their granules.

In most of the above-described experiments no trace was seen in the fresh material of precipitated vesicles, but these were observed in one or two specimens. It is, I think, probable that precipitation of the granules takes place during life in old vesicles; but it seems clear that the young stages of these bodies consist merely of a homogeneous fluid.

While the above reagents produced precipitation of the vesicles, others dissolved them completely. 90 per cent. alcohol at once dissolves them without precipitation, and the same effect is produced by 30 per cent. alcohol, which does not affect the yellow granules above described in other parts of the tentacles. The vesicle is indicated as an empty space in the tentacle after the addition of alcohol. Nitric acid dissolves the fresh vesicles, giving rise to a red-brown mass. Dilute acetic acid has no effect.

When the vesicles have been precipitated, their behaviour towards reagents is entirely different from that of fresh vesicles. Alcohol, even weak alcohol, readily dissolves the latter, but it has no effect on vesicles which have been precipitated with corrosive sublimate, as is obvious enough from the fact that they are seen in sections.

The vesicles of some sublimate-material which had been kept in 70 per cent. spirit for seven or eight months were ex-

traordinarily resistent. After decalcification of the colonies with dilute nitric acid the vesicles were practically unaffected by the following reagents:—sulphuric acid (dilute and pure), nitric acid (even when the strong acid is heated), strong ammonia, 4 per cent. potash, ether, chloroform, benzole. Gmelin's test for bile-pigments was tried without any results, and the murexide test for uric acid was also negative.

The freshly precipitated vesicles, on the contrary, were less resistent. Thus, after precipitation with distilled water, 90 per cent. alcohol had some effect on them, while a 4 per cent. solution of potash at once dissolved them. After the action of corrosive sublimate (15 minutes), followed by distilled water (25 minutes), even strong potash had practically no effect.

In some cases, though not in all, ammonia added to the vesicles, freshly precipitated by distilled water, first turned them purplish, and then dissolved them. Certain pigmented granules or cells observed in the terminal membranes of the zoœcia of T. plumosa also turned purplish on adding distilled water.

T. phalangea and T. liliacea.—Although the tentacles of these species do not usually contain homogeneous vesicles, they possess in their places structures which consist of a large number of minute vesicles (fig. 28). Each compound vesicle probably corresponds with a single cell, and its reactions are different from those of the homogeneous vesicles, which occur in the terminal membranes of the same species, as in T. plumosa. The homogeneous vesicles are precipitated by various reagents, and then take on the form seen in sections, whether they occur in the tentacles (T. plumosa) or in the terminal membranes. The compound vesicles of T. phalangea and T. liliacea under no circumstances give rise to what I may call the "formed" excretory vesicles.

The tentacles of T. phalangea, as of other Cyclostomes I have examined, contain pigmented granules in various parts of their external epithelium. Two of these (*p.*) are seen in the distal part of the tentacle shown in fig. 28. They do not give the reactions characteristic of the other structures.

The fresh compound vesicles of the tentacles of T. pha-

langea are dissolved by strong ammonia, by 4 per cent. potash, by 90 per cent. alcohol, and by nitric acid. Osmic acid also destroys them.

They are not affected by corrosive sublimate, nor are they broken up by acetic acid. After being fixed with corrosive sublimate, they are no longer affected by osmic acid, nor by distilled water.

On adding distilled water the fresh compound vesicles lost the boundaries of their constituent vesicles, and turned a diffuse red-purple, that colour becoming soon restricted to numerous granules, which disappeared on adding ammonia. The homogeneous vesicles of the terminal membrane were precipitated by distilled water; some of these (probably the more delicate ones) were dissolved by ammonia, while others remained and turned purple inside.

Several facts in the above experiments suggest that there is some connection between the well-known purple colour of certain species of Tubulipora and the homogeneous or compound vesicles. The change from a greenish to a purplish colour was specially marked on adding distilled water to the compound vesicles, and it also occurred in the homogeneous vesicles, under certain circumstances, as the result of treatment with ammonia or potash. The purple colour is, however, not entirely due to the action of reagents. The branches of T. liliacea, for instance, are commonly purple during life, the colour occurring as a purple pigment in the cells of the terminal membrane, as correctly stated by Smitt (39, p. 22), and in other parts. This purple pigment was not affected by the successive action of distilled water, ammonia, and potash, nor by acetic acid.

It seems to me probable, however, that the purple colour often seen in dry preparations of species of Tubulipora may in many cases be due to post-mortem changes of the excretory vesicles. This view was confirmed by an observation on a colony of T. liliacea which, when alive, had no trace of purple, but assumed that colour after being washed with fresh water and dried. The colour is not seen in colonies preserved

in spirit (which dissolves the vesicles), nor in those treated with corrosive sublimate (which precipitates the homogeneous vesicles); but colonies which have been allowed to dry, after washing with fresh water, are commonly of a characteristic purple colour. This colour is usually not noticeable during life in T. plumosa, healthy colonies of which are yellowish; but it is conspicuous in specimens which have been dried without being put in spirit.

I have found very pale greenish, homogeneous vesicles in Diastopora patina in the brown bodies, and apparently in the body-cavities. These were precipitated by sublimate, ammonia, or iodine in potassic iodide; but the details of the reactions were not altogether similar to those in Tubulipora. I have also seen structures somewhat resembling the compound vesicles of T. phalangea in the tentacles of a Stomatopora, perhaps S. major.

Experiments made with indigo-carmine, carminate of ammonia, and Bismarck-brown on living specimens of Tubulipora gave only negative results. The vesicles did not appear to take up any of these pigments, although control-experiments, made with species of Bugula, gave results similar to those I had previously arrived at (14). The cells which take up indigo-carmine in B. flabellata are the same as the "gelbe Tropfen" described by Claparède (4, pl. viii, fig. 1B, *t.*). Since these are normally of a yellow or yellowish-green colour, they are not unlike the homogeneous vesicles of Tubulipora. They are not precipitated, however, by distilled water, iodine, sublimate, or ammonia; and taking this fact in conjunction with the difference in their behaviour to indigo-carmine, it may be concluded that they do not closely resemble the vesicles of Tubulipora.

The result of the previous reactions seems to be that there is some substance in solution in the homogeneous vesicles which is precipitated by any reagent (not being a solvent of that substance) which alters the density or constitution of the solvent. The action of distilled water may be due to osmosis from the vesicle, while it is possible that substances like cor-

rosive sublimate have some specific action on the substance itself. The precipitate, once formed, behaves to reagents quite differently from its antecedent state in solution in the vesicle. Alcohol, for instance, dissolves the contents of the fresh vesicles without precipitation; but the precipitated contents become entirely resistent to alcohol, except immediately after their formation by distilled water.

Different colonies vary a good deal in the extent to which the vesicles are developed; but those obtained in the summer appear to have a much greater development of the vesicles than those of the same species found in the spring. I have examined summer specimens in preserved material only; but in many of them the excretory vesicles are very much more conspicuous than in the spring colonies. Their walls become very dark, and may be so thickened as to greatly diminish the lumen, while the contents may appear as a single solid concretion. The number of the vesicles may often be very greatly increased in the summer. This was observed especially in T. plumosa, in which summer specimens had almost the entire length of the tentacle occupied by a single row of closely packed vesicles.

These facts seem to indicate that the vesicles are not normally discharged to the exterior, although it would not appear difficult for their fluid contents to escape through the epidermis of the tentacles. In many of my preparations excretory vesicles appear to have been forced to the outside of the epithelia which normally cover them. I am inclined to regard this as an artefact, the fluid vesicles being squeezed out as the result of the contraction induced at death by the action of reagents, and being precipitated outside the body by those reagents. This is indicated by the fact that I have never seen them on the morphologically outer side of the ectocyst of the vestibules of the zoœcia or of the terminal membrane of the ovicell.

The increase of the number of the vesicles in the later part of the year is a reason for regarding them as excretory, and this view is confirmed by their dark colour and by the con-

cretion-like contents which may appear within them. Their occurrence in the terminal membranes of buds (even of young ones) as well as of the polypides and ovicells might suggest the view that they were nutritive.

Davenport (7, pp. 34—, pl. vi, figs. 54, 56, 57, 59) has described certain mesoderm cells (which have also been observed by Braem) in the budding regions of Paludicella. These contain refractive bodies which are not altogether unlike the vesicles which I have described above, and they are regarded by both Braem and Davenport as nutritive. It appears to me that these bodies are not comparable with the vesicles of Tubulipora; but there is enough appearance of similarity to make it worth while to call attention to the resemblance.

I can hardly doubt, however, that the Tubulipora vesicles have a close similarity to the "Exkretbläschen" which have been described by Eisig (8, pp. 725—) in Capitellidæ. The reactions of these bodies, and particularly the resistent way in which some of them behaved to mineral acids and potash, led Eisig to the belief that they contained chitin as one of their constituents, and that this substance was to be regarded as one of the normal nitrogenous excreta. The reactions which I have described agree so well with Eisig's results that the view that the vesicles are of an excretory nature appears to me to be greatly strengthened thereby. If the insoluble substance which is precipitated by various reagents is really chitin, or some similar body, the normal homogeneous vesicles may perhaps be considered to contain a substance which could be called chitinogen, which, though itself soluble, readily passes into an insoluble state. The vesicles might conceivably be employed in giving rise to the chitinous parts of the terminal membranes or vestibules of the zoœcia, although their number would appear disproportionately large on this hypothesis. It is perhaps more likely that their occurrence in the tentacles and terminal membranes is due to a tendency to deposit the excretory substances in the peripheral parts of the colony, and to the advantage of removing waste products of metabolism from the polypide-buds and embryo-

phores, where specially important processes of growth are taking place.

It would be interesting to know the fate of the vesicles when a brown body is formed. I have not as yet obtained any satisfactory evidence on this point, the colonies found in the spring having been almost completely devoid of brown bodies. It is not improbable that the vesicles found in the tentacles, at least, are in some way got rid of when a brown body is formed.

V. Description of the Development.

The ovicell of Tubulipora is in its development, in certain respects, intermediate between that of Crisia and that of Lichenopora, a conclusion which might be expected from a consideration of the adult characters of the three genera. The account given in my preliminary note (17) of "Idmonea serpens" really refers mainly to what is here termed Tubulipora plumosa, though I now find that the sections on which that account was based were partly prepared from T. phalangea.

The following account refers indifferently to T. plumosa and T. phalangea if no species is mentioned. Where statements are made with regard to T. phalangea, it must be understood that the evidence for the specific determination was, unless the contrary is stated, the absence of excretory vesicles in the tentacles. This negative evidence is not so satisfactory as the positive evidence, afforded by their presence, that a given specimen belongs to T. plumosa; since it is possible to overlook in the sections vesicles which are not very numerous. It may be noted that the material used for the study of the development of these two species was derived almost entirely from a single haul of the dredge which brought up Rhodymenia ciliata, on which T. plumosa and T. phalangea alone were discovered.

The specimens of T. liliacea were from Hydroids in deeper water at Plymouth, and the species was determined before they were decalcified.

The account of the development will, as far as possible, be described as a series of stages corresponding with those which I have formerly described in Lichenopora (16, p. 99). Those stages were characterised partly by the degree of development of the embryo and embryophore,[1] and partly by the condition of the fertile polypide. This latter degenerates at an earlier stage in Tubulipora than in Lichenopora, and the correspondence between the developments is therefore not exact.

The stages selected for descriptive purposes are thus as follows:

Stage A. Formation of the definitive egg (fig. 10).

Stage B. Division of the egg, and degeneration of the fertile polypide (figs. 11—13).

Stage C. [Not represented in Tubulipora.]

Stage D. Formation of the embryophore. The fertile zoœcium is still cylindrical, or slightly expanded distally, and its brown body becomes closely surrounded by a distinct cellular investment (figs. 14—18, 21).

Stage E. The investment of the brown body becomes vacuolated, so as to give rise to a cavity. The ovicell expands distally (figs. 19, 20, 22, 25, 32).

Stage F. Commencement of embryonic fission.

Stage G. Fully formed ovicell (figs. 1—8, 33).

Stage A.—Formation of the Definitive Egg.

The eggs are developed at a very early stage by the polypide-buds, as in Lichenopora and Crisia. This precocious occurrence of the eggs appears to be in some way correlated with a colonial habit, and cases of this kind are familiar to every student of colonial Ascidians and of Hydroids. The

[1] The term "embryophore" will be used below to denote the structures, in relation with the primary embryo and its derivative secondary embryos, which are on the proximal side of the vestibule. The principal contents of the ovicell are thus the embryophore, containing the embryo and the fertile brown body, the vestibule, and the terminal membrane.

phenomenon has been described in Cheilostomes (Bugula) by Claparède (4, p. 166).

The eggs appear as part of the outer or mesodermic layer of the polypide-bud. In some colonies they are very numerous. Thus in one case (T. plumosa) a young bilobed colony contained twenty-three individuals in which an egg or eggs had become unmistakable. Some of these were fully formed polypides, others very young buds, the youngest belonging to a stage when the bud consisted of a two-layered cell-plate which had not yet become vesicular. All stages intermediate between these extremes were represented.

In most of the zoœcia there is only a single egg; in six cases there are two, and in one case three eggs; the position being at or near the proximal end of the polypide-bud or the cæcum of the polypide, as the case may be. Each egg (whatever the number in a zoœcium) is surrounded by a very distinct follicle (fig. 10) of cells, in which a follicle-cavity is usually visible.

There can be no doubt of the normal occurrence of eggs in the manner above described, since they are repeatedly noticed in the sections. I think, moreover, that there is clear evidence that the eggs occur especially in young lobes. When a lobe possesses an ovicell belonging to one of the later stages, although eggs may not be altogether wanting, they cease to be the conspicuous feature that they form in young lobes during the breeding period. It is thus probable that the successful development of an egg, with the resulting modification of the fertile zoœcium into an ovicell, diverts the energies of the lobe from the production of fresh eggs to the nutrition of the embryos, although new lobes may be formed from the same colony and give rise to new ovicells.

The mature egg reaches a diameter of 24 μ or even 28 μ (T. plumosa), and this measurement is larger than those previously recorded for Crisia (17·6 μ) and Lichenopora (16 μ). It possesses a distinct germinal vesicle and germinal spot, and it commonly has a paranuclear body lying in its protoplasm. This body might possibly be a male pronucleus, against which is to be set the fact that the nucleus of the egg

shows no sign of alteration; or it might be a centrosome, a view which hardly appears likely, from the fact that it stains readily with hæmatoxylin. I have not discovered the nature of this body.

In stage B (fig. 13) I have seen what I believe to be spermatozoa in the follicle cavity; but I have not observed the fertilisation of the egg.

I have never found a testis in an ovigerous zoœcium, contrary to what may happen in Lichenopora. The testes appear in the majority of zoœcia, and usually in all those which produce no eggs, in the same position as the ovaries of the female polypides; the colony being thus monœcious. The young testis is distinguishable from the young ovary owing to the fact that it consists of a small group of nuclei at the proximal apex of the cæcum of the polypide. The testes grow concurrently with the polypide to which they belong, and they occur at all stages of the development of the ovicell. Owing to the great size they reach, they form a very conspicuous feature of sections, and can readily be seen in colonies mounted whole. They fill up the proximal end of the zoœcia with a great mass of developing or mature spermatozoa, and they may reach the length of 670 μ (T. plumosa). When the testis is mature, the ripe spermatozoa may be seen extending up the side of the polypide, and may even be massed between its tentacle-sheath and body-wall. I have not ascertained the mode of escape of the spermatozoa.

The testes are thus produced in the great majority of the zoœcia, while ovaries are developed in but few, and an embryo in an extremely restricted number. This seems to make it probable that cross-fertilisation takes place, the great number of spermatozoa that are produced being probably in some way discharged into the surrounding water before fertilisation is effected.

The occurrence of large testes had no relation to the age of the corresponding ovicell; whilst in Lichenopora (**16**, p. 127) there was evidence of the disappearance of the testes with increasing age of the ovicell. The apparent difference

between the two genera may be due to the fact that in Lichenopora the ovicell dominates the entire colony, whereas in Tubulipora new fertile lobes can be developed in colonies which possess an old ovicell (fig. 1). It must further be noted that the specimens of Tubulipora were collected early in the year, and therefore early in the breeding season.

Stage B.—Division of the Egg, and Degeneration of the Fertile Polypide.

The number of polypides which become actually fertile is a strictly limited one, as in Lichenopora. The bilobed condition so often characteristic of the young colony is usually correlated with the development of two ovicells. These are at first ordinary zoœcia, as in Lichenopora; and in T. flabellaris and T. aperta the proximal end of the ovicell not uncommonly has the external characters of a zoœcium, the increased number of pores which denote the ovicell beginning in some cases suddenly when the zoœcium has reached a certain length. But in all species it is easy to see, by looking down into the young ovicell at a time when it is commencing to expand, that the dilated part of the ovicell is continuous with a prismatic or pyramidal cavity which runs proximally into the general series of zoœcia. A convenient way to demonstrate the fact that the proximal end of the ovicell is morphologically a zoœcium is to stain a colony which possesses a young ovicell without decalcifying, and to embed it in paraffin. By scraping away the lower or basal wall of the colony, and then dissolving out the paraffin and mounting whole, a view of the lower surface can be obtained without having the details obscured by the relatively thick, calcareous, basal lamina. In preparations of ovicells of a suitable age made in this way (fig. 30) the young embryophore may be demonstrated in a part of the ovicell whose floor is the basal lamina, and in fact in a cavity which in no way differs from an ordinary zoœcium.

The fertile zoœcia of Lichenopora are usually differentiated at an extremely early stage in the life of the colony. In

Tubulipora the entire colony cannot be looked upon as an individual of the third order to the same extent as can that of Lichenopora; but a certain amount of individuality may be recognised in each of the ovicell-bearing lobes. The fertile zoœcium is, in fact, differentiated at a very early stage in the development of a lobe. This may be understood by reference to fig. 1, in which a young ovicell is beginning to develop at the right of the figure. The development of the first ovicells of the colony begins in T. plumosa in the immature stage which was considered by Thompson and Johnston as the adult condition of this species.

The phenomena of the selection of the fertile zoœcia are probably more primitive in Tubulipora than in Lichenopora, in which the differentiation of the ovicell may have been thrown back to an early stage in the development of the colony, in correlation with the high degree of individuality which is possessed by the entire colony in that genus.

Internal evidence that the ovicell is at first a zoœcium is afforded by the invariable presence in it of a brown body, indicating the previous existence of a polypide. This may be called the "fertile brown body," as in the case of Lichenopora.

In the latter the fertile polypide is not the first inhabitant of its zoœcium, as is indicated by the simultaneous presence in it of a brown body, a functional polypide, and an embryophore. In Tubulipora, on the contrary, the first polypide of the fertile zoœcium becomes fertile, and no new polypide is developed in the normal ovicell after the first brown body is formed. In cases of abnormal development, however, the entire embryophore may degenerate, and a new polypide-bud may make its appearance. In a single case only I have found a brown body (in addition to the normal brown body) on the proximal side of an embryophore in stage E. This may be interpreted as evidence of the degeneration of a polypide before the formation of the definitive fertile polypide.

It is not easy to obtain evidence showing the exact stage at which degeneration of the fertile polypide takes place in

Tubulipora, and this is probably because the stage is of short duration. Two possibilities have to be considered,—(*a*) that degeneration takes place while the polypide is still a bud; (*b*) that it occurs after the polypide has become functional. The latter view is probably the correct one, and if this is so, Tubulipora occupies an intermediate position in this respect between Crisia, in which the polypide degenerates while it is still a two-layered bud, and Lichenopora, in which two functional polypides successively occupy the young ovicell.

The question clearly turns on the observation of the stage at which the egg begins to develop. No trace of development is found in the eggs of polypide-buds, and on the assumption that the brown body is formed by the degeneration of a bud, it would be necessary to assume that the large eggs which are so commonly noticed in the ovaries of polypides have missed their chance of developing, and would later have degenerated. Positive evidence that the fertile brown body is formed from a polypide, and not from a mere bud, is afforded by the fact that the youngest embryophores found with a brown body and partially developed embryo occur in fully formed, long zoœcia, and not merely in immature zoœcia still in process of development at the growing edge.

Even more direct evidence is, however, afforded by the stage shown in fig. 11 (T. plumosa), which represents what is certainly a case of the division of the egg. The darkly stained bodies marked *f* probably represent degenerating eggs or their follicles. This would indicate that in the event of more than one egg occurring in a single ovary, only one of the eggs actually develops. The figure shows the cæcum of the fertile polypide, which from its size, and from the avidity with which its tissues have taken up hæmatoxylin, was clearly only just mature. Its rectum contains Diatoms, a fact which proves that the polypide had commenced to feed. A second, precisely similar polypide, containing Diatoms, and provided with an egg in the same condition as that of the first specimen, also occurred in the same colony. Most of the buds and young polypides in this colony, if not all of them, possessed either

one or two eggs, and one unmistakable ovicell was also present; no other development had taken place.

This is the only colony in which I have obtained certain evidence of the period at which the egg begins to develop; but taken in connection with the next described series of cases, the conclusion may be drawn that the degeneration of the fertile polypide normally takes place shortly after the polypide has become functional. This is in accordance with the fact that ovaries or eggs are not found in the older polypides of a colony, indicating that they are in some way absorbed or degenerate if development does not begin in them shortly after their parent polypide has become mature.

The occurrence of more than one polypide in the fertile zoœcium of Lichenopora is associated with the fact that each of the ordinary zoœcia of the young colonies also possesses a brown body and a polypide. This is not the case in Tubulipora, in which there are normally no brown bodies (in the species investigated) in the young colonies. It is not impossible that this may have something to do with the season at which my material was collected. My specimens were obtained during March and April, a time when growth is taking place with great energy, probably after a period of winter rest. I have found brown bodies commonly in colonies of Tubulipora obtained in Devonshire during the summer months. It is not impossible that the excretory vesicles which have been described above provide a means of eliminating excretory matters which would otherwise accumulate in the stomachs, and induce the formation of brown bodies. Whether this be the case or not, the entire absence of brown bodies in a large proportion of the specimens of Tubulipora, even in quite large colonies, is a fact in striking contrast with the normal occurrence of brown bodies in the young zoœcia of Lichenopora.

Evidence of the degeneration of a fertile polypide was obtained in ten cases, four of which appear to belong to T. plumosa and six to T. phalangea. The results of the examination of these cases were quite concordant, all of them

pointing to the degeneration of a polypide at a stage when the embryo consists of about 2—4 blastomeres, at first enclosed in the original follicle of the egg, the follicle ceasing to be distinct in the later stages. In two of these cases additional evidence that the degeneration was a normal stage in the development of the ovicell was afforded by the fact that it was in the position relatively to an obvious ovicell which it might have been expected to occupy, from the fact that two ovicells are so commonly found in young colonies. Fig. 12 shows the beginning of the degeneration of the fertile polypide. The section cuts the edge of the mass of tentacles, which in the next few sections are obscurely defined, showing that degeneration is taking place. Parts of the alimentary canal are visible in the section figured. The nuclei of the follicle are not so regularly arranged as in the case of unfertilised eggs. The embryo has two cells, which lie in a distinct follicle-cavity.

Fig. 13 (T. phalangea) shows a later stage, drawn with a higher power. The brown body, which is only indicated in the figure, still shows some traces of the parts of the alimentary canal of the fertile polypide, whereas a fully formed fertile brown body shows no such traces, and consists of a granular pigmented mass, containing a few nuclei. The embryo certainly possesses three blastomeres, while the elongated nuclei of two of the cells of the egg-follicle are clearly seen. The follicle contains a minute deeply stained body, which is almost certainly the remains of a spermatozoon.

The terminal membrane of the fertile zoœcium plays an important part in the development of the future ovicell. The evidence afforded by this and the next stage appears to be that the orifice of the vestibule closes at the degeneration of the polypide, and that the terminal membrane retires some little distance within the calcareous orifice. It is, however, difficult to be certain how far this regression is normal, and how far it is an artefact.

[Stage C (functional polypide + embryophore) of Licheno-pora is not represented in the development of Tubulipora.]

Stage D.—**Definitive Formation of the Embryophore.**

The appearance of the ovicell in this stage is very characteristic, and from the frequency with which it occurs in my sections I conclude that the stage is of relatively long duration. I have examined about seventeen ovicells in this stage of T. plumosa, twenty-seven of T. phalangea, and one of T. liliacea. It appears to me that the embryophore of the first species is normally distinctly larger than that of the second, while that of the third species differs from the other two in its great length.

Fig. 15 shows an embryophore of T. plumosa at the beginning of this stage. The brown body is fully formed, but appears young, and it is not yet surrounded by any very definite investment of cells. The embryo has two obvious blastomeres, and the follicle-cavity in which they lie is in the immediate neighbourhood of the brown body. The cord of cells running proximally from the follicle is the shrunken remains of the somatic mesoderm of the fertile polypide. The terminal membrane is thickened, and its staining properties indicate that it is in a state of active growth. It is continuous internally with a mass of cells which extend from it to the brown body. This mass contains four excretory vesicles, though a larger number were visible in some of the other sections of the same ovicell.

The length of the embryophore, from the proximal end of the follicle to the distal tip of the terminal membrane, is 200 μ.

Fig. 14 (T. phalangea) illustrates the condition in which the terminal membrane is usually found in this stage. The distal end of the embryophore projects into the cavity of the young ovicell as a knob, which appears to be quite free from the wall of the ovicell. The brown body is fully formed, and has a more compact appearance than that of the former specimen.

Fig. 18 is a slightly older embryophore of T. plumosa, from a colony in which excretory vesicles were specially

numerous. In the section drawn they form a large axial mass, lying in the solid tissue on the distal side of the brown body. The details of this tissue are not shown, but it does not differ in any essential respect from the similar region of fig. 16.

Two embryonic cells are seen, the two large nuclei shown to the right of the follicle-cavity probably belonging to the original egg-follicle. While in the younger ovicells shown in figs. 14 and 15 the follicle is in the immediate neighbourhood of the brown body, it is here separated from it by some intervening tissue, the existence of which, and the occurrence of a definite cellular investment to the brown body, are indications that the embryophore is more advanced in its development than was that of the former figures.

The investment of the brown body has in fact become a perfectly definite structure, sharply marked off from the surrounding tissues. The part of it which lies distally to the brown body has become thickened, and the nuclei are here arranged in several layers, while they occur in a single layer at the sides of the brown body. Distally to the thickened part is a slit-like cavity, which will be termed the vestibule. The morphology of these parts will be considered in the final part of the paper.

Fig. 16 is a still later stage (T. phalangea), as is shown by the more advanced development of the embryophore and of the embryo. The latter now consists of a considerable number of cells, still lying in a follicle-cavity. The embryophore is rather longer than before, and the investment of the brown body is now thickened laterally and proximally, as well as distally. A special growth of the investing tissue is taking place proximally to form the "nutritive tissue," destined hereafter to form the reticulum in which the secondary embryos lie. As it appears probable that this reticulum is directly or indirectly concerned in the nutrition of the rapidly growing mass of embryos, the term here suggested may conveniently be used for descriptive purposes. The investment of the brown body in fig. 16 shows some signs distally of

being continuous with the vestibule. The terminal membrane is deeply invaginated medianly; and this is in fact a general feature of ovicells in this stage. In sections which are not median the invagination may not be seen; and in some cases, as in fig. 18, it is obscured by the great development of the excretory vesicles. The existence of the invagination may, however, be looked on as the general rule; and it is probable that the vestibule is really continuous with, and has been developed by invagination from, the terminal invagination, though it is not easy to demonstrate this continuity. The wall of the vestibule, during the beginning of stage D, is closely surrounded by the other tissues of the solid distal half of the embryophore, so that the whole of its limits cannot, in most cases, be made out with certainty. Excretory vesicles were but slightly developed in this ovicell (fig. 16).

Fig. 17 is a somewhat oblique longitudinal section of the distal half of an ovicell in about the same stage as fig. 16. Part of the median invagination of the terminal membrane is seen as a slit-like cavity. The section shows the investment of the brown body, and the way in which it is connected with the vestibule, the junction being constricted like the neck of a flask. This is a normal arrangement, as is also (in T. phalangea at least) the oblique position of the neck of the flask-like connection.

Fig. 24 (Pl. 10) represents an ovicell of T. plumosa in an unusual condition. While the embryo and the proximal half of the embryophore are in stage D, the remainder of the ovicell has the form characteristic of stage E.

It can hardly be doubted, from the sections of stage D, that the ovicell at this time does not differ, or hardly differs, externally from a zoœcium. The young ovicell lies completely in series with the ordinary zoœcia, and there is no indication of a distal dilatation of its cavity.

In Fig. 24, however, the distal end of the ovicell is clearly dilated, and this expansion has been accompanied by the vacuolation of the previously solid or semi-solid distal half of the ovicell, which now contains a spacious cavity, with excre-

tory vesicles and a few cellular contents. The vestibule has well-defined walls lying in the body-cavity of the ovicell. The terminal membrane has become distinct; it closes the body-cavity at its distal end, and is somewhat thickened, except at the middle.

Fig. 21 represents an ovicell of T. liliacea in stage D. Although I have only one series of sections of this species in the stage in question, its characters correspond so closely with those of the succeeding stage (fig. 20) that I regard it as a normal ovicell. If this be admitted, it follows that there is a marked difference between T. liliacea and the other two species (T. plumosa and T. phalangea), its embryophore being much longer than anything which occurs in them, and being in fact as well developed as that of Lichenopora. Fig. 21 is drawn to the same scale as figs. 16 and 18, so that comparison is easy.

The other features of the section resemble those found in the other species. The distal part of the ovicell is still solid, and contains but few excretory vesicles (not seen in this section); the vestibule can be made out, and the brown body has a thick investment continuous proximally with the embryophore, and having distally a split leading to the vestibule. The existence of the nutritive tissue shows that the ovicell is at the end of stage D, a conclusion which is also indicated by the length of the embryophore.

The general features of stage D are thus as follows:—The egg-follicle becomes replaced by what may be termed the embryonic follicle, or simply the follicle. This becomes separated from the brown body by intervening cells, which are specially well developed in T. liliacea. The brown body, at first without any distinct cellular investment, becomes surrounded by a mass of cells which mark it out sharply from the other parts of the ovicell; and the proximal part of this investment gives rise to a special "nutritive tissue." The vestibule makes its appearance, at first surrounded by a nearly solid mass of cells containing excretory vesicles, into which a median terminal invagination of the ectoderm projects. The

ovicell is still in the main cylindrical (or really pyramidal), and does not yet differ materially from an ordinary zoœcium.

Stage E.—Development of the Cavity of the Embryophore and Enlargement of the Ovicell.

The general external features of the ovicell in this stage are seen from figs. 31 and 32, both taken from one colony. The embryophore of fig. 31 is shown in back view in fig. 30, and is a rather late representative of stage D, as can be seen by comparison with fig. 16, the nutritive tissue being clearly indicated on the proximal side of the brown body. The combination of an embryophore in stage D with an ovicell in stage E has already been seen in fig. 24. The embryophore of fig. 32 is in stage E.

The cavity of the ovicell is beginning to expand distally in figs. 31 and 32, in preparation for the great increase in the size of the embryophore which is to take place during the succeeding stage. The distal end of the embryophore in both ovicells lies at the level marked *Emb.*, and it is thus interesting to note that the embryophore is still in the part of the ovicell which represents its zoœcium-stage.

The growing margin of the ovicell has already acquired the characters of stage E, consisting of a deeply staining mass of tissue which extends round the growing edge of the ovicell.

Fig. 32 shows that the growing edge is uniformly curved in the upper wall of the ovicell, while below it has an undulating course, due to the fact that the floor of the ovicell is ridged by the upgrowth of the young zoœcia in the way that has already been described (p. 79).

I believe that the position of the growing edge shown in figs. 31 and 32 is not completely normal, but that the action of reagents has caused it to shrink away from the edge of the calcareous parts. This seems to be indicated by fig. 31, in which the protoplasmic growing edge practically coincides with the distal margin of the roof of the ovicell on the left of the figure, though it has shrunk away from it to the right. Sections show that the stained line seen in the figures is

really the thickened edge of the terminal membrane, which is deeply invaginated in the middle, as was the case in the earlier stage. A longitudinal section of the ovicell thus has the form seen in fig. 22, in which the middle of the terminal membrane is greatly depressed, so as to be widely removed from the chitinous part of the membrane (ectocyst), which is tightly stretched across the open part of the calcareous funnel. I am doubtful how far this is normal. Some amount of retraction of the living tissues from the orifices of the zoœcia almost certainly takes place when the animals are killed, and it is possible that the protoplasmic terminal membrane is normally in close contact with its chitinous ectocyst. It is not easy otherwise to see how the ectocyst is formed, unless the terminal membrane is capable of attaching itself from time to time to the ectocyst, which, it must be remembered, increases in extent so long as the ovicell continues to expand.

Decalcification is a further source of alteration. Bubbles of gas accumulate during this process in various parts, and are responsible for a good deal of distortion of the protoplasmic structures. The bubbles often find much difficulty in making their way through or past the ectocyst, and an accumulation of gas between the latter and the living part of the terminal membrane may be responsible for a considerable amount of depression of the former. As, however, the tissues were well hardened before being decalcified, I think it probable that the gas would rather tear the tissues than alter the entire position of an epithelium which had lost its flexibility as the result of long immersion in spirit.

I have examined sections of nearly seventy ovicells in this stage. About half that number belonged to T. plumosa, eight to T. liliacea, and the remainder to T. phalangea.

Fig. 19 is a longitudinal section of an ovicell of T. plumosa at the beginning of stage E. The terminal membrane is deeply invaginated in a neighbouring section. The distal part of the ovicell is still nearly solid, and contains numerous excretory vesicles. The vestibule is somewhat more distinct

than before; the brown body has diminished in size, but the embryo is not greatly changed. The most noticeable difference between this and the earlier stage is in the embryophore, the proximal part of which is much more developed than before. The nutritive tissue is, in fact, now much increased in amount, and the embryo is thereby further removed from the brown body.

The nutritive tissue is becoming vacuolated in fig. 19, and further spaces are originating between it and the outermost wall of the embryophore. This leads to the condition of fig. 22, a considerably older stage (of the same species) drawn to the same scale.

Fig. 22 is from a distinctly bilobed colony, each lobe of which contains an ovicell in stage E. The two ovicells converge proximally, as can be seen from the sections, which are parallel to the basal lamina of the colony. Their proximal ends extend far down into the part of the colony which is common to the two lobes, so that it is clear that the colony was in an unlobed state when the development of the ovicells commenced.

The most important difference between fig. 22 and fig. 19 is that the part of the embryophore containing the nutritive tissue consists in the former of a long, cylindrical, thin-walled portion, containing a loose inner mass of cells which do not nearly fill its cavity. There is still no great increase in the development of the embryo.

Fig. 20 is a section of an ovicell of T. liliacea in the same stage, and it confirms the conclusion drawn from fig. 21 that T. liliacea is characterised by the great length of its embryophore. The ovicell here figured is about as much developed as fig. 19 (T. plumosa). The distance of the brown body from the embryo is very different in the two cases.

The condition of the ovicell towards the end of this stage is illustrated by fig. 25, representing an ovicell, probably of T. phalangea, cut horizontally, as is shown by its considerable breadth. The embryo has now enlarged to a marked extent, and measures 80 μ in its greatest length, which is about three

times the length of the embryo in fig. 19. Several giant-cells are seen in the immediate neighbourhood of the embryo, as in Crisia and Lichenopora. The function of these is not certain, but there is no evidence that they take any direct part in the future development.

The cavity of the embryophore has greatly enlarged, and now fills up most of the ovicell. Parts of the nutritive tissue are seen in its proximal region, and a few scattered cells belonging to this tissue occur in the middle of the cavity. The brown body is at the distal end of the cavity; and this is its usual, though not its invariable position. In some cases, during this or earlier stages, it may lie partly in the cavity of the embryophore and partly in the vestibule, demonstrating the existence of a communication between these two cavities. The vestibule is much shorter than before, so that the distal end of the cavity of the embryophore is now very near the terminal membrane. The point where the vestibule joins the terminal membrane will become the future oœciopore, the morphological "orifice" of the ovicell.

The terminal membrane is still a good deal thickened and folded at its edge, particularly on the left side of the section, and it contains the usual excretory vesicles.

The general features of stage E are thus as follows:—The fertile zoœcium becomes definitively an ovicell, and becomes obvious externally by the dilatation of its distal end. The part of the embryophore immediately distal to the follicle becomes more or less hollowed out, so that a passage is prepared by which the embryo can pass into the nutritive tissue, which is developed from the proximal part of the investment of the brown body. The part of the embryophore containing the nutritive tissue becomes vacuolated, and finally forms a wide space.

The brown body is generally left at the distal end of this space, surrounded by a mass of cells in close connection with the vestibule; but as by the vacuolation of the surrounding tissue it becomes free from other tissues, its position towards the end of this stage is variable. It may pass into the proximal

part of the cavity of the embryophore, and in one or two cases it was found in the vestibule.

The series of developmental stages grouped under the heading of stage E is really a very long one; and there are great differences between ovicells at the two ends of the series. This difference is marked in the measurements of all the parts of the ovicell.

In the early part of stage E the embryo remains for some time in the position in which it was found in the preceding stage, and its size does not at first materially increase. It is now more or less spherical, and has a diameter of about 20—25 μ. Later in stage E the follicle becomes continuous with the cavity of the embryophore. The embryo now begins to elongate in the direction of the main axis of the ovicell, and soon reaches a length of 40—50 μ, its transverse diameter being at first small. With the increase of this diameter it becomes ovoid, and then rapidly increases in size. Measurements of the embryo fairly late in stage E amounted to 80 μ for the major axis, and 50 μ for the minor axis of the same embryo. The oldest ovicell which I have found in this stage had a pear-shaped embryo 135 μ long, with its narrow end situated proximally. The ovicell had reached a considerable size, its greatest transverse diameter, as measured in a series of horizontal sections, being 1·25 mm. (= 1250 μ). The cavity of the embryophore was spacious, and was cylindrical for about its proximal half, the distal end dilating into the form of a trefoil consisting of three lobes, the middle lobe being connected with the vestibule.

In most of the later ovicells studied in this stage the cavity of the embryophore was either cylindrical (as in fig. 22), or was somewhat dilated distally without being distinctly lobed. Beginning this stage with a length[1] of about 150 μ, the embryophore may reach a length of 500 μ by the end of this stage; and after the establishment of its cavity, its transverse diameter may become as much as 450 μ at its distal end.

[1] This measurement is taken between the points C and B in the figures on Plate 9.

In a case of rapid and continuous growth like that of the ovicell of Tubulipora there is of course no special significance in the measurements quoted, but they will give some idea of the dimensions of the parts during the stage in question. The measurements here given refer to T. plumosa.

Throughout stage E the embryo is found at the proximal end of the embryophore. At first in its follicle, the embryo begins to extend into the adjacent part of the embryophore as it begins to lengthen. Later in the stage it lies somewhat more distally in the embryophore, its long axis coinciding with that of the ovicell. The distinctness of the follicle becomes lost towards the end of this stage, and the fertile brown body diminishes in size.

Stage F.—Commencement of Embryonic Fission.

I have not given any figure of this stage, which does not differ materially from that of Lichenopora. The primary embryo increases largely in size, and divides in much the same way as in that genus (see **16**, pl. x, figs. 32—35).

The details of the lobing of the ovicell are not always the same, as is shown by the varying position of the oœciostome in the adult ovicell; but in several cases which I have observed in stage F the distal end of the embryophore was merely a later stage of the oldest embryophore, with a trefoil-like lumen, described in stage E. This trifid division of the embryophore is certainly a common arrangement, the median lobe being connected with the vestibule, and therefore later with the oœciostome; while the other lobes form the more lateral parts of the ovicell. The lobe connected with the vestibule may be termed the "axial lobe," because, although not necessarily in the axis of symmetry of the ovicell, it is part of its morphological long axis. Other lobes will be termed "lateral lobes."

Ovicells in stage F may exhibit no subdivision of the primary lobes, but in other cases the end of each of the lateral lobes becomes bilobed. This subdivision of the embryophore is in

all cases due to the upgrowth of a zoœcium which interrupts the growth of the ovicell.

The ovicell contains (as in stage E) two principal cavities,—firstly, the cavity of the embryophore; and secondly, the cavity in which the embryophore lies. The latter may be regarded as the body-cavity of the original fertile zoœcium, and it is constantly extending itself between the series of zoœcia by the growth of the edge of the ovicell. The growth of this cavity is more energetic during this stage than that of the embryophore; so that each lobe of the latter merely enters the base of the corresponding lobe of the entire ovicell.

In one ovicell in stage F where this arrangement was obvious, the axial lobe of the entire ovicell had elongated greatly, while the corresponding lobe of the embryophore only just entered its base. The vestibule had accordingly been greatly elongated (to 400 μ), so as to retain its connection both with the terminal membrane and with the embryophore. The vestibule is lined with a chitinous ectocyst, and opens to the exterior by joining the terminal membrane. The fertile brown body lies freely in the middle of the large cavity of the distal end of the embryophore. The embryo, about 225 μ long, lies in the proximal cylindrical part of the embryophore, and is clearly dividing into a number of secondary embryos. Giant-cells, similar to those of Crisia and of Lichenopora, occur in the position of the original embryonic follicle.

The total length of a trifid embryophore during this stage was 800 μ, and the distance from tip to tip of its lateral lobes was 720 μ. The brown body remains distinguishable during this stage, usually lying freely in the cavity of the embryophore. The partially divided primary embryo in one case formed a large, more or less spherical mass, with a diameter of 305 μ.

Stage G.—Fully formed Ovicell.

This stage, which corresponds with the figures given of the mature colonies of the several species, is illustrated by fig. 33, from a decalcified preparation of T. plumosa. The embryo-

phore is seen to be considerably lobed in a more or less palmate way. The proximal part is cylindrical, and occupies the cavity which represents the fertile zoœcium before it became an ovicell. This part, together with the greater part of the digitate lobes, is practically solid; and consists, as in Crisia and Lichenopora, of a reticulum of nutritive tissue, containing a very large number of secondary embryos in all stages of development. The distal ends of some of the lobes can be seen to be hollow, although the thickness of the colony makes it impossible to make out all the details. The oœciostome is at the end of the lobe marked *o.*, and this is hence the axial lobe. The greatest length of the solid part of the embryophore, from the proximal end to the distal tip of the most projecting lobe, is 2·5 mm.

Sections through ovicells in this stage are readily intelligible by comparison with stage F. A comparatively young ovicell, which had a transverse diameter of about 1·5 mm. (measured in the sections), had a five-lobed embryophore. The middle lobe was in connection with the vestibule, and the two lobes of each side had resulted from the bifurcation of the primary lateral lobes. The distal end of each of the five lobes of the embryophore was hollow, while the proximal part was filled up with a mass of young secondary embryos. Excretory vesicles were numerous in the growing edge.

The fertile brown body can still be discovered, lying freely in the cavity of the embryophore, in some of the earlier ovicells belonging to this stage.

The mass of embryonic tissue increases very greatly, and in the younger ovicells it is easy to see that embryonic fission is or has recently been taking place. In the most satisfactory series I possess illustrating this point, the ovicell contained (in addition to numerous young secondary embryos) an embryonic mass, elongated in the direction of the axis of the ovicell, and measuring about 160 μ in length. This mass was very similar to the corresponding structure which I have described at the beginning of embryonic fission in Crisia (15, pl. xxiii, fig. 11). Karyokinetic figures were clearly seen in a large

number of the nuclei, and the mass was constricting off secondary embryos on all sides, and was further surrounded by a good many young secondary embryos of about the same size as those which were still in connection with itself.

Demonstrative evidence of the occurrence of embryonic fission during the early part of this stage was obtained in both T. plumosa and in T. phalangea. In the case of the best series of sections obtained of the latter, the species was not merely inferred from the absence of excretory vesicles in the tentacles, but had been determined, before decalcification, by the characters of the oœciostome. It appears to me that the nutritive tissue is much more abundant during this stage in T. phalangea than in T. plumosa.

The younger ovicells in this stage contain a large number of young secondary embryos, all in about the same stage as those which are being constricted off from the larger embryonic mass already described. Comparing the entire embryophore to a hand, the secondary embryos at first occupy the part which corresponds to the palm, where they form a dense mass, composed mainly of secondary embryos, which are separated from one another by a certain amount of nutritive tissue. Measurements made of this mass, in sections cut in a suitable plane, gave 250—560 μ as its greatest transverse diameter in particular cases.

As in Lichenopora, I am not able to say how far a secondary embryo, once formed from a larger embryonic mass, is capable of further fission. In some cases this process seemed to be clearly indicated. But even in the oldest ovicells, containing mature larvæ, ready to escape, and even actually in the tube of the oœciostome, I have in one or two cases found larger masses of embryonic tissue clearly giving rise to more than two or three secondary embryos. One of these masses noticed in an old ovicell was 90 μ long, which is about the same as the average length of a larva ready to escape from the ovicell. The dividing embryonic masses are similar to those of Crisia, consisting of a more or less clearly defined outer layer of cells, surrounding a central solid mass containing specially large

nuclei (8 μ). These nuclei appear to be characteristic of the growing tissue of the primary embryo, since they occur at a part where no separation of secondary embryos is taking place. When traced to a part where a secondary embryo is being constricted off, these large nuclei are seen to become smaller by division, and to form the smaller nuclei (3·5 μ) of the inner layer of the secondary embryos. Evidence of fission was not obtained in all the old ovicells.

The young secondary embryos are always embedded in nutritive tissue, whatever the age of the ovicell. In many cases the occurrence of a group of young secondary embryos in close proximity to one another probably implies recent embryonic fission. As development continues, the secondary embryos become ciliated externally, and may then become quite free in the cavity of the embryophore, the wall of which may be reduced to a thin nucleated film of protoplasm in old ovicells.

The cavity of the ovicell is ultimately almost completely filled by the embryophore, the lobes of which may be several times divided. Even the axial lobe may give off secondary lobes (fig. 33), and it is clear that this must take place in many cases from the external conformation of the ovicell. The growing edge of the ovicell is usually conspicuous at the end of any lobe which is still incomplete, but it disappears with the complete closure of the lobe by a calcareous wall. Some of the older ovicells suggest as a possibility that the number of excretory vesicles may be reduced in some way during stage G, but others were provided with numerous vesicles.

VI. The Morphology of the Internal Parts of the Ovicell.

There can be little doubt that the embryophore of Tubulipora corresponds in general with that of Crisia and Lichenopora; but it is not easy to decide how far the homology is an exact one.

In Crisia (15) the ovicell is at no period of its life an ordinary zoœcium. This would almost follow from the fact that its proximal end has not the character of a simple, cylin-

drical zoœcium, but begins to widen from its commencement (cf. **13**, pl. xii, fig. 6). The ovicell develops a polypide-bud, which does not become a polypide, but gives rise to the tissues immediately surrounding the primary embryo. There is thus no fertile brown body. In Tubulipora the ovicell is at first a zoœcium with a functional polypide. Degeneration of the latter takes place at the beginning of the development of the embryo, and at a time when the polypide is still moderately young; and a brown body results. In Lichenopora (**16**) also the young ovicell is a zoœcium, but it has two successive polypides, the second of which is present until the primary embryo has developed for some time, and the embryophore has been formed.

The investigation of Crisia appeared to show that the rudimentary polypide-bud of the ovicell gave rise to a tentacle-sheath, which communicated with the exterior by means of a vestibule (described as "aperture" in **15**). The cavity of the embryophore in Tubulipora is so similar to the "tentacle-sheath" of the ovicell of Crisia that at first I had no doubt that the two spaces were homologous. The cavity in Tubulipora is, however, a completely new formation, formed after degeneration of the fertile polypide. If then it is a tentacle-sheath at all, it must be that of a newly formed bud.

In my paper on Lichenopora (**16**, p. 114) I have alluded to this possibility for that genus. The evidence afforded by Tubulipora seems to be in favour of the hypothesis that the parts of the embryophore correspond with parts of a polypide-bud. This view is supported, for instance, by the great resemblance of the vestibule of the ovicell to that of the zoœcia, in its structure and in its relation to the terminal membrane. The polypide-bud would be represented by the cellular investment which appears round the brown body. This mode of origin is probably similar to those cases which have been recorded by Smitt and Hincks (**18**, p. lvii) among Cheilostomes, in which the young polypide-bud appears to grow out of the brown body.

The relations of the cavity of the embryophore to the vesti-

bule at first sight seem to indicate that the walls of the space represent the tentacle-sheath. There is, however, one fact which suggests a different explanation. The distal end of the wall of the embryophore in stages D and E is invariably reflected inwards, as shown in figs. 22 and 25, the reflected part usually terminating in cells which are loosely connected and lie in the cavity of the embryophore. This relation is very similar to that of what I have called above the somatic mesoderm of the young polypide-buds (fig. 23, *s. m.*). If the wall of the embryophore-cavity can really be compared with this layer, the general history of the ovicell would appear to be somewhat as follows. The ovicell is at first an ordinary zoœcium, whose first polypide develops a functional alimentary canal and an ovary. An egg begins to develop, probably while the polypide is still functional, but still comparatively young. The polypide then degenerates and forms a brown body. Certain cells arrange themselves as a definite investment round the brown body; and these cells, whose origin is obscure, probably represent a polypide-bud. A vestibule makes its appearance distally to the brown body. The body-cavity of the ovicell-bud appears as a space just inside an outer epithelial layer, which represents its somatic mesoderm. This layer lies freely in the old body-cavity, and its outer surface may be more or less covered with cells belonging to that cavity. The greater part of the rudimentary polypide-bud of the ovicell becomes a mass of nutritive tissue, in the meshes of which the secondary embryos are afterwards contained. The brown body comes to lie freely in the new body-cavity by the breaking up of the nutritive tissue. By the enlargement of the body-cavity, its somatic mesoderm is brought close to the outer calcareous wall of the ovicell, and the new body-cavity thus replaces the old one. The cavity becomes lobed in correspondence with the lobing of the entire ovicell. The lumen is visible distally, even in advanced stages, while it becomes filled up proximally by the great development of the nutritive tissue and of the secondary embryos. One of the lobes of the ovicell, the "axial lobe," is connected with the exterior by means of the

oœciostome, where the vestibule opens to the exterior; and the secondary embryos ultimately escape by this passage.

The junction of the embryophore with the vestibule is somewhat complicated, and from stage D onwards there is a cellular plug between the brown body and the vestibule. This is shown in fig. 20 (*y.*), where it is still solid. In a later stage (fig. 22, *y.*) it becomes hollow, its cavity sometimes appearing completely closed, sometimes opening towards the brown body, and sometimes communicating with the vestibule. These different conditions appear to be found in ovicells of one and the same stage, and the first of them is shown in fig. 25. This figure illustrates the way in which the wall of the embryophore is reflected inwards, and the similarity with a polypide-bud (fig. 23) is marked.

It is possible that this plug of cells is the morphological representative of a tentacle-sheath. On this view, the brown body of fig. 25 does not lie freely in the tentacle-sheath (which would be a somewhat anomalous position for it to occupy), but in the body-cavity.

Sooner or later the vestibule becomes continuous with the "tentacle-sheath" (*y.*), and this with the body-cavity; the communication between the two latter being uninterrupted after the migration of the brown body to some other part of the body-cavity. A communication between the body-cavity and the exterior is not a very unusual occurrence in the Polyzoa, since the reproductive bodies escape more or less directly from the former to the outside in several cases. This is shown, in Phylactolæmata, by the escape of the statoblasts after the decay of the polypide has left an open passage to the exterior, and in certain Gymnolæmata by the occurrence of a special passage, the intertentacular organ.

The cavity of the embryophore in Lichenopora is probably comparable with the cavity in Tubulipora. The stage shown in pl. ix, fig. 27, of my former paper (**16**) is very similar to a Tubulipora at the end of stage D. The vestibule is represented by the invagination there marked "orifice;" the brown body with its investment requires no special explanation, while

the "suspensor," constituted by the inner cells between the brown body and the embryo, is probably represented in Tubulipora by the nutritive tissue.

The comparison with Crisia is less easy, but the main difference—the absence in the ovicell of that genus of a functional polypide degenerating to a brown body—has been already commented on.

Fig. 15 of pl. xxiv (15) shows a young ovicell with a bud consisting of vestibule (distally), "tentacle-sheath," and thick proximal portion, corresponding with the alimentary canal and tentacles of an ordinary polypide. Fig. 1 of the same paper is probably younger, the part there marked "tentacle-sheath" being more probably the vestibule, which is developed in Cyclostomes before the tentacle-sheath. Fig. 2 is considerably later, and its "tentacle-sheath" corresponds with the similarly marked space in the later stages; this is quite evident from the fact that a vestibule like that of fig. 3 is present in the ovicell which fig. 2 represents. Although fig. 2 is a good deal later than fig. 1, it will be noticed that its egg is in much the same state. The later ovicells formed a perfectly uninterrupted series. It thus appears to me to be sufficiently established that the vesicular bud of the ovicell of Crisia (15, fig. 1) gives rise to the "follicle" (figs. 3, 5, 6) of later stages. If this is the case, the cavity into which the follicle projects may be really the tentacle-sheath, which would, on this hypothesis, be much more developed than in Tubulipora. It seems to me more probable, however, that the cavity of the embryophore of Tubulipora is identical with the cavity of the "tentacle-sheath" of Crisia, as is indicated by a comparison of fig. 25 of the present paper with fig. 8 of my former paper (15). The apparent difference in the early stages is probably due to the fact that the development of the so-called "tentacle-sheath" of Crisia was not observed.

The junction of the vestibule and embryophore in the ovicell of Crisia (15, fig. 8) is marked by a thickening, which is by no means unlike the junction between the vestibule and the embryophore in Tubulipora. I can hardly doubt, therefore,

that the part which was described as " tentacle-sheath " in the ovicell of Crisia corresponds with the thin-walled embryophore of Stage E of Tubulipora (fig. 25), whatever may be the morphological character of this space.

The separation of the genera of Cyclostomes is notoriously difficult. In Crisia, Tubulipora, and Lichenopora we have three extreme cases, which there can be no difficulty in recognising as distinct. The fact that their ovicells belong to three entirely different types renders the ultimate definition of the genera of recent Cyclostomes a more hopeful task than it is sometimes supposed to be.[1] Crisia may be characterised as a genus in which the ovicells are modified zoœcia dilated into a pear-like form, the region of the oœciostome, as in other Cyclostomes, not sharing in this dilatation. The ovicell is from the first an ovicell; and although its morphology is indicated by the appearance of a polypide-bud, the bud never becomes a functional polypide.

In Tubulipora the dilatation of the ovicell is usually much more marked, and the ovicell is commonly lobed. Its lobes are developed owing to the formation of zoœcia distally to the ovicell, and the latter is accordingly obliged to divide into two portions which grow round the sides of the zoœcium or series of zoœcia. The degree of lobing corresponds with the extent to which young zoœcia are developed distally to the ovicell. Two lobes may become contiguous on the distal side of a zoœcium, but probably do not fuse; and the embryophore also is composed of a series of divaricated lobes which do not unite (in the species examined). The young ovicell differs from that of Crisia in beginning life as an ordinary zoœcium; and the duration of this period is accurately indicated by the extent of the proximal cylindrical (or pyramidal) portion of the ovicell.

In Lichenopora, as in Tubulipora, the ovicell is at first a zoœcium, but has more than one functional polypide (in L. verrucaria). The principal characteristic of the ovicell is, however, the mode of growth by the addition of peripheral

[1] Cf. Gregory, No. 12, p. 21.

"alveoli," which are at first distinct from the cavity of the ovicell. The lobes of the ovicell, and even of the embryophore, unite with one another on the distal side of the zoœcia, which thus pass through the cavity of the ovicell as completely free columns.

Further investigations will be necessary in order to ascertain how far the distinction of genera and species of Cyclostomes can be based on the characters of the ovicells.

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EXPLANATION OF PLATES 8—10,

Illustrating Mr. Sidney F. Harmer's paper "On the Development of Tubulipora, and on some British and Northern Species of this Genus."

PLATE 8.

The figures on this plate were all drawn to the same scale (camera lucida, Zeiss A obj., with front lens removed; afterwards × $\frac{2}{3}$). The pores of the zoœcia are in most cases not indicated.

Fig. 1.—Tubulipora plumosa, W. Thomps. (p. 105). Fertile lobe, with one ovicell and the beginning of a second ovicell; from a bilobed colony (proximal ends of oldest zoœcia, at the bottom of the figure, obscured by foreign substances). Salcombe estuary, 4—5 fathoms, March—April, on Rhodymenia ciliata.

Fig. 2.—T. aperta, n. sp. (p. 101). A small colony with a single ovicell, which is abnormal in possessing four accessory oœciostomes, numbered 1, 2, 3, 5. 4 is a similar structure, but its terminal membrane is completely

calcified with the exception of a minute central pore. The colony is seen to originate in the "primitive disc," the calcified body-wall of the larva. Godösund, Björne Fjord, Norway, June, on Laminaria saccharina.

Fig. 3.—T. aperta. Distal view of the same colony, to show the tube of the oœciostome. Two of the accessory oœciostomes (numbered as in fig. 2) are also seen.

Fig. 4.—T. flabellaris, Fabr. (p. 99). A stunted colony, in which the proximal ends of the oldest zoœcia were obscured by foreign substances. The lateral parts of the colony are bent backwards round the Cellularia peachii on which the specimen is growing. 1—6, oœciostomes of the six mature ovicells, oœciostome 4 being concealed from view by the zoœcia. The ovicells to which the oœciostomes 5 and 6 belong are seen not to have completed their growth. 7 is a young ovicell. Barents Sea, 50 fathoms, July 1; dredged by Colonel H. W. Feilden. Greatest diameter of colony, 2·36 mm.

Fig. 5.—T. phalangea, Couch (p. 94). Fertile lobe with a single ovicell. s. septum between two contiguous lobes of the ovicell. Salcombe estuary, 4—5 fathoms, March—April, on Rhodymenia ciliata.

Fig. 6.—T. phalangea. Zoœcium, with the oœciostome, broken off from a colony, and placed in such a position as to show the oœciopore. Salcombe estuary, 4—5 fathoms, March—April, on shell.

Fig. 7.—T. liliacea, Pall. (p. 90). Part of ovicell, with two series of zoœcia and an oœciostome. Plymouth, 30—40 fathoms, March—April, on a Hydroid.

Fig. 8.—T. liliacea. Fertile lobe seen from its distal end, to show the oœciostome, the upper lip of which projects so as to conceal the oœciopore, which opens horizontally. The proximal end of the lobe is the lower side of the figure. Plymouth, obtained with the last specimen on a Hydroid.

Fig. 9.—T. liliacea. Lobe in which no ovicell is yet apparent, to show the biserial, Idmonea-like arrangement of the zoœcia. Plymouth, obtained with the last specimen on a Hydroid.

PLATE 9.

The sections figured are all longitudinal. Fig. 10 was drawn with Zeiss F, figs. 11 and 13 with $\frac{1}{12}$ oil-immersion, figs. 12—22 with DD. All the figures were afterwards reduced $\frac{2}{3}$.

[The microscopical sections which are referred to as T. plumosa are those in which excretory vesicles were discovered in the tentacles; those referred to as T. phalangea are the specimens in which no excretory vesicles were seen in that position. Unless otherwise stated, the discrimination of these two species in sections depended entirely on this character.]

Fig. 10.—T. plumosa. Ovarian egg, in follicle (stage A).

Fig. 11.—T. plumosa. Stage B, the embryo consisting of two blastomeres. The cæcum of the fertile polypide (which has not yet degenerated) is seen; *f*, and the corresponding structure to the left are probably the degenerating follicles of eggs which are not developing.

Fig. 12.—T. phalangea. Degeneration of the fertile polypide (stage B). The tentacles, which are better seen in neighbouring sections, have lost their distinct outlines, and are obviously degenerating. A B = 300 μ.

Fig. 13.—T. phalangea, stage B. Three blastomeres are seen in the follicle-cavity, which also contains a spermatozoon. The brown body still shows traces of the alimentary canal of the fertile polypide.

Fig. 14.—T. phalangea. Early stage D. A B = 165 μ; C B = 75 μ.

Fig. 15.—T. plumosa. Early stage D. A B = 200 μ.

Fig. 16.—T. phalangea. Late stage D, as shown by the considerable development of the cellular investment of the brown body, and particularly by the development of the nutritive tissue on the proximal side. The terminal membrane is deeply invaginated, and the vestibule is visible. A B = 250 μ; C B = 110 μ.

Fig. 17.—T. phalangea. Late stage D; obliquely longitudinal section of the distal end of the ovicell, to show the oblique, constricted junction of the vestibule with the embryophore. The invagination of the terminal membrane is not cut so as to show its opening to the exterior.

Fig. 18.—T. plumosa. Rather early stage D, with very numerous excretory vesicles. The vestibule is small. The cellular investment of the brown body is thickened distally (X). There is at present no nutritive tissue. A B = 225 μ, C B = 85 μ.

Fig. 19.—T. plumosa. Early stage E. The nutritive tissue is largely developed, and the embryophore is becoming vacuolated. The embryo has increased in size. The section passes on one side of the median invagination of the terminal membrane. A B = 460 μ, C B = 165 μ.

Fig. 20.—T. liliacea. Early stage E. The embryophore is longer than in the other species. *y*. Plug of cells between vestibule and embryophore (see p. 148). A B = 630 μ, C B = 255 μ.

Fig. 21.—T. liliacea. Younger ovicell (late stage D). The embryophore is much elongated, as in Fig. 20.

Fig. 22.—T. plumosa. Ovicell at middle of stage E. *y*, corresponding with the similarly lettered part in figs. 20 and 25, has now acquired a lumen. A B = 960 μ, C B = 355 μ.

PLATE 10.

Fig. 23 was drawn with Zeiss DD, and was not afterwards reduced. The other figures were reduced two thirds after being drawn. Figs. 24 and 25 were drawn with DD; Figs. 26—28 with F; Figs. 30—32 with A; Fig. 33 with A, the front lens of which was removed. Fig. 29 was not drawn with a camera lucida.

Fig. 23.—T. plumosa. Polypide-bud, partly diagrammatic; for comparison with the ovicells shown in Figs. 22, 25, &c. *s. m.* Somatic mesoderm, reflected on to the very thin tentacle-sheath, which becomes continuous with the tentacles near their proximal end. The epithelium of the stomach (below the tentacles) is derived from the inner layer of the bud, and is covered by the outer layer. The distinction between the two layers is not easily made out in the mass marked "tentacle."

Fig. 24.—T. plumosa. The embryophore is in stage D, and the distal part of the ovicell is in stage E. Numerous excretory vesicles occur distally. A B = 460 μ, C B = 110 μ, D E = 275 μ.

Fig. 25.—T. phalangea. Advanced stage E. The growing edge is much lobed and thickened on the left side of the figure. The vestibule is much shortened; it is separated by the space *y* (cf. Figs. 20 and 22, and p. 148) from the brown body, which is now almost free in the cavity of the embryophore. The outer wall of the embryophore is reflected over the wall of the space *y* (cf. the polypide-bud, Fig. 23). The embryo has greatly enlarged, and three giant-cells are shown. C B = 360 μ, F G = 385 μ; greatest length of embryo = 80 μ.

Fig. 26.—T. plumosa. Tentacle, fresh, with homogeneous excretory vesicles and pigment granules (*p.*).

Fig. 27.—T. plumosa. Another tentacle belonging to the same polypide, after the addition of iodine in potassic iodide. The contents of the homogeneous vesicles have been precipitated.

Fig. 28.—T. phalangea. Tentacle, fresh, with compound vesicles and pigment granules (*p.*).

Fig. 29.—T. plumosa. Orifices of living zoœcia. The terminal membrane has been somewhat retracted.

Fig. 30.—T. plumosa. Not decalcified (see p. 136). View of a colony from below, after the basal lamina has been scraped away. X and Y are zoœcia, which are similarly marked in Fig. 31. G is the thickened edge of the growing margin of the ovicell (in stage E), and corresponds with G in Fig. 31. The embryophore (in stage D) lies in the proximal undilated part of the ovicell. The fertile brown body, the embryo, the beginning of the nutritive tissue, and the distal thickening of the investment of the brown body, can be made out. A B = 900 μ.

Fig. 31.—T. plumosa. Upper view of the same lobe. *Emb.* Leve of the distal end of the embryophore. X, Y, and G are the parts similarly marked in Fig. 30.

Fig. 32.—T. plumosa. A similar preparation of an older ovicell in stage E. The zoœcia D project upwards into the floor of the ovicell, and the thickened edge of the terminal membrane is looped over these projecting parts. *Emb.* Level of the distal end of the embryophore.

Fig. 33.—T. plumosa. Decalcified preparation of an old ovicell (stage G), with nearly solid embryophore containing numerous secondary embryos. The axial lobe of the embryophore ends in the oœciostome at *o.*, and gives off another lobe to the right. The main lateral lobe of the right side is only obscurely bifurcated; that of the left side is divided into five lobes. *Z.* Proximal part of ovicell, corresponding with a zoœcium. Greatest length of solid part of embryophore to tip of most projecting lobe, 2·5 mm.

Postscript.—Braem's interesting work, "Die geschlechtliche Entwickelung von Plumatella fungosa" ('Zoologica,' Heft 23, 1897), did not appear until after my MS. was in the hands of the printers; and I am unable to refer further to his observations and conclusions on the present occasion.

The Molluscs of the Great African Lakes.
I. Distribution.

By

J. E. S. Moore.

The present paper forms the first instalment of the zoological report of an expedition which, through the generous support of the Royal Society and the British Association, I was able to make to Lake Tanganyika during 1895 and 1896.

The primary objects of this expedition were—

1. To study the unique fauna of Lake Tanganyika on the spot;

2. To make what observations were possible in the Nyassa region while I was en route; and—

3. To bring back properly preserved material for the complete morphological investigation of the more remarkable lake organisms after I returned.

Before proceeding to the purely zoological matters with which I propose to deal, it is appropriate that I should here express my sincere thanks to Professor Ray Lankester, to whom I have been indebted for the primary suggestion of the whole inquiry, and for much kindly help since my return. I have also to thank Professor G. B. Howes for the use of the Huxley Laboratory and invaluable advice, without both of which I should never have been able to get the subject through, while I have been very materially indebted to Sir John Kirk, who procured for the expedition the necessary introductions to the administrative gentlemen through whose districts it had to pass. And last, but not least, I have to

thank Sir Harry Johnston for the very effective support he lent the expedition, and without which it would have been impossible for me to attain the objects which I had in view.

Excluding the polar regions proper, there exists in the fresh waters of the different continents a type of fauna which in the character of its constituents is essentially the same. Certain forms are added and others are omitted as we pass from the more temperate to the equatorial zones, but beneath these changes there exists a substantial similarity, so easily recognisable and so marked that geologists have not hesitated to distinguish between fresh-water and marine fossiliferous deposits wherever they may be found. On the other hand, that there is a hard and fast demarcation between fresh-water and marine faunas is not true, for there are many instances of animals—for example, of prawns and crabs, which in this country are purely oceanic—having made their way up the rivers into inland and elevated fresh-water lakes. Further, there are a number of animals that belong neither to salt nor fresh water, but are inhabitants of the brackish regions which lie between inland fresh waters and the sea. That the well-established and more permanent fresh-water organisms of the present day are descended from older phyla that were once marine, is an accepted truth. This view is necessitated by the theory of common descent, and it is supported, as in the case of the ganoid fishes, by the similarity of numerous living fresh-water organisms to older oceanic types. It is significant, however, that with few or no exceptions, all the well-established fresh-water organisms of to-day are not directly referable to the earliest oceanic forms, but rather to those which in their temporal distribution stand intermediate between then and now. It seems that the fresh-water molluscs of the present day first make their definite appearance in Tertiary times, for much doubt has recently been raised as to the genuineness of the so-called carboniferous Unio, Tichogonia, and Planorbis; these forms being now regarded as more nearly related to Anthracosia, Avicula, and

Serpula respectively. The family Limnæidæ, which is now so universal in its distribution, does not certainly extend further back than the Jurassic period. The same is true of the fresh-water Melaniidæ and of the Paludinidæ, but I need scarcely point out that it is necessary to use the greatest caution in drawing any inferences respecting the date of origin of the true fresh-water forms from these apparent facts. It may, however, be taken as approximating to the truth to say that although the typical and universal fresh-water molluscs of the present do not appear upon the stage of life as such before the Jurassic period, they almost certainly originated from a series of marine types which had become completely differentiated from their oceanic associates long before this time. Some of these antecedent organisms are probably represented in the palæontological record by those extinct genera with which the earliest known modern fresh-water types are usually associated.[1]

The facts of morphology are themselves in harmony with such a view, for in their anatomy the living fresh-water molluscs do not approximate to any of the more modern marine genera; they hark back to those more permanent marine types which were in existence long before Jurassic times. They certainly bear no resemblance to the generalised conceptions or archetypes of the more modern marine genera which appeared during Tertiary and post-Tertiary times, such as Strombus, Pteroceros, Rostellaria, Conus, Mitra, Chenopus, and the like. It is this fact that is of first importance to us here; for if it should be found that in some district at the present time there exists a fresh-water fauna which departs from the normal and universal type in the possession of genera which approximate to those that are undoubtedly modern and marine, we shall have very strong primâ facie evidence for regarding these organisms as recent

[1] It is quite possible that many of the old so-called fresh-water deposits are in reality marine, since the forms which became exclusively fresh water as time went on probably made their appearance in the sea first, as so many of the more recently derived fresh-water types have done,—prawns, for example.

importations from the sea. Now such a fauna is presented to us in that of Tanganyika at the present day, for in this lake there have been known to exist ever since 1859 what appear to be the shells of some six genera of Gastropods, which are entirely unlike any known fresh-water forms, while their shells at the same time simulate several modern oceanic types. The interest in these strange molluscs, which have been known hitherto only by their conchological characters, was greatly augmented when in 1883 Boehm found jelly-fish in the lake; and during my recent expedition I have been able to add deep-water crabs, prawns, sponges,[1] and Protozoa to this anomalous list of organisms, all of which appear to possess the same marine affinities.

It is my object in the present paper, to ascertain to what conclusions as to the nature and origin of this anomalous series the facts of distribution lead; while in those which follow I shall deal with the morphological affinities of the hitherto unknown individual forms, and thus determine whether the conclusions to which these facts of distribution seem to point are really sound. Almost no definite observations have been hitherto available for the study of this subject, and consequently the material contained in this and the following papers will be mostly new. I would, therefore, invite particular attention to the positive character of the evidence which I shall bring forward in support of the recent marine origin of a number of the animals contained in Tanganyika, as compared with the wholly negative character of that upon which the geological speculations of Murchison respecting the "permanence of terrestrial conditions" in the African interior at present rest.

To believe that the marine animals of Tanganyika are among the few remaining indications of a sea that once extended to the very heart of the African continent is to come into the most uncompromising conflict with the theory put forward by

[1] The affinities of the deep-water sponge which I obtained in Tanganyika have not yet been determined, but its striking external character and its remarkable deep-water habitat have inclined me to regard it as a member of the anomalous section of the fauna which the lake presents.

Sir Roderick Murchison in 1852. Yet such a view is now supported by the strongest kind of zoological evidence it is possible to get.

In order to arrive at a sufficiently complete comprehension of the general type of the molluscan fauna which characterises the individual lakes, it is quite unnecessary and even prejudicial to discuss the question of the occurrence or non-occurrence of many of the so-called specific forms, since a large number of these are merely geographical varieties, while others have been based on minute and often purely fanciful conchological distinctions. The four species of Hylacantha, described by Bourguignat in 1890, for example, are certainly nothing more than the rather remarkable polymorphs of the original Typhobia Horei described by Smith in 1881. But what is true of the polymorphic Typhobia is equally true of the polymorphic Paramelania and Neothauma. I shall therefore consider the distribution of the genera alone, or the main issue will become lost in the pursuit of really non-existent types.

In Nyassa, which was the first great lake I reached, and which has hitherto been better known than all the rest, there have been recorded some sixteen genera of molluscs, namely, Limnæa, Isodora, Physa, Physopsis, Planorbis, Ancylus, Ampullaria, Lanistes, Vivipara, Cleopatra, Bythinia, Melania, Spatha, Iridina, Corbicula, and Unio.

In the smaller lakes occurring in this district, such as Shirwa, there have been found a fewer number of the same genera. In Kela, which is within twenty miles of the south end of Lake Tanganyika, I found Planorbis and Limnæa. In Mwero there have been recorded Unio, Ampullaria, Lanistes, Vivipara, Cleopatra, Bythinia, and Melania; while in Bangweolo, according to Lieutenant Weatherly, there are no shelled molluscs, but it is hardly credible that their absence in this lake will be maintained. It is thus certain that the generic forms occurring in Nyassa completely cover the mollusca in a very large number of African

[1] 'Ann. des Sci. Nat.,' septième série, ix, x, 126, 1890.

lakes indeed. In the Victoria Nyanza all the Nyassan genera have been recorded, and more or fewer of the constituents of the same Nyassa list constitute the faunas of the remaining members of this more northern group of lakes,—as, for example, the Albert Nyanza, the Albert Edward, Beringo, and the like.

To facilitate comparison I have arranged the names of all the lakes about which anything definite is known in the tabular form given on p. 166. On the left-hand side will be found a list of all the genera hitherto known to be contained in each. From this table it will be seen that more or fewer members of the Nyassa list of genera are contained in every lake, but that there is a curious reduction of the number of the genera as we pass from the greater lakes to the less. This is probably due to the impermanence of the conditions in the smaller lakes, for we find in Shirwa, which is salt, and in Kela, which has periodically dried up, only those forms which can stand a wide amount of change. If we pass momentarily, however, from the study of the genera among the lakes to that of the specific forms, it will be found that there is a certain amount of variation in the specific representation in the genera contained ; and that when the lakes are widely separated—as, for example, Nyassa and Lake Mwero—such specific variations are often strongly marked. Judged, however, by the genera alone, it will be seen that there is a remarkable uniformity in the character of the African fresh-water molluscs over an immense area of ground. To this rule of uniformity in type which characterises the molluscan fauna of all the lakes about which anything is known, Tanganyika seems to form a solitary and striking exception. But the differences which this lake presents are in one sense more illusory than real, for on inspection of the table it will be seen that Tanganyika does contain, and fully represented, the great lake list of molluscs found in the Nyassa to the south, and the Victoria Nyanza to the north. It differs from the other lakes in there being here added to the otherwise universal list a number of entirely new forms. The genera which compose this superadded series

comprise among others the six genera of Gasteropods which have been known hitherto only by their empty shells, namely, Typhobia, Paramelania, the so-called Lythoglyphus of Tanganyika, Syrnolopsis, Nasopsis, and Limnotrochus. I was, during my recent expedition, enabled to add to this isolated series at least two entirely generic forms, for which I have proposed the names Bathanalia and Bythoceras. We have, therefore, now in Tanganyika some eight genera of Gasteropods which are not found in any of the other lakes, and to this isolated list of molluscs there should probably be further added among the Lamellibranchiata the so-called Unio Burtoni, and one of the Tanganyika Spathas. Consequently there are now known to exist in Tanganyika ten genera of molluscs, which appear to be restricted to the lake in which they were originally found.

Although I am concerned here primarily with the distribution of the molluscs in these lakes, it must be clearly understood that the marine organisms, such as jelly-fish, crabs, prawns, sponges, and Protozoa, with which the above molluscs are associated, share equally the same geographical limitation. The ten quasi-marine molluscan genera being, in fact, only one section of a complete fauna, containing widely separated types, which in Tanganyika exists along with the normal fresh-water stock the lake contains. The fauna of Tanganyika is thus a double series, and to distinguish its apparently marine constituents from the more normal lake animals I shall speak of them in future as the Halolimnic group.

Now it can be confidently affirmed that there are no Halolimnic animals in Nyassa, Shirwa, or Kela, all of which I visited and dredged; and they are certainly not present in Bangweolo or Mwero. Yet these organisms are so conspicuous and common, when they do occur, that they would certainly have been recorded from the Victoria Nyanza, the Albert Edward, and the Albert, if any Halolimnic animals had existed in these more northern lakes. So far as is at present known, then, the Halolimnic fauna is entirely restricted to the confines of Tanganyika, in which lake it was

originally found. The remarkable isolation and independence of the Halolimnic fauna which these facts disclose is

TABLE I.—Showing the Molluscan Genera which have hitherto been recorded in the Principal African Lakes.

	Rukwa.	Rudolph.	Stephanie.	Chad.	Kela.	Bangweolo.	Baringo.	Mwero.	Shirwa.	Albert Edward Nyanza.	Albert.	Nyassa.	Victoria.	Tanganyika.	
Unio	...	...	...	...	...	...	...	—	...	...	—	—	—	—	Normal Lacustrine Series.
Spatha	...	...	...	...	...	...	...	...	...	...	...	—	—	—	
Corbicula	...	...	...	...	...	...	...	...	...	—	—	—	—	—	
Iridina	...	...	...	...	...	...	...	...	...	...	...	—	—	—	
Limnæa	...	...	...	...	...	...	—	...	...	...	...	—	—	—	
Isodora	...	...	...	...	...	...	...	...	...	...	...	—	—	—	
Physopsis	...	...	...	...	...	...	...	...	...	...	...	—	—	—	
Planorbis	...	...	...	...	—	...	...	..	—	...	—	—	—	—	
Ancylus	...	...	...	...	...	...	...	...	...	...	...	...	...	—	
Ampullaria	...	...	...	...	—	...	?	—	—	—	—	—	—	—	
Lanistes	...	...	...	...	...	...	...	—	...	...	...	—	—	—	
Vivipara	...	...	...	...	...	...	...	—	—	—	—	—	—	—	
Cleopatra	...	...	...	...	...	...	...	—	...	...	—	—	—	—	
Bithynia	...	...	...	...	...	...	...	—	...	—	—	—	—	—	
Melania	...	...	...	...	...	...	?	—	—	—	—	—	—	—	
Neothauma	...	...	...	...	...	...	...	...	...	...	...	...	...	—	Halolimnic Group.
Typhobia	...	...	...	...	...	...	...	...	...	...	..	...	...	—	
Nasopsis	...	...	...	...	...	...	...	...	...	...	...	...	...	—	
Limnotrochus	...	...	...	...	...	...	...	...	...	...	...	...	...	—	
Syrnolopsis	...	...	...	...	...	...	...	...	...	...	...	..	...	—	
Lithoglyphus	...	...	...	...	...	...	...	...	...	...	...	...	...	—	
Bathyanalia	...	...	...	...	...	...	...	...	...	...	...	...	...	—	
Paramelnia	...	...	..	...	...	...	...	...	...	...	...	...	...	—	
Bythoceras	...	...	...	...	...	...	...	...	...	...	...	...	...	—	

Lake Dumi contains Ampullaria and Mutela. Lake Elmentella, Limnæa and Physa. I have not thought it worth while including these small lakes in the above list.

of the first importance when we attempt to ascertain from what source they may have sprung; it is extremely important, therefore, that the conclusions which result from the study of the geographical distribution of these forms can be corroborated from another point of view.

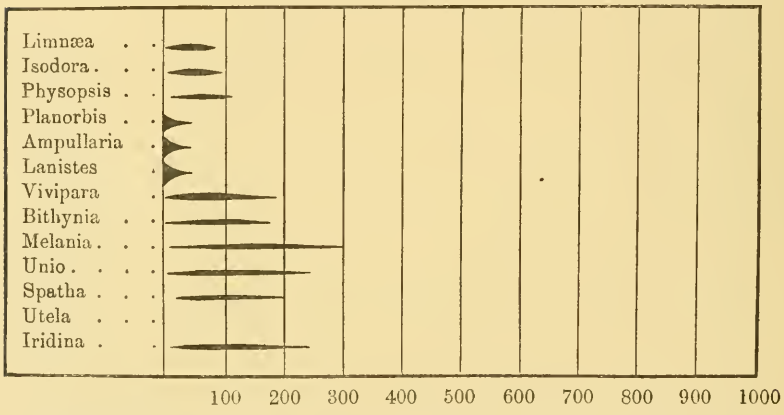
When comparatively examined, the observations which I was enabled to make respecting the bathymetric distribution of the molluscs in the lakes will be seen to show quite as clearly as the facts of their geographical distribution that the Halolimnic series is something entirely distinct from the normal fresh-water population of the lake. Through the kindness of Sir Harry Johnston I was enabled to attach myself to one of the Nyassa gunboats, and thus to become acquainted with the facts of molluscan distribution in Nyassa before I reached Tanganyika on my way north; and the observations made on this voyage have been of the utmost value as material for comparison with what I subsequently saw.

Nyassa is a relatively narrow lake of great and unknown depth; soundings of 300 fathoms, no bottom, having been obtained throughout a great proportion of its area. In total length it covers some 340 miles, and it varies from 20 to 40 miles across. The shores are of the most varied description, steep and precipitous in some places, in others bounded by extensive flats. The lake has a free outlet down the Shire River and the Murchison cataracts, the water being consequently clean and fresh. Owing to the lake's great extent, the shores are often swept by a heavy surf, and the fairly strong currents which are observed are probably produced by the trade winds, for they seem generally on the surface to set from south to north. The fauna of such a lake is exposed to the same conditions as those to which it would be on an open oceanic coast. The enormous depth of the lake in many places rendered it impossible to dredge, and whether with an efficient deep-water apparatus anything further could be obtained from the abysmal recesses it is impossible to say. But it was very soon apparent that the molluscan portion of the population rapidly thinned out with increasing depth and distance from the coast, and that beyond 100 feet one could often dredge for miles over rocks and sand and mud without securing a single shell.

In many places the lake was floored with compact drifted masses of shells and shell-fragments, consisting chiefly of the

Nyassa Viviparas. No sponges grew upon these shells, and beyond an occasional Melania such stretches of the lake were without life of any sort or kind. The curious diminution of the molluscs of Nyassa beyond the immediate coast-line is a striking feature of the lake throughout, and a tolerably correct idea of the bathymetric distribution of the individual genera will be gathered from the accompanying table, which contains an epitome of the observations made.

TABLE II.—Bathymetric Distribution of Molluscs in Nyassa.



The numbers at the foot indicate the approximate limits of depth in feet at which the molluscan genera are found, and the thickness of the lines shows in what depth of water they most abundantly occur.

The lines representing the bathymetric extent of each genus are thickened so as to show where it is found in the greatest abundance, and approximately at what depth it ceases to exist.

From this table it will be seen that the purely fresh-water molluscan population of Nyassa is more or less completely restricted to a littoral band along the shores, and that the great majority of the genera do not extend in depth beyond 200 feet.

Thus Lake Nyassa, so far as molluscs go, is thinly peopled, and great extents of its shore and deep bottom are altogether uninhabited. The above facts of distribution, indeed, give the

impression that the molluscan population of Nyassa retains its character of an importation from the ponds and streams which in the vast lake is, as it were, completely out of place.

Let us now turn to Lake Tanganyika, and compare the distribution of the molluscs in this lake with the observations I have just described in relation to Nyassa. But let me first point out that the physiographical features of Lake Tanganyika are slightly different from those obtaining in the great Nyassan valleys. Tanganyika is 2700 feet instead of 1500 feet above the level of the sea; but notwithstanding this greater elevation, the climate of Tanganyika is appreciably hotter than that of Nyassa.

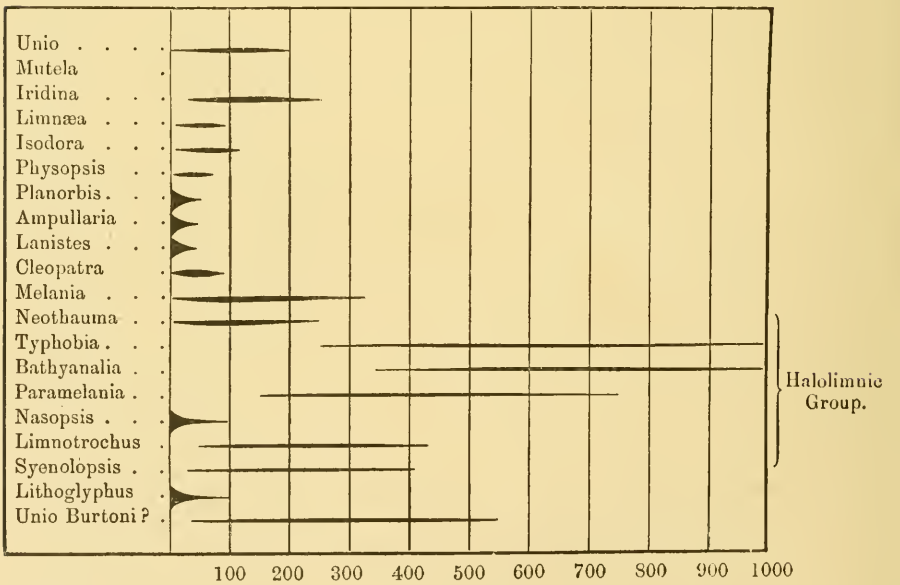
The shores of Tanganyika are, perhaps, on the whole more precipitous, and are certainly less extensively fringed with the broad coast belts of modern alluvium so characteristic of the margin of Nyassa. The southern half of Lake Tanganyika is not nearly so deep as Nyassa, the water being generally little more than from 900 to 1200 feet in depth; and it is not so pure, being always impregnated with an appreciable taint of several salts.

These slight physiographical differences which exist between Nyassa and Tanganyika cannot, however, be considered as having any potency to modify the individual fauna which the lakes contain. For there is a far greater difference between the physiographical features of Nyassa and the Victoria Nyanza than between Nyassa and Tanganyika. Yet the molluscan population of both the Victoria Nyanza and Nyassa are essentially the same. We cannot, therefore, regard the less amount of difference which is perceptible between Nyassa and Tanganyika as in any way responsible for the wide faunistic differences which exist between these lakes. This view is also in accordance with the very important fact that all the genera found in Nyassa exist in Tanganyika also. There is no more difference between the normal, non-Halolimnic molluscan population of Tanganyika and Nyassa than there is between that of Nyassa and Victoria Nyanza—a state of things which seems to indicate clearly that the difference in physiographical

features which exists between these lakes are incapable of greatly modifying the forms they contain. Therefore whatever wide difference in type and mode of distribution is apparent between the faunas of Nyassa and Tanganyika must be the expression of the difference of the animals themselves, and not of the slight differences of condition under which they live.

In confirmation of this view, we find that all the Nyassan molluscan genera are distributed both in Tanganyika and Nyassa in a similar way, i. e. they are more or less restricted to the coast-line and the shallow, sheltered places, such as creeks and bays. For comparison my observations on the bathymetric distribution of the molluscs in Tanganyika have been

TABLE III.—Bathymetric Distribution of Molluscs in Tanganyika.



The numbers at the foot indicate the approximate limits of depth in feet to which the molluscan genera known to occur in Tanganyika extend, and the thickness of the individual lines shows at what depth each genus most abundantly occurs.

epitomised in the same tabular form I used while speaking of Nyassa. From this table it will be clearly seen that if we exclude the Halolimnic fauna altogether, the bathymetric distribution of the molluscs both in Nyassa and Tanganyika is approximately the same. In both cases the fresh-water molluscs are restricted to the sheltered, shallow portions of the lakes. But directly we pass from the consideration of the normal fauna to that of the Halolimnic forms the most striking changes are at once observed. Instead of the Halolimnic molluscs being restricted to the shallow creeks and bays about the coast, they swarm on the rough surf-swept rocks and on the open beach. And what is more remarkable than this, they extend in great profusion to the deepest portions of the lake. Thus, dredging in water which varied in depth from 800 to 1200 feet, I always obtained plenty of Typhobia, Paramelania, Bathynalia, and Bythoceras among the Gastropods, as well as the so-called Unio Burtoni among the Lamellibranchiata; and how far these genera extended beyond these depths I cannot say, but they showed no signs of dying out, but rather the reverse. On the lake floors which were not so deep as this, from 200 to 300 feet below the surface, but which were yet deep enough to have yielded nothing by dredging in Nyassa, there was an abundance of Limnotrochus, Syrnolopsis and Neothauma, together with those varieties of Melania which inhabit Tanganyika. It is thus rendered apparent by these observations that the Halolimnic molluscs are all either surf-swept rock dwellers, or entirely deep-water forms. Unfortunately we are as yet entirely ignorant of the distribution of the molluscs in any of the great lakes besides the two which I have named. But as the normal fresh-water fauna of Nyassa and Tanganyika have the same bathymetric distribution, it is probable that these same genera inhabiting the remaining lakes which have not yet been investigated will be found to be similarly disposed.

It is thus apparent that the Halolimnic molluscs are completely dissociated from the normal fresh-water forms, along with which they exist in Tanganyika, not only by their singular

geographical isolation, but by their bathymetric distribution also; the conclusions to which the facts of their geographical distribution seem to point being thus completely substantiated from another point of view.

There are, however, yet other ways in which the fact that the Halolimnic fauna is entirely distinct from, and unconnected with the more normal series becomes clear. For in many branches of biological inquiry we are often rightly guided by impressions which, like the types of human physiognomy, are real enough, but quite incapable of definite expression. Impressions of this character are at once produced on reaching Tanganyika, as I did, after studying the fauna of several neighbouring lakes. For there is a singular and oceanic profusion of life in Tanganyika, which is quite peculiar, and it quickly becomes evident that this numerical increase in the aquatic population does not affect the normal fresh-water stock, it is solely produced by the astonishing abundance of the members of the Halolimnic group. In contrast with the shallows of Nyassa, the creeks and bays of Tanganyika swarm with crabs and prawns, and the open sandy beaches are strewn with empty Halolimnic shells; dead detached fragments of the deep-water sponges are tossed up by hundreds on the shore. And on the extensive rocky coasts the barely submerged stones are covered with the so-called Lithoglyphus and Nasopsis, just as the half-tide rocks swarm with Natica and Litorina on an English beach. Further, on putting out into the lake itself, the deep open water is filled and discoloured with clouds of pelagic Protozoa (chiefly Peridinia and Condylostoma); and during the dry season swarms of the lake jelly-fish are seen pulsating at all depths.

Recapitulating, it may be said, then, that the facts of the geographical and bathymetric distribution of the great lake molluscs lead to the following results:—That among all the fresh-water lakes of the African continent which have hitherto been explored there exists a type of fauna which is curiously similar throughout. It differs only in the specific representation of the same genera which these lakes contain.

This generalised African lake fauna contains only those families and genera of molluscs which would be regarded as typically fresh-water, lake, river, and pond dwellers, in whatever continent the fresh water might occur. In one African lake, however, but in one lake only, there have been found to exist, superadded to this normal lacustrine stock, a number of Gastropods which do not closely resemble any other forms either living or extinct; these molluscs are also completely dissociated from the remaining normal series of the lake in which they occur by their modes of life. Together these molluscs constitute the molluscan section of a whole faunistic series, which in Tanganyika is added to the normal fresh-water stock the lake contains. This fauna forms what I have called the Halolimnic group, and the tout ensemble of all the Halolimnic genera is marine.

To account for the presence of the Halolimnic organisms in Tanganyika, only three hypotheses which are even temporarily tenable can be found. It may be supposed—

1. That they have arisen as modifications of the ordinary fresh-water fauna through prolonged isolation in the lake;

2. That they are the surviving representatives of an extinct fresh-water stock; or—

3. That they are comparatively recent importations from the sea.

Let us examine each of these three possible explanations in the light of the new facts of distribution which have just been detailed. Unless the conditions affecting the fauna of Tanganyika have been permanent for a greater period of time than has been the case with any of the other lakes, they could not have produced the Halolimnic fauna which this lake now presents. Unless we make the further suppositions (1) that the conditions in Tanganyika have been permanent, while those affecting the fauna in all the other lakes have changed so much as to kill off the Halolimnic forms they once possessed; or (2) that all the other lakes are much younger than Tanganyika, and that therefore the Halolimnic fauna has not had time to develop in them yet. There is no evidence for either of these

views, and there is direct evidence to show that Nyassa has been a fresh-water lake longer than Tanganyika. On the shores of Nyassa there are old raised beaches, forming white limestone cliffs which contain the fossilised remains of the shells now living in the lake. But in these old lake beds there are no traces of any Halolimnic forms, and this is all the more conclusive as the shells of the Halolimnic molluscs are much more solid and durable than those of the fossilised fresh-water forms.

The second hypothesis, that which suggests that the Halolimnic fauna may be the surviving representative of an ancient fresh-water stock which has become extinct, has great attractions, as it conforms to a famous geological speculation, and has at first sight the appearance of a certain modicum of positive support. For the shells of the Paramelanias of Tanganyika have been independently supposed by White and by Tausch to be identical with the extinct estuarine or brackish Pyrguliferas of cretaceous Europe, America, or Africa.[1]

The type of shells possessed by these forms has been, however, repeated so often by so many widely separated molluscan types, such as in the Melanias, Litorinas, Pur-

[1] On further examination it appears—(1) That the genus Paramelania of Tanganyika is similar to the cretaceous Pyrgulifera; (2) but that the genus Pyrgulifera, so far as some of its representatives go, is conchologically indistinguishable from the old marine Jurassic genus Purpurina, and that the Nanopsis of Tanganyika corresponds to one section of this genus, the Paramelania to the other. (See Hudleston's figs., Plates i and ii, and text p. 85—95, 'Jurassic Gasteropoda,' Palæontographical Society, vol. xli, 1887.) It would thus appear that the marine genus Purpurina became a fresh-water form, as so often happens in Cretaceous times. We find, however, that other Halolimnic Gasteropods, Bathanalia, the so-called Lithoglyphus, and Limnotrochus, are also indistinguishable from marine Jurassic forms, which are not found in any Cretaceous formation, fresh-water or marine. Consequently the geological evidence on this matter distinctly favours the old marine origin of the Halolimnic fauna; but it places their original marine existence much further back than I had even dared to suggest. I shall discuss this most interesting line of investigation fully in a special memoir.

purinas, and the like, that it is pardonable if zoologists require something more than merely conchological characters to establish an identity among these forms.[1] But this supposed homology between the shells of a living and extinct species of Gasteropod (about the anatomy of neither of which up to the present anything whatever has been known) is the one fragment of positive evidence which can be produced in favour of the relation of the Halolimnic fauna to an extinct fresh-water stock.

The hypothesis, moreover, is combated by the same objections emanating from the facts of distribution of the Halolimnic animals that were fatal to the first hypothesis, and they have here equal force. If the Halolimnic fauna of Tanganyika is the remnant of an old African fresh-water stock, it must have been present at one time in all the lakes which are as old as Tanganyika; but we have seen that with respect to Lake Nyassa this does not appear to have been the case. It is very improbable that many of the remaining so-called rift-valley lakes are not as old as Tanganyika, yet we have seen that they do not contain the Halolimnic forms. Therefore, in order to support this second hypothesis, we shall be obliged to have recourse to hypothetical catastrophes which must be supposed to have destroyed the Halolimnic fauna in every lake but one. Hypotheses of this sort spring, however, from the carcass of a theory only after it is dead, and our second hypothesis is therefore opposed to the facts of distribution as they at present stand; its acceptance would, moreover, be revolutionary to many zoological conceptions of the present time. It would necessarily lead us to believe that deep-water crabs may be indigenous fresh-water forms; that deep-water Gastropods and sponges were common in Cretaceous times; that jelly-fish were once fresh-water organisms, and so on through a number of consequences, which, when the nature of the evidence supporting the original hypothesis is weighed, must seem little better than grotesque. On the other hand, all the facts of

[1] I am quite aware that this statement cuts at the roots of many geological determinations; but I am prepared to maintain that the criticism is sound.

distribution and the like, as well as the superficial character of the Halolimnic animals themselves, are absolutely in accord with the third hypothesis, i. e. that the Halolimnic fauna is a relatively recent importation from the sea.

But before accepting this conclusion, as the natural teaching of the facts and observations which we have been discussing, it is absolutely necessary to be quite sure that in the nature of the country itself—that is, in the past geological history of Africa—there is nothing which renders impossible the realisation of such a theory in fact. Now, on turning to the geological aspect of the questions we have just discussed, it is apparent that there is an accumulation of negative evidence drawn from what is now known to geologists of the nature of the African interior, which, although it does not specifically favour the view of the ancient fresh-water origin of the Halolimnic forms certainly renders evident a gap in the confirmation of the theory of their marine origin.

In 1852 Sir Roderick Murchison[1] advanced the hypothesis that Africa, south of the Sahara, was a continent of great antiquity and simplicity, the greater part of which has never been changed or covered by the sea, at any rate since the age of the formation of the new red sandstone. This theory has appeared to be supported by the discoveries of Livingstone, Burton and Speke, and Speke and Grant, and it was finally re-advanced and summarised by Murchison in 1864,[2] when he described this part of Africa as geographically unique "in the long conservation of ancient terrestrial conditions." But he immediately fell into the now exploded error of assuming that "this impression is further supported by the concomitant absence throughout all the larger portion of this vast area, i.e. south of the equator, of any of those volcanic rocks which are so often associated with oscillations of the terra firma." This latter speculation is now shown to be in no sense true, for there is abundant evidence of volcanic action and of volcanic materials all the way from Kilimanjaro and

[1] Murchison, President's Address, 'Journ. Royal Geog. Soc.,' vol. xxii, 1852.

[2] 'Journ. Royal Geog. Soc.,' vol. xxxix, 1864, pl. xxxvii, pp. 201—205.

Ruwanzori in the north, to the little group of volcanic cones near the coast of Lake Nyassa. "The first part of Murchison's theory, however, which affirms that Central Africa has never been below the level of the sea, is still in harmony with the known geological facts, for no deposits of a certainly marine origin have as yet been discovered in the interior." The sedimentary rocks described by Burton and Speke to the west of the Victoria Nyanza have yielded no fossils to indicate the conditions under which they were formed. The triassic Ganoids and Gastropods unearthed by Drummond[1] at the north end of Lake Nyassa have been generally regarded as fresh-water forms.[2] The great red sandstones and shales which stretch from the north of Nyassa far up the coasts of Tanganyika, which were examined by Joseph Thomson, and more recently by myself, have not yet been found to contain any animal forms; the only indication which might lead to a belief that fossiliferous rocks occur in these regions being the fact that the natives of the west coast of Tanganyika are said to wear necklaces of beads which they dig out of limestone rocks, and which, if this statement is true, are probably the disarticulated segments of crinoid stems.

Marine, Jurassic, triassic, and probably carboniferous deposits have been found along the coast at many points from Mombasa to the Cape, but these have never been shown to extend any distance inland, and they seem to have no connection with the great sedimentary deposits of the interior, such as those north of Lake Nyassa, which underlie Drummond's fresh-water triassic (?) beds. There is thus at present no geological evidence of the sea, or of even an arm of the sea, ever having been in the region of Tanganyika within reasonable geological times.

There has, however, been steadily accumulating a mass of observations relating to the formation of the so-called rift valleys, the general tenor of which has been to reveal a

[1] Drummond, 'Tropical Africa.'

[2] It appears, however, that these fossils have been by no means satisfactorily described.

great instability of the regions in which Tanganyika lies; an instability which has been quite sufficient to conceivably account for any amount of upheavals and depressions which may have been requisite for the marine contamination of that lake. The valleys in which the north of Nyassa, Lakes Tanganyika, Albert Edward, Albert, Baringo, Rudolph, and about twenty-seven minor lakes lie, are really part of a connected series of depressions formed by faults which run approximately north and south through an immense distance, and can be traced as far as Berbera on the Red Sea, thence north along the Red Sea shore itself, the coasts of which are to a great extent of similar formation, and they terminate finally in or about the Dead Sea, and the valleys of the tributaries of the Jordan.[1] All the country traversed by this immense series of faults from the north of Nyassa in the south, to the ancient sites of Sodom and Gomorrah in the north, is filled with native traditions of catastrophes, of floods, of earthquakes, of volcanic outbursts, and the like; and the geological investigations of Gregory [2] and others have shown that much of the above faulting and volcanic activity must have occurred, geologically speaking, in quite recent times. The existence of these singular rift-valley faults has divided the African lakes into two distinct series; one series of lakes being always, like Nyassa, Tanganyika, and Rudolph, long, narrow, and deep; the other, like the Victoria Nyanza, Bangweolo, and Shirwa, broad, shallow, and round.

We have, therefore, evidence of great geological instability in the very regions in which the Halolimnic animals now live; but, like the palæontological record, it affords no insight as to how or when, if Tanganyika ever was connected with the sea, this connection could really have been made; but it is a singular fact that the one lake in which the Halolimnic animals now live is that which lies at the bottom of the biggest and most conspicuous inland rift.[3]

[1] See Suess, 'Die Brücke des Oust Afrika.'

[2] The Great Rift Valley.

[3] The southern two thirds of Nyassa is not in a rift; and in contrast to

From all this it will be seen that, unless we are to assume that the Halolimnic group came into Tanganyika from the sea in very ancient times indeed, and that they are far older than their characters in any way appear to warrant,[1] we are without any direct evidence from geology that the sea, or even an arm of the sea, has ever been in the Tanganyika region of the interior. So far as positive evidence goes, geology is absolutely silent upon this subject, it offers no evidence of any sort; and the theory of marine contamination—if it occurred, let us say, during a later period than Jurassic times—is thus diametrically opposed to a geological theory of the nature of the African interior which is at present accepted by many competent authorities.

The only way in which the nature and origin of the Halolimnic group can be really satisfactorily determined is, therefore, through a minute knowledge of the morphology of the individual members of the group themselves, and the best types belonging to the Halolimnic group for this kind of work are the Gastropods, because these organisms, unlike the lake Medusæ, can be more or less directly compared with all sorts of analogous organisms, ancient and modern, fresh-water and salt. If it can be shown from the study of their morphology that the Halolimnic Gastropods in Tanganyika are really morphologically most closely related to the fresh-water Gastropods at present known, then the theory of the ancient fresh-water origin of the Halolimnic group is probably true. If, on the other hand, it turns out that the Halolimnic Gastropods are really most closely related to typically marine genera, then there will be little doubt that the Halolimnic group originated in the lake through marine contamination, and geological conceptions will have to make room for the fact of the interior of Africa having been connected with the sea as best they can.

In arriving at the conclusions contained in the preceding

Gregory I do not believe that the north of Nyassa lies in the main eastern rift, but in one which through Lake Rukwa is continuous with the western Tanganyika series. See Gregory's 'Rift Valley,' p. 7; also my paper in the 'Journal of the Royal Geographical Society,' September, 1897.

[1] See foot-note on page 161.

paragraphs I have virtually fulfilled the object which I had before me in collecting and examining the facts concerning the distribution of the African lake faunas, before entering upon any detailed examination of the evidence which can be gathered from the study of the morphology of the Halolimnic animals themselves. We have seen that the collateral evidence afforded by the facts of distribution and the like at once clear away the likelihood of the Halolimnic group having originated at any time or in any manner, de novo, in Tanganyika, and that there is finally brought on a more or less direct issue between the supposition of an ancient marine contamination of Lake Tanganyika and the ancient fresh-water origin of the Halolimnic group. All the facts of distribution which we have examined appear to me to strongly favour the former of these hypotheses; and although we are at present ignorant of the precise manner in which the marine contamination of Lake Tanganyika may have been effected, there is no positive geological objection to the view that it has occurred, while there is the certainty of a sufficiently great geological instability throughout the very districts in which Tanganyika lies to have easily accounted for it.

The Molluscs of the Great African Lakes. — II. The Anatomy of the Typhobias, with a Description of the New Genus (Batanalia).

By

J. E. S. Moore.

With Plates 11—14.

No entire specimen of Typhobia has hitherto been described, and we have consequently remained entirely in the dark as to the real morphological character of what is probably the most remarkable fresh-water Gastropod at present known.

Presumably from the characters of its empty shell this genus has been classed by the conchologists[1] with the Melanias, as a new sub-section of that group.[2] But into what serious error determinations of this sort may lead, when based on conchological evidence alone, the present paper, which contains the first anatomical description of the mollusc, will suffice to show.

It will be seen that the structural features of the Typhobias, so far from establishing the above conchological anticipations, in every way confirm the conclusions at which I arrived respecting the marine origin of these molluscs from a study of the distribution of the African lake fauna in general.[3] Hence the actual facts of anatomy are, as I anticipated from

[1] Smith, 'Proc. Zool. Soc.,' 1881, p. 276.

[2] Fischer, 'Manuel de Conchyliologie,' p. 705. These determinations have been particularly unfortunate, as they have masked the marine, and consequently intensely interesting character of the molluscs of the lake.

[3] "The Molluscs of the Great African Lakes." I. "Distribution," 'Quart. Journ. Micr. Sci.,' present number, p. 159.

the facts of distribution that they would be, directly in conflict with all those geological speculations respecting the interior of Africa that have been hitherto more or less generally held. In the paper to which I have referred[1] it was seen that the Typhobias belong to, and are, one of the most remarkable constituents of the *quasi*-marine or Halolimnic section of the Tanganyika fauna. They consequently share, along with the other members of this group, the strange geographical isolation which is its distinctive mark.

Like nearly all the Halolimnic animals, Typhobia is found pretty abundantly in Tanganyika, occurring in some places in the most astonishing profusion, but, so far as it is at present known, the mollusc is found living nowhere else in the world. I first obtained the empty shells of T. Horei on the long sandy beaches near the south-west corner of the lake, and subsequently on the southern shore of the deep Kituta Bay. They were readily recognised by the head men of the villages, who told me they had never seen the Gastropod alive, but only the shells when washed up empty along the beach. From this statement of the natives, and from the spinous character of the shells, I thought it probable that they would be found living on mud, but I was unable to find them in the muddy reaches among the Kinyamkolo Islands, in depths of fifty to one hundred feet, nor indeed in any portions of the lake that were of similar depth. It was not until I had extemporised a primitive deep-water dredging apparatus that I obtained the Gastropod alive.[2]

In June, 1896, we were on the west coast of Tanganyika and on the southern shores of Cameron Bay, and here I was able to obtain the strong bark rope used by the Wafipa fishermen for their nets. As these nets are hauled by rows of men on the ropes at either end, the ropes themselves are strong enough to drag a heavy net with all its weights and stretchers over several hundred yards of ground; and with them I was

[1] Loc. cit

[2] My ordinary dredges were smashed almost at once by the sharp rock-ridges which protrude through the muddy floor of the lake. For this deep water I used a native basket, weighted down with stones.

consequently enabled to dredge in water that varied from 500 to 850 feet in depth. Eventually in this manner, during the months of June and July, about a hundred Typhobias were obtained alive. Of these some were examined on the spot, some preserved in various ways, stored in spirit, and eventually brought back. The living Typhobias were associated with another deep-water Gastropod, also alive, a brief description of which will be found at the end of the descriptive part of this paper.

Except in the characters of the shell, this new genus is almost identical anatomically with T. Horei, consequently it is unnecessary that I should do more than point out in what it differs from the form already known.

External Characters.—The general appearance of a living Typhobia is seen in fig. 1. They are always very active when brought up from the deep water they inhabit, probably being uncomfortable through the decreasing pressure. The tentacles are very long and slender, and the eyes completely at their base; the snout is wrinkled and very much pigmented on the upper surface, and it is so long and slender as to suggest the ordinary protrusible snout or introvert of Prosobranchs; on dissection, however, it is seen to be simply elongated externally, very retractile, but, like that of Pterocera, not introvertible in any sense. The foot is very broad, and of the same pale semi-transparent yellow as that of Anodonta. The mantle is prolonged into the well-marked anterior and posterior siphons (fig. 2).

As is the case with many other fresh-water Gastropods, the shells of Typhobia Horei vary to a remarkable degree; indeed, the extreme forms when isolated, and not linked together by the innumerable intermediate forms which actually occur in Tanganyika, differ so widely that the French conchologist[1] Bourguignat regarded these differences as sufficient to split the genus up into four species, under the new name of Hylacantha; his four so-called species being respectively H. Horei, H. Bourguignati, H. longirostris, and H. Jubertii. Had this author, however, been able to obtain

[1] 'Ann. des Sci. Nat.,' septième série, ix, x, 1890, p. 125.

large numbers of these shells on the spot, and to have made collections of the extreme and intermediate varieties, it is hardly conceivable that he would have ever regarded their variations as specifically distinct. Be this as it may, however, it is quite evident, when large numbers of these shells are studied, that their varieties cannot be regarded as specific forms. The shells of Typhobia Horei are, however, undoubtedly polymorphic, for there are about four well-marked varieties, into one or another of which the great majority of the specimens I collected tend to fall (figs. 13, 16, 23, 25). When the shells are very young, about the time of birth (figs. 27, 28), they are destitute of all but the merest trace of spines as well as of the pronounced rostral beak, which is a marked structural characteristic of the majority, but by no means of all the older shells. Now the fact that in some adult shells the rostral beak is entirely wanting (figs. 16 and 20) shows that, in this respect, such shells have not deviated from their embryological character. All the four extreme types of variation graduate off into forms which, in the more or less complete absence of a rostral beak, approximate to the shells represented in figs. 16 and 25. Such shells, therefore, may be said to represent the extreme of least specialisation. The remaining extreme polymorphs (figs. 13, 23) can be all traced through successive stages from the types represented in figs. 12, 17, 18, and 21. Thus between the extremes, figs. 13 and 16, there are intermediate forms, such as figs. 12 and 17; the extreme, fig. 23, has intermediates, such as figs. 18 and 21, while the extreme, fig. 25, is connected up by forms similar to that represented in fig. 20 and perhaps 21. Any number of intermediate stages could have been represented for each series; but for obvious reasons those only have been selected which seemed to best express the transition from any one type to the next. It would thus appear that Bourguignat's four species were formed in the absence of an adequate quantity of material to work upon, and this conclusion is finally clinched by the fact that the anatomical characters of the soft parts of the extreme variations are indistinguishable from one another.

We may, therefore, conclude that the name Typhobia Horei, as given by Smith,[1] stands rightly for but one species after all.

The nervous system.—The nervous system of Typhobia, both in its general relationships and in the details of its different constituent parts, is almost entirely unique. Viewed from above, it is at once obvious that there is a great condensation and fusing together of the chief ganglionic masses, no commissure being visible externally between the cerebral ganglia (fig. 5, *5*). Each cerebral ganglion gives off anteriorly two sets of nerves, one obliquely above the other (fig. 34, *1, 3*); the upper and external arises from a prolongation of the ganglion comparable to the "saillie labiale." These nerves are distributed to the tentacles and the eyes. From each cerebral ganglion below the "saillie" there arises another set of buccal nerves, the two innermost of which pass forward, enlarge into the buccal ganglia (fig. 34, *18*, and fig. 5, *1*), and unite again below the mouth. The remaining members of this set of nerves are distributed to the buccal mass, and to the parietes of the anterior portion of the head and snout. Ganglionic cells extend along the buccal nerve trunks as far as the buccal ganglia. Laterally each cerebral ganglion gives off a number of small nerves distributed to the head (figs. 34, *19*). Towards the posterior upper surface of the cerebral ganglion the paired otocyst nerves arise, and pass obliquely backward over the pleuro-pedal commissures to the enormous otocysts (fig. 35, *6*).

Below, the cerebral ganglia are connected with the pedal ganglia by the rather long cerebro-pedal commissures (figs. 6, and 35, *20*). Immediately behind these there is found on each side a second commissure, which at first sight appears to pass from the cerebral ganglion also (fig. 35, *11*). In reality this is the pleuro-pedal commissure, the pleural ganglion being displaced forward, so as to lie closely applied to and immediately beneath the cerebral ganglia. In order to understand the relations of these ganglia it is necessary to examine several sections, as they cannot be seen by the ordinary methods of dissection. Fig. 9 represents a section taken through a point marked *X* in

[1] 'Proc. Zool. Soc.,' loc. cit.

the general figure of the nervous system given in fig. 35. It shows the cerebral ganglion (*a*), separated from the pleuro-pedal commissure (*b*), while the fore-part of the pleural ganglion is seen as a continuation of this commissure (*c*). Fig. 10 is a little further back, and shows the pleural ganglion (*a*) and the posterior portion of the pleuro-pedal connective (*b*) But the section is still in front of the connection between the pleural and cerebral ganglia, both ganglia appearing separate. Fig. 11 is slightly further back again, and shows the pleuro-cerebral connective (*a*). There is visible also the posterior portion of the cerebral ganglion immediately before it passes downwards and is merged in the pleural ganglion itself. The pleural ganglia are thus seen to be displaced, and their real position is indicated by shading in the general arrangement of the nervous system (figs. 34 and 35). The pleuro-pedal connective is consequently shorter than it would be if the pleural ganglia were in their normal positions, while the pleuro-cerebral connective, as such, may be said to be almost entirely wanting.

On the left, the pleural ganglion is continued below and very slightly across the subœsophageal space (figs. 5, *6*, 6, *3*, 34, *16*), as an enormous ganglionic trunk, in the course of which the subintestinal ganglion is superficially quite indistinguishable, but the locus of this ganglion is marked by the great pallial nerve (fig. 5, *7*). The ganglionic character of this left cord continues, as is shown by the presence of ganglionic cells, for a long distance, the appearance it presents at the point marked x′ (fig. 35) being represented in section at fig. 37, *1*. The right pleural ganglion gives off a relatively small nerve, which, after passing obliquely over the œsophagus, carries the supra-intestinal ganglion, from which nerves branch to the gill, osphradial ganglion, and to the left pallial anastomosis (figs. 34, 35). This anastomosis is formed in the usual way by a rather large nerve passing out from the left pleural ganglion, and meeting the branch from the supra-intestinal ganglion near the angle of the gill (fig. 35, *16*). The nervous system is, therefore, dialyneurous on the left, and the relations of the nerves here give no indication of the

extraordinary state of asymmetry which is encountered in the same region on the right. On this side the pleural ganglion gives off a nerve (fig. 34, *8*) which appears at first sight as if it would form the right pallial anastomosis, either in the region of the subintestinal ganglion or along the course of the right pallial nerve. This nerve, however, after passing directly outwards for some distance bends sharply forwards, branches once, and each of the twigs diminishes rapidly, and dies out in the parietes of the mantle and the body-wall. The great mantle nerve which is given off from the subintestinal ganglionic trunk (fig. 34, *10*) passes outwards, and is also distributed, without forming any connection with the pleural branch, to the mantle of the right side. Neither is there any connection between the pleural and subintestinal ganglion beneath the œsophagus. We have thus on the right side a condition of things which is almost unique among all the Streptoneurous Prosobranchiata which have hitherto been investigated. It is neither zygoneurous nor yet dialyneurous, and the condition of these parts finds its only analogy in the rather unsatisfactory descriptions given by Bouvier [1] of the nervous system of Solarium and the Scalarids. In general arrangement, and apart from the above singular feature, the character and arrangement of the cerebral, pleural, and intestinal ganglia with their nerves and connections show a marked and indisputable similarity to those of the corresponding parts in such forms as Strombus, Pterocera, Cancellaria, Voluta, and their associates. The wide distribution of the ganglionic cells in the nerve-cords of Typhobia is a remarkable and undoubtedly primitive feature; while the fact that the nervous system of Typhobia foreshadows and is similar to those of several rather widely separated modern marine genera is direct and incontrovertible evidence, so far as it goes, that these molluscs are old and modified marine forms.

The pedal ganglia project forwards and are curiously extended in front by two colossal nerves (fig. 35, *13*), which at their points of origin possess ladder-like connections one with

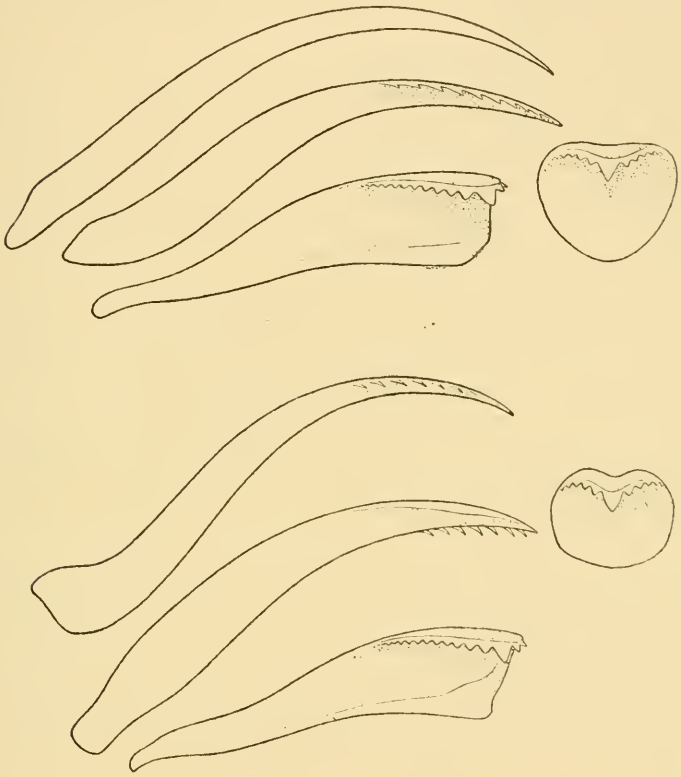
[1] 'Ann. des Sci. Nat.,' iii, iv, 1887, pp. 156—167.

another (fig. 35, *14*), and thus approximate to the primitive type of pedal nerves possessed by the Helicinidæ. The pedal ganglia themselves are united together by a great transverse commissure (fig. 35) which contains ganglionic cells; and on their postero-lateral surfaces they give off four or five large nerves which pass into the foot. The pedal ganglia are connected with the cerebral, and the pleural ganglia by the cerebro-pedal and pleuro-pedal connectives already described.

The otocysts of the Typhobias are relatively immense, and each is innervated by two fine nerves springing from the upper portion of the cerebral ganglia (fig. 35, *6*). The position occupied by the otocysts is very anomalous, being completely above and separated from the pedal ganglia, close to the cerebro-pleural ganglionic mass (fig. 35, *10*). The otoliths are numerous and small (fig. 36), and the otocyst is lined by a well-marked sensory epithelium, from the free internal surfaces of the cells composing which there are given off fine "sensory processes" projecting into the cavity of the sac (fig. 36, *1*). The position and character of the otocysts, and the prolonged pedal ganglia, are features in keeping with the generally primitive characters which the other parts of the nervous system seem to possess.

The digestive system presents points which, like those appertaining to the nerves, are at once interesting and new. The buccal mass is exceedingly small, and the radular sac is very short. There are no horny jaws, and the radular dentition is unique and peculiar in the extreme. A single transverse row of teeth is represented in fig. 43; also in the upper figure on page 189. The salivary glands are long and branched, the œsophagus being long, slender, and longitudinally folded. Internally it is lined by ciliated and glandular cells. Posteriorly the œsoph agus opens into the right side of the stomach, which is divided into an anterior and posterior chamber, the œsophagus opening into the latter. This posterior chamber of the stomach is traversed by several marked folds, the most conspicuous of which extends longitudinally (fig. 44, *7*). On the floor of the stomach, to the right of this fold, are found the openings of the

" bile-ducts " (fig. 44, *2*, *3*). The anterior chamber is separated from the posterior chamber of the stomach by a constricted annulus, and the anterior chamber encloses and is almost filled by a



Central and three lateral teeth of the radula of Typhobia (upper figure) and of Bathanalia (lower figure). Three lateral teeth on each side and a central tooth constitute the unit, which is repeated row after row along the radula.

large crystalline style, represented in fig. 44, *5*). The whole arrangement of the stomach, the position of the folds and apertures, the separation into a posterior and an anterior chamber or cæcum, and the presence in the latter of a crystalline style are all similar to the condition of things obtaining in Pterocera. The style and its sac are undoubtedly homologous with the

structures described by Collier,[1] Huxley,[2] Haller,[3] and others, and the morphological conclusions which can be drawn from the nature of the stomach are in harmony with those which I have pointed out in reference to this Gastropod's ganglia and nerves (see p. 187).

On leaving the stomach the intestine bends twice in the manner represented in fig. 42, and towards its rectal extremity it is considerably enlarged (fig. 49, *1*). This enlargement contains the curious glandular fold represented in fig. 46, 1. The anus is carried on a slight projection of the rectum from the mantle wall, and during life is slightly in advance of the margin of the mantle (fig. 2, *7*).

The "Liver" occupies the lower portion of the upper whorls of the shell (fig. 2, *6*), and has the usual characters of a digestive gland. The "bile-ducts" open by two orifices in the floor of the stomach, behind the pyloric aperture.

The Kidney occupies the region behind and to the left of the heart (fig. 3, *6*), and opens by a single minute pore, quite at the posterior extremity of the mantle cavity.

The Heart and Gills.—The heart is simple, lying rather obliquely at the end of the mantle cavity. There is a large pericardial chamber (fig. 3, *5*). The ventricle tapers from before backwards, and is surmounted by a large, rather thin-walled auricle, which in turn receives the pulmonary vein. There are well-formed valves between the auricle and the ventricle (fig. 48, *1*), and between the ventricle and the aortic trunk (fig. 48, *4*). From the aortic trunk the anterior and posterior aortæ diverge in the usual way (fig. 48, *7*, *8*).

The gill in Typhobia (fig. 3, *7*) is very long, extending from the base to the margin of the mantle cavity. It is composed of simple broad-based triangular leaves, the apices of which are elongated. The osphradium lies at the base of a groove; it is long and simple, not fimbriated or gill-like, in fact a mere ridge (fig. 3, *8*). This ridge is innervated by a

[1] Collier, 'Edin. New Phil. Journ.,' vol. vii, 1829, pp. 230, 231.

[2] Huxley, 'Phil. Trans.,' 1853, p. 10.

[3] Haller, 'Morph. Jahr.,' Bd. xix, 1893, pp. 582—584.

nerve which springs from the small osphradial ganglion. Externally the ridge is covered with ciliated and glandular cells, the relations of which and the characters of the osphradial nerve are shown in section in fig. 8. There is nothing peculiar about the gills except their great length.

The Reproductive Apparatus.—In Typhobia the sexes are distinct and the female viviparous, the whole reproductive apparatus being simple but somewhat peculiar. The ovaries and testes occupy the upper surface of the last two whorls of the spire, and in the female the eggs, with their bright green yolk, pass directly into the simple oviduct (fig. 47, *1*). From this they reach the lower expansion of the oviduct (fig. 47, *2*); and in this sac, which functions as a brood chamber or uterus, they go through the greater part of their development. The walls of the sac are very thin, and while the animal is alive the bright green yolk of the eggs is distinctly seen through the delicate semi-transparent shell, so that the sexes can be distinguished at a glance. The sac opens near the rectum, at the junction of the mantle and the body wall (fig. 3, *3*). The mollusc breeds during the months of June and July.

The testis (fig. 54, *6*) opens by several small collecting channels into the simple vas deferens (fig. 54, *4*). This tube becomes somewhat but not much convoluted on its way, and ultimately expands into the curious enlargement represented in fig. 54, *2*. On opening this it was seen in every case to bear about six singular parallel folds (fig. 49, *6*). Beyond this expansion there is a curious finger-like outgrowth, extending from the duct into the mantle wall; this process contains a muscular mass, which has all the appearance of, and probably is, an introvertible penis (figs. 45 and 46, *4*). The lower extremity of the male genital duct opens by an elongate slit (fig. 49, *5*). The possession of a penis in the mantle wall is a most curious fact, which would seem to indicate that that organ is analogous to, though very likely not homologous with, the penis of the Ampullariæ and other pulmonate Prosobranchs. In Typhobia the male genital gland is extremely interesting from a cytological point of view, as this genus is one of those Prosobranchs

which, like Murox and Paludina, possess two forms of spermatozoa.

The small normal variety appear to arise one division after the heterotype which terminates the synaptic phase, and the cells out of which these normal spermatozoa are directly formed are, as in most cases, extremely small when the actual characters of the spermatozoa are taken on. On the other hand, the cells which directly metamorphose into the large spermatozoa, or megasperms, are very large, being similar in size and character to the synaptic (growing-cells) themselves. As in a former paper[1] I have advanced the view that after the synapsis any cellular generations which may exist are to be considered as potentially ova or spermatozoa, as the case may be, this fact that during the course of the spermatogenesis in Typhobia the small spermatozoa appear to arise two divisions after the formation of the synapsis, while the megasperms appear to be produced directly from the synaptic cells themselves, is extremely interesting.

Bathanalia.

The new generic type among the Gastropoda for which I propose the name of Bathanalia is at present represented by one specific form, Bathanalia Howesi, Pl. 12 , figs. 29, 30, 31, 33. As the name implies, this species is found in association with T. Horei in the deep water of Lake Tanganyika, while in its anatomical features it is so similar to Typhobia that no special anatomical description is required.

There is only one point in Bathanalia that needs mention. I found after great difficulty, and by the help of sections, that in this genus there is a very slight but quite distinct pallial anastomosis on the right side.

It is entirely on account of the remarkable characters of the shell that I have thought it necessary to separate Bathanalia as a distinct genus from Typhobia.

The shell, as will be seen from fig. 29, is conical, composed of eight angular whorls, which from apex to base carry numerous

[1] 'Quart. Journ. Micr. Sci.,' vol. xxxviii, p. 292.

short spines. The whorls are strongly sculptured, and the columella is open (fig. 33).

The mouth is ovoid, somewhat angular, and the mantle during life is prolonged into the last spine, forming a kind of false siphon (fig. 30, *1*). The radula is shown in the process block, p. 189, and also in Pl. 12, fig. 32.

Comparative.

In establishing by comparison the true affinities of the Typhobias, I have purposely left undeveloped all those questions respecting the validity of the current systems of classification which such a comparison will inevitably raise. This course has been taken because I am now confident that the Halolimnic molluscs are among the few remaining indications of an ancient sea that once extended to or near the Tanganyika region of the present day. The anatomical features of the Halolimnic molluscs when studied together should therefore throw a most important light on the inter-relationships of those more modern marine genera and families which it has hitherto been so hard to solve. It would be premature and most unsatisfactory to view these questions from the anatomy of a single Halolimnic type; for this reason I have reserved a discussion of these wider matters until I have had time to publish anatomical descriptions of the remaining Halolimnic forms.

The Typhobias have been classed by the conchologists among the Melanias, being regarded by Fischer[1] as a section of this group equivalent to Faunus or Melanopsis. Judged by their conchological characters alone it is by no means easy at first sight to understand why such a classification was ever made, as the Typhobia shell is almost as unlike any known Melania as that of a Pteroceras or a Cone. In assigning a systematic position to any animal concerning which the morphological study is incomplete, investigators are, however, always influenced, and often rightly influenced, by whatever collateral evidence respecting the habitat or modes of occur-

[1] Fischer and Smith, loc. cit.

rence of such a form may be to hand. It was thus well known when the strange Typhobia shells were first described, that they came from a great equatorial fresh-water lake, and it appears to me that the early investigators only did the best they could with the purely conchological material they had before them, in concluding that although the shell of Typhobia had few characters in common with those of the Melaniidæ, they probably belonged to this group all the same. The Typhobias, however, happen to be one of those rare organisms in dealing with which, unless there is ample morphological material to draw upon, common sense anticipations such as the above are almost certain to be wrong. There was no reason when the Typhobias were originally described to suppose that Tanganyika, the great fresh-water lake in the centre of the African continent, had ever been connected with the sea. It was not known then that jelly-fish inhabited the lake, or that the Typhobias were only one in a long series of Gastropods which are not known to be living anywhere else in the world. Progressive zoological exploration has completely changed our views. The study of the distribution of the molluscs in the great African lakes points strongly, as I have shown, in the direction of the marine origin of the Halolimnic group of animals. We might, therefore, now with reason tend to be prejudiced in the opposite direction, i. e. in favour of the marine affinities of all the Halolimnic forms. Such a conception will, however, as I pointed out in my paper on the distribution of these forms,[1] require the very strongest morphological support, since it comes into the most uncompromising conflict with all those geological speculations respecting the character of the interior of Africa which were started by Murchison, and which affirm that the African interior has never been beneath the sea, at least since the period of the New Red Sandstone.[2] It is necessary, therefore, to use the greatest caution in determining what the affinities of the

[1] This Journal, p. 159.

[2] See also Gregory's re-statement of this view contained on p. 214 of his work 'The Great Rift Valley,' published in 1896.

Typhobias and the other members of the Halolimnic group may really be.

It will have been seen from the foregoing anatomical description that unless the family of the Melaniidæ[1] is to be considered as an utterly heterogeneous group, the Typhobias are structurally near, if they are not at, the opposite end of the whole Tænioglossate series. The Melanias, as they stand at present, are certainly by no means homogeneous, and as Bouvier very justly remarks, "La famille est une des plus mal etablies dans tout le groupe des Prosobranches," but they do contain a substratum, possibly a majority of naturally associated forms, and although it will be most important, when dealing with other Halolimnic molluscs, to set limits to this group, the question of its heterogeneity does not obtrude upon the present discussion, the Typhobias being sufficiently distinct to be at once dissociated from all those Melanian forms which have up to the present time been anatomically examined. Whether they may have relations among those numerous so-called Melanias, the anatomy of which is utterly unknown, need not be discussed.

The unique and characteristic nervous system of Typhobia at once dissociates this form from all the ordinary fresh-water types. The great subintestinal ganglionic cord presents no analogy even to the zygoneurous types of nervous system, such as those of Potamides, Cerithidea obtusa, and Pyrgus sulcatus, which have been rightly regarded by Bouvier and others as representing the transitional links between the Paludina, Bythinia (?), and true Melanian types of nervous system on the one hand, and Haller's generally marine "longicommissurate" families on the other. In Typhobia Horei the pleuro-subintestinal cord is in a most extraordinary condition, at once primitive, specialised, and unique. It is specialised in having lost the left pallial anastomosis, being thus neither zygoneurous nor dyaloneurous on the left side, a condition of things which finds its only parallel in the rather doubtful

[1] See Bouvier's description of nerves of Melania, 'Ann. des Sci. Nat.,' sér. 7, 1887, pp. 125—131.

descriptions given by Bouvier[1] of the nervous systems of the Scalarids and Solarium. It is unique in the enormous development of the pleuro-subintestinal cord, the whole of this side of the nervous system being so disproportionate to the other as to distinctly foreshadow the secondarily acquired orthoneury of the Helicinidæ. The almost complete fusion of the cerebral ganglia in Typhobia, and the reduced and shortened-up pleuro-cerebral connectives, are conditions undoubtedly analogous to those obtaining in the Strombi, the Pteroceras, the Cancellaridæ, and other forms. The displacement of the pleural ganglia and the almost complete disappearance of the cerebro-pleural commissure on both sides, appear to be characters peculiar to Typhobia alone, while the position of the otocysts in the head, and not in the foot, is most primitive, but may have been accentuated by the forward displacement of the pleural ganglia, and the consequent necessity for the otocyst nerves to pass over these ganglia before they reach the otocysts. On the other hand, the otocyst nerves are very short, and even if the pleural ganglia were in their normal position, the otocysts would still be very high up in the head. The complete fusion between the pedal ganglia, and the presence of ganglionic cells in what remains of the pedal commissure, lead to the same inferences as do the characters of the cerebral ganglia. The great forward prolongation of the pedal ganglia, and the ladder-like connections between the proximate portions of the great anterior pedal nerves, are far more primitive characters. These do, in fact, suggest that the approximation in the posterior portion of the nervous system to that condition, of secondarily acquired orthoneury witnessed in the Helicinidæ, may not be altogether illusory after all. The characters of the nervous system of Typhobia show thus in a manner which does not appear to be capable of serious disputation, that this Gastropod has no relation to, nor indeed any but the most remote phylogenetic connection with, the hitherto recognised fresh-water forms. Nor has the nervous system any of those characters which could be regarded as possibly pos-

[1] Loc. cit.

sessed by the forerunners of the Melanias, the Paludinas, the Bythinias, or indeed any of the recognised fresh-water types. Therefore, so far as the nerves go, the anatomy of the Typhobias gives a flat contradiction to the view that these Gastropods may be the survivors of any extinct fresh-water stock.[1] The nervous system of Typhobia exhibits, on the other hand, the characters of some ancient but more especially of several modern marine genera. Therefore the evidence which can be gathered from the anatomy of the nerves is exactly in accord with the deductions which were drawn from the study of the distribution of these Gastropods, the Typhobias appearing to be among the survivors of some old, but not geologically ancient, marine types.[2] What is true of the nervous system is, however, true of the remainder of the soft parts. Beginning with the digestive organs, it will be seen on reference to fig. 43 that the Typhobia radula, although very singular and self-contained, is still comparable to that of several marine Tænioglossa. Thus in the massive characters of the admedian teeth, the long slender character of the laterals, as well as the form of the median tooth, this radula approaches to those of Chenopus, Zenophora, Trochiformis, Pteroceras, Strombus, and Pustularia, while it has many characters in common with the radulæ of Crepidula, Trochita, Hyponix, Turritella, and Cassis, and it resembles in a less degree those of Vermetus, Triton, Ranella, and Natica.

The characters of the salivary glands, the relation of the stomach to the œsophagus, of the intestine to the stomach, the position of the apertures in the stomach as well as the character of the pronounced median fold in the posterior stomachic chamber, are all characters which are strictly analogous to those obtaining in the Strombi and Pteroceras. The crystal-

[1] The view that some of the Halolimnic forms are the remains of an old fresh-water stock, as advocated by White, Tausch, and others, will be found discussed in my paper on distribution, loc. cit.

[2] I beg that I may not be misunderstood in this: it is one thing to say that the Typhobias are old, since the lake in which they now live must have been cut off from the sea for a great many years; it is quite another thing to say that the Typhobias were contemporary with geologically ancient forms.

line style which I found in Typhobia and in certain other Tanganyika Gastropods, and more especially the pyloric cæcum, in which this style is contained, requires more attention than it has hitherto received. The existence of these structures in connection with the alimentary canal has long been known in the Lamellibranchiata, and it was formerly supposed to be confined to them. It is further well known that in the Lamellibranchiata the style has by no means the same relations in them all. In Anodonta and Mutela it is free in the intestine and not contained in a pyloric cæcum as in Lutraria and many other forms, nor is the cæcum present in the former types. When, however, the style and the cæcum are both present, the latter structure apparently has invariably the relations represented in fig. 53, the cæcum being a long stomachic appendiculum. This cæcum does not necessarily contain a style, and thus of the Lamellibranchiata it may be said that in some the style has indefinite relations, and there is no pyloric cæcum present; while in others the cæcum is present, but does not necessarily contain a style. It was long ago pointed out—first, I believe, by Collier[1] in 1829—that in several Gastropods, the Strombidæ, also in species of Trochus and of Murex "there is an organ, the crystalline styletto, confined erroneously by a celebrated naturalist (Cuvier) to the bivalves. It is enclosed in a sheath that passes parallel to and by the side of the œsophagus to the stomach, into which the styletto enters, leaving its coverings."

This interesting observation was subsequently confirmed and extended by Huxley[2] to Pterocera, and the organ was again more completely described by M. F. Woodward, the relations of the style and cæcum to the stomach as this author describes them being shown in fig. 52. Haller[3] has confirmed Collier's observation especting the existence of this structure in Strombus, and has extended them to Rostellaria, but he did not recognise the significance of the structure in relation to that of the Lamellibranchs; nor does he appear to have been aware of Collier's and Huxley's obser-

[1] Loc. cit. See p. 190. [2] Loc. cit. See p. 190. [3] Loc. cit. See p. 190.

vations on this point. Lastly, I have found the style and its sac to be present in Typhobia, where it has exactly the same relations to a stomachic cæcum as in Strombus, Pterocera, or in those Lamellibranchiata in which this structure is present. I may also remark that the crystalline style and its cæcum are present in the so-called Lithoglyphus of Tanganyika, the affinities of which Gastropod have been entirely misinterpreted.

From the complete similarity of the style, and more especially of the stomachic cæcum, in those Lamellibranchiata which possess it, and in those Gastropods where it is also present, there can be little doubt, as Collier, Huxley, and Woodward have already clearly seen, that the structures are in reality strictly homologous throughout. But the great importance and suggestive character of this conclusion has been much obscured by Fischer[1] and others who confuse the true crystalline style in its sac with the doubtful structure known as the "Flêche tricuspide." There is little doubt that the "Flêche" has in the majority, if not in all cases been merely the cuticular lining of the stomach which has become detached, as it most readily does. With the appearance thus produced are to be classed the bodies described by Fischer in the stomachs of Cyclostoma and Paludina. Young[2] also describes in Helix pomatia a cuticular lining to the intestine, which he erroneously compares with the crystalline style of the Lamellibranchs. There appears to be no similarity between the cæcum described by Cuvier and Keferstein[3] in Buccinum and that in Strombus and the Typhobias. Further investigation is, however, undoubtedly required.[4]

[1] 'Manuel d. Conchyliologie,' p. 41.

[2] 'Mém. Cour. Acad. Belg.,' 4to, t. xlix, No. 1, p. 34.

[3] Bronn's 'Klassen u. Ordnung. d. Thier-Reichs,' Bd. iii, Abth. 2, Malacozoa, 1862–66.

[4] Apart from their bearing on the affinities of the Typhobias, the above observations show that the generally taught hypothesis which originated with Meckel and Garner, and depicts the cæcum in the Lamellibranchiata as homologous with the radular sac of the Gastropods, and the style of the former with some part of the odontophore of the latter, must be utterly unsound.

It will be seen from all this that the Typhobias and other Tanganyika Gastropods possess crystalline styles and cæca which have identically the same relations, and are structures which are undoubtedly homologous with the similar formations present in numerous Lamellibranchiata, and in a few other Gastropods as well. Now the practical importance of these facts to the present inquiry is this, that the particular Gastropods in which as yet the cæcum has been indubitably recorded, are Strombus, Pterocera, Rostellaria, Murex vertagus, Trochus turritus, the two species of Typhobia at present known, and the so-called Tanganyika Lithoglyphus. It may also possibly be present in Bythinia. Once more, then, the Typhobias in the characters of their stomachs and their related cæca are structurally near to those marine families with which by the character of their nerves and radulæ they were seen to be akin. In the possession of a style and its sac they further exhibit anatomical features possessed by the Lamellibranchs on the one hand, and by the connecting link between the Lamellibranchs and the Prosobranchs, the diotocardiate Trochi, on the other.

The gills in Typhobia are very similar to those in Strombus and Pterocera, and the osphradium resembles completely the same structure in all those Strombi which I have examined. The heart, as will be seen from reference to page 190, possesses the characters which are exhibited by nearly all the Tænioglossa.

The siphon possessed by the Typhobias is a structure of doubtful value from a classificatory point of view, and even in its narrower application it is by no means to be trusted, as both Bouvier and Haller have already shown. An interesting example of the impossibility of separating the holostomous from the siphonostomous Prosobranchs has come before me during the present investigation, for while examining one of the Melanias which the authorities of the British Museum generously placed at my disposal for comparison, I found in one, the exact species of which was doubtful, and which had been collected by Mr. Cuming in the Philippine

Islands, the small but quite apparent siphonal extension of the mantle represented in fig. 4. This Melania had in every other respect the true characters of the group, but from the existence of the siphon it would, according to the old arrangement, have to be removed from the Melaniidæ and associated with those families of the Tænioglossa to which it most certainly does not belong. The distinct but small anterior prolongation of the mantle in Typhobia (fig. 3, *1*), does not therefore appear to be of primary morphological importance, but its existence is undoubtedly another indication of the general similarity of the Typhobias to the forms which I have named.

The reproductive apparatus in the Typhobias has been probably much modified through changed conditions, and the peculiar position of the penis is possibly more the result of extreme specialisation than the retention of any primitive condition.

From all this it will be seen that the Typhobias can hardly be said to be archaic forms, but they do, as in the character of the nerves and the otocysts, possess some undoubtedly archaic characters. They are far less specialised in the characters of the foot and mantle than Strombus and Pterocera, to which in other respects they appear to be closely allied. They certainly possess none of those characters which would suggest that they can by any possibility be regarded as the persistent representatives of an old fresh-water stock. They do, however, simulate and retain the characters of the nerves of the Solarium and the Scalarids, and they probably indicate the road by which the more modern marine genera of the Strombidæ and their associates have been evolved. But to my mind the most remarkable features which they present are those which I have pointed out as indicating an approximation to several forms which have been generally regarded as recent productions; that is, they distinctly bridge the gap between several twigs which are well up in the phylogenetic tree.

Lastly it will have been seen that in many ways the

Typhobias are self-contained, and have undoubtedly undergone individual specialisation of their own. It will therefore be most expedient, most natural, and most expressive of the actual anatomical facts, to separate these two genera of Typhobias as a family by themselves, the members of which have affinities with, and stand in the relation of forerunners of, those more modern forms which group themselves about the Strombidæ. They have been seen also to exhibit more or fewer of the characters of a wider range of forms, more especially of the Aporrhaidæ, Xenophoridæ, Cypræidæ, and that ill-defined group the Ptenoglossa.

For this family I propose the name Typhobiidæ; Typhobia Horei and Bathanalia Howesi represent the two generic forms at present known.

The Typhobias are intensely interesting forms; their affinities show that they have without doubt been cut off from an exclusively marine stock at what is, geologically speaking, no very remote period of time.

DESCRIPTION OF PLATES 11—14,

Illustrating Mr. J. E. S. Moore's paper on "The Molluscs of the Great African Lakes."

PLATE 11.

Fig. 1.—Living Typhobia. *1.* Tentacles. *2.* Eyes. *3.* Operculum.

Fig. 2.—Animal removed from shell. *1.* Anterior, *2.* Posterior siphon. *3.* Embryos seen through the thin wall of the ovisac. *4.* Stomach. *5.* Ovary. *6.* Liver. *7.* Anus. *8.* Gills.

Fig. 3.—Interior of the mantle cavity. *1.* Siphon. *2.* Anus. *3.* Genital aperture. *4.* Ovisac. *5.* Heart. *6.* Kidney. *7.* Gills. *8.* Osphradium. *9.* Muscles of mantle wall.

Fig. 4.—Mantle cavity of Melania, species? from Philippine Islands. *1.* Siphon. *2.* Gills. *3.* Osphradium.

Fig. 5.—Nervous system of Typhobia Horei dissected from above. *1.* Buccal ganglion. *2.* Buccal mass. *3.* Tentacular nerve. *4.* Pedal

ganglion. *5*. Cerebral ganglion. *6*. Left pleuro-subintestinal ganglionic trunk. *7*. Pallial nerve. *8*. Œsophagus. *9*. Superintestinal ganglion. *10*. Osphradial nerve. *11*. Siphon.

Fig. 6.—Nervous system dissected from the right side. *1*. Buccal mass. *2*. Buccal ganglion. *3*. Cerebral ganglion. *4*. Pedal ganglion. *5*. Pleuro-subintestinal trunk. *6*. Otocyst.

Fig. 7.—Section through cerebral ganglion, showing—*1*. Cerebral ganglion. *2*. Pleuro-pedal connective. *3*. Œsophagus. *4*. Anterior otocyst nerve. *5*. Calcareous bodies in connective tissue. *6*. Otocyst with otoliths.

Fig. 8.—Section through osphradium. *1*. Osphradial nerve. *2*. Osphradial epithelium. *3*. Osphradial ganglion.

Figs. 9—11.—Sections showing relation of the cerebral and pleural ganglia (see text).

PLATE 12.

Figs. 12—26.—Variations and polymorphs of shell of Typhobia Horei (see text).

Figs. 27, 28.—Typhobia shells at time of birth.

Figs. 29, 30.—Shells of Bathanalia Howesi.

Fig. 31.—Animal of Bathanalia removed from shell. 1. Operculum.

Fig. 32.—A single row of teeth from the radula of Bathanalia.

Fig. 33.—Base of shell of Bathanalia, showing the open columella.

PLATE 13.

Fig. 34.—Nervous system of Typhobia Horei, viewed from above. *1*. Buccal nerves. *2*. Tentacular nerves. *3*. Optic nerves. *4, 5, 6*. Cerebral ganglion. *7*. Otocyst. *8*. Nerve from pleural ganglion, which does not form a right pallial anastomosis. *9*. Pleural ganglion. *10*. Right pallial nerve. *11*. Visceral nerve. *12*. Superintestinal ganglion. *13*. Branch of visceral nerve. *14*. Left visceral nerve. *15*. Superintestinal commissure. *16*. Columella nerve. *17*. Left pleural nerve going to form left pallial anastomosis.

Fig. 35.—Nervous system of Typhobia Horei, viewed from the side. *1*. Buccal nerve. *2*. Cerebral ganglion. *3*. Pleural ganglion. *4*. Superintestinal commissure. *5*. Right pallial nerve. *6*. Otocyst nerves. *7*. Columella nerve. *8*. Pleural nerve, going to form pallial anastomosis. *9*. Left pleural ganglion. *10*. Otocyst. *11*. Pleuro-pedal connective. *12*. Lateral pedal nerves. *13*. Great anterior pedal nerves. *14*. Ladder-like connections between the bases of the great anterior pedal nerves. *15*. Superintestinal ganglion. *17*. Osphradial nerve. *18*. Right visceral nerve. *19*. Left visceral nerve. *20*. Cerebro-pedal connective.

Fig. 36.—Otocyst in section, showing sensory epithelium and otoliths and sensory processes, *1*.

Fig. 37.—Section through, showing ganglionic character of the right visceral cord at the point marked $\times'$ in Fig. 35.

Fig. 38.—Section through cerebral ganglion in the region of the otocyst nerves.

Fig. 39.—Sensory epithelium of the otocyst in surface view.

Fig. 40.—Section through anterior pedal nerves and ganglion, showing the ladder-like connection between the roots of the anterior pedal nerves.

Fig. 41.—Section through snout, showing the buccal ganglia.

PLATE 14.

Fig. 42.—Semi-diagrammatic representation of the alimentary canal of Typhobia Horei. *1*. Œsophagus. *2*. Opening of the oviduct. *3*. Ova in ovisac. *4*. Rectum. *5*. Stomach. *6*. Opening of the œsophagus into the stomach. *7*. Pyloric aperture. *8*. Crystalline style.

Fig. 43.—A single row of teeth from the radula of Typhobia Horei.

Fig. 44.—Dissection of the stomach. *1*. Bristle passed through the opening of the œsophagus into the stomach. *2* and *3*. Ditto, passed through the opening of the bile-ducts into the stomach. *4*. Bristle passed through the pyloric aperture into the stomach. *5*. Crystalline style. *6*. Intestine. *7*. Median fold in stomach. *8*. Smaller fold. *9*. Constricted annulus dividing the stomach proper from the cæcum containing the crystalline style.

Fig. 45.—Rectum and genital aperture in the male. *1*. Buccal mass. *2*. Anus. *3*. Genital aperture. *4*. Penis. *5*. Glandular folds in the cavity of the rectum.

Fig. 46.—Same. *1*. Penis opened to show the muscular core.

Fig. 47.—Illustrating the course of the oviduct in a female.

Fig. 48.—Heart dissected. *1*. Cavity of ventricle. *2*. Auricle. *3*. Auricular ventricular valve. *4*. Valve between the ventricle and the aortic trunk. *5* and *6*. Openings into the anterior and posterior aorta. *7* and *8*. Anterior and posterior aortæ.

Fig. 49.—Dissection of male, showing:—*1*. Enlargement of rectum. *2*. Gills. *3*. Penis. *4*. Anus. *5*. Genital aperture. *6*. Enlargement of lower extremity of vas deferens, with parallel folds. *7*. Upper portion of the vas deferens.

Figs. 50—53.—Semi-diagrammatic representation of the stomachs and crystalline styles in Lithoglyphus, Typhobia, Pterocera, and Lutraria.

Fig. 54.—Male genital apparatus dissected out. *1*. Aperture. *2*. Anus. *3*. Penis. *4*. Vas deferens. *5*. Collecting tubes. *6*. Testes.

The Segmentation of the Ovum of the Sheep, with Observations on the Hypothesis of a Hypoblastic Origin for the Trophoblast.

By

Richard Assheton, M.A.

With Plates 15—18.

Introduction.

A search for the earliest stages of the development of the sheep has been attempted several times, but, so far as I know, those efforts have not been rewarded with much success.

For instance, von Baer writes of some previous attempts: "Haller verband sich zu diesem Zwecke mit seinem Schüler Kuhlemann, und beide untersuchten Schaafe sehr häufig und von Tag zu Tage mehrere, fanden aber zu ihrer und der anatomischen Welt Verwunderung vor dem 12ten Tage gar nichts, dann etwas Schleim, der sich mehrte, am 17ten die ersten Spuren des Eies, und am 19ten schon ein sehr grosses Ei mit dem Embryo." But von Baer himself succeeded in finding an ovum in the oviduct towards the end of the first day. Of this specimen he merely says, "Die Keimschicht war sehr aufgelockert und verringert."

The following description I believe also applies to the sheep, although it is rather uncertain to what animal he is referring, whether to the sheep alone or to Mammalia in general, (vol. ii, p. 183):

"Schon im Eileiter wird, während das Ei durchgeht, etwas mehr Feuchtigkeit ergossen, als gewöhnlich. So kommt es in

den Fruchthälter. Es ist noch immer eine blosse Dotterkugel, doch scheint der Dotter schon etwas Feuchtigkeit aufgesogen zu haben, da er weniger gefärbt ist. Die Haut, die den Dotter umgiebt, ist zwar ziemlich dick, doch, wie der Erfolg lehrt, nur Oberhaut zu nennen. Es liegt wenigstens bei Hunden und Schaafen noch ein ganz unregelmässiger Stoff darauf, den man für einen Rest der Keimschicht ansehen muss. Unter der Dotterhaut is wahrscheinlich ein Keim, denn die Dottersubstanz klebt nicht an der Oberhaut an, und das Mikroscop lässt auch erkennen, dass an der Oberfläche die Dotterkörnchen continuirlich zusammenhängen. Das ist der Charachter eines Keimes."

I do not think that there is any other description of these early stages than this of von Baer.

Bonnet made an attempt in 1884 to secure the stages of segmentation, but failed to find anything before the twelfth day.

He writes, "Ich habe neun Schafe und viel Zeit vergeblich geopfort und wünsche eventuellen Nachuntersuchern meine Geduld und—mehr Glück."

"Mehr Glück" I may at any rate claim to have had. There are, of course, some gaps in my material, but I have succeeded in obtaining a very fairly perfect series of specimens from the time of fertilisation up to shortly before the stage with which Bonnet's account begins.

The ova pass very rapidly down the oviduct and enter the uterus at an early stage of segmentation without having acquired any mucous or albuminous coat.

While in the Fallopian tube they may be obtained very easily by scraping off the whole of the mucous membrane with a scalpel, and spreading it out on a glass slide, where the ova can be found under the microscope.

It is as well to search the Fallopian tube during the first four days, but it is not likely that any ova will be found after the first three days. If the actual point of rupture of the Graafian follicle is still present in the corpus luteum as a minute bright red spot, the ovum will be in the Fallopian tube.

When the ova have reached the uterus, which is usually early on the third day, the surest way of finding them is the adoption of the method I made use of in the acquisition of the early embryos of the pig. A ligature was placed round the lower ends of the Fallopian tubes, a cannula was inserted and tightly tied into the mouth of the uterus, both horns of which were then slowly filled with ·25 per cent. or ·5 per cent. chromic acid.

The uterus, when distended to its uttermost and its lower end ligatured, was left in chromic acid of the same strength for one to three days.

The contents were then let out and the uterus thoroughly washed, and the contents and washings were searched through under the microscope.

This is an excessively tedious process, because during the fourth to seventh days the uterus contains more or less of a milky secretion, which renders the contents turbid, and greatly increases the difficulty of finding so small an object as the mammalian ovum.

In this way about three out of five may be obtained.

I received the uteri about three quarters of an hour to one and a half hours after the death of the sheep, and filled them at once with chromic acid.

The specimens thus obtained were stained in carmalum, hæmalum, Kleinenberg's hæmatoxylin, or borax carmine, and passed through the usual grades of alcohol, and through cedar oil into paraffin and cut into series of sections, which varied from ·005 mm. to ·01 mm. in thickness.

The dates which I give are only approximate. I am not sure that there is so much variation in the time that may elapse between the moment of sexual union and fertilisation as in the case of the pig. It is, however, very difficult to obtain any one given stage. Circumstances did not permit of a continuous watch being kept upon the flock of sheep. The dates which I give are not from my own observations, but are derived from the information given me by the shepherd.

For the purpose of this paper I killed forty-one sheep, and

obtained from them forty specimens. Of these thirty-one were perfect embryos, and nine were apparently unfertilised ova.

From fourteen of these sheep I obtained two specimens in each case, from twelve only one apiece. In the remaining fourteen I was unable to find any embryo, although I think the majority probably were pregnant. The approximate ages of these were as follows:

	Embryos.	Unfertilised ova.
2 days . . .	3	0
2½ „ . . .	3	0
3 „ . . .	6	1
4 „ . . .	2	0
5 „ . . .	2	4
5½ „ . . .	2	0
6 „ . . .	5	2
7 „ . . .	3	0
8 „ ? . .	2	0
9 „ . . .	1	0
10 „ . . .	1	0
11 „ . . .	1	2
Total .	31	9

Segmentation of the Ovum.

In one respect I have been very unlucky. I tried with six sheep to obtain the earliest stages of segmentation. I have, however, not succeeded in getting any stage between the fertilised but unsegmented ovum, and a stage with six segments. The earliest specimens I have are two taken from the oviducts of sheep killed during the second day. Each ovary showed a small bright red corpus luteum. In each side an ovum was found about halfway down its oviduct. In each case the ovum was slightly retracted from the zona radiata, leaving a space at one side. The ovum of the sheep is large, measuring as much as ·15 mm. in diameter, or including the zona radiata ·18 mm. while fresh.

These specimens were preserved in ·5 per cent. chromic

acid stained with carmalum, cut and mounted in series of sections. Each specimen proved to be on the point of completion of the process of fertilisation.

Pl. 15, fig. 2, is a section of one of these two. The section passes through both male and female pronucleus.

I have not been able to identify a polar body. The contents of the ovum are for the most part of a very uniform texture. At three points, however, the granulations are coarser and more concentrated and are more deeply stained. The section passes through two of these. The third, which is the least conspicuous, is at the periphery opposite, and the next section passes through it.

The accumulation of deeply staining particles envelops the inner face of one pronucleus only. The opposing face of the other pronucleus seems to be surrounded by a less concentrated area. Close to this latter nucleus is a small, round, deeply staining body, which does not occur in the section from which fig. 1 has been drawn. The other specimen mentioned above is in exactly the same condition, and presents the same features, with the exception that the small deeply staining body is not present. This specimen is cut in a plane at right angles to the previously described section.

In this specimen and in others there are certain spherical bodies near to the periphery of the cell, which stain rather deeply and homogeneously. They appear to be of the nature of yolk granules, and are shown in my drawing of the next specimen which I shall describe.

This one (fig. 3) was presumably about two days old, and was found halfway down the oviduct. It was cut into sections, and was found to be in the act of dividing. The plane of the section is, I think, at right angles to the direction of the spindle. The chromatin threads are arranged near the centre of the ovum. The spherical bodies mentioned above, two of which can be seen at Y in my figure, are not scattered so evenly over the surface of the ovum as in the former stage. They are all close to the surface, and are aggregated round the two poles. There are rather more in one hemisphere than in the other—

sixteen to twelve. A curved band of them connects the two polar groups.

The six-segment specimen which I possess was found in the upper part of the Fallopian tube of a sheep killed upon the third day. It was examined in the liquid from an ovarian follicle and drawn while fresh (fig. 25). It was composed of two large segments and four small segments.

I could not make out that there was any difference in the nature of the segments, either while fresh or after treatment with Flemming's fluid and carmalum. The four small segments were nearly of equal size. One of the large segments was appreciably larger than the other (v. fig. 25).

The relative sizes of the six segments may be gathered from the following figures, the result of measurement while it was still fresh in the follicle liquid :

Four small segments { 15 × 16, 15 × 16, 15 × 15, 16 × 16 } Two large segments { 21 × 21, 22 × 23 }

Fig. 4 is a section passing through two of the small and the two large segments. The two latter appear to be upon the point of division.

I cannot clearly recognise any yolk-like spheres such as those described in fig. 3. Unfortunately the specimen is more deeply stained than the other. In the two large segments there are some larger looking bodies of more irregular shape, which may be something of a similar nature.

From the same oviduct I obtained another embryo, which was in eight segments. This was placed at once into chromic acid, where it remained for four hours. It was then stained with carmalum, cut, and mounted.

As regards size the segments do not differ greatly ; there is, however, one segment which differs most markedly in texture and in colour from all the remaining seven. The whole appearance is of very much lighter colour. The minute structure is finer, and the protoplasm has a tendency to shrink from its walls, which in other segments it has not.

Fig. 7 represents a section passing through the centre of this segment. This separation of one segment differing from the other seven is very remarkable, because there is no trace of such a difference in the former specimen (fig. 4).

Unfortunately I had preserved the six-segment specimen in Flemming's solution, and so a comparison between the two is not so perfect as might be.

I have another specimen in the eight-segment stage taken from another sheep. It was found in the upper end of one horn of the uterus of a sheep killed on the fourth day. It was in chromic acid for forty-eight hours.

This specimen was stained with borax carmine and mounted in two groups of four cells in Canada balsam, as shown in fig. 24.

The variation in size of the several segments may be recognised in the drawing. I could see no difference at all as regards colour. In one segment, which is slightly larger than any of the others, there is a group of spherical bodies which are stained a little more deeply than the protoplasm in which they are embedded.

It is a noteworthy fact that in two specimens of the eight-celled stage of the embryo one of the segments differs from all the others. On the other hand, I have two specimens in the eight-cell stage taken from the same horn of a uterus, neither of which shows any sign of difference between the several segments. Both, however, were stained differently from the beforementioned specimens.

Fig. 5 represents the middle section of a series cut through one of them. The cells are arranged so as to leave a distinct cavity, which may be called a segmentation cavity. This cavity, which would seem to persist for some time, always contains spherical and otherwise shaped masses of a substance in every way similar to the cytoplasm of the segments.

I have specimens of 15, 15, 16, and 17 segments respectively, all of which show features similar to the eight-celled specimen shown in fig. 5. They may be described as "blastulæ," with the segmentation cavity filled with fragments of cells.

In three of the specimens there may be seen a deeply stained spot, resembling the chromatin of the nuclei, either in one of the spherical masses or amidst the general detritus.

Fig. 6 represents the above condition in its most obvious phase. This is a section of a specimen with sixteen segments. There was nothing in its outward appearance to suggest that it was in any way abnormal. The segments are approximately of the same size and of similar colour. The chromatin-like granules in the interior resembled a nucleus more closely than in the other specimens.

In the other three specimens the condition of the zona led me to think that the preserving fluid had not perhaps reached them, or had not acted efficiently for some other reason, although the general form of the embryo itself seen as a whole presented no feature of abnormality. Fig. 23 shows the appearance of one of those which were made up of fifteen segments.

I cannot give any explanation of the fragments of cells seen within the segmentation cavity, nor of the origin of the nucleus-like body. Nor can I offer any suggestion for the absence of the difference in colour between the several segments which is so marked in fig. 7, and again in a later stage containing thirty segments (fig. 8), to which I shall refer presently.

I had one other specimen between the specimens just described and this one which had about twenty-five segments. In this there was no central cavity; but I do not think that there was any marked difference either in the size or the colour of the segments. It was unfortunately very feebly stained, and in trying to remedy the defect I lost it.

Instead of the uncertainty which surrounds the interpretation of the last four specimens, namely, those between the eight-segment and twenty-five-segment stages, I have now to deal with a short period in which the embryos are quite diagrammatic in their clearness.

In a thirty-cell specimen obtained from the uterus of a sheep of four days there can be no question as regards the difference between certain segments. Six are larger than the rest,

and stain more lightly, and occupy a more central position. The darker smaller cells, which partially surround the inner core of lighter cells, number twenty-four. The segments are not now so spherical as they were; they do not leave spaces between each other, but are indeed pressed closely up against each other (v. fig. 8). The difference in texture is exactly the same as it was in the case of the eight-segment stage described above (fig. 7). The light inner cells are left exposed over about one fifth of the surface.

The next stage is, I believe, represented by an embryo obtained from the uterus of a sheep of six days. A median section is represented in fig. 9. Here, again, there can be no doubt about the different nature of certain of the segments. A layer of darkly stained cells, which are on the whole rather smaller, completely surrounds a group of lightly stained cells. The light cells no longer appear on the surface at any point. The small dark-staining cells number thirty-three, and the inner light-coloured cells are five in number. Two of these, however, are so nearly divided that one may say they number seven. The outer layer of dark cells is decidedly thicker at one point than elsewhere. In the preceding figure (fig. 8) the middle cells of the outer layer are just a little larger than those at the edge. If these two thickened parts correspond, it may be concluded that the part of the inner core which remained longest uncovered is that part immediately opposite the thickened part of the outer layer.

My fig. 10 represents the middle section of a series through a specimen taken from the same sheep as that represented by fig. 9. It was taken from the other horn of the uterus. This specimen is made up of forty-four small dark cells and nine or ten rather larger inner light cells. The outer dark layer of cells has become doubled at one part, which I think we may presume to be the part which had previously shown a thickening (fig. 9). It was stained with hæmalum, which for the purpose of differentiating between the two kinds of cells is less favorable than some other stains. It would seem to be in about the same stage as the preceding specimen (fig. 9), or perhaps

it shows a slight advance. The light-coloured cells are smaller and rather more numerous.

In my next embryo, which is from a sheep of six days, the inner mass of light-coloured cells is quite unmistakable. Its cells number six. Of the dark outer layer there are now forty-six.

As to the last three specimens, there can be no difficulty in determining to which group any cell belongs, but from this time forwards the work of tracing the lightly coloured cells is far more intricate and the result less certain. I have tried to represent in my figures as accurately as I can typical sections of these stages.

The all-important question to decide is, whereabouts in a specimen such as those represented in figs. 10 and 11 does the cavity of the blastodermic vesicle arise? I have two specimens in which this cavity is just beginning to be formed. One was from a sheep of about six days; of the age of the other I have no record. My figs. 12 and 13 are both drawn from sections of the former embryo.

There is without doubt a marked difference in colour between the several segments; but there is no longer a sharply defined boundary between the dark cells and the light cells, as there is in figs. 8, 9, and 10. And yet, on the whole, the light-coloured segments are aggregated and pressed up against one part of the wall of the vesicle (x)—a thin part of the wall—much as they are in fig. 11, at the spot marked x. Amongst the light cells are undoubtedly scattered darker ones. As an explanation it may be said that, where the knife happened to pass parallel to the surface of a cell boundary which becomes included in the section resulting therefrom, darker areas will appear among the lighter.

In this specimen many strands may be seen passing between the separating cells, which suggests that the cavity was only just beginning to be formed at the moment of preservation. It indicates also that there is protoplasmic connection between at any rate certain of the segments at this time.

Another specimen of unknown age, but seemingly a little in advance of the one just described, shows similar features.

In this specimen the strands of protoplasm seen in the former are less numerous. The cavity is rather larger (fig. 14). The light cells are not so numerous nor so well differentiated as in the former case; they form an irregular mass, partially surrounded by the darker cells. I cannot be sure that there are more than eight or nine of the lighter cells. The dark cells number about seventy-five. It is, however, very difficult to count them.

Fig. 15 is a drawing from a section of a rather more advanced specimen. In this the cavity of blastocyst is well developed, and the connecting strands of protoplasm are only found in the narrow space round the embryonic pole.

There is now very little difference in colour between the several segments. I think my figure represents very fairly accurately such difference as there is. This is a very slightly lighter colour of the segments lying between the outer wall and the innermost layer of the inner mass.

The embryo now fills the whole of the space inside the zona radiata, the thickness of which has not materially altered since it left the ovary. There has been no additional investment.

From this moment the internal pressure increases rapidly, and the vesicle expands, and causes the zona radiata to become very much attenuated. Such is the condition of my next embryo, which is shown in fig. 16. This embryo was probably eight days old; the age was not noted. The embryo formed a spherical, transparent, perfectly typical mammalian blastodermic vesicle. It differs from the foregoing specimen in the great attenuation of the zona radiata and outer layer of cells, and in a general reduction in the size of the inner mass.

It is now impossible to distinguish any lighter coloured area extending over more than a very limited space. There are no visible boundaries to the cells. I am not sure that the outer layer is distinct from the inner mass.

Fig. 17 has been drawn from a section of a specimen taken from the same uterus, but not the same side as the preceding specimen. It is undoubtedly slightly more advanced. The zona radiata had ruptured or had been absorbed, and the

embryo was lying naked and rather crumpled in the cavity of the uterus. In the embryonic mass an oval body is sharply marked out, which probably gives rise to the epiblast of the later stages. I have, unfortunately, not been able[1] to obtain the stages between my fig. 21 and Bonnet's earliest stage, in which there was no trace of the trophoblast layer over the embryonic epiblast. I cannot see that there can be any reasonable doubt as to the fate of the mass E. The more loosely arranged masses lying at the sides and lining the inner surface of the epiblastic knob can be recognised as the hypoblast.

Fig. 17 probably represents a stage only very little older than the former embryo (fig. 16), for both are from the same sheep; yet the change in appearance of the inner mass is very marked. One cannot help thinking that the very decided change is due in some way to the loss of the zona radiata, and that in all probability some such delimitation as seen in fig. 17 really already exists in fig. 16, but that, owing to the greater tension under which the whole structure must be, the delimitation is masked for the time being.

In my account of the development of the pig[2] I have laid some stress upon the sudden splitting up of the embryonic epiblast and hypoblast into distinct layers, and I noted the coincidence of this phenomenon with the loss of the zona radiata. In this respect the sheep and pig correspond.

If it is possible, as I believe it to be, to identify the oval knob of fig. 17 with the lighter cells of fig. 15, and again these with the extremely plainly defined lighter cells of fig. 11, we have here a most striking developmental history. The hypoblast

[1] Since writing the above I have obtained a twin specimen of the sheep, i. e. a blastocyst with two embryonic masses, each apparently normal, with the exception of being only half the usual size. In one of these the trophoblast has disappeared over the centre of the epiblast, which at this spot shows indications of a pitting in which recalls the condition of other mammals (Tupaia, Sus) accompanying the rupture of the trophoblast over the epiblastic knob. A description of this specimen is given in the 'Journal of Anatomy and Physiology,' vol. xxxii, April, 1898.

[2] This account is already in print, and will be published in the next number of this Journal.

and trophoblast are shown to be derived from the same group of segments, which are at an early period differentiated from those which give rise to the epiblast. The trophoblast may, therefore, be said to be hypoblastic, and the hypoblast may be said to completely surround the epiblast at one period of development.

Fig. 18 is a drawing of an embryo found in the uterus of a sheep of nine days. This specimen is very much larger than any hitherto described. The vesicle was only slightly crumpled. It was oval in shape, and the embryonic mass was plainly visible on the longer face.

Fig. 19 is a drawing of a section taken along the line *a—b*. The trophoblast is quite plainly distinct from the embryonic epiblast, which has grown very considerably, and forms a solid lenticular mass. The hypoblast is stretched tightly against the inner surface of the epiblast, and even extends some short distance beyond the limits of the epiblast. It must be remembered that fig. 19 is taken rather obliquely, and so gives the impression of a further extension of hypoblast than really exists. The hypoblast is of a remarkably close texture, which is very unusual at such an early stage.

The condition of the hypoblast as seen in fig. 19 may very readily be derived from that seen in fig. 17 by the expansion of the walls of the blastocyst round the embryonic pole. At ten days there is no great change. I have only one specimen of this age. It was considerably crumpled (fig. 20). The embryonal area has a longer and shorter axis.

Fig. 21 is a section of the embryonal area. The trophoblast (T) is very thin, and so much stretched that not more than six nuclei overlie the epiblast (E).

The only change to be noted in the epiblast is a greater activity apparent, and the lower cells seem to show a tendency to become arranged in a rather columnar or stellate fashion. There is a layer of smaller cells on the inner surface of the epiblast, and one of very much larger cells near the outer surface, which may be compared to the state of things in the corresponding stages of the pig or tupaia. The hypoblast is a

continuous sheet extending nearly halfway round the inner surface of the vesicle (v. fig. 22).

This is the oldest stage which I shall describe. My next specimens are twelve and thirteen days old, and already much elongated.

The specimen, fig. 20, is younger than the youngest described by Bonnet. I regret very much that I have nothing between this and Bonnet's, for in Bonnet's specimen the Rauber layer had gone.

That the layer H is the same as Bonnet's layer E1 (fig. 3) there can be no reasonable doubt. Nor can there be much doubt that the mass E in my fig. 16 gives rise to most if not all of the "ectoblast" E in Bonnet's fig. 3. But of the fate of the Rauber cells overlying this in my fig. 16 I have no record (vide note on p. 216).

In the complete absence of zona radiata I cannot think that their fate can be very different from what it is in the pig or tupaia, with whose development the sheep has many points in common. The separation between the trophoblast cells and epiblast is quite complete in my specimens (figs. 19, 21).

Observations on the Mammalian Blastocyst.

The interpretation which I believe may be placed upon the specimens described above and in my paper on the pig may be briefly stated. At an early stage, in the sheep perhaps as early as the eight-segment stage, the future epiblast and hypoblast are differentiated.

The hypoblast surrounds the epiblast, so that at the morula stage the embryo consists of a few epiblast cells surrounded by hypoblast cells, which at one pole form a thicker investment than elsewhere. In the middle of this thickened mass of hypoblast the cavity of the blastodermic vesicle arises.

Subsequently, by the rupture of the hypoblast over the epiblast, the latter comes to the surface.

Therefore the greater part of the wall of the blastodermic vesicle is hypoblast. The hypoblast is, owing to mechanical

and physical causes, double over part of the wall in the majority of forms.

The cavity of the blastocyst is not the segmentation cavity, but the archenteron. It is bounded on all sides by hypoblast, though at the part where it stretches across the epiblastic disc it is perhaps a network of cells rather than a continuous sheet at the time when the blastocyst cavity is very rapidly expanding, as during the stages of figs. 12—16.

There would seem to be nothing in the pig to cause a differentiation in staining, but in the early stages there is a distinct suggestion of a growth of smaller cells round a group of larger ones.

The bursting through of the epiblast and rejection of the pieces of trophoblast torn apart in the process are particularly clear in the pig. I regret very much that I have not got the stages which may include the corresponding process in the sheep (vide foot-note on p. 216).

Concerning the homologies of the several parts of the segmented ovum and the blastodermic vesicle of mammals very diverse views have been held.

It is only because the facts which I have discovered in the early development of the sheep, which are corroborated by events in the development of the pig, seem to give promise of an interpretation in some ways more satisfactory than any hitherto advanced, that I venture to enter upon a short discussion of the subject.

I am perfectly well aware that there is much to be said in favour of the views expressed by embryologists far more competent to deal with the subject than am I. As recently as during the last two years important papers dealing with the question of the segmentation of the mammalian ovum have appeared from the pens of such noted embryologists as Professors Hubrecht and Duval. Although the respective views of these two authors were quite different, yet in each case most plausible explanations were offered for many of the problems of mammalian embryology.

But to my mind neither of their views can be maintained

for the sheep; and apart from this, there seems to me to be such a strong primâ facie case for the hypoblastic blastodermic vesicle theory that I believe it to be deserving of more attention than it has received. Hitherto no mammal with a typical blastodermic vesicle stage has been examined which in any way hinted at the explanation which is suggested by the study of the segmentation of the sheep's ovum.

While my hypothesis is not entirely in accord with that of any former author, it resembles those of Minot (43) and Robinson (46), and indeed owes much to the work of the latter upon the rat and mouse, and to the theoretical conclusions of his suggestive essay in vol. xxxiii of the 'Quarterly Journal of Microscopical Science.'

The chief feature of my hypothesis is that (like Minot and Robinson) I regard the main wall of the blastodermic vesicle as entodermic. I differ from them, however, in that I regard as entodermic a greater portion of it than they do.

I differ from Minot in considering the inner layer of the inner mass as entoderm as well as the whole of the subzonal membrane (v. Minot's 'Human Embryology,' pages 107 and 108). Hence the cavity is not, as he regards it, the segmentation cavity, but the archenteron, which arises as a split within the entoderm as shown in my diagrams 2 and 3, and is not comparable to the segmentation cavity of amphibians.

Haddon's (27) theory, although called by Minot a similar explanation to his, seems, however, to me to be very different. Haddon regards the subzonal epithelium, including "Deckenzellen," as the equivalent of the extra-embryonic epiblast of the bird's egg; so that the cavity of the blastodermic vesicle, which he compares with the yolk-sac, is supposed to be bounded on all sides except the embryonic pole by "extra-embryonic" epiblast, and not by hypoblast. Keibel's (38) suggestion is practically the same as that of Haddon. With Minot I should agree in so far as supposing that, to quote his words, "there is, then, a complete inversion of the germinal layers in all placental mammals." This I imagine to occur in most forms at a very early stage (pig, sheep, rabbit, mole,

&c.), but rather later in some (hedgehog, bats). According to my hypothesis the epiblast never grows round the blastocyst in Monodelphic Mammals. From Robinson I should differ in supposing that the whole of the subzonal epithelium is hypoblastic instead of only the "greater part" of it, and further in denying that the epiblast ever extends round a previously existing hypoblastic vesicle. Both layers of the extra-embryonic part of the wall of the didermic vesicle are on this hypothesis hypoblastic.

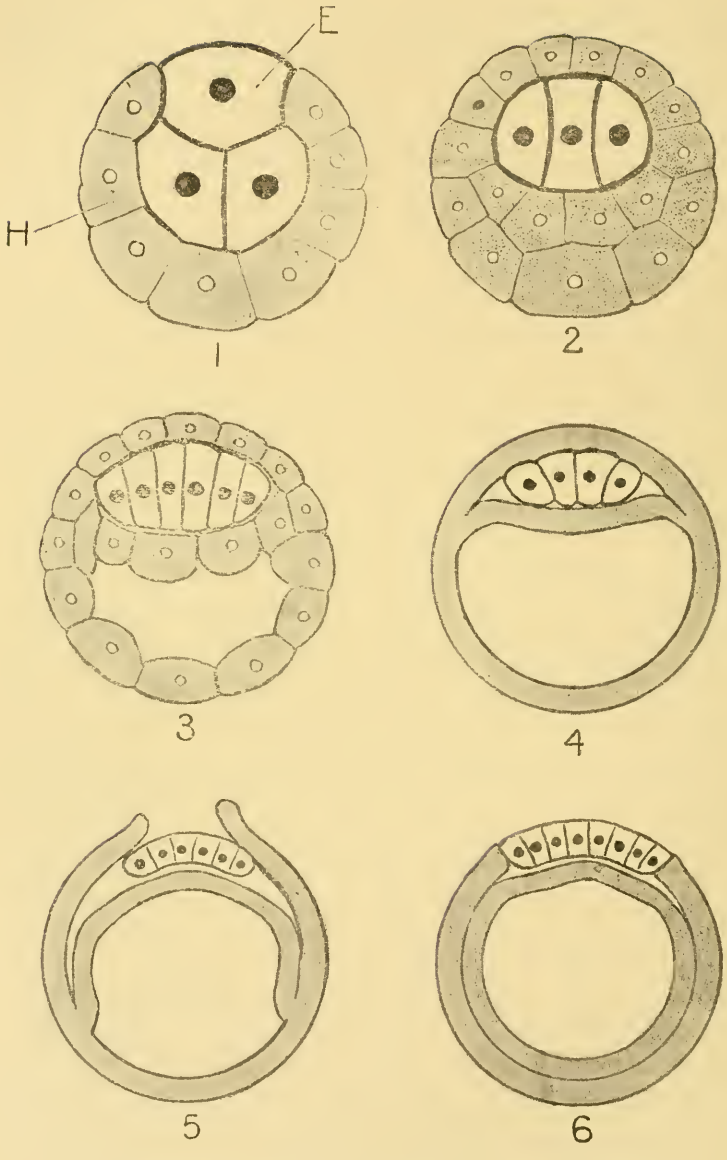
The accompanying diagram (p. 222) will render clear what I believe to be a perfectly legitimate interpretation of the facts derived from the study of the segmentation of the ovum and formation of the embryonal area in the sheep and pig.

Such a conception of the early embryo of the mammal is very closely in accord with the meroblastic egg of the Sauropsida.

If we regard the cavity of the blastocyst as the segmentation cavity, it is then impossible to compare the blastocyst with the egg of the Sauropsida. But if the cavity is regarded as the equivalent of the subgerminal cavity the comparison is complete. The origin and the fate of the subgerminal cavity of the bird and reptile are exactly the same as the origin and fate of the cavity of the blastocyst of mammals.

In each case it begins as a split amongst hypoblast cells, and becomes enormously enlarged and distended by fluid, and provides ample space for the development of the embryo free from pressure. A portion of it in each case becomes the gut cavity of the embryo. The diagrams A, B, C, on Pl. 18 represent the bird's egg during the first few hours of development according to Duval's description.

Fig. A represents an early stage of segmentation. The segmentation cavity is seen as a narrow slit between the epiblast, which is coloured pink, and the hypoblast, which is distinguished by a green tint. The segmentation cavity does not occur in the placental mammals, except perhaps in the case of the rat (Robinson) and sheep; otherwise figs. A and C are exactly comparable with the first and last of the diagrams upon the following page.



The light cells are epiblast (E), the darker cells are hypoblast (H).

In the bird's egg at the time of laying, and for the first few hours of incubation, there is said to be a discontinuity between the hypoblast and the "yolk" (B). The yolk at this time is nevertheless laden with nuclei (Duval, 23). After the first few hours of incubation the yolk again becomes continuous with the hypoblast, as in diagram C.

The passage which is caused by this temporary loss of continuity, and which extends round the blastoderm, has been regarded as blastopore, and has been said to become converted into the primitive groove. Some experiments made with a view of testing this point by myself (4) proved the assumption that it becomes converted into the primitive groove to be untenable.

It is, I think, reasonable to suppose that this loss of connection between the two portions of hypoblast is in some way related to the act of laying of the egg, and to the cessation of development attendant on it.

Fig. B, Pl. 18, can be compared with the second diagram (p. 222) as regards the doubling of the hypoblast cells, amongst which, in the next stage, the great cavities—the subgerminal cavity, and the cavity of the blastodermic vesicle—subsequently appear. In most mammals, however, the hypoblast temporarily extends over the epiblast, so that the latter is completely withdrawn from the surface during a certain period of development.

The exact method by means of which this overgrowth in the mammalian ovum is effected, and the method by which it is subsequently rectified, so as to bring the epiblast again into its proper position, seem to vary with almost every species. The series of small diagrams on Pl. 18 illustrate some of these diverse methods, and their relations to each other.

Fig. D, Pl. 18, represents the segmenting ovum of possibly all placental mammals.

The pink represents epiblast, the green hypoblast. The black ring is the zona radiata, and other investing coats which may be present.

Immediately below this is the next stage (X) of a certain

type of development in which a central core of epiblast is surrounded by a single layer of hypoblast cells. This group contains Ovis, Sus, Tupaia, Talpa, Sorex, Lepus, and probably Cavia. These all have a strong zona radiata.

Fig. Y shows the next stage of all these forms, in which the hypoblast has become doubled over the ab-embryonic pole. At this point one of the number, Cavia, loses its zona radiata, and branches off on a line of development of its own (C_{1-4}.)

Continuing down the page is a figure (Z) which may be supposed to be a stage common still to the remaining members of this group, which, however, after this point take three rather distinct courses. On the right side of the plate are shown Lepus and Sorex, L_{1-4} and S_{1-2}.

These two are characterised by the looser arrangement of the epiblast, which condition results in a gradual expansion and formation of a flat epiblastic disc. Both forms retain the zona radiata until after the growth of the epiblastic disc has caused the rupture of the overlying hypoblast cells (Rauber layer). This leads to the inclusion (either permanent or temporary) of these fragments among the epiblastic disc cells.

On the left of the page, diverging also from Z, is another group containing Sus and Tupaia (Su_{1-5} Tu_{1-2}), and probably Ovis. These are characterised by the formation of a more solid epiblastic knob, which does not become drawn out, but undergoes a process of doubling up previous to assuming its final disc-like form. The zona radiata is thrown off, so that when the rupture of the overlying hypoblast cells occurs there is nothing to prevent the fragments from being lost. Accordingly none become incorporated in the epiblastic disc.

Intermediate between these is placed Talpa (T_{1-4}), which is like Tupaia and Sus as regards its epiblastic characteristics, but like Lepus and Sorex retains the zona radiata, with the result that the fragments of overlying hypoblast become incorporated with the epiblastic disc (Heape, **30**).

On turning again to the first figure, D, there will be found upon the right of the plate a series of five figures, M_{1-5}, which

I have taken with some modification from Robinson's paper on the rat.

I take the trophoblast to be hypoblastic instead of epiblastic. The true epiblast is cut out from the surrounding trophoblast at an early stage (v. Robinson's [46] fig. 8). In the majority of his figures he certainly shows the trophoblast cells to be darker than the hypoblast and more like the true epiblast. But this is not so in all. It is not so in fig. 6. I do not see why the thin side walls of the "segmentation cavity" of this figure should not be derived from the hypoblastic mass, in which case, since they subsequently give rise to the trophoblast (figs. 8, 9), the trophoblast may be said to be hypoblastic. Selenka (49) and Duval (25) have given very different accounts of the rat's development. That there is much to be said on both sides is evident from Weysse's (56) remark that, from his own work upon the subject, he is "still undecided."

Sobotta (54) describes and figures a separation of the segments into groups of small and large segments, but he does not trace their fate.

In this case we see that there is no zona radiata. There is a segmentation cavity, which, however, quickly becomes obliterated. The very early loss of zona may account for there being no growth round by the "hypoblast" during the early stages. If Selenka's or Duval's description is adopted, it would be open to a similar interpretation to that suggested in diagram C_{1-4} for the guinea-pig. The same may be said for Mus sylvaticus (Selenka, 50). In this the epiblast is seen to be cut out very early as a definite knob (fig. 36).

On the left of the plate, also diverging from D, are shown two developmental histories, which seem to form a rather special group. In these the envelopment of the epiblast by the "hypoblast" is supposed to take place rather less quickly. Figs. V to V_7 represent what seems to be a possible interpretation of the ontogeny of the bat. In this case the surrounding does not occur until after the appearance of the cavity of the blastocyst.

Fig. V_1 is taken from van Beneden and Julin (13), the

remainder from Duval's (26) account of the bat. As, however, Duval's explanation is quite different from the above, I have given below at some length my reasons for dissenting from his views.

Erinaceus on this hypothesis would be in some respects similar.

Unfortunately the earliest stages of the hedgehog, with the exception of a two-segment stage described by Keibel (37), are not known. Fig. E is therefore quite hypothetical for the hedgehog, and should probably be drawn without a zona, and possibly at this stage the hypoblast should be shown already extended over the epiblast.

If the increase of the hypoblast cells is very much greater proportionally to the epiblast than it is in the sheep, it is quite possible that when the cavity of the blastocyst arises its walls may be more than one layer thick, some of the inner cells giving rise to the inner sac described by Hubrecht (33).

In Hubrecht's earliest figures there is certainly no distinction between the trophoblast and epiblast, but in the slightly older ones which are to be compared with my diagram E the distinction is clear, as a glance at Hubrecht's figs. 39, 8, 8 *a* will show. Figure E may be compared with fig. 15 or 16.

I have drawn Figure E_3 according to Hubrecht's figs. 20 *b* and 51, although I am not clear whether he describes the formation of the true amnion as the advance of a free edge, as would appear from fig. 51, or whether, as the wording of the text seems to indicate, (see pp. 289—296, and again 374, where he says, "We have seen that after the completion of the germinal area its epiblast remains attached to the trophoblast [from which it has split off] along a circular line of insertion. At the time of the formation of the amnion this line of attachment travels upwards, the circle becoming smaller and smaller, until at the completion of the amnion no connection between embryonic epiblast and trophoblast any longer exists," it is really a process of splitting off from the overlying trophoblast).

If, therefore, it is really a case of splitting, the amnion cavity shown in Figures E_2 and E_3 should have been drawn within

the pink mass instead of between the pink and green. In this case the amnion formation of Erinaceus would resemble more closely that of Pteropus or Cavia; whereas, as I have interpreted it, it is more like Vespertilio.

Such is briefly the hypothesis to which I venture to draw the attention of those who are interested in the problems of mammalian embryology. I cannot deny that there are difficulties, but these do not seem to me to be formidable.

Let it be noted that we have now to face the fact, based upon actual sections, that there is in certain mammals a clear separation of segments at an early stage into two groups, one of which eventually completely surrounds the other. Until the last few years this has not been so. It is true that van Beneden in 1880 described this process in the rabbit. His interpretation, however, led to many difficulties, which were only partially removed by his subsequent modification of it. I showed in 1894 that van Beneden's description, derived from surface views and optical sections only, was not supported by my actual sections, and urged that the phenomena of the development of the rabbit's ovum were open to a more strictly epigenetic explanation. Since then Duval's work on the bat and Hubrecht's on Tupaia, and my own observations on the sheep, have convinced me that such a process does occur in certain mammals.

What does this phenomenon mean? It must surely have some most profound significance. Van Beneden and Duval maintain that it is the division into epiblast and hypoblast, the former being the external layer. Hubrecht considers it is a division into epiblastic trophoblast and embryo. Sobotta (54) connected the lighter appearance of certain cells in the early stages of the mouse's development with the state of division of the cells. It is, I think, quite impossible to explain the differences in my specimens in this manner. The present hypothesis supposes that in cases where this differentiation does clearly occur it is a division into epiblast and hypoblast, the latter being the external layer; and that if this is or at some time has been the mode of development in all placental mammals, it is then

suggested that the phenomenon is capable of interpretation in some such way as that indicated by my Plate 18.

The obvious difficulties which arise from this last hypothesis may be stated thus:

(i) The alternative explanations given of individual de-developmental histories by other authors.

(ii) The double-layered condition of the hypoblast.

(iii) (*a*) The inference that the chorion of placental mammals is formed by the fusion of allantois and extra-embryonic hypoblast (trophoblast), whereas in birds and reptiles the corresponding membrane is formed by the fusion of the allantois and extra-embryonic epiblast, with, of course, in each case the intervening mesoblast. (*b*) Although the true amnion may in every case be either wholly or partly formed of true epiblast, in all cases the false amnion would appear to be formed of hypoblast.

These difficulties are discussed below. The chief features of my hypothesis which seem to me to be most likely to commend it to the attention of embryologists may be stated thus:

1. It is an explanation of the early differentiation of the segments into two groups of cells, which avoids the difficulty which attends that of van Beneden.

2. It is an explanation of the van Beneden blastopore.

3. It suggests a reason for the rejection of the Rauber layer cells by the embryonic epiblast, which is so evident a phenomenon in some cases.

4. It demands no change in function of the two primary layers during the substitution of the yolk granules by maternal fluids. The hypoblast cells maintain their function as a nutritive organ, entering into direct connection with the maternal tissues, in nearly all animals, in the various forms of träger, ectoplacenta, and villi, and thereby supporting the embryo until the placenta is established.

5. The morphological relation of the epiblast and hypoblast and the cavity from which the future gut is formed agree on this explanation absolutely with the conditions of the Sauropsidan and Monotreme egg.

It suggests an explanation of the formation and origin of the Eutherian amnion, which leads to the same conclusion as Hubrecht's view, namely, that the more primitive form of amnion is probably that which is found in mammals like Erinaceus, Cavia, or, as I think more truly, in Vespertilio, and is of a different origin to the amnion of the Sauropsida and the Monotremata; but it does not, on that account, demand the removal of the placental mammals any further from those groups than any other known anatomical differences do.

Consideration of the Objections which may be urged to this View of a Hypoblastic Origin for the Trophoblast.

Tupaia.

Hubrecht (33) in Tupaia javanica recognised an early arrangement of some cells round others. He finds no differences during the first two generations, but when there are apparently about a dozen cells or more he finds a differentiation into an inner core of one lightly staining cell, which is completely surrounded by an outer layer of darker cells. "Auf dieses vierzellige Stadium folgt sehr rasch die Gruppirung der weiteren Furchungsderivate, nicht um eine centrale Höhlung, sondern um eine centrale Zelle. Diese centrale Zelle fällt in mehreren Schnitten durch ihre hellere Farbe auf, gleichsam alsob ihr Protoplasma in etwas anderer Weise auf das Tinctionsmittel reagirt habe, wie dasjenige der peripheren Zellen." This rather later differentiation into two kinds of cells is very like what I have described for the sheep, in which case I gave some evidence to show that the differentiation occurred at the third generation.

According to Hubrecht, this differentiation marks embryo from "trophoblast." The central cell, which soon multiplies, gives rise to the whole of the epiblast and hypoblast, while the outer layer is only "trophoblast," and is destined to form no part of the embryo proper. As appears in the latter part of

the paper, Hubrecht considers the trophoblast to be of epiblastic origin, and he compares it to the outer layer of epiblast of certain amphibians. He derives the mammalian egg from a small egg like that of amphibians, with comparatively little yolk.

Apart from all question of homology and mammalian descent the term trophoblast seems to me most useful in describing the events of mammalian development, and as such I have adopted it in my descriptions of the sheep and pig; but I do not adopt his hypothesis, because—

(1) It offers no explanation of the mode of origin of the trophoblast. On Hubrecht's hypothesis we should have expected a process of delamination, whereas in the sheep and bat, where the two groups of cells are the most clearly defined, it is an essentially horizontal division, as in the meroblastic eggs of birds and reptiles.

(2) It does not account for the throwing off of the trophoblast cells over the embryonic area, which is so evident in certain forms; which is the more surprising since in the Anura (to the epidermic layer of epiblast of which these cells are supposed to owe their origin) there seems to be permanent fusion between the epidermic and nervous layers, as described by myself (2).

(3) It is not applicable to my specimens of the sheep.

In order to make my observations accord with Hubrecht's hypothesis it is necessary to suppose that the inner layer of dark cells in my fig. 11 has been derived from the light-coloured cells. The difference is so great that I think it is almost impossible to ascribe such an origin to them. Or else we must suppose that these cells subsequently pass into the outer layer again after the stages of figs. 12 and 15. On the other hand, it seems easier to make the description and figures of Tupaia agree with my description of the sheep.

Hubrecht's figures in his pl. 1 show only a small difference in colour between the inner cell and the outer wall. For instance, in the figs. 19—25 there is very little evidence to prove that none of the inner cells were derived from the outer

wall. As far as it is possible to judge from these figures there is nothing to absolutely condemn such an interpretation.

The Bat.

An attempt to reinstate van Beneden's (8, 9) metagastrula theory for the development of the mammalian ovum has quite recently been made by Professor Matthias Duval in his work upon bats in the 'Journal de l'Anatomie et de la Physiologie' for the years 1895, 1896.

Duval found in the early stage of the development of Vespertilio murinus a stage exactly comparable to that which van Beneden described for the rabbit.

Quite in accordance with van Beneden's earliest account of the segmentation of the ovum in the rabbit, Duval believes that in the bat the whole of the inner cell mass becomes hypoblast, and the outer layer alone becomes epiblast. Like van Beneden he traces the origin of the distinction between the hypoblast and epiblast to the first division in the process of segmentation of the ovum.

From the first two segments a group of larger and more slowly dividing cells arises, and forms eventually the whole of the inner mass, which is surrounded by the smaller and more rapidly dividing descendants of the other primary segment.

The outer layer is from the moment of its first formation a single cell in thickness, and it remains so until a cavity appears between the inner mass of cells and the outer layer, and converts the embryo into a typical blastodermic vesicle. The vesicle expands, and the inner mass is almost entirely flattened out, so that at one time a condition is attained when the vesicle at its upper pole consists of an outer layer of a single cell in thickness—the epiblast, and an inner layer also of a single layer in thickness—the hypoblast.

The outer layer now begins to thicken, and forms the embryonic knob. In this knob an irregular cavity subsequently appears, portions of the roof of which are thrown off and absorbed, and the floor remains as the permanent epiblast.

This description as regards the segmentation of the ovum

and origin of the epiblast is exactly comparable to that of van Beneden for the rabbit, which, however, has been abandoned by van Beneden himself. Duval believes that it is nevertheless true for the rabbit. It follows in that case that the permanent epiblast in that animal has a different origin from that which at present it is held to have.

Duval suggests that its true origin has been missed by all the workers on the rabbit hitherto, and that the whole of the inner mass becomes flattened out as hypoblast (v. Duval [26], fig. 28, p. 163).

The rabbit has formed an object of research for so many persons that such an important stage is very unlikely to have been missed. Duval suggests that it is a stage which occupies so short a time that no one has chanced to catch it. But think what the process must involve! Cells which are extremely attenuated and under considerable tension must suddenly grow so rapidly as to bud off large cells in a direction contrary to that in which they have hitherto given rise to cells, and then again resume their attenuated form; and while this is going on, the large rounded cells previously existing between the outer layer and the hypoblast have to pass into the hypoblast and become suddenly stretched and flattened, like those already there. These would surely be changes more profound than anything that occurs during the first seven days of development! It is difficult to believe that they could have escaped notice if they had existed. If there is a true "metagastrula" in the rabbit, and if the outer layer of this metagastrula gives rise to the epiblast in a way similar to Duval's description of the process in bats, the thickening of the outer layer to form the permanent epiblast must occur at a much earlier stage, and possibly before the formation of the cavity of the blastodermic vesicle,—as, for instance, the thickening of the outer layer does in the similar stage in the sheep (fig. 9).

Here is, at first sight, another possible interpretation of my sheep specimens, if the differences in colour indicated in my drawings of the later specimens (figs. 12—15) are held to

be of insufficient evidence to prove the descent of the light and dark cells of the later stages from those similarly coloured in the earlier figs. 8, 9, 11. This would be in accordance with Duval's view.

But if I attempt to take this interpretation, and say that the dark cells of figs. 8, 9, 11, are all epiblast and the light cells hypoblast, I find that, as may be seen in the figs. 12—15, there is no tendency whatever for the dark cells to become grouped together into a solid mass, such as the embryonic epiblast is in the later stages (figs. 17, 19). On the contrary, the darker cells of the inner mass in every section show a looser and a more peripheral arrangement to the inner mass. I find it is not possible to trace either the dark outer layer cells of fig. 11 into a compact inner mass, or to trace the lighter inner mass cells of fig. 11 into a loose investing sheath as at fig. 17. Whereas for the reverse course there is, as I have shown in the earlier pages of this paper, not a little evidence.

Is it possible to put an interpretation upon the ontogeny of the bat similar to that which I believe should be placed on the ontogeny of the sheep, such as suggested in my diagrams (V—V_7 on Pl. 18)?

I believe that a closer examination of Duval's and van Beneden and Julin's works indicates that such an interpretation is possible, as I will now attempt to show.

The account given by Duval is by far the most complete description of the development of any bat hitherto published. In some most important features it differs from that given by van Beneden and Julin (13).

The authors are, however, agreed that there is from the first division of the ovum a distinct difference, by which the segment which will ultimately give rise to the epiblast cells may be recognised from that which will give rise to the hypoblast.

They agree that the four-segment stage consists of two larger spheres and two smaller spheres.

Van Beneden and Julin say that two are smaller, darker,

and more granular and duller than the other two. This description refers to two specimens of Vespertilio murinus examined in the fresh state. The same holds good for specimens of Rhinolophus ferrum equinum.

On the other hand, Duval, who describes two specimens of the same species Vespertilio murinus and speaks from preserved and stained material studied in section, asserts that the two larger spheres are the darker ones, "deux plus petits et plus clairs; deux plus gros et plus foncés."

According to Duval the two small clear spheres give rise to the ectoderm. Van Beneden and Julin had unfortunately no stages between the four-segment stage and the completed blastodermic vesicle. It would seem, however, that they considered the large clear cells to be ectodermal, and the smaller darker cells hypoblast.

Again, there is a disagreement as to the position of the inner mass on the wall of the blastodermic vesicle. Duval concludes that the inner mass is attached to the base of the cup formed by the enveloping outer layer, whereas van Beneden and Julin find it at the mouth of the cup, which two accounts are quite irreconcilable. In Duval's case it is Vespertilio murinus which is being described, and in van Beneden and Julin's it is Rhinolophus ferrum equinum. But the difference is so great that I can hardly think it should be accepted as a generic variation. It is impossible to derive van Beneden and Julin's figs. 5 and 6 from Duval's fig. 24, or vice versâ.

Van Beneden and Julin's account is derived from two living specimens, and Duval's from one specimen which had been subjected to the process of preservation and staining, and had been made into sections.

Duval unfortunately never shows the presence of zona radiata in any figure, and I do not think that he ever makes any mention of it. It is difficult to imagine such an embryo existing in a uterus unless there is a zona present. Van Beneden and Julin, however, show the zona in all their figures.

One could have felt more confident that this specimen was

quite normal and uninjured if Duval had mentioned whether the zona radiata was present and quite sound.

Of course Duval notices this all-important difference, but cannot explain it. He says (p. 155), "Quant au blastopore primitif (ombilic ombilical) du Murin, nous devons aussi remarquer que nos préparations nous le montrent avec des rapports différents de ceux observés par van Beneden. Cet auteur l'a decrit et figuré comme placé au centre de la région où la masse endodermique adhére a la face interne de la sphère ectodermique. Nous l'avons vu, ou, pour mieux dire, nous avons vu un orifice, précisément dans la région opposée, au centre de l'autre hémisphère. Nous ne saurions pour le moment expliquer cette contradiction. Nous sommes bien convaincu de la valeur et de l'exactitude de la fig. 24 (pl. i), l'œuf que l'a donnée, étant conservé en coupes, qui ont pu être étudiées à diverses reprises. Mais nos observations ne sont pas assez nombreuses, car, entre le stade F et la stade H nous n'avons qu'une observation."

It seems to me that there is a greater weight of evidence in favour of van Beneden's view.

Taking the combined results of Duval's and van Beneden's papers, the interpretation offered in my diagram, V—V_7, Pl. 18, seems to be by no means an impossible one. W is Duval's fig. 15, 17, 18, or 20, reversed, E V is derived from fig. 21, and the description of the same on p. 140. Duval concludes that his fig. 21, H., is a section taken through such a specimen as his fig. 20, at right angles to the direction of the section fig. 20, close to one end, and that it cuts the ectodermal cells only. Now according to Duval this ectodermal layer is only one cell thick. Is the section Duval has drawn (fig. 21, H.) taken as close to the surface as he supposes? Is it not possible that the ectoderm is really two layers thick (or perhaps more) at this end of the embryo like my diagram E V? Fig. 21, H., undoubtedly shows an inner core of cells, and when we consider how convex a surface it is which is supposed to be cut, it is remarkable that in no less than fifteen out of seventeen cells through which the section passes the nuclei should have

been cut, if it is only one layer in thickness. The diagram V_1 is derived from fig. 6 of van Beneden and Julin's work, or from fig. 32 of Duval's work.

If there is such a distinct difference in colour between the outer layer and inner layer of the inner mass, as shown in Duval's figures, my interpretation can hardly hold good. Duval, however, explains that his figures are to a certain extent diagrammatic. He writes on p. 134, "Sur nos figures, nous avons exagéré et schématisé ces différences d'aspect entre les segments petits et clairs (cellules ectodermiques) et les segments plus foncés, plus granuleux (cellules endodermiques), et à cet effet nous avons, d'une manière conventionelle, donné aux cellules ectodermique un corps clair, avec un noyau foncé et aux cellules endodermiques un corps foncé avec noyau clair, de façon a permettre de saisir au premier coup d'œil la manière dont se comportent ces cellules les unes vis-à-vis des autres dans les stades successifs que nous figurons."

Another extremely important stage from Duval's point of view is that of his fig. 36.

Here the outer layer consists "généralement d'une seule couche de cellules cubiques." At the embryonic pole, however, these cells tend to become arranged in two layers. It is, however, a very slight thickening. The inner mass is very much flattened, and extends halfway round the walls of the vesicles.

At the embryonic pole the inner mass is, although distinct from the outer wall, two layers thick, which thickness, however, extends over a short area only.

Duval assumes that the whole of this inner mass is hypoblast, and that it eventually becomes completely flattened out into a single layer, as he shows in his diagram B on p. 106 (vol. 1896). But he apparently has not found this stage—it is not shown in any of his figures.

May we not, on the other hand, suppose that in the double-layered condition of the inner mass of this stage the inner layer is the true hypoblast, and the cells lying between this and the outer layer are the true epiblast cells? May not

fig. V_3, Pl. 18 be as likely an interpretation of his fig. 36 as that given by his diagram B, p. 106?

This stage is at once succeeded by one in which a rapid increase in the mass occurs at this point, which Duval considers to be entirely due to the activity of the outer layer. So great is the increase that it is impossible to distinguish between it and the hypoblast; there is "une soudure des deux feuillets avec engrènement des cellules de l'un dans celles de l'autre." Is it decisively proved that the intermediate cells take no part in this increase? I hardly think it is, and suggest that his fig. 41 is equally open to the interpretation given by my fig. V_4.

The subsequent history tends to support my interpretation. In speaking of the increase in bulk of the general embryonic mass Duval says, p. 430, "La cavité amniotique du Murin prend naissance d'une manière singulière. Il se forme d'abord un épaississement massif de l'ectoderme, et ce processus rapelle la masse amniotique pleine du cochon d'Inde; mais au lieu que cette masse se creuse, comme chez ce rongeur, d'une cavité centrale close de tous côtés, elle se disloque irrégulièrement, chez le Murin, et s'ouvre à la surface, de l'oeuf, figurant une bourse largement etalée, dont les bords se relévent alors selon le type classique de replis amniotique et produisent l'occlusion de l'amnios par leur rapprochment et soudure."

He then explains how after the roof of the amniotic cavity has been separated off in small fragments from the solid floor (figs. V_5, V_6, Pl. 18) the true amnion is eventually formed by the advance and fusion of the free edges of the true epiblastic mass as shown in diagram V_7.

On my interpretation, the rapid growth of cells which produces the "amniotic mass" is due chiefly to the inner mass cells, which cause the rupture of the overlying trophoblast cells which now grow rapidly for a time, and form irregular pieces which ultimately are thrown off and lost, leaving the epiblast exposed on the surface. It has no connection at all with the amnion formation, which is brought about subsequently by folds of the true epiblast, and not by trophoblast.

The Rabbit, the Mole, the Shrew.

The growth of an outer layer of cells round an inner core of cells of rather different texture was in the first instance described by van Beneden, 1875, which process he regarded as a kind of gastrulation, and to the form produced thereby he gave the name of metagastrula. The outer layer he called epiblast, the inner core hypoblast. Van Beneden's description is very complete, and was at first accepted, and his figures passed into all text-books.

From the researches of Rauber and Kölliker, however, it was seen that this interpretation could not be maintained, because, as they showed, and as was confirmed by van Beneden also in a later paper (van Beneden and Julin, **14**), the greater part of the inner core is transformed into the epiblast of the actual embryo, while only a small portion becomes hypoblast. But although the interpretation of the metagastrula as given by van Beneden was thus shown to be incorrect, does it necessitate the total abandonment of the description given by van Beneden? Does a "metagastrula" stage not exist at all? Duval, on finding in his bats an identical process to that described by van Beneden for the rabbit, re-adopts the metagastrula for both rabbit and bat.

He writes (p. 153) : "Cette gastrulation est celle, sauf une différence à préciser plus loin, que van Beneden a décrite chez le lapin sous le nom de Métagastrula, et qu'il a ensuite admise pour les Chéiroptères, d'après l'étude d'une vésicule blastodermique du Rhinolophe, vésicule formée d'une sphère externe d'ectoderme et d'une mass interne d'endoderme. Or, depuis ses deux mémoires de 1880, van Beneden a abandonné sa conception de la métagastrula des mammifères. Nous devons déclarer purement et simplement que nous reprenons cette conception, et que nous nous préparons à la défendre."

The evidence in favour of the occurrence of the metagastrula in the rabbit may be briefly tabulated.

(1) Foremost is van Beneden's (**8**, **9**) original account. The

difference between outer and inner cells dates from the first division of the ovum.

(2) Keibel (38) states that he has been able to observe in a number of cases in the rabbit the blastopore of van Beneden.

(3) Heape (29) has seen a similar appearance in the segmented ovum of the mole.

(4) Duval has described the formation of a metagastrula as a result of observations by sections in the case of the bat's egg.

(5) In the sheep, also, I have found a stage exactly comparable to the metagastrula, though I place a different interpretation upon it.

On the other hand, the value of van Beneden's and Heape's observations must be to a certain degree discounted, because they were based upon optical sections only.

Keibel does not state whether his observations are from real or optical sections.

Then, again, although Heape finds a metagastrula stage at the end of segmentation, he does not confirm van Beneden's account of its formation. On the contrary, he considers that it is formed by the migration of yolk granules from the outermost segments to the innermost at the close of segmentation.

He writes (p. 168): "I have myself examined segmenting ova of the rabbit, and have isolated the segments one from the other, in order the more clearly to compare them, and in no case have I been able to distinguish the slightest difference in the density or constitution of these segments. If my observations are correct, then the differentiation of the segmentation spheres into two layers in the fully segmented ovum is not a primary differentiation, such as Beneden discerns, but a secondary differentiation, due to the peculiar circumstances of nutrition and development attending the formation of the Mammalian embryo."

According to Heape, it is only at the close of segmentation that the segments become divided into two kinds. "A single layer of cubical hyaline segments completely surrounds, except at one point, an inner mass of rounded or polygonal densely granular segments." Thus Heape will not admit that there is

any such process as a growth of one group of cells round another group in the rabbit.

In 1894 a renewed study of this interesting stage was undertaken by myself (1). I examined a very large number of segmenting ova, both fresh, and by actual section. I was able to confirm Heape's account of the irregularity of the segmentation process, and quite failed to find any decisive evidence of the metagastrula stage at all. In fact, the balance of evidence was all against it, and I came to the conclusion that van Beneden's description was not supported by a study of the egg by means of sections.

When, therefore, I read Hubrecht's paper on Tupaia and Duval's on bats, and made my own observations upon the sheep, I was not a little puzzled to know what to think of the rabbit.

To accept van Beneden's account, as Duval proposes to do, leads us again into all the old difficulties regarding the fate of the inner mass, and, as explained above, it is, I think, impossible to admit Duval's method of escape from them.

Let it be supposed, on the other hand, that we admit van Beneden's view as far as the formation of the metagastrula is concerned: is it not possible that this stage is succeeded by a stage in which the outer layer becomes doubled, as in my fig. 11 of the sheep, and that the cavity of the blastodermic vesicle appears really between the cells of this doubled outer layer? For instance, van Beneden's (9) pl. iv, figs. 4, II, is very much like my figure of the sheep (fig. 9). The outer layer is distinctly thicker at one side than the other. May not this be the commencement of a reduplication of the outer layer?

In the absence of any difference in colour or other recognisable characteristics of the two groups of cells it is not possible to affirm that this is the course of events, but it is equally impossible to deny it.

I have again gone over the same ground in the rabbit with new material, and have treated it as far as possible in the same way that I treated the sheep embryos.

On examination of these embryos I am forced to come to the same conclusion to which I came three years ago. In the

majority of specimens there is no trace of a metagastrula stage. In two specimens I find that some cells are distinctly darker than others; these cells are mostly internally placed, but they come to the surface at more than one place and at nearly opposite poles of the embryo. I have drawn similar sections in my former paper (1, figs. 18, 19).

So, although I would not now assert that a metagastrula stage does not exist in the rabbit, I must reiterate that I have been unsuccessful in finding satisfactory evidence of it. But if it does exist, I believe it to be open to the same interpretation as that which I have placed upon the similar condition in the sheep.

In the same way it is not difficult to explain the development of the mole as described by Heape. In the absence of any very marked difference between the characters of the two groups of cells it is not easy to deny that any of the cells of the inner mass have been derived from the outer wall of cells. In the sheep the characters are markedly different. The doubling of the outer layer at one point is clear.

In the mole (as in the pig) there is no such marked difference. Hence it is not possible to deny or to affirm such a course of events.

Heape gives the subsequent separation of the hypoblast in these words:—"Certain of the cells bordering the blastodermic cavity become separated off from the main portion of the inner mass, and form a single layer of cells bordering the mass on the inner side. This layer is the hypoblast. The hypoblast is therefore derived from cells which result from the multiplication of the inner cell mass present in the fully segmented ovum."

But is it not possible that these cells may have originally been derived from the outer layer? Unfortunately Heape had no real sections of any stage younger than his fig. 17, in which the cavity of the blastodermic vesicle had attained a great size.

The difference which Heape detected between the "single layer of cubical hyaline segments" surrounding "an inner

mass of densely granular segments" (29, p. 166) had, according to his description, a strictly physiological significance only, and was due, he suggested, to the transmission of yolk contained in the outer segments to the inner segments, this transmission being performed in order that the changes about to "take place in the constitution of the ovum may more readily be performed" (p. 167). And the inner mass as it appears in his figure of this stage (fig. 1) gives rise to definite epiblast, definite hypoblast, and a part of the walls of the blastodermic vesicle (vide 30, p. 418).

It is, however, possible that a stage similar to my figs. 8, 9, 11, of the sheep may have been missed, and that the metagastrula stage of the mole may be open to the same interpretation as that which I offer for the sheep.

In Sorex the earliest specimen described by Hubrecht (34) is one in which the cavity of the blastocyst is well established. The segmentation stages are unfortunately not known.

It is, I think, by no means difficult to interpret Hubrecht's youngest stage (Pl. 36, fig. 5) in the way that is indicated in diagram Z (Pl. 18). There is, moreover, a distinct difference in size between the cells comprising the embryonic knob. Two are very much larger, and two more are larger still than the others. Are these the epiblast, and all the rest hypoblast? (vide Hubrecht's remarks upon it, pp. 506, 507).

The Rat, the Mouse.

Robinson's hypothesis, to which I have already alluded, was based chiefly upon his interpretation of rat and mouse embryos. I have suggested only a slight modification of his views in connection with these animals. His idea with regard to other animals was that the epiblast subsequently grew round a pre-existing hypoblastic vesicle. It was in connection with this inference that he met with so little sympathy.

In a former paper on the rabbit (1) I wrote, "There is no evidence in support of Robinson's speculations concerning the existence of a hypoblastic wall to the blastocyst surrounded subsequently by the epiblast."

In making that statement I was confident that Robinson was not right in the manner of application of his suggestions to the rabbit. His remarks on the blastocyst of the rabbit will be found on pp. 420—422 (**46**), part of which I must quote here. "It is stated that the flat cells on the outer surface of the inner mass either disappear entirely, or they fuse with the cells immediately beneath them to form the epiblast of the germ; and in either case, after the flat cells over the outer surface of the inner mass have disappeared, it is just as possible that the remainder of the vesicle wall hangs in continuity with the peripheral flattened cells derived from the inner layer of the inner mass—that is, with the hypoblast, as with those which constitute the epiblast; but in the case of the rabbit there is no evidence which will completely substantiate a statement that either the one or the other of those possibilities occurs. It is certain that the portion of the vesicle wall, which is at first formed by a single layer of flattened cells eventually becomes didermic, and that the change from the single to the double layered condition commences in the vicinity of the inner mass, whence it gradually extends to the opposite pole of the ovum. It is stated that the didermic condition is produced by the extension of the hypoblast round the inner surface of the primitive wall, but no satisfactory proof has been brought forward in support of this statement. The cells of the extending layer are from the first flattened, like those over which they are extending, and nuclear division has not been clearly demonstrated either in the inner or the outer layer."

"It appears to me, therefore, that the evidence which has been obtained from the study of the rabbit's ovum does not conclusively substantiate the statement that the outer wall of the primitive blastocyst is epiblastic in nature, and that the hypoblast extends round its inner surface."

I paid much attention to this, and I can have no doubt that the rabbit's blastocyst is not capable of receiving the interpretation that Robinson suggests. The cells, which apparently "extend round" the blastocyst, are beyond all doubt on the inside.

Hubrecht (35) says in reference to Tupaia javanica, in speaking of Robinson's "ingenious speculations," "According to these views, the outer layer of the monodermic blastocyst is in reality a hypoblastic layer. The didermic phase essentially originates out of this by a gradual spreading of epiblast cells outside the more primitive hypoblastic wall. However ingenious these speculations may be, the author holds them to be erroneous." He then proceeds to give an account of the development of Tupaia javanica "as an example of a mammal, the early development of which furnishes us with decisive evidence in this respect."

So, although Robinson's conclusions cannot be maintained for the rabbit when applied in the way he did, there seems to me to be no great objection to placing an interpretation upon a much earlier stage, in accordance with my discoveries in the sheep, as explained above.

I would, therefore, suggest a modification of Minot's and Robinson's theories of a hypoblastic blastocyst as follows:

1. The whole of the subzonal epithelium is entodermic.

2. The central portion of the inner mass of the mammalian blastocyst is ectodermic, but the surrounding layer is entodermic.

3. "The cavity of the mammalian blastocyst does not correspond with the segmentation cavity of the lower Vertebrates, but with the archenteron" (Robinson).

4. The archenteron is at first a closed vesicle; there is no blastopore.

In criticising Robinson's suggestions with regard to the rabbit, it must be remembered that he has described a stage in the development of the ferret (47), in which the blastodermic vesicle of the eleventh day is said to be a thin-walled vesicle of hypoblast, bearing at one pole and on the outside, i. e. between the main wall of the vesicle and the zona radiata, a small disc of cells—the epiblast.

Thus Robinson finds in the ferret what he expected to be the case in the rabbit.

If the blastocyst of the ferret becomes, as we may suppose,

converted into a didermic vesicle, like those of other Carnivora, we must conclude that it is brought about by the growth round of the epiblastic disc cells. The process in the ferret is, then, diametrically opposed to that by which an extremely similar condition is brought about in the rabbit, Tupaia, Talpa, &c.

It is, of course, just possible that Robinson may have been deceived by the appearances of the sections he examined. The stage which he had is an extremely difficult one, and in this case was quite isolated; for the author had neither older nor younger material. He had as many as seven specimens of this one stage.

If we are to suppose that Robinson was quite right, we must conclude that in the ferret the course of development seems to be very different from that of any other mammal. But a more extended research in the ferret should be made before this conclusion is adopted.

The Two-layered Condition of the Hypoblast.

With reference to the difficulty of the double-layered condition of the hypoblast, I would point to my figs. 15 and 17 on Pl. 16, and ask what else but a two-layered condition could result upon the occurrence of the expansion of the blastocyst without a corresponding extension of the epiblastic knob? (v. figs. 17 and 19).

The simple expansion of the blastocyst would be sufficient to set up the two-layered condition by drawing out the angles as shown diagrammatically in the figure on p. 222. The two-layered condition once set up, the more complete separation of fig. 19 might arise owing to difference in tension and so forth, under which the layers might reasonably be supposed to exist. In some cases where the expansion of the blastocyst does not occur in this way there is no double layer of hypoblast, e. g. Cavia, Mus (Robinson).

If, as suggested in the sequel, it is thought to be better to compare the trophoblast more strictly with the yolk-bearing

hypoblast of the meroblastic egg, then a distinction might perhaps be drawn between true hypoblast and trophoblast, and compared with the true hypoblast and yolk nuclei of the bird's egg, by whose union in the bird's egg the bourellet endodermique is formed (Duval).

The Relation of the Allantois to the Amnion.

In a former paper (1) on the early stages of the development of the rabbit I ascribed the apparent extension of the hypoblast round the inner surface of the blastocyst to the presence of a region of special activity in the extra-embryonic epiblast, or, as I would now rather call it, the trophoblast, which begins to be manifest as early as the fifth day, and culminates at last "in the production of the ectoplacental area, or part of it" (p. 146). When discussing in a subsequent paper (3) the mode of extension of the primitive streak mesoblast, I was not able to understand how the extension of that layer in the form of a wide-meshed reticulum, such as it is in its peripheral parts, could be produced except by its being dragged away from the primitive streak by the expanding walls of the blastocyst in a way analogous to that assigned for the spreading of the hypoblast.

Accordingly I thought that the overlying layer must also have come from the primitive streak, and wrote, "We may take the outline of the primitive streak mesoblast as indicating also the outline of the epiblast derived from the primitive streak, at any rate for the stages up to about the one drawn for fig. 6. At this time cells are separated from the hypoblast, both at points where there is already existing primitive streak mesoblast, and at points where, up to now, there has been no mesoblast." So far, I think, I was right; but in drawing the diagrams on Pl. 22, and in writing the description of them, I seem to have neglected to consider the mesoblast of hypoblastic origin in the region round the primitive streak (originally described by Hensen), and so fell into the error of taking the extreme outline of the mesoblast as an indication of the limit of cells of primitive streak origin at this

more advanced stage. If this were so, the greater part of the ectoplacenta would be of primitive streak origin, and not trophoblastic, which would be adverse to the hypothesis I have been tempted to put forward as the result of my observations on the segmentation of the sheep's ovum.

From a renewed examination I am quite convinced that the outskirts of the mesoblast surrounding the hinder end of the embryonal area of the rabbit about the time the primitive streak attains its maximum elongation, are in part made up of cells of hypoblastic origin, though whether to such a large extent as Bonnet describes for the sheep, and Hubrecht for Sorex, I cannot say.

In the sheep Bonnet says the first formed mesoblast cells are those of hypoblastic origin, and in this case it is clear that the primitive streak mesoblast does not extend far beyond the embryonic area; whereas in the rabbit the first formed cells are undoubtedly those of epiblastic origin, and if these are very quickly removed from the immediate region of the primitive streak, as seems probable, it is impossible at present to determine the actual boundary between the two sets of cells.

If the greater part of mesoblast which underlies the ectoplacenta is of hypoblastic origin, and if the true primitive streak mesoblast does not really extend beyond the inner limits of the ectoplacenta—i. e. not further than the circle 4 or 3 in fig. 44 of the paper referred to above (3),—there would then be no reason for supposing the ectoplacenta to be anything but trophoblast, and the rabbit would agree with other mammals in this respect.

Dr. Robinson tells me that in the ferret the ectoplacenta is formed, and attachment to the uterus thereby effected, before the mesoblast has appeared beneath the primitive streak. In this case there is, therefore, still less reason for doubting a trophoblastic origin for the ectoplacenta.

In my diagrams on Pl. 18, I have shown how the true amnion is in most cases formed entirely of true epiblast; that is to say, it can be shown to have been derived from the embryonal area, and not the trophoblast.

This is pretty obvious in cases like those of the guinea-pig and Pteropus, in which the amnion is formed by the hollowing out of a portion of the embryonic epiblast, and not by folding.

Duval's account of the development of the amnion in the bat almost as clearly proves it to be of true epiblastic origin.

In the rat also it is clearly the embryonic epiblast which forms the amnion.

In all these cases the subzonal membrane which takes part in the formation of the chorion is trophoblastic, and the true amnion alone is epiblastic. But how is it in such cases as those of the rabbit, pig, and mole? Is the amnion in these cases also true epiblast?

As regards the rabbit, I have nothing further to say, beyond what I have written above. The amniotic folds arise between the hinder end of the embryo and the ectoplacental region. Where exactly the junction between epiblast and trophoblast lies cannot be determined. In my diagram (L_4) I have left it uncertain.

In the pig, however, I believe I have been able to trace the junction. In an embryo thirteen days old, which exhibits the earliest sign of amnion formation, the boundary between the embryonal area and the trophoblast is just recognisable.

The embryonal area cells are still slightly lighter in colour, the nuclei are on an average larger, and at many places the remains of the membrana hypoblastica limitans are still visible (figs. 26, 27).

From this specimen it appears that the amniotic folds arise at about the junction of the embryonal area and the trophoblast. It gives one the impression that when completed the true amnion will be formed of embryonal area epiblast and the false amnion of trophoblast.

But, although it is exceedingly difficult to follow, I do not think that this is exactly what happens. I fancy that in a specimen in which the amnion, with the exception of the "amnionnabelstrang," is completed I can trace the junction between epiblast and trophoblast within the limits of the true amnion, as shown in my diagram Su_4, and figs. 28, 29, T, E.

I have no doubt at all that the true epiblast takes some part in the formation of the true amnion, but I am not at all sure that it forms the whole; in fact, I think it does not.

Since I hold that the trophoblast is probably of hypoblastic origin in all these forms, it follows that the true chorion in mammals is formed by the fusion of the allantois with hypoblast (i. e. trophoblast), whereas the analogous membrane in birds is formed by the fusion of allantois with epiblast. To some this may appear to be a fatal objection, as it implies that the false amnion of a bird is not strictly homologous with the false amnion of the pig.

To this I would reply, firstly, that the more primitive form of amnion for mammals may be that which we find in the guinea-pig or hedgehog, in which the parts which serve as false amnion are developed quite apart from the production of the true amnion. From these we get through stages such as are found successively in the bat, pig, and rabbit, to a method of production very similar to that of the bird, as the condition of development became more and more alike.

Secondly, the essential feature of the hypothesis is that the epiblast never grows round the ovum in placental mammals, and with the exception of the true amnion it takes no part at all in the formation of any part of the fœtal membranes. In birds the epiblast grows round the ovum, ultimately forming a firm wall to a sac which enables the all-important organ, the allantois, to become rapidly and effectively developed and extended.

In mammals the sac is sufficiently well produced at an earlier stage, and the conditions necessary for the growth of the allantois are provided without need of the extension of the epiblast.

The epiblast cells which grow round the yolk in the bird do not take any part in the formation of the actual embryo; their function ceases at the time of hatching, and they are thrown off or absorbed.

The progress of the epiblast over the yolk in the bird is described as being due to the multiplication of the epiblast

cells themselves. It is an actual sliding of the free edge of the epiblast over the yolk, and is therefore quite different from the extension of the epiblast over the amphibian egg, where it is not a sliding of one layer over the yolk, but a conversion in situ of nuclei from the yolk into epiblast nuclei.

Let us, on the supposition that the mammalian ancestor had a large yolked egg like that of a reptile or bird with vitelline membrane, consider the probable steps which converted such a condition to the one suggested in my paper.

Firstly, it must be remembered that the epiblast is cut off from the rest of the ovum at a very early period—in the bird about the time of laying. From this moment it never receives any nuclei or cells from the yolk, but spreads by its own interstitial growth. It does not of itself form a closed vesicle until quite late in embryonic life.

On the other hand, the lower layer cells, after their separation from the epiblast, become continuous with yolk along the germinal wall (bourrelet entodermo-vitellin—Duval), and form at once a closed vesicle, which now rapidly fills with fluid and expands.

This closed vesicle, which is formed by true hypoblast and yolk-containing hypoblast, has two functions in addition to that of supplying actual cell material,—namely, of providing nourishment, and of supplying a roomy cavity for the reception of the embryo while forming.

If the same condition appertained in the large-yolked egg of the mammalian ancestor, is it not more likely that the same group of cells should have continued during the substitution of maternal fluids for yolk the offices of supplying nutriment as before, and of the maintenance of a large cavity for the reception of the embryo, than that the other group of cells should acquire them ?

Duval (24) has described a thickening, and a series of villous processes on the edges of the extending epiblast of the bird's egg, which according to him form with the allantois a "placenta," by means of which nourishment is obtained from the albumen towards the end of incubation. Nevertheless by far

the greater portion of nourishment is obtained from the yolk contained in the hypoblast cells.

The difference in size between the fertilised ovum of a reptile or bird, and of a mammal is very great; but the difference in size between the embryo of, say, a bird, with one pair of mesoblastic somites, and of a mammal of the same age, is comparatively small. This means that nearly the same space is required for the production of the mammalian embryo as of the Sauropsidan, and has to be provided.

As the egg diminished in size, owing to the loss of yolk, a corresponding increase in activity of the cells of the wall of the vesicle must have taken place to keep up the size of the vesicle. The smaller the egg became, the longer would be the time occupied in the attainment by the vesicle of the requisite size.

Now, as the epiblast plays the more prominent part in the formation of the bulk of the embryo during the earliest stages, it clearly would be useless for the embryonic part to exhibit much energy of growth until the old conditions were to a certain extent regained; hence the lethargy exhibited by the embryonic epiblast in mammals during the first week of development. No feature of the early stages of the mammalian embryo is more striking than this inertness of the embryonic epiblast—or, as I should now prefer to call it, simply epiblast —during the first few days.

A need for the extension of the edges of the epiblast as a protective layer, as in the Sauropsida, has been obviated by the development among mammals of the strong zona radiata; and any tendency which existed for it to spread may very easily be conceived to have been prevented by the increased activity of the hypoblast cells, now no longer laden with yolk. This in many cases resulted in a temporary overflow—as it were— round the epiblast, aided perhaps originally, if not actually in present embryos, by the restricted confines of the zona radiata. Some such intermediate stage is shown in fig. D on Pl. 18.

It is probable that for the more primitive history of the relation of trophoblast to the uterus and placenta we ought to turn to the smallest mammals; and it is there that we see

the closest connection between the walls of the blastocyst and the mother before the formation of the placenta.

In large animals, such as the sheep and pig, it is doubtful whether there is any connection quite comparable to the ectoplacenta or träger eating its way into the maternal tissue. The energy is present, but shows itself in an enormous growth of the vesicle, which no doubt answers the purpose of acquiring nutriment, but in a rather different way.

In many of the smallest mammals we find also great differences in the mode of formation of the amnion. For instance, the amnion of Erinaceus, Mus, Cavia, Vespertilio, differ much from each other and from the Sauropsidan type. In the larger animals, Sus, Ovis, and in those smaller ones which retain the zona radiata for some considerable time, Lepus, Sorex, Talpa, the amnion formation is apparently more like that of the bird.

Do the smaller mammals really show us the more primitive type of amnion development for the mammalia?

One is tempted to ask, did a continuance of the retardation of the epiblast and acceleration of the hypoblastic mass with its tendency to surround the former, lead to a curving upwards of the edges of the epiblast, which, by means of their subsequent expansion, gave rise to an amnion which is essentially different from the amnion of the Sauropsida,—an amnion dependent in no way on the force of gravity, and less on the action of the internal fluid than in the Sauropsida, and therefore less likely to be so much affected by the frequent alteration of position which must occur in the mammalian uterus? Such an amnion formation is actually found in the case of Vespertilio (Duval).

On this view the free edges of the advancing amnion fold, as described by Duval, would be both morphologically and physiologically equivalent to the advancing free edges of the epiblast of the blastoderm of the Sauropsidan egg.

A hastening of this process might lead to the condition in Pteropus (52) or Cavia in which the amnion is from the first appearance perfect.

A delay in the accomplishment of the formation of the amnion until after the complete attachment of the edges of the true epiblast to the trophoblast subsequent to its rupture would cause a condition as in the pig or sheep, when the first starting of the amnion seems to be due to the bending up of the edges of the embryonic area, but the full completion of the amnion to be due to other causes, such as the pressure of the enclosed fluids. Thus a state analogous to the Sauropsida is attained. In this case (pig), as I have shown in my figs. Su_4, Su_5, Pl. 18, the true amnion is only partly formed by the true epiblast. If this is a correct view, it is interesting to note that there is not the same tendency for the edges of the amnion to fuse in this case, pig (also sheep), as where the free edges of the epiblast coalesce.

It seems not improbable that in the rabbit this latter process has gone even further. It differs much in its formation from that of the pig. It arises much later, and is practically formed entirely from the hinder fold.

It is much to be regretted that we have not yet a detailed account of the development of the monotreme blastoderm. Semon's account of the segmentation of the ovum does not extend as far as the separation of the true hypoblast as a permanent layer. There is no trace of an archenteron in his oldest stage on pl. ix of his work.

I shall not attempt to reconcile this hypothesis with the description of the opossum given by Selenka. The development of this animal is very unlike that of any placental mammal. Although it has what at first sight seems to be a typical blastodermic vesicle (v. Selenka [51], pl. xix, figs. 1, 5), this, nevertheless, differs from all forms hitherto observed by the absence of a Rauber layer over the formative epiblast. It is clear from this fact, and from the presence of a large "segmentation cavity" which never disappears, the evident "blastopore," and the peculiar origin of the entoderm, that the Marsupials in their development differ more widely from the placental mammals than the members of the latter group differ among themselves. Selenka's account is by no means com-

plete, and in the absence of a more extended research into the process of segmentation of the ovum of the Marsupialia and Monotremata it is useless to discuss at any length the present hypothesis with regard to those groups of mammals. As far as we can gather from the little which Caldwell and Semon have published concerning the segmentation of the Monotreme egg, we must expect that the eggs of these mammals resemble those of the Sauropsida, and that in them the epiblast does grow round the yolk, producing a double-walled sac consisting of an outer layer of epiblast, and an inner layer of large yolk-laden hypoblast, as described by Hill and Martin (31) in a specimen obtained from the uterus of an ornithorhynchus, in which seventeen mesoblastic somites were present, How this sac is formed we do not know.

Whether the Marsupial development resembles that of the Monotreme or that of the Placentalia more closely cannot at present be decided.

Whichever view is taken of Selenka's description of the opossum, many obvious difficulties remain, for the solution of which no satisfactory suggestion can as yet be offered. If, as I believe, the development of the placental mammals shows us, the protomammalian had a large-yolked egg, the Monotreme's development would be of the greatest possible interest. Hubrecht has, in the very interesting paper to which I have frequently referred, most ably advanced the opposite view that the protomammalian egg was a small egg of the amphibian type, and that the Monotreme is an aberrant offshoot. If I prefer to support the other view I am only adhering to the opinion expressed by Balfour in his great work on 'Comparative Embryology,' 2nd ed., p. 189 :—"The features of the development of the placental Mammalia receive their most satisfactory explanation on the hypothesis that their ancestors were provided with a large-yolked ovum like that of the Sauropsida. . . . The embryonic evidence of the common origin of Mammalia and Sauropsida, both as concerns the formation of the layers and of the embryonic membranes, is as clear as it can be. The only difficulty about the early

development of Mammalia is presented by the epibolic gastrula and the formation of the blastodermic vesicle. . . . No satisfactory phylogenetic explanation of the mammalian gastrula has, in my opinion, as yet been offered."

Although my inferences as to the homologies of the amniotic folds cannot be said to have been contemplated by the writer of these words, I hope it may be thought that my work upon the segmentation of the sheep might have removed from his mind the difficulty of the supposed epibolic gastrula of the mammal by showing that such a phenomenon does not occur, and that the appearance which has given rise to the misconception is capable of quite a different interpretation in complete accord with his view that the development of the mammalian ovum bears traces of a descent from an ancestor with a large-yolked egg.

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DESCRIPTION OF PLATES 15—18,

Illustrating Mr. Richard Assheton's paper "On the Segmentation of the Ovum of the Sheep, with Observations on the Hypothesis of a Hypoblastic Origin for the Trophoblast."

List of Reference Letters.

E. Epiblast. H. Hypoblast. M. Mesoblast. M. H. L. Membrana hypoblastica limitans. T. Trophoblast. Y. Spherical bodies found near the surface of the unsegmented egg. Z. Zona radiata.

PLATE 15.

Fig. 1 (Ovis 3, No. 1).—Unfertilised ovum from the uterus.

Fig. 2 (Ovis 36, No. 1).—Age 1½ days. Section of the fertilised ovum of a sheep, showing male and female pronucleus. Found in the Fallopian tube. Preserved in ·5 per cent. chromic acid. Stain carmalum. Thickness of section ·0075 mm. × 420.

Fig. 3 (Ovis 35, No. 2).—Age 3 days. Section of the fertilised ovum of a sheep. Fertilisation completed, and the division of the ovum is taking place. Preserved in 1 per cent. chromic acid. Stain, Klein. hæmatox. Thickness of section ·0075 mm. × 380.

Fig. 4 (Ovis 33, No. 1).—Age 2½ days. Section of a segmented ovum of a sheep with six segments. Preserved weak Flemming. Stain carmalum. Thickness of section ·0075 mm. × 380.

Fig. 5 (Ovis 49, No. 1).—Age between 4 and 6 days. Section of a segmented ovum of a sheep with eight segments. Stain carmalum and picric acid. Thickness of section ·006 mm. × 380.

Fig. 6 (Ovis 63, No. 1).—Age 4 days. Section of a segmented ovum of a sheep with sixteen segments. Stain carmalum. Thickness of section ·005 mm. × 380.

Fig. 7 (Ovis 33, No. 2).—Age 2½ days. Section of an ovum of the sheep with eight segments. Preserved in ·5 per cent. chromic acid. Stain carmalum. Thickness of section ·0075 mm. × 420.

Fig. 8 (Ovis 18, No. 1).—Age 4 days. Section of the ovum of a sheep with thirty segments, six of which are large and light in colour. Preserved in ·5 per cent. chromic acid. Stain carmalum. Thickness of section ·005 mm. × 420.

Fig. 9 (Ovis 14, No. 1).—Age 6 days. Section of the ovum of a sheep in which the large light-coloured cells have been completely surrounded by the

small darker segments. Preserved in chromic acid. Stain borax carmine. Thickness of section ·005 mm. × 420.

Fig. 10 (Ovis 14, No. 2).—Age 6 days. Section through an embryo in which the dark outer layer has become doubled at one pole. Preserved in chomic acid. Stain hæmalum. Thickness of section ·01 mm. × 380.

Fig. 11 (Ovis 6, No. 1).—Age 7 days. Section of the segmenting ovum of the sheep in which the doubling of the outer layer of dark cells is very distinct, and the same layer has become thinner at the opposite pole. Preserved in chromic acid. Stain carmalum. Thickness of section ·0075 mm. × 420.

Fig. 12 (Ovis 4, No. 1).—Age 5¾ days. Section through an embryo of the sheep in which the cavity of the blastocyst is beginning to be formed. It apparently appears amongst the dark cells. Preserved in chromic acid. Stain carmalum. Thickness of section ·0075 mm. × 380.

PLATE 16.

Fig. 13 (Ovis 4, No. 1).—Another section through the same embryo as fig. 12. × 380.

Fig. 14 (Ovis 43, No. 1).—Age uncertain. Section through the embryo of a sheep with cavity of the blastocyst well established. Preserved in chromic acid. Stain carmalum. Thickness of section ·0075 mm. × 380.

Fig. 15 (Ovis 4, No. 2).—Age 5¾ days. Section through the embryo of a sheep slightly more advanced than the above. Preserved in chromic acid. Stain carmalum. × 380.

Fig. 16 (Ovis 34, No. 1).—Age unknown. A section through the embryo of a sheep just before the rupture of the zona radiata. Preserved in chromic acid. Stain carmalum. Thickness of section ·0075 mm. × 380.

Fig. 17 (Ovis 34, No. 2).—Age unknown. A section through the embryo of a sheep immediately after the rupture of the zona radiata. Thickness of section ·0075 mm. × 380.

Fig. 18 (Ovis 42).—Age 9 days.

Fig. 19.—Section through above along the line *a*—*b*.

Fig. 20 (Ovis 8).—Age 10 days.

Fig. 21.—Section through above specimen (Fig. 20) along the line *a*—*b*. Preserved in chromic acid. Stain carmalum. Thickness of section ·0075 mm. × 380.

Fig. 22.—Camera outline drawing of above section to show the extension of the inner hypoblast layer.

PLATE 17.

FIG. 23 (Ovis 40, No. 1).—Age 6 days (?). Segmenting ovum with fifteen segments, which surrounded a central cavity comparable to that shown in fig. 6 on Plate 15.

FIG. 24 (Ovis 40, No. 2).—Age 6 days (?). An eight-segment embryo separated into two groups of four cells. One segment contained certain round bodies which are not found in any of the others. Stain borax carmine.

FIG. 25 (Ovis 33, No. 1).—Age 2½ days. Six-segment stage of sheep. A section of this is shown in fig. 4, Plate 15.

FIG. 26.—Section of the side of an embryonal area of the pig of thirteen days; to show the difference in the character of the epiblast and trophoblast, at the place where the first sign of the amnion fold can be detected.

FIG. 27.—Another section of a similar embryo of the pig.

FIG. 28.—The whole section, from a part of which Fig. 29 was drawn. Pig of about 17 days. The amnion and false amnion folds are seen.

FIG. 29.—A section of a portion of the amnion fold of a pig of about 17 days. A very sudden alteration in the character of the epiblast of the amnion between the letters T and E is supposed to indicate a transition from trophoblast to true epiblast of the embryonal area.

PLATE 18.

In the diagrams on this plate, pink indicates epiblast, green hypoblast. The black ring is the zona radiata.

FIG. A represents the ovum of a bird while in the oviduct, showing the segmentation cavity. Compare Duval (**23**), fig. 29.

FIG. B.—The ovum of a bird about the time of laying. Compare Duval's 'Atlas,' fig. 33.

FIG. C.—The ovum of a bird after a few hours of incubation. The subgerminal cavity is now well established. Compare Duval's 'Atlas,' figs. 49—55, and Duval (**23**), 50—53.

FIG. D.—A diagram to illustrate a supposed intermediate condition of a protoentherian ovum in which a tendency to grow over the epiblast is exhibited by cell masses of hypoblastic origin.

FIG. W.—The segmenting ovum of a sheep and bat, and by hypothesis of all placental mammals.

FIG. X.—Another later stage of a segmenting ovum of Ovis, Tupaia, and by hypothesis of some other mammals. The hypoblast has completely surrounded the epiblast.

FIG. Y.—A stage found in Ovis, and by hypothesis common to many other mammals. The hypoblast is doubled over one pole.

FIG. Z.—A typical mammalian blastodermic vesicle interpreted according to the specimens figured on Plate 2 of this paper, supposed to be common to many mammals besides the sheep.

FIGS. T_1—T_4.—Four diagrams illustrating the interpretation according to the hypothesis of some of the succeeding stages of the development of the mole (Talpa europæa). Compare Heape (**30**), figs. 18, 23, 26, 27.

FIGS. TU—TU_2.—Three diagrams illustrating the stages subsequent to Z of Tupaia javanica. Compare Hubrecht (**36**), figs. 16, 23, 33, 41, 62, 68, with diagrams X to TU_2.

FIGS. SU—SU_5.—Six diagrams illustrating the stages subsequent to Z of Sus scrofa. Compare Assheton (**5**), figs. 19, 31, 39, and Keibel (**40**), and figs. on Plate 17 of this paper.

FIGS. L—L_4.—Five diagrams illustrating the stages of the rabbit (Lepus cunicula) subsequent to Z.

FIGS. E_1—E_4.—Four diagrams illustrating the stages subsequent to W of Erinaceus interpreted according to hypothesis. Compare with Hubrecht (**33**), figs. 25, 39, 15, 20 *b*, 51, 49, &c.

FIGS. V—V_7.—Eight diagrams illustrating the development of Vespertilio. Compare figs. W, V, with Duval (**26**), figs. 15, 21, fig. V_1 with Van Beneden and Julin (**13**), fig. 6, and V_2—V_7 with Duval (**26**), figs. 33, 36, 41, 50, 76, 102.

FIGS. M_1—M_2.—Five diagrams of Mus. Compare with Robinson (**46**), figs. 4, 6, 8, 10, 13.

FIGS. C_1—C_4.—Four diagrams of Cavia. Compare with Selenka (**49**), pls. xi, xii, figs. 1, 3, 9, 13.

On the Heart-body and Cœlomic Fluid of certain Polychæta.

By

Lionel James Picton, B.A.

With Plates 19—22.

[This work was done at Naples during my tenure of the Oxford Scholarship there in 1896–7.

Besides the subjects mentioned in the title, there is a description of a new bow-shaped corpuscle in Notomastus profundus on p. 268, footnote; and an appendix at the end of the paper concerning intra-vitam staining of nuclei of the gut-wall with carmine in sea-water.—L. J. P.]

In several groups of Polychæta there is found in the dorsal vessel or "branchial heart" a rod-like structure usually attached anteriorly and posteriorly, and sometimes at other points, to the vascular walls; but otherwise lying in the lumen of the tube freely. It is in many instances of a deep brown colour, due to the presence of numerous pigment granules deposited in it. The cells in which these are contained are small and thick-walled. They have little protoplasm, but well-marked nuclei. This organ, under the various names "corps cardiaque," "Herz-Körper," "second œsophagus," "cæcum gastro-œsophagien," and others, has been discussed by several authors.

Claparède (9, A, p. 399)[1] mentions in the dorsal vessel of Terebella multisetosa "une substance d'un noir profond, distribuée en cordons irréguliers;" and describes a similar structure also in the Cirratulidæ. He further notes (9, B) that chloragogen, the so-called "liver" tissue of Annelids, which usually clothes the gut and the exterior of the chief blood-vessels, is entirely absent in those forms in which "les masses intravasculaires" are known to exist.

Salensky (28) describes its state in the larva of Terebella Meckelii. Though not observed at the earliest period, at a certain stage an opening in the wall of the vessel was noted, which led into the cardiac body. Salensky does not state the exact position of this orifice, but says, "Il s'ouvre d'abord dans un petit tube bien étroit et se continue ensuite dans le corps cardiaque, qui à ce stade du développement représente un organe de forme cylindrique." For the rest, the state of the heart-body is described as being much the same as in the adult.

In the Chlorhæmidæ many naturalists have observed the peculiar dorsal vessel which appears to spring from the wall of the gut, and is of a very deep colour on account of the heart-body contained in it. Horst (16) describes the mistaken views of Otto, who thought it was a second œsophagus; of Dujardin, who regarded it as a mere blood-vessel; of Claparède, who thought it was a gland opening into the pharynx, and of others; and himself considers that it is not merely the heart, nor a gland only, but that it is a gland contained in the heart. He thus combines the views of Dujardin and Claparède. He further holds that the heart-body is derived from a pouch of the gut comparable with that of Enchytræidæ.

Kennel (20) considers a structure in the dorsal vessel of Ctenodrilus as the homologue of the heart-body of Terebella.

Steen (32, p. 42), in the case of Terebellides Stroemii, thinks the heart-body has a valvular function, preventing a

[1] The numbers refer to the Bibliography at the end of the paper.

back-flow caused by the contracting gills. He describes it as enclosed in a fine membrane.

Eisig (**12**, p. 691) mentions the statements of the earlier writers on the heart-body. First, ordinary chloragogen is absent in the forms which have a heart-body, whilst the heart-body is very similar to chloragogen tissue. He therefore proposes to call it "intra-vascular chloragogen." Secondly, he considers that Salensky's statements make it very probable that it is derived from the wall of the dorsal vessel and from peritoneal tissue, from which the cœlomic corpuscles[1] are also produced. This is equivalent to saying that chloragogen, which is a modification of peritoneal tissue, instead of remaining on the outside of the dorsal vessel, becomes folded so as to occupy its interior. Both to cœlomic corpuscles and to chloragogen excretory functions have been attributed, and this view regards them as having a morphological connection.

Cunningham (**10**) describes the heart-body in Cirratulidæ, Chlorhæmidæ, and Terebellidæ, and mentions its occurrence in Amparetidæ and Amphictenidæ. He is inclined to regard it as a ductless gland of mesoblastic origin, and he suggests its possible homology with the "notochord" of Balanoglossus.

E. Meyer (**24**, B) describes the heart-body of Chætozone sætosa, and expresses the opinion that its function consists in the preparation of the pigment which is dissolved in the red blood-fluid.

Jourdan (**17**), in his work on Siphonostoma diplochætos, gives an account of the "cæcum gastro-œsophagien," or heart-body. He speaks of it as "communiquant avec le tube digestif," which is denied by Bles (**5**), who supports the view that the heart-body is of mesoblastic origin, remarking that chloragogen is peritoneal, and that the connections of the

[1] "Haemolymph" is the word used, but it evidently refers to the cœlomic fluid. Would it not be better to restrict the term "hæmolymph" to such fluids as represent the combined blood and lymph, such as that of the Capitellidæ, to which Eisig previously applied the word? It was first used by Professor Ray Lankester for a fluid consisting, like Vertebrate blood, of both "hæma" and "lymph," when discussing the presence of corpuscles in what was then called the "pseud-hæmal" system of Chætopods (this Journal, 1878).

heart-body with the vascular walls suggest a similar source of origin for it. He regards the organ as a blood-pigment gland.

Miss Buchanan (6, A) found a heart-body in Hekaterobranchus Shrubsolii; and also (7, B) a structure, which is wanting in the adult, in the dorsal vessel of the developing Magelona. She regards it as a larval heart-body, and supposes that on its dissolution it forms the blood-corpuscles.

Cuenot (9″), who examined chiefly Nicolea venustala, describes cells in the heart-body as enclosing a number of refringent green granules. "J'ai vu," he says, "plusieurs fois les cellules vertes de la glande émettre de courts pseudopodes et faire saillie au-dessus de leurs voisines pour être, sans aucun doute, entraînées plus tard par les courants sanguins; le corps cardiaque est donc une glande lymphatique parfaitement caracterisée." Further, he agrees with Meyer in regarding it as also effecting a transformation of colourless albuminoids into hæmoglobin.

Schaeppi (29), in his interesting paper on the chloragogen of Ophelia, though his chief purpose is a study of the remarkable chitinous rods which occupy certain of the cœlomic fluid corpuscles of that worm, yet gives in addition a careful description of the heart-body, and also of certain peculiar intra-vascular connective tissue which occupies the perivisceral sinus posterior to that organ. This latter tissue begins where the heart-body ends; and, though the author shows that it contains different substances from those in the heart-body, the description suggests very strongly that it is an accessory part of the heart-body prolonged backwards from the short anterior dorsal vessel or heart into the sinus which surrounds the gut. The heart-body itself is a flattened band, of a bluish-white colour, occupying the cavity and attached to the walls of the heart. It consists of a homogeneous groundwork strewn with connective-tissue cells. Besides these there also occur cells occupied with peculiar green pigment granules, similar to others found in the thoracic sinus, but only rarely in the rest of the blood system. There are green granules also in some

of the cœlomic fluid cells; and Schaeppi found that in both cases they resist the action of alkalies and cold acids. He therefore regarded them as consisting of chitinous chloragogen. They gave no uric acid reaction. The cells of the intra-sinus connective tissue, however, he found to contain guanin granules, which, he suggests, are passed through the sinus wall into the peritoneum, the cells of which he describes as falling off in clusters from time to time. This process would give an opportunity for the guanin granules to be carried into the nephridia and excreted. With regard to the function of the heart-body itself, on the other hand, he remarks that the central position of the granules which occur in it, and the absence of a secretory epithelium, are objections to any theory of glandular action. He considers that the organ, by swelling up at systole, owing to the pressure of the blood into its meshes, acts as a valve.[1]

[1] Some such mechanical function may very well belong to the organ, though it seems unlikely that the blood should enter its meshes and swell it up; but still the presence of the chloragogen granules remains to be explained. In the first place their material is evidently drawn from the blood; but though Schaeppi admits its excretory nature, yet he objects on several grounds to the inference that it is taking part in a blood-cleansing process. He leaves the question in this dilemma, that the granules are neither accumulated in the organ, for in old individuals it is often comparatively free from them; nor found in its periphery, as if in the course of being removed into the body-cavity by leucocytes. Nevertheless the bulk of the evidence, and a comparison with other animals, seem to point to the removal of the granules, not necessarily intact, into the body-cavity, by a process similar to that which Schaeppi describes for the guanin chloragogen of the intra-sinus connective tissue. That there, in the cœlomic fluid, they or their débris should be absorbed by the lymph-cells, the green granules of which are shown to have the same properties, appears a natural theory.

Schaeppi gives an account of the way the chloragogen granules of the small lymph-cells fall into an alignment, fuse, and form the well-known rods which characterise the large lymph-cells. On account of their proximity to the nucleus, however, he considers that the granules are originally derived only from katabolic products of the nuclein; which is equivalent to saying that the admittedly excretory function of the rods, which it must be remembered reach such a size as to be visible to the naked eye, is limited to dealing with the nuclear refuse of the single cells which contain them. Comparing the

Beddard (4) supports Horst's view that the organ is hypoblastic, and suggests comparisons of it with the thyroid and spleen of Mammalia.

parallel case of the "ciliated pots" of Sipunculus, which collect excretory matter from all sides, this view is surely untenable. The granules must have some other origin; and their source from chloragogen, such as that of the heart-body and thoracic sinus, is at any rate not disproved.

Concerning the fate of the rods, the only evidence he adduces is that a collection of them in the body is nowhere found, though clear signs of degeneration are noticeable (p. 298). But since Ed. Meyer has shown, in Terebellidæ, that lymph-corpuscles degenerate and are found in the lumen of the nephridium [**24**, B, p. 648], and as such bodies as the rods could not be carried through the nephridial tubes, to maintain their excretory function it is necessary to suppose that they degenerate. Schaeppi mentions cell-heaps full of granules, and three times the size of the rod-cells, floating in the cœlomic fluid, and occurring also in the cavity of the nephridium itself. He regards them as portions of the chloragogen-bearing peritoneum from around the intestinal sinus, which have fallen off into the body-cavity. Some of them probably bear this explanation; but in cases, which he overlooks, when they contain rods identical with those of the lymph-corpuscles, it is evident that they are not derived from the peritoneum. In such instances they may consist of a plasmodium of rod-cells, and the granules may be formed by degeneration of the rods. Further—and this can be seen in single corpuscles—the rods appear to undergo a peculiar method of degeneration. Often in larger and older corpuscles the ends of the rods are notched. This notching is such a frequent occurrence that it would appear to have some special significance (fig. 61). Occasionally the part beyond the notch has fallen off, and may be seen lying in the cell-body (fig. 62). This is perhaps a regular process, and the rod having reached full size continues to grow at the ends into projections which from time to time are detached. The projections are often of a paler colour than the rest of the rod, which may be taken as an indication of recent growth. Degeneration in plasmodia may be the final stage of the rod-cells, when their life-history is complete.

Corpuscles containing Chitinous Bow-shaped Rods in Notomastus profundus (fig. 60).

Hitherto the chitinous rods of Ophelia have been unique, no structures resembling them having been found elsewhere among the Annelida. In the hæmolymph of Notomastus profundus, however, I found a cell containing a refringent bow-shaped rod, which had a strong general resemblance to the rod-cells of Ophelia. It is not mentioned in the excellent account of the hæmolymph in Eisig's monograph on the Capitellidæ; but the description there deals only with the hæmolymph in its fresh state,

Guido Schneider (31, B) shows that in the heart-body of Pectinaria hyperborea iron is present, and increases in quantity after injection of "Ferrum oxidatum saccharatum" into the body-cavity; but only the preliminary notice of his work is published, and his interesting researches may be expected to throw much light on the subject.

Monticelli (25) gives a very minute description of the heart and heart-body of Polyophthalmus, correcting the earlier description by E. Meyer (23, A, p. 815).

Finally, in a recent paper on the Ampharetidæ (13), Fauvel deals with the heart-body of that group. He describes it as a solid organ bifurcated at the base, where it is inserted into the dorsal face of the stomach. He regards it as fulfilling several functions. It supports the heart in the first place, especially when the pharynx is everted; and it also acts as a valve. It

whilst I first noticed the bow-shaped corpuscle in a fixed and stained film. In such preparations they are much easier to find than in fresh hæmolymph, though I subsequently found them in the fresh state. I may say that I found at least one bow-shaped corpuscle in every specimen of hæmolymph of each individual of Notomastus profundus which I examined.

The bow is of a pale yellow colour, and highly refringent. Dr. Paul Mayer kindly examined it with polarised light, and pointed out that it was always of a different colour from the rest of the field. Its shape is that of a strung bow, the tips being slightly recurved. Like the large brown granules which occupy the hæmoglobinous corpuscles, it is insoluble in cold caustic potash, whether in 25 per cent. solution or weaker; nor was it soluble when heated in potash solution up to 120° C. It is therefore probably of a chitinous nature, if not actually chitin. The protoplasm completely encloses the rod, forming, however, a very thin layer over the ends and the convex side. It is colourless, often slightly vacuolated, and frequently exhibits long filamentous projections, which may be seen in the fresh state making slow movements. It is noteworthy that these projections extend from the protoplasm on the convex side of the rod, as well as from the body of the cell, which, of course, lies on the concave side. The nucleus lies on the concave side, and slightly lateral to the rod. It is stained, though usually not strongly, with hæmatoxylin. The rest of the protoplasm is almost unstained, except at the ends of the rod, the cap which covers the latter being often intensely coloured.

Small fusiform corpuscles containing a clear straight rod occur also in the hæmolymph, and may be the young stages of the rod-cells. Dr. Eisig, however, was inclined to think that the rod-containing corpuscles are parasitic.

removes, he thinks, waste products from the blood, and stores them up as pigment. The observation from which he draws this conclusion is that pigment is more abundant in the organ in older than in younger individuals. Another function which he suggests it may perform is the secretion of chlorocruorin, the colouring matter of the blood. The embryology of the group is at present unknown; but the connection which he notes of the heart-body with the wall of the gut, leads Fauvel to conclude that Horst's view of the hypoblastic origin of the organ holds good for the Ampharetidæ. He considers, however, that the heart-body may have a different origin in different groups; that is to say that the heart-body is not homologous throughout the families of Polychæta in which it occurs.

In the Ampharetidæ, then, Fauvel considers the heart-body as a gland annexed to the digestive tube, and performing a function more or less hepatic. Contrary to Cuénot, he thinks it has no connection with amœbocytes.

It will be evident from this recapitulation of the history of the subject, that both on the morphology and functions of this curious organ widely divergent views are held.

In the present paper a description of its anatomy and histology in some of the chief groups in which it occurs will be given, with some observations on the chemical nature of the granules, and on their ultimate fate in connection with the cœlomic corpuscles. Matters about which a good account is already existing will be treated briefly. A description of the organ in the Cirratulidæ, where it reaches its maximum development, will afford the best opportunities for pointing out significant features which reappear in the different forms. Finally, an account of its principal characters, and of those of the cœlomic fluid, in the Chlorhæmidæ, Terebellidæ, and Amphictenidæ, and an instance of its development, will be given.

Besides in the groups just mentioned, the heart-body occurs also in the Ampharetidæ, Spionidæ, Magelonidæ, and Hermellidæ. Its degree of development is very unequal, in Magelonidæ the organ being merely larval and transitory, whilst in

the Cirratulidæ the dorsal vessel, which often reaches a diameter as great as that of the gut, is almost blocked by it.

The Heart-body of the Cirratulidæ.

The blood-system of a Cirratulid has been well figured by Ed. Meyer (24, B), and his observations are confirmed by an examination of young living individuals, and specimens dissected and afterwards fixed and cleared. In Audouinia filigera, as in all the group, the dorsal vessel is not confined to the anterior end of the body, but runs back into the abdominal region. At about the fifth segment it gives off a pair of lateral recurrent vessels, which supply branchial vessels in each gill-bearing segment (fig. 1). In an adult stained specimen several vessels are also seen going off directly to the gills, whilst the dorsal vessel itself continues anteriorly as a much-diminished trunk. The heart-body extends forwards to the point of origin of the lateral vessels. There it usually ends in irregular projections which appear on the point of becoming detached; but it may continue as a broken cord extending for the space of a segment into the fine anterior dorsal vessel. The three brown cords, of which it is typically composed, are constricted at the intersegmental septa, and branched, united, and folded irregularly (fig. 2). Sections reveal the fact that they are connected with the heart-wall at many points by means of fine processes. The greatest development of the heart-body is at about one third of its length from its anterior end. In the small transparent Cirratulus chrysoderma it may be seen with the low power that at this point, on systole, it almost entirely blocks the lumen of the vessel, the action of which as a blood-propelling organ must be greatly modified. The suggestion by Schaeppi in the case of Ophelia, and by Steen in that of Terebellides Stroemii, that it has a valvular function has been mentioned above. Beddard, in his monograph on Oligochæta, refers to Michaelsen also as saying that it "serves to ease the contractions of the dorsal vessel." Michaelsen's view seems rightly applicable to

the heart-body generally. The mechanical principle here involved is the same as in the case of the right ventricle of the mammalian heart, which contracts upon the convex solid wall of the left ventricle. In the same way the dorsal vessel of the worms in question contracts on systole, so as practically to obliterate the lumen between its wall and the solid heart-body, so that the whole of the blood which the vessel contained at diastole is expelled. In the gill-vessels of Sternaspis cellular rods are found which confer on them a similar mechanical advantage.

Between the cords of a piece of a fresh heart-body, flattened under a cover-slip, the minute, fusiform, non-nucleated blood-corpuscles are often observed with a high power. Claparède states that they are of the same colour as the plasma in which they float, that is, yellow. Lankester (18) says that they are colourless. They appear to me a bright pink, unlike hæmoglobin.

The histological structure of the heart-body shows points of great interest, but has been for the most part neglected in the literature of the subject. Three layers may be distinctly recognised (fig. 5),—an endothelium clothing each cord, a cortex, and a central or medullary tissue. The outermost layer, or endothelium, is directly bathed by the blood. It is composed of a single layer of cells, the nuclei of which are seen at intervals. The cortex is composed of cells, elongated in shape, the walls of which are very well marked (figs. 2 and 5). The nuclei frequently have a deeply-stained nucleolus; but, except for some refringent yellow granules, there are few cell-contents. In the periphery of the organ the cells are closely set together, with their long axes at right angles to its surface, and the nuclei are towards the outer side. Beneath the peripheral layer the cells are irregular and nuclei are rarer.

The development of the medulla varies in different individuals; whilst in the same individual it is reduced in amount, or absent, at the intersegmental constrictions. It consists of a highly granulated tissue. In this, although at some points nuclei can be recognised with a comparatively low power (D, Zeiss, figs. 5, 8), yet the numbers of granules which occupy the structure render

it very difficult to distinguish the cell walls. On examination with an oil immersion, however, a framework of cells is seen to support the granules (fig. 11), some of which are enclosed in these medullary cells, whilst others appear to be intercellular. The granules are greenish yellow in fresh tissue; in sections they remain unstained with hæmatein, and are of a bright yellow colour. They very frequently occur in heaps, constituting larger granules (*b*). Occasionally a granule may be noticed set in a vacuole (fig. 5, *a*). Besides these there are large homogeneous granules, often kidney-shaped, which stain intensely with Ehrlich's hæmatein.[1]

There still remain to be mentioned the most remarkable structures in the heart-body of this worm, and which, on first looking at a section of the organ through a microscope, at once arrest the attention by their striking appearance.

The medullary tissue contains numerous spherical cavities (figs. 2 and 5), the majority of which are occupied by from one to eight round or oat-shaped bodies. In those which are round a "nucleus" is frequently well marked; and were it not for their large size, which gives them a resemblance to ova, and the fact that the "nucleus" is the only spot stainable with most dyes, there would be little need for hesitation in pronouncing them to be single cells. Those which are oval in shape are much creased and folded; they stain irregularly with Ehrlich's hæmatein or with fuchsin, some folds colouring intensely, others hardly at all. Picric acid, used after hæmatein, stains the blood, and also stains parts of these bodies; but eosin, which likewise stains the blood, does not affect them.

Some of the spherical spaces are empty, whilst others again contain a colourless refringent, unstainable mass, dotted with numerous dark points. The mass does not quite fill the cavity, but the remaining space is partially occupied by a fine crumpled membrane which appears to ensheathe the colourless bodies. Smaller masses of the same unstainable material are seen in some sections embedded in the groundwork of the organ.

[1] I.e. Ehrlich's hæmatoxylin, with the substitution of hæmatein for hæmatoxylin, as Mayer recommends for all cases.

The line of demarcation between the central granular or medullary part of the organ and the external or cortical tissue is fairly well defined, and at some points a split between the two occurs. This is usually crossed by branching and highly granular trabeculæ (fig. 5). Sometimes it is considerably widened (fig. 2, *y*.), though in no case is there any sign of blood within it. On the other hand, the heart-body is frequently so folded as, in section, to show spaces surrounded by a proper superficial cortical tissue, which contains blood (fig. 2, *x*.); nevertheless it may be said that the blood never penetrates the organ,[1] but only flows between its folds and strands.

Between all the granules occurring in the heart-body a series of steps can be traced, so that it would appear probable that they represent different stages in some process which it must be the chief function of the heart-body to carry on. An examination of the shapes and properties of these granules will help to throw some light on the nature of that process. The methods used in this study, besides that of sections, were teasing fresh tissue and also tissue that had been macerated in Bèla-Haller's fluid, tests of solubility in different reagents, and various micro-chemical tests, such as those for iron, fat, glycogen, chitin, and uric acid.

In the following description the granules are treated in the order in which they appear to pass step by step into one another. But whether the history indicated be in fact correct must be left an open question. Certain unstainable refringent bodies have been mentioned above as occurring in the medulla of the heart-body, embedded in the groundwork (fig. 5, *e*); they are not conspicuous, and are apt to be overlooked, but as they increase in size a space appears around them, which renders them very noticeable (*f*). This is the origin of the spherical spaces so characteristic of the medulla. Under an oil-immersion objective these bodies are seen to consist of a

[1] Except in a few doubtful cases, where I have noticed in sections a small patch of what appears to be coagulated blood in a cavity in the medullary tissue.

stained spot, sometimes surrounded by a ring (fig. 8), which may represent the nucleus, embedded in a spherical granular mass composed of globules of a colourless material, in the centre of each of which is a minute refringent granule, unstained, but naturally of a yellow or pink colour (fig. 10). In teased preparations it is well seen that each spherical space containing such a mass is enclosed in a definite membrane (figs. 6 and 7). Frequently from two to eight masses occupy a single space, in which case they are smaller than single masses, by the division of which they are probably formed (fig. 9). The "nuclei" are frequently well marked at this stage (fig. 11). About this stage also the bodies begin to show a slight general stainability with Ehrlich's hæmatein. The limits of each mass, as well as of the spaces, are defined by a membrane, which is often developed unequally in several masses in the same space (fig. 10); the appearance of these membranes is very striking,—indeed, they are most conspicuous objects in a section of the heart-body (fig. 2). They are oval in shape, and much creased and folded along the long axis; the surface is often scattered with granules (fig. 12), and some of the folds or contents of the folds stain very intensely. Meanwhile the granular mass itself round which the membrane was formed has disappeared. Minute holes in the membrane, revealed, as will be seen later, by the action of caustic potash, may be the way of exit for this matter (fig. 13); at any rate, it appears to be extruded into the surrounding space (fig. 14), and eventually gets into the groundwork, where it undergoes a transformation which gives rise to the rich granulation that characterises the heart-body. The refringent globules, colourless or with a slight tinge of green, form a morula-like mass (figs. 15 and 16), in which some of them are turning brown. Eventually the whole mass becomes a deep greenish-brown colour, and forms a large composite granule (fig. 17). The minute yellow points, which were mentioned above as occurring in the centre of each globule, probably survive in the form of a dark spot, to be seen in fresh teased tissue in the centre of some of the larger granules (figs. 18 and 19). Besides brown,

minute pink and green granules are found, and also large bright red masses, that probably have some office in connection with the blood-pigment, possibly that of reserve. One more structure remains to be spoken of—the large, deeply stained, homogeneous granules mentioned above (fig. 5, *c*). The fact that they most frequently occur in richly granulated places suggests that they are other products of the same activity that gave rise to the granules. And indeed, although their mode of origin is obscure, some cases are found in which they appear to be in a state of formation from the deeply staining portions of several of the crumpled, ovoid bodies. This is at any rate possible, seeing that the chief portions of the latter require to be accounted for, since spherical spaces are often found empty, except for a scrap of degenerating membrane.[1] The homogeneous bodies occur in considerable numbers, and occasionally one is found in the cortex of the organ.

The reactions of the brown granules serve to differentiate them from the sorts of chloragogen described by Schaeppi. In the following tests for solubility the reagents were applied directly, and not drawn under a cover-slip. Absolute alcohol had but little effect on the granules, except to slightly contract them. After the addition of a saturated solution of oxalic acid to fresh teased tissue, in three or four minutes those granules which naturally contain a central dark spot surrounded by a lighter portion had become homogeneous and somewhat reduced in size. There was no further change. When ether is poured over a hand-section of material preserved in alchol, as evaporation goes on some large yellow drops appear, a pigmented fat having been dissolved out of the tissue. Strong acetic acid, whether cold or boiling, has no effect. The same is true in the case of hydrochloric acid diluted to 50 per cent. Strong hydrochloric acid, on the other hand, dissolves the brown granules in the cold when allowed to stand for some time; boiled on a slide or in a test-tube the granules are at once dissolved by it. Sulphuric acid gives a

[1] On the other hand, it is possible that the empty spaces have lost their contents during manipulation.

brown solution on boiling. Nitric acid completely dissolves the granules after standing in the cold, and at once on boiling; the solution on evaporation leaves a yellow residue, which gives the xantho-protein reaction, turning yellow on addition of ammonium hydrate; but in many trials I never saw the purple coloration of the murexide reaction.

On standing in the cold in caustic soda the brown pigment granules are dissolved, giving a fluid of a brownish-yellow colour. On boiling a piece of fresh heart-body with caustic potash, the pigment having been dissolved, some masses of insoluble material remain floating about in the liquid. Continued boiling has no further effect on them; and on removing them to a slide and examining them microscopically they are shown to be composed of some irregular and granular débris, but chiefly of clusters of bodies of an irregularly oval shape, considerably creased and folded, translucent, in places colourless, but at some points brown, especially where they are creased (fig. 13). Sometimes brown dots or circular markings are scattered over their surface, whilst occasionally there is a clear appearance of small tubular perforations of the walls resembling the transverse striations of some egg membranes. These bodies are evidently the crumpled oval envelopes so conspicuous in the spherical spaces of the heart-body; but the appearance of perforations is a new feature in them, revealed by the action of the boiling alkali. It has already been suggested that the openings thus shown may be the ways by which the granular contents of the bodies are extruded. The remarkable resistance of these bodies towards boiling alkalies at once refers their material to a group of organic substances of a chitinous nature.

I asked Prof. Paul Mayer whether any further tests could be applied to solve the nature of this substance, which he was so good as to examine, and he pointed out that its effect on polarised light corresponds with that of chitin. He referred me to Ambronn's paper on the cellulose reaction of chitin (2), and was so good as to perform the test there recommended himself. I collected the insoluble remnants of half a dozen

heart-bodies after boiling in caustic alkali solution, and by his direction bleached a portion by his chlorate of potash and hydrochloric acid method. The acid was introduced by a pipette at the bottom of a tube of alcohol, so that the problematical bodies, which were suspended in the tube in a piece of silk gauze, could be but little affected by it. Dr. Mayer tested both bleached and unbleached material, the unpigmented portions of the latter being quite sufficient to indicate any colour reaction. The substance was first washed in distilled water, and then a drop of the double salt, chloride and iodide of zinc, was put upon it. Some of the bodies turned deep indigo-blue, others took a bluish tinge, whilst others again did not show the least stain. In a cluster of three, two might be markedly coloured, and the third not at all. With the double salt of calcium, in the case of unbleached material, some of the bodies turned light violet, others yellow. These reactions show that the nature of the substance is closely similar to that of chitin, but in some of the bodies it is in a different state from that in others, since the stain does not occur uniformly. This fact helps to confirm the view that these structures, which may now be referred to as "chitinous bodies," are in process of formation in the heart-body. The occurrence of the chitin-cellulose reaction in Annelida is interesting, since Ambronn, who used Spirographis, did not find it in this group.

Osmic acid darkens many of the granules, but in a pigmented structure such as the heart-body it shows nothing conclusively. Ranvier gives as a reaction of fat a blue coloration with quinoline blue, and its preservation in glycerine [26], and Rosa confirms his statement [27]. When a piece of a heart-body is teased, treated on the slide with alcohol, and then with an alcoholic solution of quinoline blue diluted with water as far as is possible without precipitation of the colouring matter, the granules are stained greenish blue, and also a number of globules are rendered intensely blue. After standing in glycerine under a cover-slip for three weeks the blue of the globules has not faded, but the colour of the granules has

become green. It appears, then, that a number of fat globules exist side by side with the granules—a state of things which accords with the effect of ether, mentioned above, of dissolving out fat from the heart-body. This view is clearly confirmed by the use of Soudan III. The reaction of this aniline colour on fat was recently discovered by Daddi [11]. Professor Mayer informed me that he had examined it, and found it appeared to be a reliable stain for all fats, rendering them bright orange. In a hand-section of material fixed in alcohol, and stained on the slide with a solution of Soudan in 70 per cent. alcohol, and then teased in glycerine into fragments, numbers of fat droplets stained a bright orange colour are seen distributed in the cells. They are especially abundant in the outer layers. In the heart-bodies of Audouinia that had been about a month in the aquarium tank, fat had entirely disappeared.

The finding by Guido Schneider of iron naturally occurring in the heart-body of Pectinaria has been mentioned above. I found that in the case of Audouinia, hand-sections of material fixed in alcohol, when laid in ferrocyanide of potash solution, acidulated with hydrochloric acid, showed a blue coloration of the gut wall at once; but in the heart-body when teased only a few insignificant fragments were bright blue. MacAllum's ammonium-hydrogen sulphide method [1] for micro-chemical detection of iron [21], although it of course shows inorganic and albuminate compounds of iron, is especially designed to reveal iron in higher organic compounds, such as nucleins, which exist, as well as in nuclei, also in secreting cells, yolk of egg, hæmatoblasts, and other such bodies. On laying a hand-section of the heart-body in a drop of 50 per cent. glycerine thoroughly mixed with two drops of ammonium-hydrogen sulphide, there was no immediate reaction except a slight general greenish tinge. The tissue was then teased by means of drawn-out glass rods with broken points, instead of needles, and, covered with a cover-slip, was left in a water-bath

[1] Since this work was done MacAllum has published a new and easier method for the micro-chemical detection of iron, in the 'Journal of Physiology,' September, 1897.

at 60° C. for ten hours. It was then found to be coloured green, and numerous black dots were scattered about it.[1] On examination with a $\frac{1}{12}$-inch objective these dots were seen to be intensely stained granules, a crescent-shaped portion round the edge of each of which was of a deeper colour than the rest. Besides these, numbers of clearly defined and minute granules were stained, especially in the outer cells of a strand of the tissue. After 3 per cent. nitric-acid-alcohol acting for some hours at 35° C. the results were the same, except that the crescentic marking of the larger granules was not shown. In tissue subjected to the action of Bunge's fluid for thirty hours no trace of the reaction takes place. On the criteria laid down by MacAllum, the facts that iron is not, or only slightly, revealed by the ferrocyanide reaction, but yet that it is shown by the ammonium-sulphide method, whilst it is easily removed by Bunge's fluid, go to indicate that it is in organic ("maskirt"), but not in very elaborate combination. The presence of iron-containing bodies and also of fat globules in especial numbers in the peripheral cells is noticeable. It has been mentioned that the fat dissolved from the heart-body by ether was pigmented, and it may be here suggested that possibly this pigment contains the iron. It is likely that the iron-holding bodies are connected with the formation of the blood-pigment.

Barfuth's method of demonstrating glycogen microchemically (3) was applied to the heart-body, his mixture of glycerine and iodine dissolved in potassium iodide solution being used. Material fresh from the sea was chosen, and also worms placed in an aërated mixture of starch and sea water. The worms took up the starch, but neither in this nor in any case was a clear red-brown colour obtained with the reagent, sufficient to prove the presence of glycogen.

The indications of all these reactions point to the following conclusions as to the nature of the contents of the heart-body: that the brown granules, being soluble in caustic alkali solu-

[1] The nuclei did not markedly show the iron reaction. No doubt iron is present in them, but for its demonstration, according to MacAllum's recipe, the cells would have to be completely isolated from one another.

tion, are not chitin; that they are not guanin, since they give no murexide reaction (in these points they differ from both kinds of chloragogen described by Schaeppi in Ophelia); that the ovoid crumpled structures are chitinous bodies; that fat is distributed in globules in the heart-body, especially in its periphery; that iron is distributed in larger and smaller granules, also especially in the periphery; and finally, that glycogen is probably absent.

Before leaving the Cirratulidæ the relation of the heart-body to its surroundings suggests several matters for consideration. On passing out of the dorsal vessel the great bulk of the blood must first go to the gills; so that if the heart-body in any way modify the constitution of the blood, that change has probably some relation with the respiratory organs. In connection with this point it is interesting to note the pigment lymph-glands described by Ed. Meyer (**24**, B, p. 701) on the blood-vessels, particularly the arteries, of the gills of Cirratulidæ. They consist, he says, of a single glandular cell-layer belonging to the peritoneal wall of the respiratory vessels, and contain brown pigment.[1] He also mentions them in Terebellidæ (p. 645) and elsewhere. His view with regard to them is that they take some fluid ingredient out of the blood, and cast it into the cœlomic fluid. This theory, however, does not account for the situation of the organs in the gills. Is it to be inferred that the excretory matter is cast to the exterior through the gill-wall?[2] At any rate, gill

[1] Iron-holding patches, scattered at some points in the epidermis of the gills, are demonstrated both by the Prussian blue and the ammonium sulphide reactions; the iron is removed by Bunge's fluid at 60° C. in an hour. No iron reaction was noticed in the pigment lymph-glands.

[2] Gill glands for excretory purposes have been demonstrated by Allen (**1**) in Crustacea; whilst amongst Annelida yellow concretions regarded as certainly excretory have been found by Miss Buchanan (**8**, C) in the gills of a branchiate Polynoid. She finds a similar phenomenon in several other worms. In sections of the posterior gills of Sternaspis I find numbers of small refringent bodies crowding the epidermal cells. It may be suggested that the important functions of the dorsal pores of earthworms, of removing waste products, may be represented in branchiate worms by an excretory activity of the gills.

excretion is a possible mode of removal for waste products thrown into the blood by the heart-body, chloragogen, or other organs connected with the vascular system.

A remark of Schaeppi's has already been mentioned, that the intra-sinus guanin chloragogen of Ophelia is excreted by being carried through the sinus wall into the peritoneal tissue, clusters of the cells of which eventually become detached and fall into the cœlom, and are removed by the nephridia. Quite independently of this, anyone glancing at sections of Audouinia filigera would suspect some connection between the heart-body and peritoneum. The latter is remarkably thickened on the mesenteries which cross the cœlom from the gut and dorsal vessel to the body-wall (fig. 2); its cells are well developed; their protoplasm is concentrated in their central part, and crowded with yellow or green granulations, and, what is here to the purpose, this tissue is often found lying on the wall of the heart, the inner side of which in many places is connected with the heart-body (fig. 20). Thus it is often found that the peritoneum and heart-body are only separated by the thin muscular wall of the heart; and that a transfer of granules takes place from one tissue to the other is not improbable. The granules of the peritoneum, it is true, are not so large as the larger in the heart-body; but it is reasonable to suppose that the small and disintegrated granules would be the most suitable for excretion. Granular heaps are found floating in the cœlomic fluid, and I have especially noticed them in the anterior part of the animal,—that is to say, in the neighbourhood of the great anterior pair of nephridia. The cœlomic corpuscles also, which are large and amœboid, frequently contain brown granules, though many are occupied by globules of a reddish-brown pigment[1] (figs. 21 and 22). These different places where brown granules are found[2]—the heart-

[1] In the season when I examined the cœlomic fluid of Audouinia it was so filled with genital products that the cœlomic corpuscles were present in relatively very small numbers.

[2] Numerous small, homogeneous, spherical, dull green granules are found also in the gut wall. But they are always on the inner side of the nuclei of

body, the peritoneum, the detached cell-heaps, the amœboid corpuscles, and finally the great anterior nephridia—trace out a path by which they may be removed from the blood to the body-cavity, and thence to the exterior.

Characteristics of the Heart-body in other Polychæta.—The organ in the Chlorhæmidæ, Terebellidæ, and Amphictenidæ has much in common with that in the Cirratulidæ, but each group shows peculiarities. Of the two other groups which are included in Dr. Benham's classification, under the head of Terebelliformia, Sternaspis has no heart-body proper, and the Ampharetidæ are rare at Naples.

Chlorhæmidæ.—The dorsal vessel is, unlike that of the Cirratulidæ, confined to the anterior end of the worm. It arises at the opposite side of the digestive tube to the point of attachment of the stomach, and the blood from the sinus which surrounds the digestive organs flows into it. It is a broad trunk, lying dorsal to the œsophagus, and after its point of origin quite separate from the alimentary canal. It narrows towards its anterior end, and eventually divides into two branchial vessels. In Siphonostoma, Cunningham (10) aptly describes the heart-body which occupies this vessel as a folded band, which in section branches dorsally; he also states that it contains no lumen, though the cells of the opposite sides are in a definite line of contact. Jourdan (17), writing the same year, 1887, stated, on the contrary, that there is a lumen, and gave a figure showing it.

Now, on examining a series of transverse sections, it is clearly seen that the "band" is formed of a cylindrical tube, the walls of which have been compressed together, and that though the single cell-layers which form the walls[1] are in

the cells that contain them, that is the side towards the gut, and are probably of a different nature from that of those discussed.

[1] If an endothelium covering the heart-body exist, it must be extremely attenuated. One or two nuclei on the surface of the organ seem to suggest its presence. Bles states that the gut wall is directly bathed in blood, but there, too, it is not impossible that an extremely attenuated membrane is interposed. All the structures of Siphonostoma are so thin, the solid jelly

places in contact, yet a considerable space is generally left between them (fig. 23). This lumen appears so constantly that I think it is natural, and not due to fixation and handling, nor has it the appearance of an artefact. Moreover it is possible in a fresh specimen to snip the heart longitudinally with a pair of fine scissors, so as partially to slit open the heart-body, so that on microscopic examination a single thickness of the wall is viewed. If this be really the case, as I suppose, then the lumen is probably greater in life than it appears in sections. There is another even more important discrepancy between the descriptions of different naturalists. Whilst Jourdan speaks of the lumen as " communiquant avec le tube digestif," Bles (**5**), on the contrary, states that it has no connection, not even contact, with the gut. As a matter of fact, a series of sections clearly shows that in its posterior part it is attached to the gut, and that this connection extends for a considerable distance; on the other hand, no open duct or passage from the one into the other is seen to pierce the gut-wall. The attachments to the heart-wall are mentioned by Bles, who considers that they point to the peritoneal origin of the organ. These occur irregularly, and consist of one or two finely drawn-out cells, much resembling the muscular cells of the heart-wall, to which they are fastened on the one side, whilst on the other they are inserted into projections of the heart-body, which they have pulled out (Fig. 34).

The cells, which are uniform throughout the organ, are columnar, though when found isolated in teased preparations of fresh tissue they have assumed a spherical shape. It may be inferred from this that their connection and packing together not only induces, but maintains their cubical form. With regard to their granulations, Bles remarks that in hardened specimens they are crowded with green granules, which are also found in clotted blood. The natural appearance of the granules, however, is very striking. There are three kinds in each cell (fig. 25). First there is an irregular homo-

in which the animal is enwrapped rendering strength unnecessary, that the usual morphological layers are very difficult to recognise.

geneous granule of a clear red colour, about a quarter of the diameter of the cell; then there are usually two emerald-green globules, each of which is somewhat less than half the size of the red granule; and finally, there are numbers of still smaller granulations, deep brown in colour, which are usually concentrated at the poles of the cell, as seen in isolated cells of macerated and teased tissue (fig. 26). The nuclei are large, and are placed towards the inside or lumen of the organ, whilst the large granulations are towards the outside (fig. 23).

The green globules look like oil droplets, but, like the red and brown granules, are insoluble in ether. They blacken somewhat with osmic acid; and with acetic both they and the red granules lose their colour. With cold 50 per cent. hydrochloric acid the red pigment is dissolved and stains the cells, and the brown granules at a certain focus look purple. The latter are not dissolved even on boiling with the dilute nor on standing in the strong acid in the cold, though they disappear on heating. The residue of a solution in nitric acid fails to give the murexide reaction. Cold caustic soda solution removes the red and green bodies, but only causes the brown to swell and turn a paler shade. On boiling, however, they too dissolve, but a small residue of insoluble bodies remains, somewhat resembling the chitinous bodies of Audouinia.[1] In some of these, which had been boiled on the slide with alcoholic potash, the double salt of chloride and iodide of lime produced a yellow, and in some cases a violet tinge (Dr. Mayer).

Fat was not found in the heart-body.

Iron is shown by the Prussian blue reaction in some of the granules. The ammonium sulphide test occurs only on heating, but after six hours at a temperature of 60° C. tissue treated with this reagent is dotted over with clearly defined greenish-black patches, which I believe to represent the red granulations. After the action of Bunge's fluid for an hour at 60° C., followed by nitric-alcohol, ammonium sulphide fails to produce any coloration, so that the iron must be in such

[1] These are not noticeable in sections, but I have found pale-staining bodies in the lumen which may represent them.

combination as to be easily removable by hydrochloric acid. In tissue treated for ten hours with nitric alcohol the granulations are very markedly stained by either method.[1]

In Stylaroides hirsutus the heart-body folds have become rounded or flattened strings, which may run for considerable distances independently of one another. The cells in sections of 4 μ are clearly defined, and usually occupy the whole thickness of a strand, though occasionally a lumen is left. Except for brown granules, some of which are large and contain a dark central spot, there are few cell-contents.

Cunningham describes the cords in Trophonia plumosa as hollow, and formed of three layers of cells, the most internal of which is composed of spherical elements which project irregularly into the lumen as in a section of a nephridium, which the whole structure much resembles.

Horst studied the heart-body in Brada (16). He is struck with the lack of demarcation between the cells: "Bei einem jungen Examplare von Brada villosa war an der Peripherie der Stränge die Zellgrenze ziemlich deutlich, der centrale Theil aber wurde gebildet von einer mit braunen Körnchen gefüllten Grundsubstanz, worin keine deutlichen Zellen nachzuweisen waren. Bei den erwachsenen Individuen zeigen die Stränge auf dem Querschnitt nur ein unregelmässiges Netz von Fasern, in dessen Knotenpuncten deutliche Kerne liegen, während in der durchsichtigen Grundsubstanz der Maschen die braunen Körnchen zerstreut sind." If "Fasern" means cellular fibres, such an arrangement would be similar to that above described in the medulla of Audouinia; but, on the other hand, if, as is more probable, the so-called fibres are only cell-walls seen in section, the arrangement agrees with that seen in Stylaroides, which is characteristic of the Chlorhæmidæ. Haswell, however, who studied Australian Chlorhæmidæ (15),

[1] In fresh heart-bodies a considerable amount of débris and a number of crystals are noticeable. The forms of the crystals were not characteristic of any special substance. Certain of them, hexagonal in shape, stained indigo blue with iodine dissolved in potassium iodide. Dr. Mayer pointed out to me that they have no effect on polarised light.

states that in Coppingeria the cellular structure is only represented by nuclei.

In Siphonostoma, Stylaroides, and Trophonia there is no such development of the cœlomic epithelium as that in Cirratulidæ. If excretory products be passed through the heart-wall, as suggested in the case of that group, they would be received directly by the cœlomic fluid or its cellular elements. The latter consist in Siphonostoma of colourless cells, containing large nuclei, in some cases with very little surrounding protoplasm, in others, however, with a considerable cell body of a pointed oval shape (fig. 37), staining strongly with eosin, usually somewhat vacuolated, and containing one or two small dark spherules. In Trophonia, besides small vacuolated fusiform cells (fig. 40), which are often drawn out to a fine point at one end, as in the corpuscles described by Goodrich in Enchytræus hortensis (14, p. 56), where the processes in an early stage of the corpuscles attached them to the walls of the cœlom; there are also somewhat larger cells (fig. 41) with nuclei of the same size, but with their protoplasm, in specimens rapidly fixed, entirely drawn out into broad flattened projections which radiate from the nucleus like the petals of a flower. In both kinds of corpuscles, when examined fresh, vacuoles and yellow granules are observed, the latter being sometimes as large as the chitinous granules in the ordinary corpuscles of Capitellidæ, and with a similar concentric marking.[1]

[1] Problematical Bodies constantly present in the Cœlomic Fluid of Siphonostoma and Trophonia.

Jourdan (17, p. 33) describes in Siphonostoma crystalline calculi contained in the mesenteries, especially that of the stomach. I find similar structures, with their cellular envelope around them, constantly present, floating freely in the cœlom (figs. 38 and 39). The calculi are usually spherical, and tend to split into segments radially. They are pale green in colour.

In Trophonia bodies occur in the cœlomic fluid which are possibly of a similar nature. They are colourless rods, usually shaped somewhat in the form of a figure of 8, and are enclosed in an envelope formed of a few cells, in the substance of which are some globules of fat (fig. 43). In the centre of the rod is an elongated dark streak. The whole structure has a striking

A very striking fact has been noticed by Bles in Siphonostoma, which, so far as I have seen, holds good in other Chlorhæmidæ: the great single anterior pair of nephridia is totally deprived of blood-vessels. Their near proximity to the heart-body, the occurrence of the similar granules in both, and in the cœlomic corpuscles, coupled with this singular fact, are very strong arguments in favour of the way of excretion of waste blood-substances proposed. On the other hand, pigment lymph-glands similar to those mentioned by Ed. Meyer in Capitellidæ and Terebellidæ are described by Haswell in the gills of Stylaroides cinctus, accompanying the branchial vessels, and containing a reddish-brown pigment; and these offer an alternative possibility as a way of excretion from the blood.

Terebellidæ.

The arrangement of the blood-vessel is similar to that in the Chlorhæmidæ, and the branchial heart, formed by the union above the gut of a pair of peri-intestinal sinuses, runs forward freely suspended in the body-cavity by a dorsal mesentery, and ends, after piercing the dissepiment, by dividing into two branches which carry blood to the three pairs of gills (fig. 3). In some species a small median vessel proceeds forwards from the point of division; but in Polymnia this is represented by a median vessel which springs from the base of the heart-body, and runs forward between that and the gullet.[1] Thus in Polymnia all the blood which flows over the heart-body goes to the gills. The heart-body itself is in the form of a cylindrical rod, extending from the posterior end of the heart to a point a short distance beyond the dissepiment. It is attached to the heart wall by fine processes, both at its extremities and sometimes at other points. Amongst the many Polymnia which I examined, a single specimen showed a peculiar abnormality of the organ. Instead of ending at the posterior extremity of the

resemblance to bodies described by Metchnikoff in the spleen of the Jerboa (22, pl. iii). In that case the figure-of-eight shaped bodies represent reactions against bacteria.

[1] Milne-Edwards' otherwise excellent description of the blood-system is wrong on this point ('Ann. Sci. Nat.,' 2e sér., t. x, p. 199).

heart, the heart-body extended in two thick cords into the peri-intestinal sinuses. In Terebellides Stroemii the organ is hollow; but in most Terebellidæ it is solid. In Lanice conchilega it is in the form of a number of cords similar to those of Stylaroides.

The colour of the heart-body in the Terebellidæ is deep brown, and varies in intensity with the general depth of pigmentation in the rest of the animal. Oval masses of coarser pigment are set in it here and there transversely to its length. The organ is enclosed in a fine endothelium, as in the case of Audouinia. Cortex and medulla are not distinguishable; but the elongated cells of which the structure is composed form a sort of network, radiating out from the central axis. There is no great lumen; but numerous intercellular spaces appear. The pigment granules are of a greenish-yellow colour, and each is usually surrounded by a vacuole (figs. 27 and 32). Each of the oval patches of granules mentioned above is contained in an envelope, and perhaps represents a much-distended cell (fig. 28).

In sections of Polymnia nebulosa numerous blood-corpuscles are to be seen in the coagulated blood in the vessels. They appear to be non-nucleated; but each contains a minute granule or crystal.

The cœlomic corpuscles of Terebellidæ may be classified into three kinds,—amœboid, fusiform, and eleocytes. To what extent these are distinct in origin I do not know; but, both in young and mature specimens, they may be clearly distinguished from one another. All three forms may contain brown pigment granules. In Polymnia the fusiform corpuscles[1] average about 25 μ in length, whilst the amœboid are of somewhat smaller diameter (figs. 57 and 54). One end of the cell-body of the former is sometimes expanded so as to contain a circular body, deeply staining with eosin, in which is placed a granule staining blue with methyl green (fig. 55).

[1] In Amphitrite the fusiform corpuscles are sometimes drawn out to a considerable length (fig. 44), recalling the statement of Lim Boon Keng (19) that the spindle-shaped cells of Lumbricus may project their ends for great distances.

The third kind of corpuscle appears in relatively small numbers in Polymnia, but is very abundant in Amphitrite (A. rubra and A. variabilis). On making a small incision in the body-wall of a fresh specimen of this worm, a thick pink liquid comes out. A drop of this examined under the microscope is seen to contain numbers of corpuscles of a striking size and appearance. About 35 μ in their greatest diameter, they are of a flattened oval form (fig. 50), and are filled with pale yellowish spheres which resemble oil droplets, and evidently are the cause of the pink colour of the cœlomic fluid in bulk. These droplets are undoubtedly fat. In a cover-slip preparation of cœlomic fluid, fixed in absolute alcohol, and stained with Soudan III dissolved in 70 per cent. alcohol, washed with dilute alcohol, and mounted in glycerine, the globules are stained a bright orange-red (fig. 52). If, instead of washing with alcohol, the preparation be washed with ether, the stained fat is removed from the corpuscles, leaving the spherical cavities which contained it empty. There is a large nucleus, somewhat eccentrically placed, and visible only on staining,[1] whilst the groundwork of the cell is occupied in the fresh condition with minute greenish granules which do not stain with Soudan III, and may be compared with the discrete granules described by Lim Boon Keng in Lumbricus. Strongly defined granules of yellow pigment also occur, especially in smaller eleocytes, with little fat (fig. 51); and, finally, the cell is enclosed in a distinct cell membrane. These large fat-containing corpuscles may be compared with the eleocytes of Oligochæta, in which Rosa (27) states, on the evidence of Ranvier's fat test, that the granules consist of fat.[2]

[1] In preparations treated with Ehrlich's hæmatein, stained curved lines were often seen radiating irregularly from the central point in the eleocytes of Amphitrite. I supposed at first that at this point there was a centrosphere, the whole arrangement being similar to that described by Rosa (27) in the eleocytes of Allobophora. In preparations treated with iron hæmatoxylin, however, no centrosphere is brought out, so that the appearance remains unexplained.

[2] I have confirmed this observation by the use of Soudan III, on the eleocytes of an Allobophora, a common worm in the Villa Reale, Naples.

With regard to the relation of the heart-body to the cœlomic fluid, the position of the dissepiment sacs must be noted. These are diverticula of the dissepiment, or great anterior septum, which extends transversely across the body-cavity behind the nephrostomes of the most posterior pair of the great anterior nephridia. The heart-body runs in the heart to a point a little beyond where the latter pierces the dissepiment. The sacs consequently occupy a position on each side of the heart—a fact which is especially noteworthy in considering the connection of the heart-body with leucocytes, since the dissepiment sacs are generally crowded with corpuscles.[1]

Some of the species of Polycirrus are remarkable among the Terebellidæ in lacking blood-vessels, and consequently also gills and heart-body. It is, therefore, not without interest to observe an accumulation of cœlomic corpuscles upon a membrane supporting the gut in the anterior dorsal region. An area for the origin of the coloured corpuscles may survive at this point.

These coloured corpuscles, as is well known, occur mingled with the ordinary cœlomic corpuscles, the resulting cœlomic fluid being termed hæmolymph (Eisig and Meyer). Meyer states (**24**, B. p. 645, note i), "Abgesehen von den eigentlichen Lymphkörperchen, noch andere auch freie Zellen in grosser Menge in der Leibesflüssigkeit umherschwimmen, welche durch und durch von einer Art Blutpigment därchtränkt sind und somit die Rolle von gefärbten Blutkörperchen zu übernehmen scheinen." The ordinary corpuscles of which he speaks are amœboid, and contain considerable masses of amber-coloured pigment insoluble in ether (fig. 29). The "blood-corpuscles" are elongated and oval, or sometimes very small and round, and are pale yellow in colour, whilst in vacuoles placed, in the case of the elongated form, at the extremities of the cell, there are often small droplets of a madder-pink colour (figs. 30, 31, 57, 58). Cuénot (**9″**, p. 414) speaks of these

[1] It is remarkable in young Polymnia that whilst numbers of elœocytes occur behind the dissepiment, they are almost entirely absent from the part of the cœlom anterior to it.

droplets as colourless. They seem to me to bear the same colour relation to the yellow colour of the cell-body as the blood-corpuscles of Audouinia bear to the colouring matter dissolved in the plasma of the latter worm.

Development of the Heart-body in Polymnia nebulosa.—The work of Salensky, who described the primitive cavity in the heart-body of Terebella Meckelii, and the communication between this and the cœlom; and also Eisig's conjecture based on that observation, that the organ is due to an infolding of the heart wall, have already been mentioned.

Although much discussion has taken place on the question as to whether the heart-body be a hypoblastic or a mesoblastic organ, no further embryological work has been published on the point.

In Polymnia nebulosa[1] the matter can be settled by an examination of transverse and longitudinal sections of the heart-body shortly after its first appearance in the larva.

As in Terebella Meckelii, the blood-system is earliest represented by a peri-intestinal blood-sinus; but whereas in that species the dorsal and ventral vessels made their appearance only in a larva of twenty segments, in Polymnia, at a stage when nine pairs of chætæ were counted, a ventral vessel was clearly defined; whilst in a larva of thirteen pairs of chætæ a dorsal vessel also had appeared in the anterior part of the body. But as yet there was no heart-body. There were, however, cells in the vessel walls, which were swollen, and contained collections of greenish-yellow pigment (fig. 33). The blood-pigment which gradually makes its appearance is probably derived from this.

The exact moment at which the heart-body forms is difficult to determine. In a larva about 1·5 cm. in length it has

[1] The eggs of Polymnia are obtainable in great numbers at Naples, and develop well in captivity. Also large numbers of the larvæ are found amongst the algæ from certain beds. The tube is very early secreted, and is removed with great difficulty. Cav. Dr. Lo Bianco, however, told me that, on allowing them to stand for a few hours in stagnant sea water, the larvæ came out of their own accord. By this means they may be obtained living and without their tube, with great ease.

appeared as a cluster of cells with large nuclei in the dorsal vessel. From the first it shows signs of pigmentation. Even in the living state a cavity can be recognised in it, whilst sections show that part at least of this cavity opens directly into the cœlom on the ventral side of the heart just anterior to its origin. In other words, the heart-body is an in-pushing of the heart-wall (fig. 36). It shows no connection whatsoever with the hypoblast. Later (fig. 35) the open connection with the cœlom appears to be narrowed, and finally obliterated.

Sections through the organ at the stage when this is going on are difficult to interpret except in the light of the earlier stage. Nevertheless the heart wall may be traced on the ventral side to a point where it is invaginated, and becomes continuous with the wall of the heart-body (*x*.). It is not clear whether the lumen which primitively exists at the posterior end of the heart-body, and which at first is open to—in fact, is a part of—the cœlom, is continuous with that in the anterior part of the organ. It is not unlikely that, an invagination of the heart-wall having taken place, proliferation of cells from the anterior end of the invagination gives rise to the anterior part of the organ, and that in this a secondary cavity is formed.

However that may be, the main fact is evident that the heart-body in Polymnia is a purely mesoblastic structure.

The Heart-body in the Amphictenidæ.

In Pectinaria, Claparède (**9**, A, p. 379) states, "Le gros vaisseau intestinal est accompagné dans toute sa longeur par un cordon jaune, irrégulier, adhérant à sa paroi, et formé de cellules larges de 11 micr., et pourvues d'un nucléus clair. Cet organe est sans doute comparable aux boyaux celluleux qui adhèrent au vaisseau dorsal chez tant d'Annélides. J'insiste sur ce fait, parceque ce vaisseau dorsal de l'intestin joue chez les Pectinaires le rôle d'un vaisseau dorsal véritable."

Wirén (**33**), in his figure of the blood-system of Pectinaria, shows that this great intestinal vessel gives off the gill vessels.

Thus in position in the circulation, as well as in appearance, this irregular cord (fig. 4) corresponds to a heart-body. It contains numerous yellowish pigment granules.

Summary and Conclusions.

It is undesirable to attempt as yet to form a theory of the action of the heart-body accounting for the details of its structure. The facts relate themselves to one another, and suggest resemblances with other animals; but the part the organ plays in the economy cannot be defined: still there are several indications of the broad outlines of its nature.

First of all, with regard to its homologies, its mesoblastic origin, shown at least in one instance, Polymnia, must be considered. The organ is not homologous with the diverticulum of the gut which projects into the dorsal vessel of Buchholzia and other Oligochæta, and the theories of Horst and Beddard to this effect fall to the ground. Nevertheless, considering the fact that in those forms the diverticulum of the gut, as it pushes into the heart, must carry the heart wall with its cœlomic epithelial covering in front of it, it is evident that the latter must form a part of the so-called "heart-body." It is, perhaps, this mesoblastic part from which the anterior part of the heart-body of Buchholzia is divided, and were this the case there would be a partial homology of the organ with that of Polychæta. Further, the heart-body described by Nusbaum and Rakowski in Fridrica (25′) appears to have no connection whatsoever with the gut, and accordingly resembles that of Polychæta in being entirely mesoblastic.

The mesoblastic origin being established, the organ may be regarded, as Eisig suggests, as of the nature of intra-vascular chloragogen,—that is, as modified peritoneal tissue, primitively clothing the outside of the dorsal vessel, but becoming folded so as to lie within it. The word "chloragogen," however, must be understood only as a descriptive term, Schaeppi having shown that the granules occurring in the tissues included under that designation are of different chemical natures.

The function of chloragogen is a much-debated question. Certain text-books speak of the tissue frankly as "liver cells." Schneider sums up the facts which point to such a conclusion: "Den Leberzellen ähneln die Chloragogenzellen in folgenden Punkten. Sie nehmen Pigmente (Kükenthal) und albuminoide Substanzen (Cuénot) wahrscheinlich aus dem Blut auf und absorbiren Indigkarmin und Eisen aus injicirten Lösungen" (30, A, p. 386). The analogy with the liver of Vertebrates is emphasised when the chloragogen is in the form of a heart-body; it is then situated in the stream of blood from the alimentary to the respiratory organs—in the portal blood, in fact; and it is reasonable to conclude that its functions play a similar part in the economy of the worm to that undertaken by the liver in as far as the latter may be regarded as a ductless gland.

The analogy cannot be followed into details. Glycogen, though occurring in Sipunculus (Jourdan), and described even in the chloragogen of Oligochæta (Cuénot, 1897), appears to be absent in the heart-body. Other substances, however, may exist in it, representing elaborated and stored products. Fat and iron have been shown to be present, and the latter, it can hardly be doubted, is associated with the hæmatopoietic function. The functions of the pigment and the chitinous bodies are obscure, and if they be excretory their mode of removal is difficult to understand. It has been suggested above that waste products may be carried through the heart wall, and thence to the nephridia by leucocytes, or emptied into the blood and removed by gill excretion; the latter alternative seems only applicable to fluid substances.

With regard to the mechanical functions of the heart-body we are on surer ground. The vessel wall contracting upon it obliterates the heart cavity at systole, the whole of the blood passing on to the gills.

In conclusion I wish to offer my sincere thanks for the assistance I have received from the Staff of the Zoological Station at Naples. Professor Paul Mayer gave me invaluable

help, and I am also much indebted to Professor Eisig. I have to thank Professor Lankester and Mr. E. S. Goodrich of Oxford for suggestions and criticism.

Appendix.

Note on intra-vitam Staining of Nuclei with Carmine in Sea-Water.

The question whether nuclei can be stained whilst alive is still undecided. Galeotti ('Z. f. wiss. Mikr.,' xi, p. 172) says that the processes of coloration in the normal condition "mai si mostrano nel nucleo." Pfeffer ('Untersuch. Bot. Inst. Tübingen,' vol. ii, p. 325) says that nuclei will only stain with methylene blue after the cells have been damaged. Bethe ('Biol. Central.,' xv, 1895, p. 141) states that the epithelial nuclei of a living piece of a Ctenophore may be stained with methylene blue. Lauterborn ('Untersuchungen über Diatomen,' Leipzig, i, 1896, p. 36) speaks of seeing moribund diatoms, the nuclei of which were stained, move along in the field of the microscope. Finally, Przesmycki ('Biol. Central.,' xvii, 1897, p. 321), writes of "intra-vitale Färbung des Kerns und des Protoplasmas," with "neutral red" and methylene blue by means of a special technique, to be explained in a later paper.

During my work on the heart-body I had occasion to cut sections of Siphonostoma which had been for a week in a jar of aërated sea-water in which some powdered carmine had been stirred up, and was surprised to find that in sections mounted without further stain the nuclei and especially the nucleoli of the gut-cells were coloured red. The fixative used was corrosive sublimate and acetic acid, and to the action of this on the carmine in the gut of the animal, Dr. Meyer attributed the stain. I found, however, that the stain still occurred after fixation in 90 per cent. alcohol; and even in living tissue[1] some nuclei were found stained pink. The majority of the gut cells are so loaded with granules in the fresh state that it is impossible to

[1] Ciliary movement was going on in the preparation.

make out the nucleus in them; but in some pale yellow cells, occurring just posterior to the stomach, it can be seen. That it was the nucleus that was stained in these cells was shown by the action of acetic acid, which revealed no other nuclei. Dr. Mayer, to whom I showed a preparation, described the appearance as a faint but distinct nuclear stain, though he was inclined to regard the carmine as in the form of granules in the nuclei.

It should be stated that carmine partially dissolves in sea-water, and this solution will stain fixed tissues; and also that the contents of the gut of Siphonostoma are faintly alkaline.

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21. MacAllum.—'Quart. Journ. Micr. Sci.,' xxxviii, 1895, p. 175. (Microchemical detection of iron.)

22. Metchnikoff.—'Leçons sur l'Inflammation.'

23. Meyer (Ed.).—(A) 'Arch. für mik. Anat.,' Bd. xxi, p. 815. (Polyophthalmus.)

24. Meyer (Ed.).—(B) 'Mitt. Zool. Stat. Neapel,' vol. vii, 1887. (Studien über den Körperbau der Anneliden.)

24'. Milne-Edwards.—'Ann. Sci. Nat. Zoo.,' 2e sér., tom. x, p. 199. (Circulation of Polymnia.)

25. Monticelli.—'Boll. della Soc. di Nat. in Napoli,' vol. x, 1897, p. 47. ("Polyophthalmus.")

25'. Nusbaum und Rakowski.—'Biol. Centralblatt,' Bd. xvii, 1897, p. 260. (Enchytræidæ.)

26. Ranvier.—'Traité technique d'Histologie,' p. 102. (Fat test.)

27. Rosa.—'Mem. Reale Acad. Sci. Torino,' xlvi, 1896, p. 149. (Cœlomic fluid of Oligochæta. Fat test.)

28. Salensky.—'Archiv. de Biol.,' iv, p. 252. (Terebella Meckelii.)

29. Shaeppi.—'Jen. Zeitschr. für Nat. Wiss.,' 1894, pp. 247—293. ("Ophelia radiata.")

30. Schneider (Guido).—(A) 'Zeitschrift für wiss. Zool.,' Bd. lxi, Heft 3. ("Chloragogenzellen.")

31. Schneider (Guido).—(B.) 'Arb. Nat. Geo. Petersburg,' Bd. xxvii, Heft 1. (On the segmental organs and heart-body of Polychæta, with a German translation.)

32. Steen.—Jena, 1883, 'Terebellides Stroemii' (Inaugural Dissertation).

33. Wirén.—'Kongl. Svenska Vetenskaps-Akad. Handlingar,' Stockholm, 1885, 21, No. 7, pl. vi.

EXPLANATION OF PLATES 19—22,

Illustrating Mr. Lionel James Picton's paper "On the Heart-body and Cœlomic Fluid of certain Polychæta."

PLATE 19.

Fig. 1.—Audouinia filigera. Diagram of a dissection from the dorsal surface. The head and posterior half of the worm are not represented. The heart is represented as cut open, revealing the heart-body within it. The arrows indicate the direction of the circulation.

Fig. 2.—Audouinia filigera. Transverse section, × 115, through widest part of heart-body. The cut strands of the heart-body are represented lying in the heart, bathed by the blood (red). *x*. Blood between folds of the heart-body. *y*. Lumen in heart-body.

Fig. 3.—Anterior portion of young Polymnia nebulosa. Dorsal view; drawn whilst alive; under low power. The heart-body is a dark rod inside the dorsal vessel (heart).

Fig. 4.—Pectinaria belgica. Transverse section of heart-body. D objective (Zeiss).

PLATE 20.

All the figures in this plate refer to Audouinia filigera.

Fig. 5.—Transverse section of a strand of the heart-body, showing endothelium, cortex, and medulla. The last is occupied by yellow granules and spherical spaces, which contain chitinous bodies. *a*. Granule in vacuole. *b*. Large yellow granule. *c*. Granule deeply stained with hæmatein. *d*. Nuclei of medullary cells (the outlines of these cells are not evident). *e*. Granular mass.

Fig. 6.—Granular mass and its envelope. (Bèla-Haller's fluid.) $\frac{1}{12}'$ objective.

Fig. 7.—Ditto.

Fig. 8.—Granular mass and its envelope, from a section stained with Ehrlich's hæmatein, showing "nucleus." $\frac{1}{12}'$.

Fig. 9.—A mass divided into two. (Bèla-Haller's fluid.)

Fig. 10.—A mass divided into three, from a section. Parts are deeply stained with hæmatein.

Fig. 11.—A portion of the medulla. $\frac{1}{12}'$. From a section.

Fig. 12.—Four chitinous bodies in a common envelope. (Bèla-Haller's fluid.) $\frac{1}{12}'$.

Fig. 13 —Chitinous bodies after being boiled in caustic potash solution. D (Zeiss).

Fig. 14.—Chitinous body, surrounded by granules. $\frac{1}{12}'$.

Figs. 15—19.—Formation of brown granules. $\frac{1}{12}'$ objective.

Fig. 20.—Heart-body, heart-wall, and adjacent peritoneum. Transverse section. $\frac{1}{12}'$.

Figs. 21 and 22.—Leucocytes with pigment, fresh. $\frac{1}{12}'$.

PLATE 21.

Fig. 23.—Siphonostoma diplochætos. Transverse section through heart-body at the origin of the heart. × 85.

Fig. 24.—S. diplochætos. Transverse section of heart-body. $\frac{1}{12}'$. The nuclei stained with hæmatein (the dark granules, red in the fresh tissue, were stained deep greenish blue with hæmatein; these are shown in the figure in black).

Fig. 25.—An isolated cell of the heart-body of S. diplochætos, in its natural colours. $\frac{1}{12}'$.

Fig. 26.—Similar cells, after maceration and teasing in Bèla-Haller's fluid. $\frac{1}{12}'$.

Fig. 27.—Isolated cell of heart-body of Lanice conchilega. $\frac{1}{12}'$. Bèla-Haller's fluid.

Fig. 28.—Lanice conchilega. Composite granule. $\frac{1}{12}'$.

Fig. 29.—Polycirrus, amœboid corpuscle, with amber-coloured granules; fresh. $\frac{1}{12}'$.

Figs. 30 and 31.—Polycirrus, pigmented corpuscles; fresh. $\frac{1}{12}'$.

Fig. 32.—Isolated cell of heart-body. Terebellides Stroemii. Bèla-Haller's fluid. Stained with methyl green.

Fig. 33.—Polymnia nebulosa. Larva (ten pairs of chætæ). Pigmented patch on wall of ventral vessel.

Fig. 34.—Siphonostoma diplochætos. Sketch showing an attachment of the heart-body to the heart-wall; fresh. D (Zeiss).

Fig. 35.—Polymnia nebulosa. Sagittal section of posterior end of heart-body in the larva, showing communication of its cavity with the cœlom in course of obliteration. $\frac{1}{12}'$. Blood red, nuclei blue. *x*. Point where the heart-wall is continuous with the wall of the heart-body.

Fig. 36.—Polymnia nebulosa. Sagittal section of posterior end of heart-body at an earlier stage than in Fig. 35. $\frac{1}{12}'$. Blood yellow, nuclei blue.

PLATE 22.

Cœlomic Fluid.

Siphonostoma diplochætos.

Fig. 37.—Oval corpuscle (fixed in alcohol; stained hæmalum and eosin). $\frac{1}{12}'$.

Fig. 38.—Problematical bodies (hæmalum, &c.). D (Zeiss).

Fig. 39.—Problematical body; fresh. $\frac{1}{12}'$.

Trophonia.

Fig. 40.—Fusiform cœlomic corpuscle, with stalk. (Methyl green and eosin.) $\frac{1}{12}'$.

Fig. 41.—Stellate corpuscle. (Formol, alcohol, and acetic mixture, &c.) $\frac{1}{12}'$.

Fig. 42.—Amœboid corpuscle; fresh. $\frac{1}{12}'$.

Fig. 43.—Problematical body; fresh. D (Zeiss). In the cellular envelope fat droplets are seen.

Figs. 44–46.—Amphitrite variabilis. Fusiform corpuscles. $\frac{1}{12}'$. Fig. 46 shows a granule stained with hæmalum.

Fig. 47.—Young Polymnia nebulosa. Cœlomic corpuscle. $\frac{1}{12}$.

Amphitrite (variabilis and rubra).

Fig. 48.—Granular corpuscle. $\frac{1}{12}'$.

Fig. 49.—Granular amœboid corpuscle; fresh. $\frac{1}{12}'$.

Fig. 50.—Eleocyte; fresh, showing fat and pigment spherules, and granular protoplasm. The cell-envelope is distinct.

Fig. 51.—Smaller eleocytes, with little fat and well-marked pigment granules. Osmic, bichromate of potash, hæmatein. $\frac{1}{12}'$.

Fig. 52.—Eleocyte. Fixed in absolute alcohol, stained with hæmalum; fat stained with Soudan iii; granulated cell-protoplasm unstained.

Polymnia nebulosa.

Fig. 54.—Fusiform corpuscle. Alcohol, hæmalum. $\frac{1}{12}'$.

Fig. 55.—Fusiform corpuscle. Stained methyl green and eosin. $\frac{1}{12}'$. The contained circular body is stained deeply with eosin, its "nucleus" blue with methyl green.

Fig. 56.—Vacuolated fusiform corpuscle. $\frac{1}{12}'$.

Fig. 57.—Amœboid corpuscle; fresh. $\frac{1}{12}'$.

Figs. 58 and 59.—"Blood-corpuscles" of Polycirrus. Hæmalum. $\frac{1}{12}'$.

Fig. 60.—New bow-shaped corpuscles in Notomastus profundus. Absolute alcohol, hæmalum, eosin. $\frac{1}{12}'$.

Ophelia radiata.

Fig. 61.—Rod-cell, showing notches in rod. A (Zeiss).

Fig. 62.—Rod-cell, showing deciduous process. A (Zeiss).

On the Hypothesis that Lake Tanganyika represents an Old Jurassic Sea.

By

J. E. S. Moore.

With Plate 23.

"For anything that geology or palæontology can show to the contrary, a Devonian fauna and flora in the British Islands may have been contemporaneous with the Silurian life in North America, and with a Carboniferous fauna in Africa. Geological provinces and zones may have been as clearly marked in the Palæozoic epoch as at present, and those seemingly sudden appearances of new genera and species which we ascribe to new creation may be simply due to migration."

If the statements contained in this remarkable passage express the truth—and no one acquainted with the forcible arguments which Huxley brought forward [1] in their support will doubt that such is actually the case—it follows as a sort of natural corollary that the existence of our modern fauna and flora may not be incompatible with the co-existence in certain places of extremely ancient types. In several former papers I [2] have laid especial emphasis upon the very singular fact that the fauna of Lake Tanganyika is a double series, that it is in reality composed of two entirely dissimilar faunas which co-exist in the great lake side by side.

[1] Anniversary address to the Geological Society, 1862.

[2] 'Nature,' July, 1897, p. 198; 'Science Progress,' October, 1897; "The Molluscs of the Great African Lakes:—Distribution." 'Quart. Journ. Micr. Sci.,' vol. 41, pp. 159—180.

I[1] have shown that one of these two faunas consists of the normal and ubiquitous fresh-water stock, which is distributed throughout the whole African continent, and indeed throughout the world. The second fauna is altogether different from this, and in the appearance of its widely divergent constituents is utterly unlike any modification of the normal fresh-water fauna that is known. It has long ago been recognised that the superficial facies of the molluscan shells belonging to this series are those of a marine rather than a fresh-water stock, and in recognition of the more complete marine affinities which a closer scrutiny of the internal anatomy of these animals has revealed, I[2] have here, as elsewhere, spoken of the whole series of forms in Tanganyika which exhibit these quasi-marine characters as members of the halolimnic group.[3]

[1] "On the Zoological Evidence for the Connection of Lake Tanganyika with the Sea," 'Proc. Roy. Soc.,' vol. lxii, 1898, pp. 452—458.

[2] "The Molluscs of the Great African Lakes.—II. The Anatomy of the Typhobias, &c.," 'Quart. Journ. Micr. Sci.,' vol. 41, 1898, pp. 181—202.

[3] If the practical distinction between fresh-water and marine faunas in general were not a well-established and accepted fact, it would have been impossible for geologists to separate, as they have done, fresh-water from marine deposits by the characters of the animals they contain. It is generally assumed that the modern fresh-water fauna has gradually originated far back in time by organisms having one by one acquired characters which have enabled them to successfully colonise fresh water in connection with the sea; but the actual phylogenetic descent of most of the true fresh-water organisms, except in a very broad sense, is lost in antiquity and hopelessly obscure. In some Crustacea, in the Ganoids, and some other fishes we have enough palæontological evidence to demonstrate their actual migration from the sea, and such evidence forms part of the ground whence it is argued from analogy that all fresh-water organisms have originated in a similar way. Further evidence of this kind is afforded by those cases, at once remarkable and few, where animals that are generally marine exhibit a wonderful capacity to migrate inland, there being every reason to believe that such organisms constitute the "modern instances" of the origin of new fresh-water types. The true fresh water fauna of any period is thus a heterogeneous assemblage of organisms, all of which have, so to speak, voluntarily acquired the habit of living in fresh-water, and, excepting in this peculiarity, they have no necessary relation with each other. The constant facies which the fresh-water fauna presents all over the world are due primarily to the universal distribution of its heterogeneous constituents, and secondarily to the direct similar effect produced on organisms by a fresh-water life.

It is perhaps needless for me here to reiterate the great importance of arriving at a final decision as to the real nature of the halolimnic forms, for it will be obvious that if they have nothing to do with the normal fresh-water series, and are to be regarded as the remnant of an ancient sea, our views respecting the past history of the African interior must be greatly changed.

Having obtained the animals, it appeared, therefore, in the first place to be incumbent on me to ascertain, by a careful study of their anatomy, whether this superficially marine appearance was real, and indicative of their common origin from the sea, or whether it was merely, so to speak, skin deep, and to be regarded as wholly the result of modification, or to the persistence of characters which belonged to some old fresh-water stock. So far as zoologists are concerned, the evidence which I have now accumulated on this point will be found to be conclusive; but since, with the exception of my paper on the Typhobias (loc. cit.), the detailed accounts of the anatomy of the Halolimnic Gasteropods have not yet been published, I will briefly recapitulate the facts. It has been found[1]—

[1] In order that the significance of the new classificatory outline, given in the text immediately below, may be fully appreciated, it should be clearly understood that before my return from Tanganyika no account of the anatomy of any of the halolimnic molluscs was in existence,—indeed, so far as I can ascertain, with the exception of the brief description by Smith of a few badly preserved Nasopses brought home by Captain Hore, the animals contained in any of these shells had never been seen before. Consequently their conchological classification was, as, indeed, Smith frankly implied in 1881, entirely provisional.

All these Halolimnic Gasteropods appear to be rigidly restricted to the confines of Lake Tanganyika, and the only molluscs from this lake, halolimnic or otherwise, of which we have had any anatomical description, is the common Reiodon, the morphological characters of which were described by Professor Pelseneer from specimens which were brought back by the officials of the Congo Free State ('Bull. de Musée Royale d'Hist. Nat. de Belg,' Brussels, 1886, iv, p. 103).

The marine appearance of the genus Nassopsis was pointed out by S. P. Woodward in 1857, but with curious inconsistency he regarded this form as a Melania belonging to the sub-genus Melanella. It is to Smith that we owe the first definite assertion of the possibility that these Gasteropods

1. That in the genera Bathanalia and Typhobia we have a type of Gasteropods which stands very much in the same relation to the modern Strombidæ that the early Equidæ do to the modern horse.

2. That in the so-called Spekia zonatus we have a form which even in its most minute anatomical details, as well as in its shell structure, is an unquestionable Naticoid of the Lamellarian type.

3. That the so-called Tanganyicia rufofilosa is closely related to the oceanic Planaxids, and that it is antecedent to a certain section of the heterogeneous Melanoid group, much in the same way that Littorina is antecedent to another.

4. That the genus Limnotrochus is really compounded

might, when their anatomy became known, turn out to be marine derivatives. Smith was unfortunate, however, in his forecast of the affinities of Typhobia as a Melania, since it is obvious, from the character of the radula of this mollusc alone, that it has no affinity with that group (see my figs., 'Quart. Journ. Micr. Sci.,' vol. 41, pt. 1, p. 189). Great credit is, however, due to Smith for his shrewd guess at the marine nature of the halolimnic shells with which he was then acquainted, and more especially so because he was not, as it were, frightened out of his better judgment as a naturalist by the existing geological preconceptions respecting the past history of the African interior. The later classification of the Tanganyika shells given by Bourguignat ('Ann. des Sci. Nat.,' t. x, pp. 1—267) is quite unintelligible either as to the means by which his endless species are distinguished from each other, or as to their affiliation in his so-called natural groups. Indeed, as an example of the utter confusion and obscuration of the facts which may be produced by the unrestrained application of the conchological method of determining molluscan affinities when the animals contained in shells are quite unknown, this work is perhaps unrivalled. In M. Fischer's excellent conchological treatise, on the other hand, there will be found a careful estimation in each case of the probable affinities of those Tanganyika shells which were known. But each of these is, of necessity, simply drawn from conchological data, and the caution with which the author proceeds in the absence of all morphological information is most marked. In order that the reader may obtain a clear conception of the points in which what may be called the newer classification given in the text of this paper differs from and extends that which could be arrived at by the study of the empty shells, I give here in parallel columns for comparison a list of the families and genera with which the Halolimnic Gasteropods are incorporated in M. Fischer's work, and those to which I should myself refer them after a study of the morphology of each. In this list I

of two distinct types, one of which, represented by L. Thompsoni, is closely similar to Bathanalia; while the other, represented by the unique L. Kirkii, is the only fresh-water Xenophora (Onustus) at present known.

5. That in the Paramelanian group, composed of the genera Paramelania, Nassopsis, and Bythoceras, we have forms

have used the family name Purpurinidæ to include the genera Paramelania, Nasopsis, and Bythoceras, and the new generic name Chytra for the old generic name of Limnotrochus, in the case of L. Kirkii.

Conchological Classification according to M. Fischer.	Redetermination from the Characters of the Animals themselves.
Fam.—Melaniidæ.	Fam.—Typhobiidæ (Moore).
Genus.—Typhobia (Smith).	Genus.—Typhobia.
T. Horei, Smith.	T. Horei, Smith.
Genus.—Paramelania (Smith).	Genus.—Bathanalia (Moore).
P. Damoni, Smith.	B. Howesii, Moore.
M. nassa, S. P. Woodw.	Genus.—Limnotrochus (Smith).
Fam.—Hydrobiidæ.	L. Thomsoni, Smith.
Genus.—? ? Syrnolopsis (Smith).	Fam.—Planaxidæ.
S. lacustris, Smith.	Genus.—Tanganyicia (Crosse).
Genus.—Spekia (Bourguignat).	T. rufofilosa, S. P. Woodw.
S. zonata, S. P. Woodw.	Fam.—Xenophoridæ.
Genus.—Tanganyicia (Crosse).	Genus.—Chytra (Moore).
T. rufofilosa, S. P. Woodw.	C. Kirkii, Smith.
Genus.—Limnotrochus (Smith).	Fam.—Purpurinidæ.
L. Thomsoni, Smith.	Genus.—Paramelania (Smith)
L. Kirkii, Smith.	= Purpurina (Hudl.).
	P. Damoni, Smith.
	P. crassigranulata, Smith.
	Genus.—Nassopsis (Smith).
	N. nassa, S. P. Woodw.
	Genus.—Bythoceras (Moore).
	B. iridescens, Moore.
	Fam.—Naticidæ.
	Genus.—Spekia (Bourguignat).
	S. zonata.

In the above list I have placed the genus Chytra among the Xenophoridæ on account of its conchological similarity to numerous fossils which are referred to this group.

The notes of interrogation in the older list are those of M. Fischer.

which, judged by their anatomical (as well as by their conchological) features, do not appear to be living elsewhere now, but their shells approximate in a most remarkable degree to those of the extinct marine Jurassic genus Purpurina, whilst at the same time they possess the nervous system of a Cyclophorus. They thus appear not only to come of a marine stock, but also to indicate the hitherto unknown road by which the Cyclophoran nervous system has been evolved. These living Tænioglossa stand in much the same relation to their extinct marine ancestors as the living Cyclostoma has been shown (by the beautiful investigations of Lacaze-Duthiers and Bouvier) to stand in relation to the common periwinkle of our shores.

No Stromboid, Naticoid, or Xenophoran molluscs have been found hitherto in any fresh water that is known; and when we remember that these truly marine Gasteropods are associated in Tanganyika with other and widely different marine forms, such as sponges, medusæ, crabs, and prawns, it is impossible to avoid the conclusion that these animals can be anything but the dwarfed and stunted remnant of a fauna that the sea has left behind.

That the halolimnic animals still living in Tanganyika are the remains of an extensive sea fauna that once existed there, is thus the plain and unequivocal testimony afforded by the morphology of the widely different types of which this fauna is composed.

This being so, in the present paper I shall attempt to show, from a variety of considerations relating to the similarity between the halolimnic shells and certain fossils, and upon more general grounds, to what old sea fauna the halolimnic series once belonged. It will probably have been seen that the above conclusion by no means exhausts the information which can be gathered from the joint study of the distribution and the morphology of the halolimnic group. We need only refer to what is now generally known respecting the gross physical features of the African continent, and especially of the regions about the great lakes, to see that it is impossible for most if not all the halolimnic forms either to have made their way up to, or to have been left in, Tanganyika in recent

times. The lake is now 2700 feet above the level of the sea, and is more than 700 miles from any coast; there is but one effluent, and the course of this river is beset with rapids and with falls even long before it reaches the lower channels of the Congo on its way towards the sea; and finally, there are no true representatives of the halolimnic fauna, except, perhaps, the universal Xenophoridæ, in that part of the Atlantic into which the Congo flows.

The physiographical features of the continent point directly, therefore, to the conclusion that the halolimnic fauna must be very old. It must have been left in the great valley of Tanganyika long before that part of the continent had attained its present altitude, and when the surface of the water was approximately at the level of the sea. In exact conformity with this indication, it will have been seen that the halolimnic animals as they now exist, although closely allied to different marine genera, are not exactly similar to any oceanic species that we know; and finally, it has been shown that the halolimnic Gasteropods, at any rate, stand in the relationship of immediate ancestors to several of our well-known oceanic forms. There thus exists evidence which appears to be practically conclusive that the halolimnic animals retain the facies of a sea fauna that has elsewhere disappeared, and consequently, unless they have become modified out of all semblance to their original marine progenitors, it is only natural to expect that on some marine fossiliferous horizon we shall again encounter in a fossilised condition similar molluscan shells. The hope that we may in this way be able actually to "locate" the halolimnic fauna of Lake Tanganyika with that particular marine stock from which it sprang is all the greater, on account of the very striking facies which the shells of the molluscs belonging to it invariably present. But in actually searching among marine deposits for the particular sea fauna to which the halolimnic animals may correspond, it is essential that we bear in mind the caution that single comparisons are likely to be of little service as affording any indication that two such faunas are the same. There must be in the old stock, to which we are

going to compare the halolimnic fauna, at least a sufficient number of types which are similar to individual halolimnic forms to correspond with a majority of the forms the halolimnic fauna now contains. I have emphasised this point because certain comparisons have already been instituted between the shells of the Paramelanias of Tanganyika and forms occurring in the fresh-water cretaceous beds.

In 1883, White,[1] in an extremely short paragraph, pointed out that, speaking conchologically, there is not much to distinguish the shell of the genus Paramelania (Smith) from that belonging to the extinct fresh-water Pyrgulifera, which he obtained from the Green River deposits of the United States. So far as the outward forms of these shells go, there are slight differences as to sculpture, and so forth (compare Pl. 23, figs. 1 and 7). But I do not know that such dissimilarities as these would justify even a conchologist in regarding the genera as distinct, and that this comparison of a single halolimnic and cretaceous shell is, in the absence of any possibility of information respecting the nature of the contained animals or their associates, "so far, so good," seems to be the total net result of the further observations made upon the subject by Tausch[2] and Oppenheim,[3] except that these latter authors appear to have had at their command more extensive and better preserved material than that which White examined. Speaking conchologically, then, there is one type in the cretaceous fresh-water deposits and one in the African halolimnic fauna which are similar in form. But even in the case of the single correspondence which presents itself Tausch's work appears to have rendered it extremely doubtful whether the two forms can be still considered as even conchologically the same. He showed, after examining hundreds of Pyrguliferas from the upper cretaceous beds from Ajka in Hungary, that

[1] 'Proc. U.S.A. Nat. Mus.,' S. 98, Washington, 1882, p. 98 (published in 1883).

[2] 'Sitz. Ber. d. k. Acad. Math. Wien,' 1885, Bd. xc, p. 57.

[3] Zeitschrift. der Deutsch. Geol. Gesell.,' 1892, Bd. xliv, p. 697; for diagnosis of Pyrgulifera see 'U.S. Geol. Surv.,' 40 parallel, vol. iv, p. 146, pl. 7, fig. 19.

their shells could be sorted out into several groups which in their extreme forms were quite distinct, but which were really indissolubly connected together by innumerable transitional types. Thus one type of Pyrgulifera agrees with Paludomus Pichleri (Hoern.) from the "Gosauformation," another with P. armatus (Math.) from the French chalk, a third with P. lyra (Math.) from the same, a fourth with Pyrgulifera humerosa (Meek) from the Laramie of North America; while to a fifth and sixth Ajka variety there seem to be no known corresponding forms. Since all these types are stated by Tausch to run completely into one another, they can but be regarded as connected polymorphs of one and the same generic type, whatever the actual organisation of this genus may have been. Tausch further points out that in Paludomus Pichleri there are certain characters at the base of the mouth which have led to this shell being described both as a Paludomus and a Melanopsis. This melanopsid "mouth" is not found, according to Tausch, in P. Stephanus (Bens), but it is present in P. humerosa and in the Paramelania Damoni of Tanganyika. Tausch therefore argues that the Paramelania Damoni, Pyrgulifera humerosa, and those forms of Paludomus which possess this peculiarity of "mouth" are, together with certain forms of Melanopsis, merely varieties of a single polymorphic type. This type embraces also in its other modifications forms approximating to Melania amarula, Lamarck's type of the genus Melania. I can fully confirm the observation of the remarkable similarity of some of the Pyrguliferas collected by Dr. Oppenheim, and now in the British Museum, to M. amarula; in fact, some of these forms approximate far more closely to the living M. amarula of Madagascar than they do to the Paramelania of Tanganyika. Thus, whatever the dead Pyrgulifera may have been, its shells in their different modifications agree with a great number of living types, and if it be really legitimate to draw any conclusion from this complexity of corresponding forms, it can only be said that Tausch's work has shown that there appears to have existed in the fresh water

of the upper cretaceous series a form which united by insensible gradations the conchological characters of Melanopsis, Paludomus, Pyrgulifera, Melania (amarula) and Paramelania. But if on this ground it should be maintained that the living representatives of these different groups have any immediate phylogenetic relationship with each other, all that can be said by anyone acquainted with the morphology of such of them as now exist must be that although a deduction of this kind from the characters of living and extinct shells may be conchologically correct, it is also at the same time morphologically nonsense; there is no sort of morphological similarity between Melanopsis and a Melania amarula. These forms, as the investigations of Bouvier have shown, should by right be placed in different families. Paludomus differs from them both, while the Paramelania of Tanganyika is altogether unlike any of the three. Thus if the genus Pyrgulifera corresponds to any of these types which now exist, it differs from all the rest which I have named. If Pyrgulifera humerosa was morphologically similar to Melanopsis, it was not a Paramelania. If, on the other hand, it was a Paramelania, it was neither a Melampus paludomus nor a Melania proper. There is thus really no direct reason why the Pyrgulifera of the chalk should not have been a Paramelania; but since the genus Pyrgulifera has been shown by Tausch to correspond equally to three widely distinct living types, it is clearly more than three to one that such was not the case.

As to the question of the identity of the entire fresh-water fauna with which the Pyrguliferas are connected in the upper chalk, and that consisting of the halolimnic group in Tanganiyka, whether we regard the Paramelanias and Pyrguliferas as similar or not, it will be obvious that as there are no other forms in these faunas bearing the slightest resemblance to one another, the question of their general identity is ipso facto out of court. Not only do the halolimnic animals differ from those of the fresh-water fauna individually, but the whole halolimnic fauna differs entirely from the cretaceous or any

other fresh-water stock in the general facies it presents. These old cretaceous beds present the facies of a true fresh-water fauna, otherwise they could not be identified as such; they contain no crabs or prawns, there are no impressions of jelly-fish in the soft grey mud of which they are generally composed; they contain no shore sponges, Lamellariidæ, no Xenophoridæ or other marine Gasteropods, all of which are still living in the slightly brackish water of Tanganyika at the present time. In fact, the halolimnic fauna differs from that occurring in these fresh-water cretaceous beds just in those features which distinguish fresh-water from marine stocks in general, and there is not the slightest doubt that had the halolimnic fauna occurred fossilised it would have been regarded as unquestionably marine.

The halolimnic fauna of Tanganyika, then, is not the remnant of a cretaceous fresh-water stock, neither is it like any cretaceous marine fauna which we know, nor is it represented in any of the upper Mesozoic beds. It is only when we compare the shells of the halolimnic molluscs with those in several of the lowest secondary formations that any substantial similarity appears.

In fig. 1 A, are represented two remarkably fine examples of the marine Jurassic genus Purpurina; the figure is copied from a specimen P. bellona courteously placed at my disposal for this purpose by Mr. Hudleston from his magnificent collection of Jurassic fossils. The genus has a somewhat curious history in literature, which will be found fully dealt with in Mr. Hudleston's[1] monograph, 'The Jurassic Gasteropoda.' As amended in this work for P. elaborata, the diagnosis of the genus runs as follows:

"Shell ovate conoidal, apex acute, whorls about five or six, posterior area tabulate, sides moderately tumid. The ornaments consist of about eighteen longitudinal costæ, which are feebly developed on the tabular area, rise up into spinous nodes on the keel, and are strong and regular on the flanks of the whorls. The costæ have a tendency to die out anteriorly on the body-whorl; the costæ decussate with regular and closely

[1] 'A Monograph of the British Jurassic Gasteropoda.' Palæont. Soc., 1887, Part 1, No. 2, p. 86.

set spirals, which extend down to the base of the shell. No spirals are seen on the flat area. Aperture oval to subquadrate, columella moderately reflexed, so as to produce anteriorly a wide and shallow groove towards the point. Umbilical slits scarcely indicated."

Side by side with these old Jurassic shells I have had drawn two corresponding views of Smith's Paramelania Damoni from Tanganyika (fig. 1), the generic diagnosis of which runs as follows:

"Shell solid, ovate, conical, imperforate, longitudinally ribbed, transversely lyrate, covered with a thin epidermis. Aperture ovate, entire, indistinctly effuse at the base, last whorl sometimes slightly prolonged inferiorly. Peristome thick, margins joined by a callosity, operculum like that of Typhobia."[1]

The striking similarity of the two shells from these descriptions will be at once apparent; in fact, as Mr. Hudleston remarked while we were examining the recent shells and fossil side by side, "they are not only generically the same, but specifically identical."

The shells of the genus Paramelania were, however, shown by the German authors I have quoted to be similar to the Cretaceous genus Pyrgulifera, and, as objects which are like the same thing are necessarily like each other, it becomes a question for the systematists and the conchologists whether or not the genus Pyrgulifera and Paramelania should be quashed, and both replaced by the older genus Purpurina. There are slight differences between the shells of the genera Pyrgulifera, Purpurina, and Paramelania when they are carefully examined side by side; but these are not at all sufficient to separate the specimens from one another as specifically distinct, and, as Dr. Woodward pointed out to me, those of the genus Paramelania approximate more closely to the shells of the Jurassic genus Purpurina than they do to the more recent Pyrgulifera type.

In Hudleston's monograph there are represented two rather

[1] 'Proc. Zool. Soc.,' 1881, p. 559.

distinct types of Purpurina shell, one characterised by the P. bellona (fig. 1A), the other by the P. inflata given in fig. 2A. Hudleston did not separate these forms as generically distinct, but figured the types of which they are characteristic on separate plates. How closely similar this inflata type of Purpurina is to the living Nassopsis of Tanganyika will at once be apparent from figs. 2 and 2A. The genus Nassopsis was separated by Smith[1] from Paramelania on account of the difference in the operculum, but it is doubtful if this distinction can be maintained from their anatomy; indeed, I should be inclined to place Paramelania, Bythoceras, and Nassopsis as species of one new family, the Paramelanidæ. The fact that there is more constant distinction between the Tanganyika Paramelania and Nassopsis now than that which used to exist between the bellona and inflata types of Purpurina is just what we might expect, since it is probable that these two forms would become less transmutable as time went on.[2]

The Tanganyika Paramelania and Nassopsis are thus identical with two forms occurring in the old Jurassic beds, and the Paramelania corresponds more closely to the Bellona type of Purpurina than it does to the Pyrgulifera of the chalk.

In the same Jurassic series there is another characteristic genus, Amberlya, which is specifically very variable in size, sculpture, and in the character of its spines. Two forms are represented in fig. 3A, the upper one from the collection in the British Museum, the lower from Mr. Hudleston's collection. The history of this genus Amberlya is peculiar and instructive, and will be found fully set forth in Hudleston's monograph.[3] The genus was originally founded by Morris and Lycett, but was subsequently modified by Hudleston, and

[1] 'Proc. Zool. Soc.,' 1881, p. 559.

[2] There is a peculiarity in the base of the columella of some of the Nassopsis shells which is not represented in those of the genus Purpurina, but which is a permanent feature of the Jurassic Monodonta. So far as Nassopsis goes this is an unimportant feature, since it is not constant in the genus.

[3] Loc. cit., part 1, No. 6, pp. 274—279.

as amended by him the diagnosis runs—"Shell turbinate, more rarely trochoid, rather thin, imperforate or nearly so; subelongate, frequently turreted; sutural space wide; ornamented with spiral bands, usually spinulous or nodular, some of which are prominent. The interspaces are finely striated, the striæ being slightly oblique to the axis; sometimes these fine lines are strong enough to represent fine axial ribs. Base rounded, spirally ribbed, and marked by fine radial striæ; aperture suboval, but varying according to age, in the adult more or less rounded, so as to become suboval or subcircular; there is usually a considerable deposit of callus; outer lip thin, often crenulate."

This description would certainly answer for that of one of the new types which I found in Tanganyika, and for which I have proposed the generic name Bathanalia (fig. 3), for although the Jurassic genus Amberlya shows a considerable range of specific variation, all its species have essentially the same characteristics as the two represented in fig. 3A, upper and lower. The thin shell, the absence of all trace of epidermis, and the character of the whorls, as well as the sculpture and the character of the mouth, are all essentially the same in Bathanalia as they are in Amberlya; the only point in which they differ is in the columella, that of Bathanalia being generally open, while that of Amberlya is always closed. I have, however, consulted Mr. Edgar Smith and others about this, and he assures me that such differences cannot be upheld as generically distinctive, more especially as the amount of umbilical opening in Bathanalia varies a good deal in extent from shell to shell. We may, therefore, conclude that conchologically Bathanalia and Amberlya are the same.

The next example of the close similarity existing between the living shells in Tanganyika and the marine Jurassic types is that afforded by the Limnotrochus Thompsoni of the one and certain so-called Littorinas of the other. In fig. 5 are represented two views of L. Thompsoni, while in fig. 5A are given similar views of the Jurassic species Littorina sulcata.

Smith's generic diagnosis of Limnotrochus runs thus:—"Shell trochoid, umbilicated, without an epidermis, spirally ribbed; body-whorl keeled round the middle; aperture non-lyrate within; with the outer lip oblique, the basal margin broadly sinuated, and the columella edge somewhat reflexed. Operculum horny, paucispiral, litterinoid."[1] This description would not do for the living Littorinas of our shores, but it covers the two forms, one from Tanganyika and the other from the marine Jurassic beds, just described.

I would next direct attention to the very obvious conchological similarity between the so-called Limnotrochus Kirkii (fig. 6) and the marine genus Xenophora (Onustus), a form which has extended in the ocean from the Devonian to the present time. This genus is not, therefore, typical of the Jurassic period, specially those which I have already described, but it forms one more remarkable example of the marine character of the halolimnic forms. I have represented side by side the L. Kirkii (fig. 6) and an example of Onustus (fig. 6 A), a typical Jurassic form.[1]

I have already stated that the so-called Lithoglyphus (Spekia) zonatus of Tanganyika (fig. 4) is unquestionably, from the characters of its anatomy, a Naticoid; and in the inferior Oolite there are forms which it would be quite legitimate to regard as coming near this genus. To illustrate this fact I have figured the so-called Neridomus (fig. 4 A) of the inferior Oolite, the affinities of which are doubtful in a high degree.

Lastly, in Tanganyika there exists a remarkable longitudinally sulcated shell known as Melania admirabilis (Smith); how closely this form corresponds to those remarkable Oolite shells known as Cerithium subscalariforme will be seen on comparing their respective shells. I did not find this species myself in Tanganyika, and as the animal it contains is not

[1] 'Proc. Zool. Soc.,' 1881, p. 285.

[2] It is needless for me to point out that the two forms of Xenophora here figured from Tanganyika and from the Inferior Oolite are not specifically the same. The so-called Limnotrochus Kirkii of Tanganyika being much more like several modern examples of the genus Onustus (Xenophora). The figures only illustrate the general similarity of such shells.

known I have not thought it necessary that I should give figures of them here.

Besides the above marine types the halolimnic fauna contains two forms, Syrnolopsis and Turbonella terebriformis, which, although they do not resemble any known Jurassic shells, nevertheless exemplify in a remarkable manner the marine affinities of the halolimnic mollusca as a whole; for the shell of the first of these species is practically undistinguishable from that of the genus Syrnola, a form found in the tropical seas, the second from that of the genus Terebra.

It is thus apparent that with the exception of Typhobia,[1] and possibly of Bythoceras, all the halolimnic genera now living in Lake Tanganyika are generically identical with Jurassic forms, while two of these, Paramelania and Nassopsis, contain forms which are specifically indistinguishable from their corresponding Jurassic types.

Curious and startling as the foregoing comparison undoubtedly appears, I might still have had some hesitation in bringing it forward as evidence of the origin of the halolimnic fauna, had not the three authors, White, Tausch, and Oppenheim, practically forced my hand by attempting the comparison of which I have spoken between the living halolimnic and the old cretaceous fresh-water stocks. Whatever may be the real value of evidence which is based upon shell structure alone (and this certainly becomes more and more questionable as time goes on), it will have been rendered clear that the amount of this kind of evidence favouring the similarity of the halolimnic and old cretaceous fresh-water stocks is utterly insignificant beside that which can be produced in favour of the similarity of the halolimnic and old marine Jurassic forms.

So far as I am concerned, therefore, this paper will have

[1] The genus Typhobia, as I have shown, is, however, closely related to Bathanalia, and there is very little doubt that it simply represents a modification of the former form. It may be that the genus Typhobia in reality represents the Jurassic form Purpuroidea.

fulfilled its purpose if it acts merely as a sort of counterpoise to the altogether disproportionate importance which has been attached to the apparent similarity between the Paramelania of Lake Tanganyika and the Pyrgulifera of the upper chalk.

Whatever opinion those competent to judge may form of the comparisons which I have just instituted between the marine Jurassic and the halolimnic faunas, it is obvious that these comparisons are nothing like so rash an undertaking as that attempted by the three authors I have named. The view that the Tanganyika fauna corresponds to a fresh-water cretaceous stock rests on nothing but the similarity of a single type of shell common to the halolimnic and cretaceous fresh-water series; and, as we have seen, the possibility of even this single point of similarity being due to anything more than mere convergence of external form has been rendered so extremely doubtful by the more extended observations which one of the authors named has already made, that any attempts to pursue the question further would be simply waste of time. Even if the far more weighty evidence for the correspondence of the halolimnic fauna with that of the Jurassic seas rested solely on the similarity of their respective shells, although such evidence would be as good as that forthcoming for many sweeping geological deductions, I should, for my part, be highly sceptical that it afforded any trustworthy indication that the hypothesis is true. When, however, we view the supplementary facts of this comparison, when we regard it in the light of what I have ascertained respecting the distribution, and especially the comparative morphology of the halolimnic forms, it is very clearly apparent that the theory of their similarity is not without much collateral support.

We know now that the morphological characters of the halolimnic fauna are those of an early oceanic stock, that they do not stand midway between the living fresh-water faunas and their marine beginnings, for they do not foreshadow any known fresh-water types; on the other hand, we have seen that they do very distinctly foreshadow many living oceanic types, each individually uniting the characters of several modern oceanic

species. We are sure, therefore, that the halolimnic group represents an old sea stock that became detached from the general oceanic fauna of which it was a part, far back in time. Like the Oolite molluscs, those of this halolimnic fauna have a striking type of shell, and when, after reviewing the facies presented by the marine fauna of the successive geological periods, we find such types represented abundantly nowhere except in the Jurassic seas, and that these seas present forms corresponding to them all, the comparison appears to be something more than a mere coincidence. It rather appears as the fulfilment of an expectation raised simultaneously by the three chief lines of search relating to their distribution, morphology, and affinity with existing types.

I offer this comparison, therefore, as the probable explanation of the singularly interesting problem presented by the mixed fauna which Tanganyika now contains, and I have all the more confidence in so doing since much study of the question, in the light of every suggestion which I could either invent or borrow, has convinced me that no other even momentarily tenable explanation is likely to be found.

DESCRIPTION OF PLATE 23,

Illustrating Mr. J. E. S. Moore's paper "On the Hypothesis that Lake Tanganyika represents an Old Jurassic Sea."

Fig. 1.—Front and back view of the shell of Paramelania Damoni (Smith), Tanganyika.

Fig. 1a.—Front and back view of the shell of Purpurina bellona (Hudl.), the corresponding Jurassic form.

Fig. 2.—Front and back view of the shell of Nassopsis nassa (Smith), Tanganyika.

Fig. 2a.—Front and back view of the shell of Purpurina inflata (Hudl.), the corresponding Jurassic form.

FIG. 3.—Front and back view of Bathanalia Howsei (Moore), Tanganyika.

FIG. 3A.—Upper figure, back view of Amberlya, sp.? from the Inferior Oolite, British Museum; lower, front view of Amberlya, sp.? from the Lias, the corresponding Jurassic forms.

FIG. 4.—Front and back view of Spekia zonatus (Woodward), Tanganyika.

FIG. 4A.—Front and back view of Niridomus minutus, var. tumidulus (Phill.), the corresponding Jurassic form.

FIG. 5.—Front and back view of Limnotrochus Thompsoni (Smith), Tanganyika.

FIG. 5A.—Front and back view of Littorina sulcata, the corresponding Jurassic form.

FIG. 6.—Back view and base of Limnotrochus Kirkii (Smith), Tanganyika.

FIG. 6A.—Back view and base of Xenophora (Onustus), from the Inferior Oolite.

FIG. 7.—Back view of the shell of Pyrgulifera humerosa (Meek), from the fresh-water deposits of the upper chalk.

On the Reno-pericardial Canals in Patella.

By

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With Plate 24.

Strange indeed, and happily unique in the annals of comparative anatomy, has been the history of our knowledge of the reno-pericardial canals of Patella. Although discovered more than thirty years ago, and investigated by many observers since, not only is their structure insufficiently known, but their very existence has been called in question, and even positively denied!

Wishing to find out definitely whether these ducts really existed or not, I undertook this work, which was carried out in Oxford, on material obtained from Plymouth and Naples. In this short paper I hope to establish clearly, and beyond the possibility of doubt, the fact that there are reno-pericardial canals leading from the pericardium to the right kidney and to the left kidney in Patella.

A communication between the pericardial and the renal cœlom of Patella vulgata was originally described by Professor Lankester in 1867. "By most careful dissection," he tells us, "Dr. Rolleston and myself detected what appears to be a minute opening from the pericardium into the supra-anal reticulated sac lying in the curve of the rectum [left kidney]. The orifice I found first by opening the pericardium, when it was seen between the bifurcation of the auricle at the right side of the cavity, and was then traced from both the pericardium and supraanal sac in other specimens." Dr. von

Jhering ten years later, in an important paper on the kidneys of molluscs, states that he was unable to find a reno-pericardial communication: "Die Pericardialöffnung sah ich nicht" (1877, Jhering). In 1881 Lankester and Bourne together reinvestigated the question, and described the canal thus:—"Its presence can be demonstrated both by injections which pass from the pericardium sometimes into the right, sometimes into the left renal sac, and by dissection. The orifice leads directly into a narrow subanal tract of the further or right renal sac, and not directly into the left or small renal sac" (1881, Lankester). It will be seen that, curiously enough, although the presence of the canal previously described as leading into the left kidney is not actually denied, yet the author seems not to be convinced of its existence. Shortly after, Mr. J. T. Cunningham undertook the study of these canals by means of series of sections (1883, Cunningham). In this paper, to the details of which we shall refer later on, two pericardial canals are described leading into the small left and large right kidneys respectively in Patella cœrulea. The fact that only a diagram of the canals is given, and that Cunningham made use chiefly of injected material, perhaps contributed to weaken the evidence brought forward. The main facts were, however, confirmed by Mr. Harvey Gibson in his studies on the anatomy of Patella vulgata (1887, Gibson). An important memoir on the kidneys of the Prosobranchs was brought out by M. Rémy Perrier in 1889; in this work the author states that although he made use of sections, and found the right reno-pericardial canal, yet he was unable to find a canal opening into the left kidney: "Je n'ai pu retrouver la communication du péricarde avec le rein gauche." Perrier concludes that the right kidney alone communicates with the pericardium (1889, Perrier).

We now come to the most sensational chapter in our story. In 1892 Dr. R. von Erlanger published an elaborate work on the 'Paired Nephridia of Prosobranchs' (1892, Erlanger), in which he positively denied the existence of any reno-pericardial duct in Patella. The author, criticising the injection method, maintained that previous observers had been misled by the

injection being forced through weak spots where the kidneys reached the wall of the pericardium. In conclusion, von Erlanger stated that in Patella there is "no reno-pericardiac duct whatever."

The absence of the communicating canals seemed now to be as firmly established as their presence had appeared to be but a short time before. The matter was not long allowed to rest in this condition. Hardly had naturalists become reconciled to v. Erlanger's view, when Dr. Béla Haller published some elaborate studies on Prosobranchs (1894, Haller), in which he describes in considerable detail a right reno-pericardial canal in Patella magellanica. A dissection is figured showing the apertures of this canal. As for the left canal, Haller denies its existence: "Wie wir wissen, hat Cunningham auch für die linke Niere eine pericardiale Mündung behauptet, darum war ich, obgleich dieses mir nach dem Verhalten bei der Monobranchen höchst unwahrsheinlich vorkam, doch bemüht dieses unbefungen zu verfolgen. Auf Totalpräparaten war dieses in Folge der subtilen Verhältnisse nicht recht möglich und darum benützte ich hiefür meine Querschnittserien, doch konnte ich bei keiner der untersuchten Formen eine Mündung der linken Niere in das Pericard auffinden. Eine solche fehlt ganz entschieden."

Having thus briefly reviewed the history of the subject, I must now give a short account of my own observations, which are founded on the examination of complete series of transverse sections. The structure and relations of the small left and large right kidney are now so well known that they need not again be mentioned. I shall, therefore, merely describe the selected sections figured on Pl. 24.

In fig. 1 is represented a section through the two kidneys, rectum, and pericardium, some little way behind the posterior limit of the mantle chamber. It will be seen that from the right ventral corner of the pericardium proceeds a diverticulum, which, in fact, is the beginning of the right reno-pericardial canal. A section taken farther forward (fig. 2), so as just to cut through the hinder region of the mantle cavity, shows the

right reno-pericardial canal separated off from the pericardium, and lying close to the wall of the subrectal portion of the right kidney. From the pericardium a second diverticulum is seen coming off at a higher level than the first,—it is the beginning of the left reno-pericardial canal. If we compare this figure (fig. 2) with fig. 25 *b*, pl. xxxvii of Erlanger's memoir, we can hardly doubt but that he actually figured the origin of the left canal without understanding its true naturc ; for nowhere else does the right wall of the pericardium become folded or pushed out at this level as v. Erlanger represented. Neither of the canals opens straight into the renal cœlom,—on the contrary, they bend forwards and extend along the walls of the kidneys for some considerable distance. The right reno-pericardial canal is especially long. If we follow the left canal to about one third of the way between its place of origin and the external aperture of the left kidney, we find that it opens into this kidney (fig. 3) through its left wall. Tracing out the right or lower canal farther forwards, we find it opening into the subrectal region of the right kidney, about two thirds of the way from its origin to the right renal pore (fig. 4). In both cases the reno-pericardial opening is situated at the end of a papilla projecting into the renal cœlom, and forming a ciliated funnel-like spout.

The excretory epithelium of the kidneys (fig. 9) is formed of a layer of very tall cells, the free ends of which are much swollen, and often broken off. They sometimes bear cilia. A round nucleus is situated towards the base, and outside it are numerous excretory granules; the swollen distal end appears almost empty—an effect due, no doubt, to the reagents. At the rim of the funnel (figs. 6 and 7, and the reconstruction in fig. 5) this epithelium changes suddenly into ordinary high columnar epithelium, provided with numerous long and powerful cilia directed towards the renal cavity. Near the base of the funnel the ciliated epithelium passes into the flat cœlomic epithelium lining the canal (fig. 8). The pericardial epithelium itself is identical and perfectly continuous with that of the canal.

Comparing this description with that given by previous observers, it may be remarked that in the main my results agree with and confirm those of Cunningham; yet neither he nor Gibson appears to have seen the well-marked funnels. On the other hand, like Gibson, I am unable to find the "triangular piece of tissue" described by Cunningham as forming "a sort of valve" over the opening. It is difficult, indeed, to see how such a flap would act in connection with the papilla. Haller describes a ciliated funnel at the right reno-pericardial aperture without figuring a section through it; but he further states that the canal itself is lined with high columnar cells. This is certainly not the case in the species I have investigated, and I cannot help thinking that he may have mistaken in this instance a branch of the ramifying kidney for the reno-pericardial canal. Both the kidneys give off numerous branches lined with epithelium similar to that of the main renal chambers.

In the four series of sections of Patella vulgata examined I have always found the two reno-pericardial canals present, and well developed. In Patella cœrulea I have observed two canals of essentially similar structure,—in fact, the description given above applies equally well to either species.

Summary.

In the foregoing pages it has been shown that in Patella vulgata and cœrulea there are two reno-pericardial canals, opening by means of projecting ciliated funnels[1] from the pericardium into the right and left kidneys respectively.

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[1] Similar but better developed structures are found at the mouth of the reno-pericardial canals of Chiton and other molluscs.

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EXPLANATION OF PLATE 24,

Illustrating Mr. Edwin S. Goodrich's paper on "The Reno-pericardial Canals in Patella."

All the figures refer to Patella vulgata.

Figs. 1, 2, 3, and 4.—Four transverse sections through the region of the rectum and pericardium, showing the course of the two reno-pericardial canals. Drawn with the camera. × 9.

Fig. 5.—Reconstruction of the opening of the right canal into the right kidney.

Fig. 6.—Section through the opening of the right canal into the right kidney. Cam. × 130.

Fig. 7.—Section through the opening of the left canal into the left kidney. Cam. × 130.

Fig. 8.—Section through the wall of a reno-pericardial canal, showing the flat cœlomic epithelium on the inside and blood space outside.

Fig. 9.—Section through the wall of a kidney, showing the large excretory cells.

The Development of the Pig during the First Ten Days.

By

Richard Assheton, M.A.

With Plates 25—28.

Introduction.

The history of the development of the roe deer, published by Bischoff in 1854, contains almost the only account there is of the early stages of the development of any Ungulate. The description which that distinguished embryologist was able to give was, however, somewhat meagre, and does not offer much assistance towards the solution of problems which occupy the attention of embryologists of the present day.

He was able to describe the external features of some of the stages of segmentation, and made the very interesting discovery, that in the deer the embryo, upon reaching the fully segmented or morula stage, enters upon a period of quiescence, and remains for some weeks unaltered.

Von Baer (2), who made most careful observations upon the later stages of development of both the sheep and pig, found the egg of a sheep in the oviduct as early as the end of the first day. His descriptions, however, of the earliest stages of these animals, although historically interesting, are of little use for our present purpose.

Between 1884 and 1889 Bonnet (10, 11) published his work upon the sheep, but in this he dealt with nothing earlier than the twelfth day.

Keibel's (18, 19) work, which treats of the pig (Sus scrofa domesticus), contains no reference to earlier embryos than those of fourteen days after fertilisation. At fourteen days the embryonal area is well defined and oval; the primitive streak is distinct, and the whole blastodermic vesicle has grown to an extraordinary extent, varying from half to a whole metre in length.

A younger stage of the pig's development has been described by Weysse (25), who obtained some thirty specimens of about the ninth to eleventh days, which he states present "phenomena unusual in the ontogeny of the Mammalia."

My specimens, which I propose to describe at the present time, are of stages prior to any described by the above authors, and date from the second day after fertilisation to the tenth.

They include the earliest phases of segmentation, the formation of the blastodermic vesicle, and the definite establishment of the epiblast and hypoblast layers of the future embryo.

Method of treating the Specimens.

The great difficulty of finding the embryos has made it almost impossible to examine many in the fresh state. The size of the cavity of the uterus of a pig is so great, and the foldings of the mucous membrane are so complicated, that a search to find them in situ proved most unprofitable. Accordingly, with very few exceptions, the whole of the material upon which I have worked was preserved in the way I am about to describe before any examination of it was made.

The ova, which, while in the Fallopian tube, can of course be very easily found, unfortunately in the pig travel very rapidly down the Fallopian tube, and pass into the uterus during about the third day, before segmentation has proceeded further than the four-segment stage.

The ova are extremely small, and receive no additional investment from the walls of the oviduct, and are quite invisible as they lie among the folds of the uterus wall. By scraping the mucous membrane of the uterus a specimen may occasionally be procured, but by far the most satisfactory

method of procedure is the slow injection of the cavity of the upper part of the uterus with some preserving fluid, and a subsequent search through the contents under the microscope.

Within a space of time after the death of the sow, varying from three quarters of an hour to three hours, I very slowly injected ·25 or ·5 per cent. chromic acid into the upper half or third of the uterus, having previously tied up the upper end of the piece of uterus. I allowed as much fluid to flow in as was possible without running the risk of bursting the uterus, in order that the folds of the mucous membrane should be reduced to a minimum. In this way the ova were caused to float freely in the fluid.

The lower end, through which the injection had been made, was then ligatured, and the whole was placed in ·5 per cent. chromic acid for two or more days. The contents of the uterus were then let out into glass vessels, and the specimens were easily found under the microscope.

Chromic acid used in this way was found to be the most satisfactory, on account of its clearness and the absence of any precipitate.

In a few cases I used Flemming's solution with equal success; but other fluids, such as Perenyi's, or solution of nitrate of silver, were unsatisfactory, owing to the great precipitation which occurs rendering the search for the embryos too laborious.

All my specimens were stained before they were cut into sections. The stains used were hæmalum, carmalum, hæmatoxylin, and borax carmine. They were embedded in paraffin by means of cedar oil.

Material.

The following account is based upon the examination of about 100 embryos obtained from sixteen sows. In about ten other sows I failed to find any specimens. The majority of those I have no doubt were really pregnant. My specimens may be tabulated thus:

List of embryos obtained arranged according to age :

Under 4 days . . .	2	From 7 to 8 days . .	15
From 4 to 5 days . .	23	„ 8 „ 9 „ . .	4
„ 5 „ 6 „ . .	9	„ 9 „ 10 „ . .	10
„ 6 „ 7 „ . .	14	„ 10 „ 11 „ . .	19

List of embryos arranged according to stage of development:

A. Prior to the 5-segment stage	13
B. The 5-segment stage to morula, with surface cells approximately equal	12
C. Commencement of blastodermic vesicle to oldest retaining zona	33
D. From rupture of zona to rupture of Rauber layer . .	20
E. Formation of embryonal area to first sign of primitive streak	22

Embryos of Stages A and B.

The dates which are given must be regarded as approximate only.

In the pig it would seem that fertilisation may occur about the end of the first day, or may be postponed two or three days after union.

I have made no observations upon the process of fertilisation, and merely draw the above conclusions from finding specimens in identical conditions in pigs killed upon the fourth, fifth, or sixth day. It is accordingly very difficult to obtain any given stage.

Embryos frequently differ very greatly in the same uterus. For instance, in one case I had ova in two segments, nine segments, and completed morulæ from the same horn of the uterus.

The youngest stage that I have is a two-segment stage (Pl. 25, figs. 1 and 2). This was taken from the uterus of a pig on the sixth day. It was preserved in Flemming's solution (weak formula). The embryo is protected by a thin zona

radiata, which is not thickened by the addition of any mucous or albuminous layer.

The slight and very thin irregular external coat to the zona radiata is seen upon higher magnification to be caused by numerous spermatozoa, which seem to have adhered to the zona radiata.

The embryo lies freely within the zona radiata. Both segments appear plainly divisible into two parts, an outer clearer layer which extends round the greater part of an inner much darker part, within which lies the nucleus.

The outer layer, which shows a very fine reticulation or granulation, is only very slightly darkened by the fixing solution. On the other hand, the inner portion is very much blackened, owing to the action of the osmic acid upon the numerous oil globules whose presence characterises this part of the segment.

In a fresh specimen these globules are rather dark and of an olive-green colour. Ordinary stains have no effect upon them. Osmic acid is the only reagent that I have found to colour them. In this respect, among others, they differ very much from the yolk granules of reptilian, avian, and elasmobranch eggs.

The nucleus, like the outer layer, has none of these oil globules, and so the centre of each segment appears more transparent. The nucleus of each of the segments of this specimen is distinctly visible, and is spherical.

Two polar bodies are present.

Fig. 2, which is a side view, shows very distinctly the relative extent and position of the outer clearer zone and the inner oil-bearing mass.

Fig. 3 was drawn from a fresh specimen taken from the upper end of the uterus within 10 mm. of the opening of the Fallopian tube. It was found with three other specimens, two of which were in the four-segment stage, the other in five segments.

The nuclei could not be distinctly seen, but their presence was indicated by the slightly greater transparency of the central

parts of each of the three segments. The same characters of clear and darker portions are recognisable as in the two-segment stage.

Fig. 4 is a drawing from a fresh specimen taken from the Fallopian tube of another sow. This and one other, which was also in the four-segment stage, are the only two embryos I have ever obtained from the Fallopian tube of the pig. The same features were present as regards inner and outer portions of each segment.

The four segments were not of equal magnitude.

The following measurements were made while the specimen was quite fresh :

Diameter of the whole specimen from outer edges of zona radiata . . .	·164 mm.	
Thickness of zona radiata	·016 „	
Diameters of the largest sphere . .	·064 „	× ·066 mm.
Diameters of the smallest sphere . .	·060 „	× ·060 „

These two segments formed one pair in contact with each other. At right angles to this pair was the pair formed by the other two segments, which were of equal size and did not quite touch each other.

Diameters of each	·060 mm.	× ·064 mm.
The diameters of one polar body were .	·015 „	× ·010 „

The second polar body was too indistinct to be measured.

I was not able to detect any difference in the colour or in the composition of the several segments, either while fresh or after the application of osmic acid and stains. There would seem to be distinct difference in size.

The following measurements from another specimen made after fixation give similar results.

Pair in contact :

Diameters of the larger segment . .	·056 mm.	× ·058 mm.
Diameters of the smaller segment . .	·056 „	× ·054 „

Pair widely separated and at right angles to the preceding :

Diameters of larger segment . . .	·050 mm.	× ·061 mm.
Diameters of smaller segment . . .	·047 „	× ·058 „

The polar bodies of this specimen were especially large. They were spherical, and measured ·025 mm. and ·014 mm. respectively.

In the five-segment stage (fig. 5) there is one segment distinctly larger and one distinctly smaller than all the others. The remaining three are approximately equal. There is no means of determining to which generation the segments respectively belong. It is, however, interesting and important to note that at this early stage there is a very great difference in size between the several segments.

The next stage I have is one of six days three hours. I had several in this stage, all of which had been treated with Flemming's fluid, and were therefore considerably blackened, owing to the action of the osmic acid on the oil vacuoles. This makes it difficult to determine the number of segments in surface view. The chief feature of all these specimens was easily discernible. This was the presence of one segment very much larger than any of the others.

One specimen was stained in carmalum and cut into sections, a drawing of one of which is given as fig. 14, Pl. 26. In this specimen there are altogether nine segments. One of these is far larger than any of the others. The nucleus of this segment is in an early stage of mitosis. The section I have drawn, however, does not pass through it. The remaining eight segments are of very different sizes. The nucleus in each is in its resting condition.

The specimen of which fig. 12 represents a section was, when seen in surface view, apparently in a similar stage to that represented by fig. 13. It seems, at any rate, possible that it was really in the two-segment stage, and that one of the segments had by some means burst.

The section which I have drawn shows one very large and complete segment, and partially surrounding this there is a mass which contains not a trace of nucleus nor any arrangement of the oil globules comparable to those of the large intact segment or to those of fig. 13. If this is really the case,

the large segment will represent very well the more minute structure of the embryo when in the two-segment stage, as in figs. 1 and 2.

Towards the periphery the protoplasm is very finely reticular, whilst more centrally the meshes are much larger. It would seem really to be vesicular, the vesicles being filled with oily "yolk," as mentioned above.

The less vacuolated cortical layer makes inroads at two points into the central portion. The nucleus is spherical and sharply defined by a very deeply staining membrane. A plate of very fine chromatin granules passes through its centre. There are no oily vacuoles in the nucleus.

In fig. 6 I give a drawing of a rather more advanced embryo. In this there were about twelve to fourteen segments. There was a very great disparity in the size of the several segments. Such a stage may be easily derived from the foregoing condition of fig. 13. The large segment has divided, as well as several of the smaller ones. This specimen, together with the next one which I shall describe, and those of which figs. 1 and 2, 12 and 13 are drawings, were all obtained from the same horn of a uterus. Their age was six days. The same uterus contained embryos in which the cavity of the blastodermic vesicle was established.

Fig. 8 represents a specimen which presented a unique stage. Segmentation had proceeded very much further than in the preceding case. At one point a single large segment protruded beyond the other. Over the rest of the embryo the outlines of the segments formed tolerably regular figures, showing that the segments on the surface were approximately uniform in size and somewhat compressed by their surroundings.

This is the only specimen I have found in this condition. It was unfortunately lost.

This stage is succeeded by one which may be described as a typical mammalian morula (fig. 7). In surface view the segments present no great disparity of size. The embryo as a whole is spherical. A series of sections shows that there is a

very considerable difference in the minute structure of the segments as compared with an earlier stage.

Although the oily "yolk" vesicles are still present, but in diminished numbers, there is certainly a tendency to the formation of other spaces or vacuoles. I cannot say whether these spaces are the commencement of the cavity of the blastodermic vesicle or not.

Fig. 14 is a median section through a specimen such as that represented by fig. 7. It was taken from the uterus at six days.

It is very difficult to detect in sections any boundaries between the cells. It would not, however, be right to say that the embryo is a vacuolated protoplasmic mass containing nuclei, for surface-view specimens treated with silver nitrate show the lines of division between the cells very clearly. Here and there, also, there is in some of the sections a trace of a cell boundary, more especially where it would mark off a peripheral from an inner mass cell.

There is also a distinctly radiate arrangement of the protoplasm round certain centres, by which the sections (v. fig. 14) are marked out into cellular areas. The arrangement of the nuclei round the periphery should be noted.

This concludes all the evidence I have to offer with regard to the process of segmentation of the ovum in the pig.

The most noteworthy feature is the very great dissimilarity of size of the segments after the four-segment stage. I unfortunately have only one certain specimen in the two-segment stage. In this I can see no difference in size or nature between the two.

In the four-celled stage there is always a slight difference in size, but at the eight- to twelve-segment stage the difference is most marked, and recalls the great difference which we find in many molluscan ova. In no case, however, have I found any difference perceptible except in size. The smaller segments form a cap upon the larger.

This great inequality is more marked than in any other mammalian segmentation. Bischoff shows nothing like it in

his figures of the segmenting ova of the dog, or guinea-pig, or deer, and only in a less degree in the rabbit. Van Beneden (4) for the rabbit described a cap of smaller segments, which ultimately surround a group of larger cells. I was in a former paper unable to offer evidence in support of this. Heape (15) found nothing of the kind in the mole.

When, however, we take into consideration the recent account given by Duval (13) for the bat, and Hubrecht (16) for Tupaia, of a growth of slightly smaller cells round a core of slightly larger cells, which is made much more obvious by reason of a difference in the affinity to stains of the two groups of segments, and an account of a similar process in the sheep, which I hope to give in an accompanying paper, together with the original description of the rabbit as given by Van Beneden (although subsequent observers have been unable to confirm his statements), it is clear that there is much evidence in favour of this original discovery of Van Beneden; and in the present case I have to consider whether the specimens I have described above of the segmentation in the pig may indicate an identical wrapping round one group of segments by another or not. It seems extremely probable that there is such a process. Its significance, however, I have discussed in my paper on the Development of the Sheep (1a), in the last number of this Journal, whose development I believe shows that it is really the hypoblast which grows round the epiblast, and not vice versâ, as Van Beneden supposed.

Formation of Cavity of Blastodermic Vesicle to Rupture of Zona radiata—Stage C.

The surrounding membrane consists still of the zona radiata, which is becoming slightly stretched (fig. 15). In this figure the vacuolation seems to be more pronounced at one part than elsewhere.

I am inclined to think that this more intense vacuolation eventually leads to the origin of the cavity of the blastodermic

vesicle, which soon after becomes established. If this is so, the embryo in this particular behaves like that of the rat (Robinson [23], Duval [12], or Tupaia javanica, Hubrecht [17]), whereas in forms like the rabbit and mole the blastodermic vesicle cavity arises as a clean cleft between the several segments, which are always more sharply separated than in the case of the former animals. In some instances I have seen strands of protoplasm connecting the inner mass with the outer wall of the ab-embryonic pole after the cavity has attained as great a size as in fig. 16.

Small spherical globules can often be found within the cavity which have the appearance of being oil drops liberated during the process of vacuolation that has resulted in the formation of the cavity of the blastodermic vesicle.

In fig. 15 the cavity is seen distinctly in two places. In fig. 16 the cavity is large and eccentrically placed. The embryo may be described as a spherical hollow ball, the wall of which is much thickened at one spot. There is as yet no distinct inner mass clearly divided from the outer wall.

As the embryo grows and the blastodermic vesicle cavity enlarges, the innermost of the nuclei of the thickened portion of the wall become, with their surrounding protoplasm, more and more free and more rounded; while the outer layer, which is more directly connected with the remainder of the wall, is found to be clearly separated off from the former, and it becomes alone directly continuous with the outer wall of the vesicle (compare figs. 17 and 18). These changes occur very rapidly, and without much increase in the number of cells.

The oily yolk globules are still present in little groups in the meshes of protoplasm, chiefly round the nuclei. The whole protoplasm is very much vacuolated, and retains this condition until after the rupture of the zona radiata.

The inner mass consists now of very few cells. In one specimen, in size between those shown in figs. 18 and 19, the inner mass was completely flattened up against the outer wall, so that the embryonic pole of the blastocyst was not more

than twice as thick as the ab-embryonic pole, instead of being six or seven times as thick as it is in fig. 17.

Pl. 25, fig. 9, is an outline drawing of the embryonic pole of this specimen. The nuclei of the outer layer are shaded with lines, the nuclei of the inner mass with dots. The solitary nucleus of the outer layer which overlies the inner mass is smaller than the others. The inner mass is there seen to consist of six cells only.

Boundaries of cells cannot be distinguished at this stage, except in silver nitrate specimens.

I have obtained a large number of specimens between the stages represented by my figs. 16 and 20. In these I find a great inconstancy in the relative size of the inner mass to the outer layer. Although the inner mass may consist of as few as six cells, it is more usually of greater size than this. I cannot say whether there is a reduction in the number of cells of the inner mass after it has become separated off from the outer layer.

Heape (14) described an occurrence of this nature in the mole, and assumed that cells passed from the inner mass into the outer layer.

Just before the destruction of the zona radiata comes about the inner mass shows a marked increase in activity, and increases in size and becomes more compact (figs. 19 and 20).

I have not seen in any specimen anything to indicate, before the rupture of the zona radiata, which cells will become the definite epiblast and which the hypoblast. The diameter of the whole blastodermic vesicle just before the rupture is about ·15 mm. Until the rupture the blastocyst is spherical. I cannot say how the rupture is effected. The zona radiata becomes exceedingly attenuated, and I am inclined to think that it becomes torn and broken in many places, and is not thrown off in large pieces.

Unless I have missed the intermediate stages, the dissolution of the zona radiata is accompanied by very marked changes in the embryo. It loses its spherical form, and is no longer tensely distended. The nature of its cells is

changed. Previously they contained many vacuoles, but now they are without vacuoles, and far more homogeneous in their minute structure. There are no longer any thinly drawn-out portions such as can be seen in fig. 19.

The inner mass has undergone a complete change. Instead of being a compact lenticular mass, it is now divided sharply into (*a*) a small group of cells, very often lying quite separated from the outer layer of cells, (*b*) a loose layer or network of cells lying apparently but very slightly attached to the inner surface of the group (*a*), and extending beyond its limits on to the "outer layer" (figs. 21 and 24). So slight is the connection between (*b*) and (*a*), that very often the two appear to be entirely separated (v. figs. 22 and 23, 25 and 26). It seems probable that the conditions of figs. 21 and 24 are normal, and that the separation of the two layers seen in figs. 22, 23, and 25, 26, may be due to the disturbances during preservation. It may therefore be accidental, but the specimens which show the separation were to all appearances quite perfect when they were examined after having been stained and cleared. In any case it seems to point to a very slight connection between those two layers. This is curious because in the immediately preceding stages (figs. 19, 20) there is hardly anything which indicates it. Fig. 19 shows a whiter core, *E.*, which may perhaps suggest such a separation.

I shall throughout this paper talk of these three separate cell areas as trophoblast, epiblast, and hypoblast, but in adopting Hubrecht's terminology—which, as he remarks, is extremely convenient—I do not adopt the conclusions to which he comes regarding the homology of the trophoblast as enunciated in his work, 'Die Phylogenese des Amnions und die Bedeutung des Trophoblastes.' In the dissertation following my description of the sheep's development (1a) I give reasons for regarding the trophoblast as really part of the hypoblast. So we may describe the embryo, shortly after the loss of the zona radiata, as a closed vesicle of very varied shape, whose wall is formed by a single layer of cells which are nearly cubical in shape. This layer is the trophoblast. At one

point on the inner surface a small group of cells is loosely attached to it, which is the epiblast, and lying along the inner surface of the epiblast is a loose network of irregular-shaped cells, the hypoblast.

In figs. 21 and 24 the hypoblast (*H.*) forms a continuous layer beneath the epiblast (*E.*). I do not find that the embryonic hypoblast in the pig has a later origin from the inner mass than that which the extra-embryonic hypoblast has in the way described by Hubrecht (17) for Tupaia.

The smallest specimen which I have, from which the zona radiata has gone, is one (figs. 22, 23) in size intermediate between fig. 19 and fig. 21. Its anatomy is similar to the latter specimen.

The increase in size between the stages of fig. 19 and fig. 22 or 23 is by no means great, which leads me to suppose that the latter specimens are only slightly advanced on the former.

The epiblast cells in the specimen represented by figs. 22, 23, number twelve or fourteen, and the hypoblast cells seven.

The actual "ages" of the specimens, figs. 18—24, are as follows:

Fig. 18 was	5 days.
„ 19 „	$4\frac{1}{2}$ „
„ 20 „	5 „
„ 21 „	7 „
Figs. 22 and 23 were . . .	$7\frac{1}{2}$ „
Fig. 24 was	7 „

We may say that, as a rule, the zona radiata is lost during the seventh day, and on the same day the separation of the inner mass into epiblast and hypoblast occurs. Even if this separation is not so sudden as I have supposed, there can be no doubt that the two inner cell layers are derived from the inner mass of figs. 19 and 20.

From this time—that is to say, during the eighth and until the closing hours of the ninth day—the shape of the blastodermic vesicle becomes extraordinarily irregular.

The degree to which the embryos become crumpled is rather interesting. No doubt the rupture of the zona radiata very greatly affects the osmotic properties of the walls as a whole. Up to this time it would seem that the accumulation of the fluid within the cavity of the blastodermic vesicle was in excess of the growth of the cell walls, and so caused a great tension to be placed upon them. Now it seems that the accumulation is unable to keep pace with the growth of the walls, and consequently the crumpling which they undergo is quite extraordinary. It reaches its maximum about the middle of the ninth day, after which the fluid gains upon the excess of wall growth, and so the vesicle becomes again distended, and by the eleventh day the creases have to a large extent become smoothed out.

During the eighth and ninth days a fluid is poured out into the cavity of the uterus from the cells lining the cavity of the uterus and the glands, which contains many corpuscles, which may often be found adhering to the walls of the vesicles. These corpuscles are clear and colourless, and do not readily take stains. The amount of this fluid is, however, slight in comparison to that poured out into the cavity of the sheep's uterus.

Eighth and Ninth Days—Stage D.

The changes that occur during the eighth and ninth days are not very remarkable. They are chiefly visible in the large increase in size of the whole blastodermic vesicle, in the spreading of the hypoblast layer, and consolidation of the epiblast.

The hypoblast at the moment of its first separation from the inner mass forms a kind of network, which lines at first the embryonic pole only of the blastocyst (fig. 21, *H.*). During the eighth and ninth days it extends its borders, and before the end of the eleventh day it practically lines the entire blastocyst. At this time the part lining the lower pole of the blastocyst is still a network, while at the embryonic pole it is

a continuous membrane (*H*, figs. 36—42). On the earlier parts of the eighth and ninth days it is, however, a network over all parts, and is still absent from the lower pole. Whether this extension is effected by an active advance of its edge, or is only an apparent advance due to the more active growth of the circum-embryonic part of the trophoblast, as I believe to be the case in the rabbit, I cannot say. During these two days the blastocyst increases in size from about ·2 mm. to about 1·5 mm. The chief interest undoubtedly lies in the fate of the epiblastic portion of the inner mass and the part of the trophoblast which at first overlies it and is quite distinct from it.

Fig. 27 may be taken to represent quite typically the condition of things at the end of the eighth day. The trophoblast cells are cubical with clearly marked boundaries, both in section and in surface view. There is no change observable for some days in the nature of these cells beyond the limits of the embryonic epiblast.

In that part of the trophoblast which covers the epiblastic portion of the inner mass the following changes occur concurrently with certain changes in the epiblast. I will describe the two together.

The diameter of the epiblast—that is to say, of the embryonal area—as seen in surface view increases from about ·04 mm. to ·25 mm. As it grows it becomes more firmly pressed against the trophoblast, which at this spot becomes distinctly thinner and more compressed, and its nuclei become much flattened (fig. 28).

The epiblast begins to show signs of more active growth, and the cells which at first were loosely arranged and equal in size, and showed no sign of differentiation, may now, in some cases, be seen to be arranged in an inner layer of small nuclei lying next to the cavity of the blastodermic vesicle, and a layer of very much larger nuclei between this and the trophoblast layer (fig. 29).

This inner layer of smaller nuclei, which also stain rather more deeply, is so very distinct in one of my specimens which I obtained from a sow killed at eight days, that I thought it

possible that this internal layer might split off and give rise to the embryonic hypoblast in the way Hubrecht describes for Tupaia.

As somewhat supporting this view I may draw attention to the fact that the hypoblast is extremely attenuated, and very reticular in the region of the embryonic area at this time. I cannot positively assert that the hypoblast in many of my specimens can for certain be seen lying against the region in question.

Also in the same sow there were two other specimens in which the epiblast seemed to be reduced to little more than a single layer of large cells (or rather nuclei). The small nuclei were, however, nowhere to be seen, and the hypoblast was not in any degree more evident.

As militating against this view is the fact that in such specimens as that drawn for fig. 28, the whole mass (*E.*) here is undoubtedly epiblast. This is proved by the presence of the thin membrane (*M.H.L.*), as will appear in the description of the older stages. In this case there is also a tendency to the formation of an inner layer of smaller nuclei. This is probably an older stage, its age being nine and a half days, but the hypoblast here is by no means a continuous layer, such as it is on the next day. A similar layer of small nuclei is also plainly visible long after the hypoblast has become a well-developed layer on the twelfth and thirteenth days. So, although there are certain features which suggest a further splitting off of the embryonic hypoblast comparable to what Hubrecht has described for Tupaia, yet, from a consideration of the evidence on both sides of the question, I am of the opinion that this phenomenon does not occur in the pig, but that the whole hypoblast is split off at the same time immediately after the rupture of the zona radiata; and that the condition shown in my figure is comparable to the condition shown in Hubrecht's (17) figures 57 to 59, or my figures of the sheep, and have nothing to do with the hypoblast formation.

The Rupture of the Rauber Layer. The Tenth and Eleventh Days.

I have now reached the stage of which we have recently had a description from Weysse (24). In this paper Weysse described a very remarkable overgrowth, which, starting from the edges which he assumed to be the posterior and lateral margin, grew forwards over the embryonal area.

According to his description both the embryonic epiblast and the trophoblast cells were concerned in this process.

It must be remembered, however, that Weysse did not regard this as the real trophoblast, but as the definite extra-embryonal area epiblast. He thought that he had evidence of a third layer, the true Rauber layer, outside this again. On this I shall have some more remarks to make.

The overgrowth, according to him, formed a complete covering, or, as he termed it, a bridge, over the hinder part of the embryonic epiblast. Ultimately the bridge was said to fuse with the underlying embryonic epiblast, and to give rise to the embryonic area.

Weysse compares this overgrowth to the apparent growth of the epiblast over the neural plate from the hinder part of the embryo of Amphioxus. To this conclusion he seems to have been brought chiefly by finding in certain sections a very narrow canal leading from the exterior under his bridge into the space between the epiblast and the hypoblast, or rather into the space between the epiblast and a non-cellular membrane, which is apparently the homologue of that which Schäfer (24) found in the cat, and called the membrana limitans hypoblastica. This canal Weysse considered to be equivalent to the neurenteric canal. The important difference that the supposed canal leads into the space noticed above, and not into the archenteron, does not seem to have influenced his opinion.

Duval (13) and Hubrecht (17) both comment on Weysse's discovery, and each interprets the facts differently.

Weysse will probably stand alone in instituting a comparison with Amphioxus, but as regards the actual facts I am able to support him to a certain extent.

It is true that what I have found is in detail extremely unlike the majority of Weysse's specimens. Yet my own specimens differ so greatly one from another that it is probable that almost any form may be assumed by the cell masses in question.

The course of events would seem to be as follows. During the ninth day the epiblastic knob is a compact mass of cells, nearly as thick as it is broad. The inner surface is characterised by the presence of many small nuclei, while towards the surface the nuclei are larger (fig. 29). (Compare Hubrecht [17], figs. 57, 59.) These features probably indicate a more rapid growth of the inner parts of the epiblastic knob. The result is that the knob acquires a convex surface on its inner face (figs. 32, 33). An exaggeration of this process might no doubt lead to a doubling up as in Tupaia or Talpa. Something of the kind does occur in the pig, but I do not think it is so evident a phenomenon as it is in those animals. Figs. 30—33 are drawings of four sections through one specimen. There are thirteen sections in all; fig. 30 is the third. In this there is a distinct doubling up, and the trophoblast is broken. In fig. 31, which is the fifth section, the epiblastic knob is curved and bent away from the overlying trophoblast, which, however, is not broken. Figs. 32 and 33 are the ninth and tenth sections, and are therefore near the other end of the series. These show that this part of the epiblastic knob is not doubled up, but forms a solid plate, which is not actually fused with the trophoblast above it. The trophoblast overlying the epiblast is broken in several places.

Fig. 35, which represents a section of another specimen from the same uterus, shows a very slight curvature of the epiblastic plate. The overlying trophoblast is broken, and pieces of it (*T.R.*) are to be seen lying upon the epiblast. In another specimen (fig. 34) there is no curvature at all. The

trophoblast is lying over the surface of the epiblast, and is broken. The age of these three specimens was nine days eighteen hours.

I have killed several animals in attempting to acquire a more perfect series of this stage, but without success. There is no possibility of obtaining with certainty embryos of a given age.

If we take the specimens of figs. 30—33 as representing the ordinary course of development, we see there is evidence of a doubling up of, at any rate, a certain part of the epiblastic plate; but I do not think that it ever amounts to such a complete folding as it does in Tupaia or Talpa. In any case it is clear that considerable pieces of the ruptured trophoblast may be left upon and more or less attached to the epiblastic knob (v. figs. 31—35), which give rise to the conditions described below, and which, I think, account for the appearances described by Weysse.

There is no regularity in the shapes of the pieces thus separated, some more and some less completely, from each other, and from the edge of the trophoblast adjoining the embryonic area.

In a certain specimen (fig. 10), the age of which was ten days two hours, the trophoblast at one spot is seen in a section to be overlying the embryonic epiblast (fig. 37, *T.R.*). The trophoblast stains more darkly than the embryonic epiblast. On following this overlapping edge through the series of sections, it is found to run towards the centre of the embryonal area as a narrow strip quite free from and rising away from it as it advances towards the centre. At the centre it expands into a fan-shaped plate, from the floor of which a pillar runs to the embryonal area below, and is clearly fused with it at this spot (fig. 36, *T.R.*).

It is evident that here is a structure even more deserving of the name of "bridge" than the lateral expansions of Weysse, which may be likened rather to balconies.

Over another part of the same embryonal area are a few cells which lie more loosely upon the epiblast.

Another specimen (Pl. 25, fig. 12, A) shows a large isolated fragment not actually fused nor continuous with the edge of the trophoblast. Fig. 39 represents a section taken through this specimen. At no point is there any complete fusion between the fragment and the underlying epiblast, as in the last case (fig. 36).

Another specimen has a long fragment, clearly a continuation of the trophoblast, running across the surface of the embryonal area (as in the case of figs. 33, 37), but in this instance it ends freely, and at no point is it attached to the embryonal area below. In the same section (fig. 39) a single cell (*T.R.*) may be seen, which from its appearance, colour, position, and prominence above the others seems undoubtedly to owe its origin to the trophoblast layer. Such cells are not rare but by no means numerous. The number of them compared with that of the cells of undoubted inner mass origin is, however, quite insignificant.

Now it seems to me that such structures as the above-mentioned irregular masses, and the appearances described by Weysse, are to be explained in this way.

After the trophoblast has ruptured during the tenth day, in the accomplishment of which process I assume that the local tension produced by the growth and lateral expansion of the epiblastic mass plays an important part, the ragged edges of the ruptured trophoblast and the isolated cells of the same layer are by no means forced to die or to degenerate immediately. On the contrary, they grow and multiply, and grow into almost any shape, and form balconies, bridges, &c. After some hours they become, for the most part, rubbed off or swept away by the action of the uterine ciliated cells, and by contact with the walls of the uterus. Some few cells which have become more closely attached, like *T.R.* in fig. 39, or those at the point of fusion (*T.R.*) in fig. 36, remain for a longer time as part of the embryonal area. Whether, however, any of them become permanently part of it I am quite unable to say. I cannot trace any further than the condition of the single cell (*T.R'.*) in fig. 38 or 39.

Some time ago (1) I supported Balfour's (3) and Heape's (14) views upon the fate of Rauber's cells in the rabbit.

It must be remembered that the conditions in the pig are very different from those of the rabbit. In the pig the epiblastic disc is a compact mass of small area, and has no investing membrane. In the rabbit the epiblastic disc is a loose layer of cells, stretching over a large area, and outside the trophoblast layer is a firm investing membrane, the albumen layer, against which all the cellular layers are pressed by the force of internal fluid. So whereas in the pig there is nothing to prevent the broken fragments from being brushed away or otherwise lost, except such original attachment as they may have to the underlying epiblast, in the rabbit these fragments are kept tightly pressed down to the surface of the epiblast, and may even be forced into the interstices between the loosely arranged cells as shown in my figures of the rabbit (1) (Pl. 16, figs. 30, 32, 33).

Although there is very good evidence (Balfour [3], Heape [14], Lieberkühn [21], &c.) that in certain mammals a fusion occurs, it is equally clear that in other cases (Verspertilio murinus, Duval [13], Tupaia, Hubrecht [17], and in the pig) most if not all of these cells are lost. It is only in those cases in which there is, at the time in question, a well-developed albuminous or other investment that we find evidence of a fusion between these layers.

So perhaps we may say that there is no inherent tendency for the "Rauber" cells to fuse with the formative epiblastic disc (except, of course, round its margin), but that in certain cases (e. g. the rabbit, mole, and perhaps Sorex [Hubrecht, 16, 17]) in which extraneous conditions in the form of zona radiata and albumen layer occur—and in the case of the rabbit, the existence of interstices between the epiblast cells—many of the cells are included with and may form part of the permanent epiblast.

It may be asked, Is the rejection of the cells in the pig, Tupaia, and bat, due to the loss of continuity caused by the physical and mechanical conditions of the earlier development,

or is there really a more profound difference in the nature of the cells which prevents trophoblast and epiblast cells from mingling? If (as I have argued in a discussion on the development of the sheep) the trophoblast is really to be regarded as hypoblast, and if there is in the pig so real a difference between the two layers, and dating from so early a period as appears to be the case in the sheep, one may well consider that the trophoblast cells overlying the epiblast are incapable of permanent incorporation as epiblast cells; and that fusions which are brought about by the presence of investing layers as described in the cases of the rabbit, mole, and Sorex are secondary phenomena, or may even be only apparent, and are not permanent fusions.

Weysse took a quite different view of the homology of the layer he termed extra-germinal outer layer. The innermost layer of cells (*H.*, fig. 36, &c.) he calls entoderm. The outer layer (*T. R.*) he did not regard as the original outer wall of the blastodermic vesicle, but called it the extra-germinal ectoderm, and considered that it was continuous at all times with the ectoderm of the germinal area (*E.*).

Outside this he believed that a third layer, the true Rauber layer, had existed during an earlier stage, and he describes the remains of it as appearing at intervals over the surface of the "extra-germinal ectoderm."

It is clear that this was a rather rash supposition, as the youngest embryo which he possessed was ten days old, —that is to say, the same age as those of which my figs. 10, 36—39, are drawings. That certain cells existed outside the "extra-germinal ectoderm" he was quite convinced, and describes and gives drawings of them. Hubrecht (17) considered that these cells must be cells derived from the uterine walls.

Weysse is perfectly right in saying that at the time of the process of "bridge" formation on the embryonic area, there are at certain places cells outside the outer wall of the vesicle. For instance, in the specimen (fig. 10) there are no less than forty-three perfectly distinct cells with nuclei, scattered at

intervals, some singly, others in groups of two, three, or, in one case, as many as seven cells, upon the lower or ab-embryonic pole.

Figs. 40 and 41 are drawings of some of these seen in section. The trophoblast layer is thick (*T. R.*), the hypoblast layer (*H.*) is very uncertain, and is probably a network, and on the outer surface of the trophoblast a few isolated cells (*T. R.''*) form conspicuous objects. These cells are in every way exactly comparable in their colour, texture, and size of nucleus with the cells of the trophoblast layer. They are never present before this time; they disappear very quickly afterwards.

I am quite sure they are not derived from the walls of the uterus. They are not leucocytes, nor are they corpuscles of the secretion of the uterine glands. Such are, indeed, present at this and an earlier period, but cannot be mistaken for embryonic cells. The epithelium of the uterus is still perfectly sound, and shows no sign of degeneration until after the fourteenth day. It becomes detached about the seventeenth day.

I have very little doubt of the origin of these scattered cells. I think they are the detached fragments of the trophoblast from the embryonal area.

The uterus at this time contains ciliated cells, whose action, no doubt, helps the passage of the blastodermic vesicles down the uterus. It seems very likely that the broken fragments are swept along by the action of the cilia, and adhere for some time to the walls of the blastodermic vesicle until ultimately swept or rubbed off altogether.

Fig. 42 represents a section taken through a larger piece, which was only a short distance from the embryonal area of another specimen. The clear line which invariably marks them off from the walls of the blastocyst makes it unreasonable to suppose that they have been budded off in situ.

I think there can be no doubt that the cells which I find and have just described are the same as those described by Weysse.

At the same time his description can hardly be said to agree with mine. He speaks of the cells being indistinct and flattened, the cytoplasm "scarcely stainable" at all. In my case they are mostly sharp and rounded, and they stain with carmalum and hæmatoxylin as deeply as any others in the embryo. A difference in methods of preservation and staining may account for this.

However this may be, there can be no question of the presence of a third complete layer outside the layer which he calls extra-germinal ectoderm, and I call trophoblast; such a layer never exists.

Before the completion of the eleventh day all trace of the loose fragments of the torn edge of the outer layer has disappeared from the embryonal area and elsewhere.

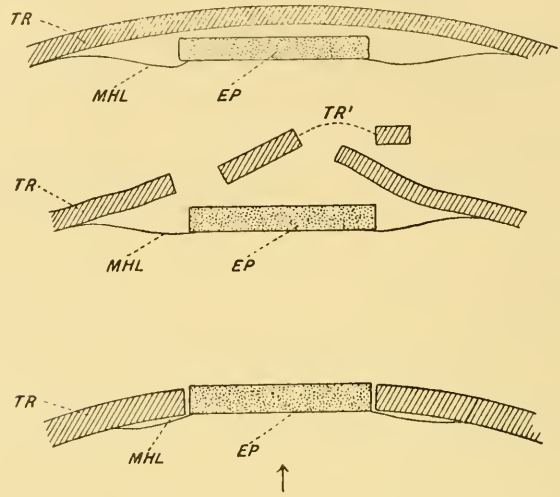
The formative epiblast is now continuous all round its margin with the trophoblast, and forms a disc-like plate, slightly thicker near its centre than at its edge.

The hypoblast immediately beneath the epiblast is thicker, and contains more nuclei than elsewhere. Its characters are shown in figs. 36—42.

There is one structure which is very puzzling. Weysse has noticed this. This is the membrane shown in figs. 30—33 and 36—39, *M. H. L.* I have not been able to determine its origin. Weysse (25) gives a correct description of it on pages 297 and 298. It certainly has in my sections the appearance of being part of the inner margin of the cells of the trophoblast and the epiblast. It is structureless, but whether secreted by the epiblast or trophoblast and the hypoblast I do not know. Schäfer (24) considers it of hypoblastic origin in the cat. Its probable function would seem to be in some way connected with the rupture of the outer layer, and the subsequent fusion of its edges with those of the epiblastic disc.

A time may very well occur when there would be weakness in the general wall of the blastocyst during this process. The membrana hypoblastica limitans seems to serve to hold the disc in its place while this fusion is effected. The membrane is tightly fixed to the inner surface of the epiblastic disc, but

more loosely to that of the trophoblast. The fluid inside the blastocyst would force the disc up into its place.



The changes of the blastodermic vesicle and its great increase in length, which occur during the twelve to fourteen days, have been related by Von Baer, Coste, and Bischoff, and more recently, together with a detailed account of the changes in the embryonal area, by Keibel. A brief account from me will suffice in confirmation of their discoveries.

From the moment of the rupture of the zona pellucida upon the sixth day of development, there has been a constant tendency for the vesicle to become elongated. By the twelfth day it is 10 or 12 mm. long and only 3 mm. broad. It now grows out with exceedingly great rapidity, and by the thirteenth or fourteenth day each embryo may measure as much as a foot in length (30 cm.).

The hypoblastic vesicle extends to nearly the same length as the outer wall. Hence the whole blastocyst can be said to be didermic.

By the seventeenth and eighteenth days the vesicles have attained to about their greatest length, and completely fill the cavity of the two horns of the uterus. The length of each

vesicle depends, it would seem, directly upon the length of the uterus, number of embryos, and the position of the embryos in the uterus.

The walls of the uterus are very soft, and the foldings of its mucous membrane very complicated, and obliterate the lumen of the tube almost completely.

The several blastodermic vesicles do not overlap each other. The highest up on each side is always longer than the others in the same horn of the uterus. The longer ones are thinner than the shorter ones. The vesicle is not folded back upon itself, but crumpled like the sides of wind bellows. In one sow killed upon the eighteenth day, the one which occupied the highest position of one side, measured when removed and unfolded forty-two inches. The length of the piece of uterus which contained it was twelve inches. The two at the lower ends of the horns of the uterus extend into the vagina, and in one case were fused with each other. I have never found any fusion between any of the vesicles within the uterus, although their ends were in contact.

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DESCRIPTION OF PLATES 25—28,

Illustrating Mr. Assheton's paper on "The Development of the Pig during the First Ten Days."

Complete List of Reference Letters.

C. Bl. Cavity of the blastocyst. *E.* Epiblast. *H.* Hypoblast. *I. M.* Inner mass. *M. H. L.* Membrana hypoblastica limitans. *S. P.* Spermatozoa. *T.* Trophoblast. *T. R.* Trophoblast cells which overlie the embryonic knob. *Z.* Zona radiata.

PLATE 25.

Fig. 1 (Sus 25, No. 1).—Embryo of the pig in the two-segment stage. Found in the uterus. Killed with Flemming's solution. The oily globules scattered throughout the inner part of each segment have been blackened by the osmic acid. Age 6 days. × 380.

Fig. 2.—Same embryo as the former, but seen edgewise. × 380.

Fig. 3 (Sus 12, No. 1).—Embryo of pig in the three-segment stage. Found in the upper part of the uterus. Examined and drawn while fresh. Age 4 days. × 380.

Fig. 4 (Sus 1, No. 1).—Embryo of pig in the four-segment stage. Found in the Fallopian tube. Examined and drawn while fresh. Age three days. × 380.

Fig. 5 (Sus 12, No. 4).—Embryo of pig in the five-segment stage. Found in the uppermost part of the uterus. Examined and drawn while fresh. Age 4 days. × 380.

Fig. 6.—Embryo of pig. Found in the uterus. Drawn after the specimen had been treated with chromic acid. Age 6 days. × 380.

Fig. 7 (Sus 25, No. 6).—Embryo of pig in morula stage. Found in uterus. Drawn after the specimen had been treated with chromic acid. Age 6 days. × 380.

Fig. 8 (Sus 25, No. 7).—Embryo of pig. Morula stage probably not quite attained. Age 6 days. × 380.

Fig. 9 (Sus 26, No. 13).—Embryo of pig. Advanced blastodermic vesicle stage. Age 5 days. × 380.

Fig. 10 (Sus 28, No. 2).—Embryo of pig. The blastodermic vesicle is now a large thin-walled sac of irregular shape. This is double-layered; and the embryonal area is a circular or sometimes slightly oval patch upon one side of it. Age 10 days 2½ hours. × 20.

Fig. 11 (Sus 33, No. 1).—Embryo of pig. The blastodermic vesicle is thrown into many folds. Age 8 days. × 20.

Fig. 12 (Sus 28, Nos. 5, 3).—The embryonal areas of two specimens showing the pieces of trophoblast, *T. R.*, lying upon the disc of true epiblast. The specimen from which the smaller embryonal area was taken measured about 3 mm. in length. The embryonal area itself measured ·215 × ·190 mm. The specimen from which the larger figure (B) was taken measured 3·5 mm. by 2·94 mm., and the embryonal area was about ·22 mm. in diameter. The two figures were drawn with the camera lucida, but under different magnifying power.

PLATE 26.

All the figures on this plate are camera drawings of sections of embryos of the pig. The magnification in every case is 380 times.

Fig. 13 (Sus 23, No. 3).—Age 6 days 3 hours.

Fig. 14 (Sus 23, No. 1).—Age 6 days 3 hours.

Fig. 15 (Sus 25, No. 10).—Age 6 days.

Fig. 16 (Sus 29, No. 1).—Age 5 days.

Fig. 17 (Sus 19, No. 2).—Age 5 days 3 hours.

Fig. 18 (Sus 26, No. 2).—Age 5 days.

Fig. 19 (Sus 31, No. 8).—Age 4½ days.

Fig. 20 (Sus 27, No. 1).—Age 5 days.

In all the foregoing the zona radiata is present, but after this point it has ruptured and disappeared.

Fig. 21 (Sus 32, No. 4).—Age 7 days.

Fig. 22 (Sus 14, No. 6).—Age 7½ days.

Fig. 23 (Sus 14, No. 6).—Age 7½ days.

Fig. 24 (Sus 33, No. 6).—Age 7 days.

PLATE 27.

All the figures on this plate are camera drawings of sections of embryos, or of the embryonal area of embryos of the pig. The magnification in each case is 380 times.

Fig. 25 (Sus 32, No. 1).—Age 7 days.

Fig. 26 (Sus 32, No. 1).—Age 7 days. The hypoblast in this specimen, as in several others, is removed from the epiblastic knob.

Fig. 27 (Sus 14, No. 2).—Age 7½ days.

Fig. 28 (Sus 14, No. 1).—Age 7½ days.

Fig. 29 (Sus 33, No. 3).—Age 8 days.

Figs. 30, 31, 32, 33 (Sus 18, No. 8).—Age 9½ days. These are four sections through one specimen, namely, the 3rd, 5th, 9th, and 10th of a series of thirteen sections.

Fig. 34 (Sus 18, No. 10).—Age 9½ days.

Fig. 35 (Sus 18, No. 9).—Age 9½ days. Diameter of embryonal area 1 mm.

PLATE 28.

All the figures on this plate are camera drawings of sections through portions of the walls of the blastocyst of the pig. All are magnified 380 times.

Fig. 36 (Sus 28, No. 2).—The same embryo as Fig. 10. Age 10 days 2½ hours. Section through embryonal area. Whole embryo measured 3·57 × 2·31 mm.

Fig. 37 (Sus 28, No. 2).—Another section through the same embryonal area.

Fig. 38 (Sus 28, No. 4).—Age 10 days 2½ hours. Section through the embryonal area. Blastocyst measured 4·06 × 2·60 mm.

Fig. 39 (Sus 28, No. 5).—Age 10 days 2½ hours. Section through embryonal area.

Fig. 40 (Sus 28, No. 2).—Age 10 days 2½ hours. Section through the abembryonic pole.

Fig. 41 (Sus 28, No. 2).—Age 10 days 2½ hours. Another section similarly located to above (Fig. 40).

Fig. 42 (Sus 28, No. 7).—Age 10 days 2½ hours. Section through the wall of the blastocyst a short distance from the embryonal area.

The Structure of the Mammalian Gastric Glands.

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With Plate 29.

In a preliminary notice, published in the 'Proceedings of the Canadian Institute,' vol. i, Part I, I gave a brief account of some new points in the structure of the gastric glands of mammals, which appear to afford a solution of the question of the morphological significance of the pyloric glands.

The view of Heidenhain,[1] Ebstein,[2] and Grützner,[3] that the pyloric glands are simply peptic glands without border cells, and that the pyloric gland cells are identical with the chief or central cells of the fundus glands, is no longer tenable.

Heidenhain[4] himself noted that in the fresh condition the pyloric gland-cells are finely granular, whilst the chief cells of the fundus glands are coarsely granular.

Langley and Sewall[5] observed the same feature, but considered that the undoubted presence in the pyloric glands of pepsin in small amount was sufficient evidence of their pepsinogenic character. They concluded, therefore, not that the pyloric gland-cells are different from the chief cells of the

[1] 'Arch. f. mik. Anat.,' Bd. vi.
[2] Ibid.
[3] 'Pflüger's Archiv,' Bde. vi and viii.
[4] Herrmann's 'Handbuch d. Phys.,' Bd. v.
[5] 'Journal of Physiol.,' vol. ii.

fundus glands, but that "pepsin formation is not necessarily connected with the formation of coarse granules;" and further, that "the chief cells of the fundus are a highly differentiated form of the pyloric gland-cells."

The introduction of new methods has made us acquainted with new points of difference between the two kinds of cells.

Schiefferdecker[1] found that the pyloric gland cells of the pig and man stained intensely in dahlia, a property which is not shared by the chief cells of the fundus glands, or by the glands of the œsophagus and mouth. He found, moreover, that the pyloric glands of the cat and dog did not stain in dahlia, a fact which is of importance as indicating a difference between the pyloric glands of different mammals, and further, that these glands differed anatomically from those of the pig and man.

Bonnet[2] also has studied, by means of aniline dyes, the staining reactions of the various cells of the stomach. He finds that the pyloric gland cells stain differently from the surface epithelium and from the chief cells in methyl violet, Congo red, and acid fuchsin.

R. Krause[3] has observed that the cells of the pyloric glands, in common with many mucous cells, stain metachromatically in thionin.

On the other hand, the results of research by purely physiological methods seem to point to a functional relationship between the pyloric gland cells and the chief cells. Of the host of observers who have examined the pyloric mucous membrane of the dog for pepsin, few have failed to find it, and its presence in the secretion of the pylorus has been shown by Klemensiewicz[4] and Heidenhain,[5] who established pyloric fistulæ, and found abundant evidence of the presence of a

[1] 'Nachrichten d. Göttingen Gesellsch.,' 1884, p. 303.

[2] 'Berichte d. Oberhessisch. Gesellsch.,' xxix, 1893.

[3] R. Krause, "Zur Histologie der Speicheldrusen," 'Arch. f. mik. Anat.,' Bd. xlv.

[4] 'Sitzungsber. d. k. Akad. d. Wissensch.,' Bd. lxxi.

[5] 'Pflüger's Archiv,' Bd. xviii.

proteolytic ferment, in Heidenhain's case even after the lapse of five months.

In his recent exhaustive compilation of the literature of this subject, Oppel [1] attempts to reconcile the conflicting results of these two lines of research. He concludes that the pyloric gland cells are cells sui generis, differing both from the surface epithelium and from the chief cells, and engaged in the secretion of pepsin holding gastric juice.

The present state of our knowledge does not permit of any comparison between the pyloric glands and the other glands of the stomach, nor is it possible to compare them with any of the gastric glands of lower Vertebrates.

Further, the researches of Edelmann[2] have shown that there exists in the cardiac region of the stomach of many mammals a peculiar kind of gland, called by him the cardiac gland, differing both from the fundus glands and the pyloric glands, and concerning which we are even more in the dark.

The application of new methods to the study of the gastric glands has convinced me that the pyloric and cardiac glands of various animals are closely allied to one another, and that the various kinds of cells one meets are but the results of differentiation along divergent lines from a single primitive type. The pyloric gland cells, furthermore, are in most mammals closely allied to, and in the cat, dog, and rabbit identical with, certain cells in the neck of the fundus gland, which, up to the present, have been regarded as ordinary chief cells.

A convenient starting-point for the descriptions which follow is afforded by the gastric glands of the frog.

A fundus gland of this animal may be divided into three portions; the duct or stomach pit, lined by mucus-secreting cylindrical cells similar to those of the surface; the neck, occupied by very large vesicular-looking cells, which, although different from the surface cells, are also regarded as mucous cells, and the body of the gland, occupied by granular proto-

[1] 'Lehrbuch der vergleichenden mik. Anat.,' 1896.

[2] 'Deutsch. Zeitschr. f. Thiermedizin,' Bd. xv.

plasmic cells, which secrete both acid and pepsin. In sections stained in the muchæmatein solution of Mayer, the mucigenous border of the cylindrical cells of the surface and the whole of the large vesicular neck cells stain intensely, indicating beyond doubt that the latter are mucin-secreting cells. Langley [1] has shown that during digestion these cells exhibit the usual secretion changes.

The pyloric glands of the frog are made up of only two kinds of cells, those of the body and those of the duct. The former bear so strong a resemblance to the large mucus-secreting neck cells of the fundus glands, that one cannot avoid the conclusion, with Partsch, that they are of the same nature. They, too, stain intensely in muchæmatein.

It is generally admitted that the two main kinds of cell of the mammalian fundus gland are the result of the differentiation of the one kind found in the body of the gland of lower Vertebrates. The view advanced by Oppel,[2] that the mucous neck cells of batrachian and reptilian glands correspond to the chief cells of the mammalian gland has been, however, the only attempt to find in the glands of mammals a morphological equivalent for these peculiar cells.

My studies have enabled me to establish what has hitherto been unsuspected, namely, that there exists in the fundus glands of many mammals cells which are morphologically and physiologically equivalent to the mucous neck cells of the batrachian gland, and that the same relationship exists between these and the pyloric gland-cells as obtains in the Anura.

These cells in the neck of the gland have received little attention from histologists owing to their small size, and to their being overshadowed by the large and numerous border cells of this region of the gland; they are generally regarded as small pepsin-secreting chief cells.

The fact that they are different from the cells lower down in the gland has not, however, entirely escaped notice. Bizzozero [3] noticed in the dog that the chief cells of the neck

[1] 'Phil. Trans. Roy. Soc.,' vol. clxxii.

[2] 'Anat. Anzeig.,' Bd. xi.

[3] 'Arch. f. mik. Anat.,' Bd. xlii.

of the gland have a more transparent protoplasm than those of the body of the gland, and a nucleus compressed against the base of the cell. Similar features have also been noted for the glands of the badger and hedgehog by Oppel,[1] who calls attention to the fact that in the hedgehog the neck cells contain less protoplasm than the chief cells of the bottom of the gland, and that this stains less readily with hæmatoxylin. Bizzozero suggests that these cells may be a transitional type between the cells of the gland duct and the fully developed chief cells of the deeper portions.

My attention was first attracted to these cells in the glands of the greater curvature of the rabbit, in sections of which, stained in hæmatoxylin, the chief cells appear as comparatively large cubical cells with deeply staining protoplasm, whilst the neck cells are small pyramidal structures which stain but feebly. The question naturally arose whether this difference was due to a different functional condition of the cells, or to the cells being essentially different, and I turned for a solution of the question to a study of the distribution of zymogen granules in the gland.

The stomach of the rabbit did not lend itself very readily to this investigation on account of the comparatively short neck that the glands of this animal possess, and because I was not then able to fix the granules in any but the lowest portions of the glands. I therefore resorted to a study of the glands of the cat and dog, in which the neck region is relatively long. I subsequently discovered that it was possible to fix perfectly the granules in all parts of the glands of many mammals by means of a modification of Foa's blood-fixing fluid, prepared by mixing equal parts of a saturated solution of mercuric chloride in 95 per cent. spirit, and a two to four per cent. aqueous solution of potassium bichromate. I was also fortunate enough to discover a means of staining in a distinctive fashion with indulin these peculiar neck cells, and the use of these methods has enabled me to extend the facts discovered in the cat and dog to the rabbit and other mammals.

[1] 'Lehrbuch d. vergleich. mik. Anat.,' 1896.

My methods are briefly as follows:—Small pieces of the gastric mucosa are snipped off with scissors, and dropped into the sublimate bichromate mixture, where they remain from one half to two hours, according to their thickness. They are then transferred to 70 per cent. alcohol, in which they remain twenty-four hours, or until all the free bichromate is extracted, then to 95 per cent. alcohol. Sections of 3—5 micra are cut after embedding in paraffin by the oil of bergamot method, fastened to the slide, and stained. The results obtained by this method of fixation were controlled by the study of pieces fixed in alcohol, in aqueous bichloride solutions, and in the osmic acid fixing fluids of Hermann and vom Rath. The staining methods employed will be indicated in connection with the special descriptions.

I have chosen for special description in the present memoir the gastric glands of the cat and dog, because these present the most highly differentiated form of the gastric gland, and because the relationship obtaining between the pyloric and fundus glands corresponds so closely to that found in the highly specialised Anura.

A. The Gastric Glands of the Cat.

The fundus glands are elongated tubular structures, opening into shallow depressions of the surface lined by mucus-secreting cylindrical cells, and called the stomach pits or gland ducts. The glands consist of two kinds of epithelial cells, the central or chief cells and the parietal or border cells, and are divisible into two portions, a narrower superficial part called the gland neck, in which the border cells are in excess, and a deeper, wider portion called the body of the gland, in which the chief cells predominate.

That the difference between the body and neck of the gland is of a more profound nature than a mere difference in relative size, or in the relative numbers of the constituent cells, may be readily determined by the study of the fresh mucous membrane in an indifferent fluid. If, in a freshly killed cat,

a piece of mucous membrane be snipped off, and as thin a section as possible prepared with a razor moistened with aqueous humour, and mounted under a cover in a drop of the same fluid, it may be observed, under a low power and small diaphragm, that the mucous membrane is divided into a superficial transparent zone and a deeper more opaque zone (fig. 1).

Under a high power (Zeiss, apo. 2 mm. and 8 oc.) the opacity of the deeper zone is seen to be due to the presence of numerous large, coarse granules of zymogen. These granules are entirely absent from the superficial zone, although many minute fat globules may be seen in both kinds of cells.

The superficial granule-free zone includes not only the pits, but a large portion of the glands themselves; and it may be inferred that the chief cells of the neck of the gland do not contain zymogen in the form of granules.

This peculiarity of the distribution of zymogen granules in the mucosa did not escape the notice of Langley and Sewall,[1] as their figure (14) of the neck of the gland of the cat clearly indicates. They, however, attributed the absence of granules from the neck of the gland to the comparatively infrequent occurrence of chief cells here.

It will be seen from what follows that the absence of the granules from the gland neck is rather to be ascribed to the fact that the chief cells are different from those of the body of the gland, and are engaged in the secretion of a quite different product. In order that the differences between the two kinds of chief cells may be clearly defined, it will be necessary to describe accurately the ordinary chief cell of the body of the gland.

In sections from the greater curvature of the stomach of a cat that has fasted one to three days, the chief cells of the body of the gland present the appearance indicated in fig. 2. They are pyramidal or wedge-shaped, and so appear cubical or triangular, according to the direction in which they are cut. The contents of the cell exhibit an exceedingly regular network

[1] 'Journal of Physiology,' vol. ii.

of large meshes, and in thick sections present a vacuolated appearance. The protoplasmic strands which compose this network are very coarse, and stain readily in hæmatoxylin, a feature which is particularly noticeable at the thickened nodal points.

In sections stained in gentian violet or safranin the appearance depends on the degree of success attained in fixing the zymogen granules. If they are well preserved they stain intensely in these dyes, and the cell is then seen to be filled with large deeply stained granules, between which may be seen running the trabeculæ of the protoplasmic framework. If the fixation is less successful the granules are found to have swollen up, so that the whole cell stains diffusely—affording, however, unmistakable evidence of the presence of zymogen.

The relation between the granules and the protoplasm may be clearly seen in sections stained in the Biondi three-colour mixture, in which the granules stain a pale blue and the protoplasm red. In sections thus stained each granule is found to correspond to a mesh of the protoplasmic network. This is, then, not a true network, but simply the optical expression of the fact that the zymogen granules occupy small cavities in the cell, which are separated from one another by thin films of the protoplasm of the cell. In hardened cells there is usually a clear space surrounding each zymogen granule, but it is to be inferred that in the living resting cell the granule completely fills the cavity in the protoplasm which it occupies.

In the base of the cell, even after a prolonged fast, there may usually be seen a small quantity of protoplasm which, on account of the peculiar properties it presents, seems to merit a more extended description than is usually accorded it.

Langley [1] observed that the protoplasmic zone of the active cell contains a substance which stains more readily with osmic acid than ordinary protoplasm, and which he inferred to be one of the earlier steps or mesostates in the formation of the

[1] 'Phil. Trans. Roy. Soc.,' vol. clxxii.

zymogen. Grützner[1] also noted this peculiar staining with osmic acid.

Some information as to the nature of the substance in question is afforded by the researches of Macallum,[2] who describes the difference in staining properties exhibited by the resting and exhausting pancreatic cell, and explains the difference as follows:—"The chromatin of the nucleus gives rise to a substance which we may call prozymogen, sometimes dissolved in the nuclear substance, sometimes collected in masses (plasmosomata), and finally diffused into the cell protoplasm, uniting with a constituent of the latter as zymogen." In a subsequent research into the distribution of assimilated iron compounds in animal and vegetable cells,[3] he found, in the outer protoplasmic zone of the pancreatic and many other gland cells, a firm organic compound of iron, which he regards as the prozymogen of his earlier investigation. A similar view is taken by Mouret of the nature of the fibrillar chromophilous element in the outer zone of the pancreatic cell, and the term "prézymogen" is applied by this observer to the substance in question.

I have made a series of experimental studies of the gastric and many other glands, with a view of determining the relation of this substance to the formation of zymogen granules, and also its source in the cell. The results of these studies will be contributed in a separate paper, and I will content myself at present with a recital of the facts that are of importance from the stand-point of determining the morphological relationships of the cells.

The prozymogen is co-extensive with the protoplasm of the cell, and, even in cells which possess only a small outer zone, usually presents quite definite staining and structural characters, which enable one to decide with ease as to its presence. The most favorable material for studying its characters is offered

[1] 'Pflüger's Archiv,' Bd. xx.

[2] 'Trans. Canadian Institute,' vol. i, part ii, 1891.

[3] 'Quart. Journ. Micr. Science,' vol. xxxviii, part ii, new series.

[4] 'Journal de l'Anat. et de la Physiol.,' année xxxi, 1895.

by the glands of animals that have been in active digestion for ten to twelve hours, and therefore exhibit a well-marked outer protoplasmic zone (fig. 3). In sections from such glands, stained in freshly prepared Mayer's hæmalum, a pure nuclear stain is obtained in all the cells, with the exception of the chief cells of the body of the gland, the outer protoplasmic zone of which also stains blue. A more vigorous stain of this portion of the cell may be obtained by the use of Ehrlich's acid hæmatoxylin, diluted for use with a 5 per cent. solution of ammonia alum in water. The sections, after staining in this fluid, are washed in tap water, then dehydrated and mounted by the usual methods. Staining in very dilute solutions of methylene blue, gentian violet, or safranin, followed by rapid dehydration in absolute alcohol, and clearing in benzole, also gives a very serviceable stain of the outer zone of the cell. In sections so stained the outer zone of the cell exhibits an obscurely fibrillated structure, which reminds one strongly at first of the striated epithelial cells in the intralobular ducts of the salivary glands (fig. 3). On closer examination it may be seen that the fibrillation in the outer zone of the chief cell is not so regular, nor are the fibrillæ so distinct from one another as in the salivary ducts.

The strong affinity for nuclear stains exhibited by the outer protoplasmic zone of the chief cell is due to the presence in it of a chromatin or firm organic compound of iron, the prozymogen of Macallum, as may be shown by the reactions for the presence of iron. If a section of a piece of mucous membrane that has been hardened in absolute alcohol be treated with ammonium hydrosulphide, or an acid solution of potassium ferrocyanide, no reaction occurs, indicating that no inorganic iron is present in the cell. If, however, the sections be first treated with a solution of pure sulphuric acid in alcohol, containing four volumes per cent. of the former, for a period of three to six hours at a temperature of 37° C., and then, after thorough washing in fresh alcohol, transferred to ammonium hydrosulphide or acid ferrocyanide solution, a strong reaction for iron is obtained, not only in the chromatin of the nucleus,

but also in the outer protoplasmic zone of the chief cells of the body of the gland. The reaction in the protoplasm is about equal in intensity to that obtained in the oxyphile nucleolus. The best method of demonstrating the presence of prozymogen is by means of the hæmatoxylin iron reaction, recently announced by Macallum.[1] In this method, after unmasking the iron by means of sulphuric acid alcohol, the sections are carefully rinsed in alcohol to remove all the free acid, then transferred to 0·5 per cent. solution of pure hæmatoxylin in water, which turns every portion of the section containing iron a peculiar slate-blue colour similar to that obtained in staining by the iron-alum hæmatoxylin method of M. Heidenhain. This is a much more sensitive test than the ammonium hydrosulphide or ferrocyanide reactions, and serves extremely well to exhibit the iron when present in only minute quantities in the protoplasm. In sections so treated the outer protoplasmic zone of the chief cell shows a distinct blue colour, indicating that it contains a considerable amount of unmasked iron. A reaction is also obtained in the protoplasm of the portion of the cell occupied by the granules of zymogen, although not equal in intensity to that in the outer clear zone.

The presence of a large quantity of masked iron in the cell protoplasm is a feature which serves to distinguish the chief cells of the body of the fundus gland from all other glandular cells in the stomach. The protoplasm of the border cells shows no reaction whatever when treated in the manner indicated above; and that of the pyloric gland cells, of the cylindrical surface cells, and of the chief cells of the neck of the gland gives only a faint reaction for iron.

The prozymogen or cytoplasmic chromatin differs from the nuclear chromatin in some respects, as is shown by its relation to stains. In sections stained in gentian violet the prozymogen takes a reddish metachromatic stain, which contrasts very well with the colder blue of the nucleus and zymogen granules (fig. 4). In Biondi solutions, which give a good basic nuclear stain, the prozymogen stains reddish—not, however, a

[1] 'Journal of Physiology,' vol. xxii, 1897.

pure rubin stain. Furthermore, washing in alcohol after staining in safranin will extract the safranin from the prozymogen, and, as Macallum[1] pointed out, from the oxyphile nucleolus, long before it is extracted from the basophile chromatin of the nucleus. I have made use of this property in connection with the hæmatoxylin iron reaction to determine the distribution of prozymogen in the glandular cells of the stomach. In working with the hæmatoxylin method alone one is often in doubt whether an apparent reaction is a real one, or simply the result of a nucleus lying in an upper or lower plane of the section, and out of focus, acting as a light filter. If, however, the section after treatment with the hæmatoxylin be well washed and transferred to a dilute solution of safranin in 30 per cent. alcohol, then extracted in alcohol until the safranin is nearly all removed, cleaned in benzole, and examined, it is found that whilst the nuclear chromatin has taken on a reddish-blue tinge from the safranin, the oxyphile nucleolus and the prozymogen have retained a slaty-blue colour, which it is impossible to mistake even in thick sections (fig. 5).

The fibrillated appearance presented by the outer clear zone of the chief cell is of adventitious origin, and not in itself of importance. A study of the mode of growth of the zone shows that the first indication of an increase of protoplasm is a thickening of the trabeculæ separating the granule-containing spaces in the outer ends of the cells. Then, as the granules disappear from the outer ends of the cells, this thickening becomes more apparent, and affects more particularly those trabeculæ which are arranged in a direction parallel to the long axis of the cell, so that these give in optical section the impression of longitudinal bars or fibrillæ.

The fine fibrillation which Eberth and Müller,[2] Mouret,[3] and others figure in the pancreatic cell may be seen in the gastric chief cell, only in the small amount of unused protoplasm which is usually seen in the resting cell. This not

[1] 'Quart. Journ. Mic. Science,' vol. xxxviii, part ii, new series.

[2] 'Zeitschr. f. wissensch. Zool.,' Bd. liii, supplement.

[3] Op. cit.

rarely presents peculiar sheaf-like and concentric forms which are not unlike the figures published by Macallum,[1] Mouret,[2] and others, of the nebenkerne in the pancreatic cells of Batrachia.

A coarse fibrillation, similar to that exhibited by the outer protoplasmic zone of the chief cell, I have also observed in the cells of the œsophageal glands of the frog, and in the serous glands of the gustatory region of the tongue of the rabbit and dog, the fibrillæ being strongly chromophile and iron-holding in each case. Solger[3] has described in the serous cells of the human submaxillary gland, and Erik Müller[4] in the submaxillary glands of the guinea-pig, rod-shaped elements, placed vertically in the bases of the cells, which stain intensely in hæmatoxylin. It appears probable that these also are small masses of protoplasm which owe their affinity for hæmatoxylin to the fact that they are strongly impregnated with prozymogen.

Between the coarse fibrils in the base of the cell may be seen small vacuoles containing fluid, and in the pepsin-secreting cells of the stomachs of Batrachia the outer zone appears rather vacuolated than regularly fibrillated. In osmic acid specimens minute fat droplets may usually be seen in the outer ends of the cells, the border granules of Langley.

The nucleus of the chief cell of the body of the fundus gland is placed near the base of the cell in the resting condition. It is spherical or slightly oval in shape, frequently exhibiting slight irregularities of contour. A well-defined chromatin network and one or two large oxyphile nucleoli may be made out in all stages of secretion. The latter are always invested by a thin layer of basic chromatin, which frequently collects in small masses at certain points on the periphery of the nucleolus, as has occasionally been observed in the nuclei of nerve-cells.

It will be seen from the foregoing that there are two salient

[1] 'Trans. Canadian Institute,' vol. i, part ii, 1891.
[2] Op. cit.
[3] 'Anat. Anzeiger,' Bd. xi.
[4] 'Arch. f. mik. Anat.,' Bd. xlv.

features in the structure of the chief cells of the body of the gland, namely, the presence of granules of zymogen in a portion of the cell of varying extent next the lumen, and the presence in large amount in the protoplasm of the outer end of the cell, and in that between the granules, of a kind of chromatin called prozymogen, which stains strongly with hæmatoxylin and other nuclear stains, and gives, after treatment with sulphuric acid alcohol, a strong reaction for iron. Further, the outer protoplasmic prozymogen-impregnated zone exhibits a coarse fibrillar structure, which is a quite frequent morphological feature of the ferment-secreting cell.

I have not been able to make out any primary structure in the protoplasm, that is any differentiation into a firmer framework and hyaline interstitial substance, although such may exist and be masked in the ferment-secreting cell by the large amount of deeply staining prozymogen present.

In the neck of the gland the chief cells are of smaller size and more pyramidal in shape, and are present to the number of one to four between each pair of border cells.

It has already been indicated in the description of the fresh gland that the neck of the gland is devoid of zymogen granules. This may be readily verified by the examination of sections prepared after fixation in the alcohol bichromate sublimate mixture, and stained in gentian violet. Such preparations exhibit exactly the same division of the mucosa into two zones as has been observed in the fresh material, due in this case, however, to the fact that the zymogen granules with which the chief cells of the body of the glands are filled stain intensely in the dye, and thus give a deep stain to the body of the gland, whilst the cells of the neck of the gland which contain no granules have only their nuclei stained. Under the high power not a single granule may be found in the chief cells of the neck of the gland.

This absence of granules is not due to imperfect fixation, as might be inferred, for one may see at the junction of the neck and body-cells of both kinds, side by side, some containing granules of zymogen, perfectly preserved and staining readily,

and others entirely devoid of these. Moreover one can readily distinguish the two kinds of cells from one another, even when, as occasionally happens, the fixation of the granules has been imperfect. In such cases the zymogen-holding cell still stains strongly, although diffusely, in gentian violet, whilst in the neck cell only the nucleus stains.

In sections from the stomach of an animal that has been digesting for several hours and stained in hæmatoxylin, the division into two zones may again be observed. In this case the extensive prozymogen-holding outer zone of the chief cells of the body of the gland stains strongly, showing that the distribution of the prozymogen corresponds to that of the zymogen. The protoplasm of the chief cells of the neck of the gland exhibits but little affinity for hæmatoxylin, and it is only by means of the hæmatoxylin-iron reaction that it is possible to demonstrate the presence of any cytoplasmic chromatin at all in these cells. In sections of alcohol-hardened material, treated with sulphuric acid alcohol for three to six hours in the warm oven, ammonium hydrosulphide or acid ferrocyanide produce a scarcely recognisable reaction in the protoplasmic portions of the neck cells. If, however, the sections be treated with aqueous hæmatoxylin, according to the method already outlined, a slight blue colour is obtained, which, however, is not to be compared in intensity with the strong reaction observed in the chief cells at the bottom of the gland. The protoplasm of the cells of the surface, and of the duct of the gland, gives a similar faint reaction for iron.

The chief cells of the neck of the gland, therefore, lack the two most important features of the chief cells of the body of the gland; they contain no granular zymogen, and they contain prozymogen in such small amount that they are rather to be compared in this respect with the mucus-secreting cells of the surface and gland duct. It might be urged that these are young or imperfectly differentiated zymogenic cells, which as yet secrete their ferment in such small amount that its antecedents do not appear in the form of granules in the cell. It is not at present possible to determine whether or not these

cells do secrete small quantities of pepsin, but a study of the positive characters of the cell reveals that they possess different staining characters from the chief cells lower down, and are engaged in a different kind of secretion.

I shall describe in the first place the cells from the upper portion of the gland neck (fig. 6, *a*). These are conical or pyramidal in shape, and wedged in between the large ovoidal border cells of this portion of the gland in such a way that in vertical sections their broad end is usually directed towards the lumen. In sections stained in hæmatoxylin and eosin the cell is divided into two zones, an outer protoplasmic zone staining readily in eosin, and an inner zone engaged in secretion which stains with difficulty. The outer zone consists of two elements, fine fibrillæ which join one another to form a network, and a hyaline substance filling up the interstices of this network. The inner zone exhibits a structure similar to that of the chief cells of the body of the gland, and for similar reasons the accumulation of droplets of secretion forces the protoplasm to simulate the appearance of a reticulum, although the bars which compose it are not nearly so thick nor so regularly arranged as in the chief cells lower down.

The secretion in the cells of the gland neck stains intensely in Bordeaux R and indulin, and these dyes, particularly the latter, have rendered me considerable service in determining the distribution of this kind of secretion in the stomach, and also in studying the secretive processes in the cells containing it. I have found the most convenient method of applying this dye to be in the form of Huber's blood-staining fluid, consisting of two grammes each of aurantia, eosin, and indulin, rubbed up in a mortar with thirty grammes of pure glycerine. This fluid is diluted with from 100 to 400 times its volume of distilled water before use, and allowed to act on the sections transferred to it from water for five to thirty minutes. The sections are then washed in water, dehydrated, cleared in benzole, and mounted. On examination it is found that the red blood-corpuscles are stained yellow, the nuclei of all cells a faint hæmatoxylin tint, the border cells and chief cells of

the body of the gland red, and the white fibres of connective tissue blue. The protoplasm of the chief cells of the neck of the gland also stains red, but the portion of these cells containing secretion stains intensely blue (fig. 6). Staining in indulin solutions alone does not give successful results, as the stain in this form is also taken up diffusely by the other cells; but mixtures containing only eosin and indulin, or orange G and indulin, give fairly good results. This colour reaction is very little affected by the mode of fixation, as it may be obtained in exactly the same features as indicated in fig. 6, in preparations hardened in absolute or dilute alcohol, or aqueous corrosive sublimate.

In sections stained in the indulin mixture the inner zone of the cell appears vacuolated, exhibiting in optical section a network of thick bars separating spaces, in which lies blue-stained secretion. The network also stains, as a rule, much more intensely than the secretion, indicating that it too is impregnated with the indulinophilous substance. Not rarely one may notice irregular clumps or flakes of deeply stained substance lying in the spaces of the cell network, giving the cell a coarsely granular appearance. This is never observed in cells fixed in aqueous sublimate, and is probably due to the rapid extraction of water by the alcoholic fixative, and a consequent precipitation in this form of the solids of the secretion.

It will be noted that the inner zone of these cells, when stained in the indulin mixture, exhibits a coarse network, while in hæmatoxylin eosin sections the network is finer than in the chief cells of the body of the gland. The reason appears to be that the solids of the secretion are precipitated along the bars of the protoplasmic network, and when intensely stained give these a triple thickness. In sections stained in concentrated solutions of the indulin mixture, one sometimes finds the true protoplasmic portion of the network stained red, and it then presents the same characters as in the hæmatoxylin eosin sections.

As one follows the neck of the gland downwards, it is seen

that (fig. 6, *b*) the portion of the cell engaged in secretion becomes relatively greater, and at the lowest portion of the neck of the resting gland two zones are no longer to be recognised in the cell, the whole of which is concerned in the formation of the indulinophilous secretion. These cells of the lower portion of the neck of the gland are in structure exactly like the cells of the mucous salivary glands in the secretion-filled phase.

Following the gland in the direction of the free surface, it is found that the cells of the uppermost portion of the neck, and of a varying portion of the duct or stomach pit, are also engaged in the formation of the indulinophilous secretion, the portion of the cell thus engaged becoming relatively less, and the protoplasmic portion greater as one approaches the surface. The cells also, as the border cells become fewer, assume the cylindrical shape and become longer. In these cells (fig. 6, *e*) the secretion is found, for the most part, as a spherical mass in the middle of the protoplasm of the cell near the nucleus. There is always, however, a small amount of indulinophilous substance diffused through the protoplasm intervening between this and the free surface of the cell, and along the free surface. Passing up the duct of the gland this mass gradually approaches the free border of the cell again, and becomes less stainable in indulin, thus passing by a gradual transition into the mucigenous border of the surface cylindrical cells, which in the cat stains but faintly in the indulin mixture.

A further difference between the cells of the gland neck and those of the free surface is that the protoplasm in the latter cells is of a denser character than that in the cells of the gland neck,—that is, it contains a larger proportion of the fibrillar element, and a smaller amount of interfibrillar substance.

Thus, although the cells of the gland neck differ from those of the free surface, both in general appearance and in staining properties, it is impossible to discover at any point intervening between the lowest part of the gland neck and the free surface an abrupt change in the character of the cells. This

in itself would suggest a similarity in the nature of the secretion products of the cells, as well as a similarity in their mode of origin. There are, however, additional reasons for regarding the secretion of the neck cells as of a mucous nature. Staining in thionin gives a faint metachromatic red stain to the secretion inside the cell, and in Mayer's muchæmatein the secretion of these cells stains even more intensely than that of the surface epithelium. I may add that in the latter fluid the chief cells of the body of the gland stain not at all.

The nuclei of these cells vary in shape with the amount of secretion present. In those cells which have a well-defined outer protoplasmic zone it is round or oval, and of regular contour. In the cells that are filled with secretion the nucleus presents the irregular, compressed, sometimes crescentic outline usually observed in mucus-secreting cells.

Mitotic divisions are, as Bizzozero [1] pointed out, most abundant in the cells of the bottom of the duct and of the upper portion of the gland neck, but they are by no means infrequent in the chief cells of the gland neck themselves, and I have frequently observed cells completely filled up with secretion with their nuclei in the various phases of indirect division.

The nature of the difference between the mucus secreted by the cells of the gland neck and of the surface epithelium is a point of some interest, but one which it is very difficult to determine. The fact that the cells in which mitoses are most frequent contain secretion which stains with indulin, and that the cells of the gland neck containing a similar secretion also divide frequently, while the cells of the surface rarely undergo division, would suggest that the difference is partly of the nature which Bizzozero [2] has found to exist between the secretions of the young and old mucus-secreting cells of the Lieberkuhnian glands of the intestine. This is not, however, a sufficient explanation, as similar differences in staining re-

[1] 'Arch. f. mik. Anat.,' Bd. xlii.

[2] Ibid., Bd. xl.

actions are found in cells which are strictly comparable morphologically. For example, the mucous border of the surface epithelium of the stomach of the rabbit stains in indulin almost as readily as the neck cells of the gland of the cat, and in the intermediary zone of the rabbit's stomach there are peculiar transparent glands, the cells of which have all the features of a mucous cell, but which stain differently from the cells of the neck of the fundus gland, from the pylorie gland cells, and from the surface epithelium. The only fluid which stains the secretion of these cells is the muchæmatein solution of Mayer, in which it becomes intensely blue.

The indulinophilous mucus-secreting cells do not cease abruptly at the lower end of the gland neck, but a few may be found among the ferment-secreting cells of the upper portion of the body of the gland, as indicated in fig. 6, *d*, and rarely one finds them even in the lowest parts of the gland. These cells in hæmatoxylin and eosin stained sections stain red, contrasting strongly with the more blue stained ferment-forming cells. It seems to me very probable that these are the cells observed by Pilliet,[1] Trinkler,[2] and others, and regarded as stages in the transformation of chief cells into border cells, or vice versâ.

The cells of the neck of the gland exhibit the usual secretion changes. In the first hours of digestion very little change is to be noticed; but after twelve hours of secretion, and more particularly when the stomach is mechanically stimulated by sponge feeding, the secretion is seen to have been largely passed out into the lumen of the gland, where it stains readily with indulin and muchæmatein, and exhibits the stringy or spongy texture usually presented by mucus outside the cell. At the same time the cell becomes reduced in size, and contains relatively more protoplasm. Secretion changes may also be observed in the cells of the bottom of the gland duct, the rounded mass of mucus moving forward to the free surface of the cell, where it is partly discharged.

[1] 'Journal de l'Anat.,' &c., 1887.

[2] 'Arch. f. mik. Anat.,' Bd. xxiv.

It has seemed to me that there is an increase of indulinophilous cells in the body of the gland during the first hours of digestion, but I have not yet given this matter enough attention to enable me to speak with certainty concerning it.

The discovery of the different nature of the cells of the neck of the gland affords a cytological basis for the division of the gland into two regions, a neck and body.

The neck of the gland, as determined by the distribution of the indulinophilous cells, varies in length in the different portions of the stomach. It is shortest in the glands of the greater curvature, where it forms about one third of the gland, and longest in the lesser curvature, where it may comprise as much as four-fifths of the entire gland.

B. The Pyloric Glands of the Cat.

These glands are, as Toldt[1] pointed out, branched tubular glands, consisting of a deep pit or duct lined by a continuation of the surface epithelium, and, opening into this, branched tubules of various lengths exhibiting a tortuous course in the deeper layers of the mucosa.

Disregarding the anatomical divisions of the gland, one may divide it, on a basis of the nature of the cells, into three regions: a funnel-shaped duct, lined by a continuation of the surface epithelium (fig. 7, *a*); the tubular body of the gland, lined by the true pyloric gland cells (fig. 7, *c*); and a short portion connecting these, and lined by cells of an intermediate type (fig. 7, *b*).

The true pyloric gland cells are, except as regards shape, identical with the chief cells of the neck of the fundus gland. They present all the characteristic staining properties of the latter, and, if one compares similar secretion phases, are of the same structure. They contain a secretion which stains intensely in Bordeaux R, in indulin and Mayer's muchæmatein, and, as R. Krause has already pointed out, gives a metachromatic red stain in thionin. This secretion I regard, for similar reasons, as of a mucous nature.

[1] 'Sitzungsber. d. k. Akad. d. Wissensch., Wien,' 1881.

The cells of the pyloric glands contain neither in the fresh condition nor in the hardened and stained gland granules of zymogen. Furthermore, the protoplasm of these cells is not chromophilous to nuclear dyes, and examination for masked iron by the methods already indicated reveals but a trace of prozymogen. The cells of the transitional portion of the gland resemble most closely those of the surface, but contain a relatively larger amount of protoplasm. By means of Mayer's muchæmatein it may be shown that these cells always contain a small mass of mucus at their free ends, but this is so small that, when using less decisive staining methods, it is readily overlooked, and the cell regarded as entirely protoplasmic. In these cells mitoses are fairly numerous, although they also occur in all parts of the gland proper.

These pyloric glands present exactly the same features as the neck and duct of the fundus gland,—that is, although the true pyloric gland cells differ both in general appearance and in structure from the cells of the surface, in passing from one to the other along the gland at no point can one discover an abrupt change from one type of cell to the other.

The secretion phases in the pyloric glands of the cat are peculiar, and are represented in figs. 8—10. Fig. 8 shows the condition of the gland after a fast of twenty-four hours' duration. The cells are comparatively long, with a spherical or oval nucleus placed near the base of the cell. The stored-up secretion is found, for the most part, in the form of a narrow zone along the lumen of the gland, although it is not uncommon to find a second mass of secretion in the deeper portion of the cell near the nucleus (fig. 8, *a*). It is also quite common to find this second mass of secretion more stainable in indulin than that along the free border, the cell then presenting an appearance closely resembling that already described for the cells of the lowest portions of the duct of the fundus glands. Sometimes, as in the neck of the fundus glands, the mucin is precipitated in the form of irregular flakes or granules. A prolongation of the fasting period even to four days does not

result in an increase of the amount of secretion stored up in the cell.

Fig. 9 shows a similar gland from the pylorus of an animal killed six hours after a copious meal of meat. The cells are increased in length, and the nuclei exhibit a tendency to become irregular in outline and flattened in a direction at right angles to the long axis of the cell. The portion of the cell between the nucleus and the lumen is now entirely filled up with a coarse meshwork, which stains strongly in indulin, and contains a similarly staining secretion, although in many cells a division of this into two masses, partly separated from one another by a band of protoplasm, is still obvious.

Fig. 10 is from an animal killed twelve hours after a meal consisting of several pieces of sponge soaked in beef juice. The lumen of the gland is now increased in size, and the cells much shorter than in the preceding phase. The nuclei have regained their spherical shape, and are situated nearer the middle of the cell. The secretion has been nearly all cast out of the cell, so that only an extremely small amount on the free border is now to be recognised.

The pyloric glands of the cat illustrate in a very striking manner the fact that in gland cells the period of greatest loading does not always coincide with the end of a normal period of rest. Here it appears that the growth that takes place in the cell during the rest period following the completion of digestion results merely in an increase of the protoplasm of the cell, and that during the first hours of digestion a large portion of this is rapidly transformed into mucigen, so that the real period of greatest loading is reached some hours after digestion begins.

Further evidence in support of the view that the indulinophilous cells of the neck of the fundus glands and the pyloric gland cells of the cat are identical is afforded by an examination of the short intermediary zone. The first change that one notices in passing from the greater curvature in the direction of the pylorus is an increase of the indulinophilous cells in the body of the gland. At the same time the neck of

the gland increases in length. As the pylorus is approached these cells gradually replace entirely the zymogen-forming chief cell, and the proximal portion of the pyloric region is occupied by short glands, consisting of indulinophilous cells and a few border cells. Ultimately the latter also disappear, and we have the true pyloric gland.

C. The Gastric Glands of the Dog.

A section of the fresh mucosa of the greater curvature of the stomach of the dog, examined in aqueous humour, reveals much the same features as a similar preparation of the cat's stomach. The superficial portion of the mucosa, including not only the pits but a large portion of the glands themselves, is entirely free from the zymogen granules that crowd the lower portion of the gland. The granules are smaller than in the cat, and much more difficult to preserve, so that I have been compelled to rely on the examination of the fresh glands for control of my results.

In sections hardened in corrosive sublimate, or in the bichromate sublimate mixture, the chief cells exhibit the same structural features as those of the cat, namely, a regular meshwork separating spaces, which one may infer to have been occupied in the fresh cell by the zymogen granules (fig. 11), and in glands from a digesting stomach (fig. 12) of an outer protoplasmic zone, which stains intensely and readily in hæmatoxylin, exhibits a coarse fibrillar structure, and gives, after treatment with sulphuric acid alcohol for three or four hours at a temperature of 37° C., a well-marked reaction for iron. In short, the cell contains both zymogen and prozymogen in large amount.

The chief cells of the neck of the gland, on the other hand, contain no granular zymogen, and only a trace of prozymogen. These cells are of the same nature as in the cat, and present an inner secretion zone of variable extent, which stains intensely in indulin and muchæmatein. As in the cat, also, a gradual change in the character of the cells is seen as the surface of the mucosa is approached.

It is an interesting fact that among the other cells of the neck of the fundus gland of the dog, cells may be found which are in every respect similar to the cells of Stöhr of the pyloric glands, as described by Hamburger.[1] These are characterised by their fusiform shape and their faintly staining protoplasm, in which wavy fibrillæ may be seen. The pointed end of the cell which reaches the lumen stains intensely red both in the Biondi mixture and in eosin.

A point of some physiological interest is the varying length of the gland neck in the stomachs of different dogs. It will be seen from the following measurements that the differences in the thickness of the mucous membrane are mainly due to variations in the length of the body of the gland:

	Thickness of mucosa.	Length of gland neck.	Length of body of gland.	Percentage of whole occupied in zymogenesis.
No. 1	·68 mm.	·28 mm.	·4 mm.	58·8
„ 2	·608 „	·276 „	·332 „	54·6
„ 3	·68 „	·3 „	·38 „	55·8
„ 4	1·118 „	·358 „	·76 „	67·9
„ 5	1·387 „	·37 „	1·017 „	73·3

The above measurements are taken from vertical sections from the middle of the greater curvature, stained in the indulin mixture, so that the junction of the body and neck of the gland was sharply indicated by the cessation of the indulinophilous cells. It is obvious that such differences in the percentage of the whole gland engaged in ferment secretion must be taken into consideration in estimating the relative amounts of pepsin in the mucous membrane in different periods of digestion.

The pyloric glands of the dog are in all essential features similar to those of the cat, the main difference being that the ducts are much longer and rather cylindrical than funnel-shaped, and the gland cells are much longer. The pyloric gland cells contain a secretion which stains intensely in Bordeaux R, in indulin, and in muchæmatein, and are in every respect similar to the chief cells of the neck of the fundus gland.

[1] 'Arch. f. mik. Anat.,' Bd. xxxiv.

The secretion phases are represented in figs. 13 and 14. The resting cell (fig. 13) contains already a great deal of reserve secretion, the nucleus being crescentic and compressed against the base of the cell. In the exhausted phase (fig. 14) the secretion is for the most part confined to the free border of the cell, although many of the cells exhibit spherical masses in the neighbourhood of the now spherical nucleus. The protoplasm is also very much increased in amount.

All observers are agreed that the fresh pyloric glands of the dog do not contain coarse granules. The only granules that may be observed are small globules of fat, which are particularly numerous in the bases of the cells. The mucous secretion of the cell, too, occasionally, as in the cat, precipitates in a granular or flaky form, although I have never observed this in secretions hardened in aqueous sublimate solutions. The protoplasm of the cell contains only a trace of prozymogen.

Conclusions.

In the cat and dog the fundus glands contain two kinds of chief cells, those of the body and those of the neck of the gland. The former are engaged in the secretion of ferment, and are characterised by the possession of a large number of zymogen granules which occupy a portion of the cell of varying extent near the lumen, and a protoplasmic outer zone of various size which stains intensely in nuclear dyes such as hæmatoxylin, and presents a coarse fibrillar structure. The staining properties of this outer protoplasmic zone are due to the presence in it of a kind of chromatin, which may stand in a genetic relation to zymogen, and which has been named prozymogen.

I regard the cells of the neck of the gland, and the pyloric gland cells in the cat and dog, to be of the same nature, for the following reasons :

The chief cells of the neck of the fundus gland and the pyloric gland cells do not contain at any period of digestion zymogen in the form of granules, and prozymogen is present only in traces.

Both groups of cells are engaged in forming and secreting a substance which stains intensely in Bordeaux R, in indulin, and in Mayer's specific mucin stain muchæmatein, and which gives the usual metachromatic mucin stain with thionin.

Both may be traced through a gradual transition to the cylindrical cells of the surface.

The assertion that the secretion of these cells is mucin rests as yet on the evidence afforded by the structure of the cell, and its staining in thionin and Mayer's muchæmatein. No method has suggested itself to me for securing the secretion of these cells unmixed with that of the surface epithelium, and testing it for mucin. It is, however, on evidence of the same character that the large transparent neck cells of the batrachian gland have been accepted as mucus-secreting cells.

The chief cells of the neck of the fundus glands, and the pyloric gland cells of the cat and dog, are the physiological and morphological homologues of the mucous neck cells and pyloric gland cells of the frog, with which they correspond in situation, structure, functional changes, and staining. It is interesting to note that these cells in the frog and other Batrachia stain intensely in indulin when treated with mixtures of eosin, indulin, and aurantia.

The cells of the lowest portion of the gland duct or pit are of a type intermediate between the surface epithelium and the mucin-secreting neck cells or pyloric gland cells, and form the fundamental type to which the origin of both must be traced. Moreover the researches of Bizzozero[1] have made it extremely probable that these, although themselves secreting cells, are constantly dividing, and so form the young elements from which both the cells of the surface and the neck cells, or the pyloric gland cells, are recruited, although it must be admitted that the two latter are partially replaced by division among themselves.

Mucin-secreting neck cells are present in the fundus glands of the mink, rabbit, mouse, rat, squirrel, ground hog (Arctomys), chipmonk (Tamias striata), pig, and sheep. The relation

[1] 'Arch. f. mik. Anat.,' Bd. xlii.

existing between these and the pyloric gland cells on the one hand, and the cardiac glands on the other, will be discussed in a second paper now in course of preparation.

EXPLANATION OF PLATE 29,

Illustrating Mr. R. R. Bensley's paper on "The Structure of the Mammalian Gastric Glands."

NOTE.—All figures are drawn with the aid of the camera lucida, and, with the exception of figs. 1 and 7, as seen under the Zeiss apochromatic objective (2 mm. apert.) and compensation ocular 8. In fig. 7 the same objective and compensation ocular 4 were employed. Figs. 2, 3, 11, and 12 are reproduced from grey pencil drawings, and do not indicate the colours of the original preparations.

FIG. 1.—A photomicrograph of a fresh unstained section of the mucosa of the greater curvature of the stomach of the cat.

FIG. 2.—Transverse sections of the lower ends of three fundus glands of the cat after a fast of twenty-four hours' duration; hæmatoxylin and eosin.

FIG. 3.—Transverse sections of the lower ends of three fundus glands of a digesting cat, stained in hæmatoxylin and eosin. *a*. Indulinophilous mucin-secreting cells. All other chief cells show a deeply staining outer zone with coarse fibrillar structure.

FIG. 4.—Fundus gland from a digesting cat, stained in gentian violet. The outer prozymogen-holding zone stains metachromatically.

FIG. 5.—Fundus gland of cat fixed in alcohol. Section treated with sulphuric acid alcohol, then with aqueous hæmatoxylin, and finally stained faintly in safranin. The prozymogen-holding outer zone gives a strong reaction for iron, which has been unmasked by the action of the acid alcohol.

FIG. 6.—Vertical section of the duct, neck, and upper portion of the body of a fundus gland of a fasting cat; indulin, eosin, and aurantia. *a*, *b*. Mucin-secreting chief cells of the neck. *d*. Ferment-secreting chief cells of the body of the gland. *f*. Mucin-secreting cell of the body of the gland.

FIG. 7.—Pyloric gland of cat after a fast of twenty-four hours' duration; indulin and eosin. *a*. Duct. *b*. Intermediate portion. *c*. Body of gland. Exhibits the gradual transition in passing from the body of the gland to the duct.

Figs. 8, 9, and 10 are sections from the lowest portion of the pyloric gland of the cat in different secretion phases, and stained in indulin and eosin.

Fig. 8.—From an animal after twenty-four hours' fast.

Fig. 9.—From an animal killed six hours after feeding.

Fig. 10.—From an animal killed twelve hours after sponge-feeding.

Fig. 11.—Body of fundus gland of dog after a fast of twenty-seven hours; hæmatoxylin and eosin.

Fig. 12.—The same from a digesting dog; the cells here contain an unusually large amount of prozymogen-holding protoplasm.

Fig. 13.—Cells from the pyloric gland of the dog after twenty-seven hours' fast; indulin and eosin.

Fig. 14.—Cells from the pyloric gland of the dog twelve hours after sponge-feeding; indulin and eosin.

On Certain Green (Chlorophylloid) Pigments in Invertebrates.

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With Plates 30 and 31.

Contents.

Preliminary.

The existence in various Invertebrates of pigments presenting a remarkable resemblance to chlorophyll has long been known. Of these pigments the most familiar are the green pigments of the worms Bonellia and Chætopterus, and the pigment described by Dr. MacMunn as enterochlorophyll. In each case an identity with chlorophyll has been asserted by different authors. The green pigment of Bonellia has been described as chlorophyll by Schmarda, Schenk, and others, although the researches of Sorby, Geddes, and Krukenberg long ago demonstrated the erroneous nature of the description. Similarly, Professor E. Ray Lankester, who discovered the peculiar pigment of Chætopterus, called it a "chlorophylloid" substance, and at one time placed Chætopterus in the list of chlorophyll-containing animals, although he no longer maintains this position. In the case of "enterochlorophyll," however, Dr. MacMunn's application of the term chlorophyll has not been seriously challenged.

During some work on the pigments of the Crustacea I came across a statement by Dr. MacMunn that enterochlorophyll occurs at least in some cases in the "liver" of these forms. Not being able to find it there readily, I resolved to study its properties in the organs in which it was first described, namely, the digestive glands of the Mollusca. Soon after I had begun this work Professor Lankester sent me some solutions of the pigments chætopterin and bonellin, and asked me to make a chemical examination of them. I found so much resemblance between the chætopterin solutions and solutions of enterochlorophyll as to make it desirable to study the two pigments side by side. In the interim Professor Lankester's own paper on chætopterin appeared, with spectroscopic observations by Drs. Benham and Engelmann.

I am much indebted to Professor Lankester for the solutions of chætopterin and bonellin which he sent, to Professor W. A.

Herdman for solutions of his new pigment, thalassemin, and to Professor W. M'Intosh for various green Invertebrates.

A. Chætopterin.

(1) General Characters.

As chætopterin occurs both in larger amount and in a purer state than enterochlorophyll, it is convenient to begin with some account of it.

It is not necessary to say much with regard to its general characters and mode of occurrence, for these points are fully discussed in Professor Lankester's paper (5). As shown there, the pigment occurs in the form of numerous minute granules in the cells of the mid-region of the gut. In addition to these small green granules, there occur in some of the gut cells much larger round vesicles of a more brownish tint. These bodies seem to me also to contain the pigment. The pigment further occurs in oily drops mingled with the contents of the gut.

An interesting microchemical reaction which indicates the presence of chætopterin, or the related pigments, is afforded by the use of strong acid, preferably hydrochloric. If a drop of this acid be placed on the gut wall of Chætopterus when spread out on a slide, the cells, previously greenish brown, turn a vivid green, or more rarely blue. The test is not absolutely diagnostic, inasmuch as certain of the lipochromes give a dirty greenish tint on the addition of strong hydrochloric acid, but the lipochromes in animal tissues are usually uniformly diffused and not granular, and they only give the reaction in the dry condition, the addition of water or alcohol destroying the colour. Chætopterin granules do not lose their colour on the addition of water or alcohol, except in so far as a green pigment dissolves out. Further, in the case of chætopterin the green colour can be removed by the addition of a little alkali, and the process repeated indefinitely, which is again impossible in the case of the fleeting lipochrome colour. In the general case also the lipochrome would be dis-

tinguished by its yellow or orange tint. The reaction, which does not seem to have been previously described, is of some importance, because it affords a readily available method of recognising the pigment in cases where the amount may be too small to make it easy to demonstrate its nature in any other way.

As shown by Professor Lankester, the pigment readily dissolves in cold methylated spirit to form a solution varying in colour according to its strength. Very strong solutions are a deep brownish-yellow colour with greenish lights; more dilute ones are grey-green, while those which are very weak may be almost pure green. All the solutions show a strong blood-red fluorescence. The want of definiteness in the colour is very characteristic, and is no doubt due to the complex spectrum,—that is, to the differential absorption. The bands are not all of equal intensity, and as those at the violet end disappear when the solution is diluted before those at the left end of the spectrum, the effect of diluting is naturally to increase the amount of green in the tint.

As shown by Professor Lankester, the spectrum of the freshly extracted solution shows four bands, a very strong one over the line C, and three others lying respectively to the left of the lines D, E, and F. In strong solutions there is also a shading at the right of the D line. For the details of the spectrum reference should be made to Professor Lankester's paper; it is figured in Plate 30, fig. 1, for convenience of comparison.

On the addition of a considerable amount of acid to the solution the colour changes to a dusky blue without loss of the blood-red fluorescence. The spectrum shows the original four bands, of which, however, the first two at least have shifted slightly to the right, and an additional band, which lies to the right of the D line, in the position of the shading already noticed in the normal solution. Besides the slight alteration in position, the original bands in the green and violet have greatly diminished in intensity. This is probably due to the dilution and the slight turbidity of the solution.

It occurs to such an extent as often to almost obliterate the F band; thus this band is figured as absent in Dr. Benham's maps, though Dr. Engelmann's chart indicates its presence.

By neutralisation with alkali the original spectrum and colour may be restored—a marked difference from the phenomena exhibited by solutions of chlorophyll.

(2) Action of Acids on Chætopterin.

The changes which solutions of chætopterin undergo on the addition of acids are of so striking a nature that they form a natural starting-point for any investigation.

The acid usually employed was hydrochloric acid in concentrated solution. The employment of gaseous hydrochloric acid produced the same effect as the solution; it did not produce a precipitate, as it does in the case of solutions of chlorophyll. It was found, further, that, as is the case with bonellin according to Krukenberg, when other acids are employed the amount necessary to produce the blue colour depends upon the strength of the acid, and not upon its chemical nature. Thus a much larger amount of acetic acid is necessary than of hydrochloric, while excess of nitric acid is apt to carry the reaction beyond the blue stage, and produce a brown solution from which the original pigment cannot be recovered. This is a product of decomposition.

Comparison with Bonellin.—In its relation to acids chætopterin shows a marked analogy to bonellin. The changes which bonellin undergoes have been studied by Krukenberg (3), and his results may be briefly detailed.

Krukenberg found that the addition of a considerable amount of strong acid to a solution of bonellin turned his bright green solution violet without destroying the fluorescence, while a large excess turned it pure blue without any trace of fluorescence. He figures three sets of spectra corresponding to three stages in the action of the acid. The first of these he describes as indicating the existence in the solution of a mixture of bonellin and an acid derivative; the other two as representing two distinct acid derivatives, which he calls

respectively bonellidin and acidobonellin. Now Professor Lankester has shown that the solution which Krukenberg regarded as that of neutral bonellin was in reality alkaline bonellin, the neutral solution being not green but dusky grey. We are thus entitled to omit Krukenberg's first stage from our comparison with chætopterin, and consider only his bonellidin and acidobonellin solutions. We have already described the colours of these solutions, and as the pigments were not isolated there remains only the spectroscopic characters. It is not necessary at present to discuss these in detail, for an account of the alteration in position undergone by the dominant band in the red is sufficient for our purpose. Bonellin, like chætopterin, exhibits in neutral solution a very strong band in the red. On the addition of acid this band, without marked diminution in intensity, shifts its position towards the right. This is an old observation. Krukenberg found, however, that a further addition of acid in very large excess caused the band to move back until it almost occupied its original position. It was this double movement, combined with the colour changes in his solutions, which induced him to reject Sorby's suggestion that the acid has merely a physical effect, and to put forward the theory of the existence of two acid compounds. The subject has not apparently been again studied.

Passing from solutions of bonellin to those of chætopterin, we find that here again acid has a duplex effect. While a small amount of acid produces the blue colour already described by Professor Lankester, I find that a very large excess turns the blue solution a pure clear green, with diminished fluorescence. Further, an examination of the spectra shows that, as in bonellin, the band in the red shifts first to the right, then with excess of acid back to its original position. The band of chætopterin does not occupy exactly the same position as that of bonellin, nor is the movement so extensive, but there is at least an analogy.

Characters of Acidified Solutions.—The question whether the changes which occur on the successive addition

of acid do or do not indicate the existence of acid derivatives may seem at first sight one which can be readily determined. When, however, it is recollected that we are dealing not with a well-defined chemical substance capable of being invariably recognised by definite reactions, but with an unstable, unknown substance, which, apart from the presence of impurities, may be a mechanical mixture of several pigments; when, further, it is found that virtually the one available test is that of the spectrum, whose validity as a test is the point to be proved, it is then possible to obtain some notion of the difficulties. It is curious to note that in point of fact, in spite of the frequent occurrence of pigments whose spectra change on the addition of acids, Schunck's beautiful work on chlorophyll (**10**) seems to be the only case where the reasons for the changes have been fully investigated. The difficulties mentioned above perhaps afford a ready explanation of the blanks in our knowledge of such pigments.

The most obvious characteristics of the acidified solutions are, of course, their spectra. The spectrum of the blue acid solution has been completely mapped by Professor Engelmann, whose method shows its peculiar characteristics in a very striking manner. My own observations were made both with a Sorby's microspectroscope and the large double prism spectroscope of the Cambridge Instrument Company. The results are recorded here (see fig. 2) only because they are essential to the course of the argument; they agree very closely with those of Engelmann, but on account of the indefiniteness of the margins of the bands are less accurate,—that is, the point given as the centre of the band does not always coincide with the point of maximum absorption.

The general characters of the spectrum of the blue acid solution have been already described. In regard to detail, the most important point is the character of the dominant band in the red. In freshly extracted solutions of chætopterin this band, as measured by a table spectroscope, has the following position :

λ 679 — λ 643, centre = λ 661 (see the first band of fig. 1).

On the addition of a considerable amount of hydrochloric acid its limits are as follows:

λ 669 – λ 637, c. = λ 653 (see the first band of fig. 2).

It will thus be seen that in spite of the fact that the solution has been very considerably diluted, the actual extent of the band has not been greatly diminished. Further, while the left-hand side of the band remains fairly sharp and well defined, the right-hand side is very indefinite, shading gradually off, so that exact measurement is virtually impossible. This peculiar appearance has been described by Dr. MacMunn (6) in the case of solutions of enterochlorophyll as "a band superimposed upon a shading." Now if excess of acid be added to this solution until it turns green, this shading at the right hand disappears, and the band recovers approximately its original position. Thus it may stand as follows:

λ 677 – λ 647, c. = λ 662.

As to the other bands of this green acid solution, they may be present as in the blue acid one, but in the general case they are exceedingly faint, and present merely as traces. Their changes, if they do change, can be followed with much less certainty than those which are undergone by the very distinct band in the red. These changes are not sudden, but take place very gradually, and can be watched step by step when acid is added to a solution suspended in front of the slit of the spectroscope. As the acid is added the band moves to the right until the movement reaches its maximum, and then on further addition of acid it moves back to its original position. Further, if alkali be added to a very strongly acid solution the band shows the same change of position as when little acid is added to a normal solution, and excess of alkali restores it to its original position, just as does excess of acid in the other case. In other words, dilute acid produces a change in the position of the band, which is reversed by strong acid; and it is unimportant whether the dilute acid is directly added to the solution, or produced by removing some of the acid from a highly acidified solution. In the case of bonellin, Krukenberg states that the strongly

acidified solution is without fluorescence. In the case of chætopterin the fluorescence ceases to be marked when the solution is very strongly acid, but when a ray of bright light is thrown upon the vessel containing the solution placed against a dark background, it rarely fails to show a trace of the characteristic blood-red colour. The more detailed characters of the blue and green solutions it may be well to study separately.

The Blue Acid Solution.—When acid is added to an alcoholic solution of chætopterin the solution becomes more or less turbid in appearance. If a considerable amount of acid be added, and then water, and the whole shaken in a separation funnel, the superficial layer of ether becomes pale green, the lower layer a pure clear blue with marked fluorescence.

(*a*) The ether gives more or less distinctly the original four bands of chætopterin without the band at the right of D. When weak the band in the red has its centre about λ 661—the position of that of the original solution; but if the ether contains much pigment the centre of the band tends to shift to the right, and to approach more closely that of the band of the acid solution. In other words, the spectrum may be that of fig. 1, but there is a tendency for the first band of it to be replaced by the first band of fig. 2, or by a band intermediate between the two. When the ether is evaporated and the pigment dissolved in methylated spirit a green solution is formed, with little fluorescence. On adding acid the solution does not turn blue; it shows at first little alteration, and later becomes brownish. The pigment is thus altered,—is not identical with neutral chætopterin. At the same time it is to be noticed that the amount of alteration varies greatly. In some cases the ether seems to give the full four-banded spectrum of fig. 1 without alteration; in other cases it may give only a band in the red, corresponding to the first of fig. 2. The green tint and the absence of the power of giving a blue colour with acid are the most constant characters.

(*b*) The blue acid solution left after shaking with ether gives the same spectrum as it did before the process. When strong it is a beautiful pure blue colour, but in some cases

nearly all the colour can be removed by successive shaking with ether.

If ammonia be added to this blue solution until it remains only slightly acid, the blue colour greatly diminishes in intensity, and on shaking with ether the ether becomes brownish green in colour, and gives beautifully the original four-banded spectrum (fig. 1). On evaporation the ether leaves a dull green pigment, which dissolves in methylated spirit to form a brownish-green strongly fluorescent solution, which turns blue with acid, and shows all the characters of the original solution. The whole of the pigment cannot, however, be extracted from the acid solution in this way, for when ammonia is added in slight excess ether does not extract any pigment, chætopterin being readily soluble in ammonia to form a solution in which the band in the red only is distinct. Instead of attempting to neutralise the acid solution, an easy method of precipitating its pigment is to add pieces of marble to it. Violent effervescence occurs, the blue colour is completely lost, and a dull green precipitate falls, leaving the solution colourless. The precipitate after washing readily dissolves in methylated spirit, and yields a solution of blue-green colour, more or less fluorescent, and giving a four-banded spectrum. The band in the red is distinct and broad. In strong solution it has the following position:

$$\lambda\,669 - \lambda\,639,\ \text{c.} = \lambda\,654;$$

that is, it has almost the same position as that of the band in the red in the blue acid solution. The other three bands have the same position as in neutral chætopterin solutions; the band to the right of D is absent. The spectrum is thus that of fig. 1, but with its first band replaced by that of fig. 2. On standing with acid this solution turns brown; the position of the band in the red does not alter, although the band at the right of D may appear. The pigment thus appears to be identical with that obtained by shaking the acid solution with ether.

In attempting to explain these reactions, the first point is to consider whether they support the view that in the case of

chætopterin there is an acid derivative corresponding to Krukenberg's bonellidin. As already seen, bonellidin is the pigment supposed to be present in a solution of bonellin turned violet by acid; it is characterised by its colour and its spectrum. The spectrum is distinguished from that of neutral bonellin by the different position of especially the band in the red, the appearance of a new band analogous to that at the right of D in the case of chætopterin, as well as by the loss of the band in the violet. Blue acid solutions of chætopterin are characterised by the (slight) change of position of the band in the red, the apparent loss of the band in the violet, and a slight change in the position of the other bands, as well as by a colour-change as contrasted with neutral solutions. But the band to the right of D is present as a shadow in neutral solutions, and is only marked when the solution examined actually contains acid. Thus, if a solution of the pigment in anhydrous ether be vigorously shaken with acid, this band, previously a mere shadow, becomes suddenly distinct. If the ether be then carefully washed with distilled water to remove any trace of acid, the band disappears. This seems to me to prove that the appearance of this band is not due to the formation of a compound, for it is difficult to believe that a true compound could be so extraordinarily unstable. Krukenberg noticed a similar fact, that an evaporation of solutions of bonellin containing a volatile acid caused the disappearance of the corresponding band; he calls this the regeneration of bonellin from bonellidin, but I cannot agree to this conclusion.

Again, the loss of the band in the violet is a point of no importance, for it may be quite easily explained as the result of the slight turbidity of the solution, and the change in position of the other bands is too slight to be of any moment. I thus dissent from the view that the spectrum and the colour of the blue acid solution are together diagnostic of the existence of a new pigment defined by these characters, and believe that the point which requires explanation is that the action of dilute acid is to produce a permanent alteration in the position of the band in the red solutions in chætopterin, and

a permanent loss of the power of giving a blue colour with acid. To these points we shall return after considering the characters of the green acid solution.

(*c*) The Green Acid Solution.—The deep green solution produced by adding excess of acid yields no pigment to ether. On adding ammonia, and again shaking with ether, unaltered chætopterin of brownish-green tint is extracted by the ether. On the addition of marble to the acid solution a precipitate falls of dull green colour. This dissolves in methylated spirit to form a green solution with a mere trace of fluorescence. The spectrum shows four bands: the band in the red is exceedingly strong; the other three have the same position as in unaltered chætopterin, and vary greatly in intensity. The position of the band in the red is as follows:

$$\lambda\,666 - \lambda\,634,\ \text{c.} = \lambda\,650.$$

The band has thus much the same position as the first band in fig. 2 would have if the shadow to the right were to become distinct. The addition of acid to this solution does not change the position of the band in the red, but it ultimately turns the solution a brownish colour.

When the dull green precipitate obtained by the addition of marble is treated with methylated spirit, there remains undissolved a brownish residue which is insoluble in ether or methylated spirit, and which is unaffected by acid or alkali. A trace of similar residue remains when the precipitate from the blue acid solution is treated with methylated spirit or ether.

General Conclusions as to the Action of Acids.

If we survey generally the action of acid on solutions of chætopterin, we see that the pigments precipitated respectively from the blue and green acid solutions by the action of marble, agree with one another, and differ from the original chætopterin in forming in methylated spirit solutions which are pure green or bluish green in colour, very slightly fluorescent, and which turn brown and not blue or green on the addition of acid. They differ from chætopterin and from each other in the

position of the band in the red, about λ 653 in the one case and λ 650 in the other, while they may or may not exhibit distinctly the other bands of the original chætopterin solution. In this last respect there is much variation, but it is unnecessary here to repeat the numerous spectroscopic observations which were made in order to find, if possible, a valid explanation of the variation. There is also variation in tint in both solutions. When the chætopterin bands are distinct the tint of the solution exhibits an approximation towards the indefinite brown-green colour of the original chætopterin; when less distinct the solution is a bright blue-green; when they are barely visible the solution is a pure dull green. These observations ultimately convinced me that both solutions contain a mixture of pigments, the two components varying in amount. After many trials I succeeded in proving this as follows.

(1) The precipitate from the blue acid solution obtained by adding marble was dissolved in ether, the ether placed in a separation funnel, and concentrated hydrochloric acid cautiously poured in. The acid became indigo-blue at the line of junction, deep green further down, and ultimately green throughout; the ether remained pure pale green. When examined with a spectroscope the ether showed two bands in the red (see fig. 3), one with the centre at λ 661—the band of the original chætopterin; and another with the centre at about λ 641, an entirely new band. If the ether be examined in very thick layer the two bands approach so near as to be distinguished with difficulty, and then appear like one broad band with a centre about λ 653 (cf. figs. 2 and 3). In addition to these two bands the ether in thick layer shows a trace of a band at about λ 601, which corresponds to the second band of the original chætopterin solution. The acid gives a spectrum showing the band in the red characteristic of chætopterin, and traces of the other bands,—that is in essence the spectrum of fig. 1.

(2) The precipitate obtained by the addition of marble to the green acid solution if dissolved in ether and treated with concentrated acid gives the same results. Again, the ether

shows a double band in the red, the one to the right being more distinct. The right-hand band is entirely new, the other is one of the bands of chætopterin. By successive treatment with acid it is possible to remove the left-hand band from the ether almost entirely, and leave only the new band at λ 641,—that is the second band of fig. 3; the solution is then pure green without fluorescence.

The changes which chætopterin undergoes on the addition of acid I therefore explain as follows:

When a small amount of acid is added to a neutral solution, a small amount of a new pigment of a green colour is formed, which shows one band with its centre at λ 641. This band, however, is so near the broad band of chætopterin that the two overlap, and in strong solution the apparent result is to shift the red band to the left, as well as to produce that indefiniteness of margin of which we have already spoken (see fig. 2). The other slight peculiarities of the spectrum of acidified solutions I believe to be analogous to the changes seen in the position of the bands of bonellin when the solvent is varied, and not to indicate chemical change.

When excess of acid is added to the solution the green derivative is not destroyed, but, owing apparently to its imperfect solubility in strong acid, its band disappears, leaving only the original chætopterin band. This is, as I believe, the explanation of that shifting of bands of which we have already spoken (p. 396). The disappearance of the band of the derivative in strong acid solution is paralleled by that of the other chætopterin bands, which become exceedingly indistinct when much acid is present in the solution, although by removal of the acid the original pigment can be recovered.

The changes which occur in the spectrum of chætopterin solutions when acidified I thus believe to be due to the blending of the spectrum of unaltered chætopterin and that of an acid derivative. The colour-change is, I believe, similarly due to the mixture of pigments present.

Characters of the Green Acid Derivative.—The green derivative formed by the addition of acid to a solution

of chætopterin is not very easy to obtain unmixed with chætopterin. If the pigment precipitated by marble from an acidified solution be treated with ether, the ether becomes green as already stated, leaving behind a brownish insoluble residue. If the ether be repeatedly treated with concentrated acid, the acid removes at least the greater part of the chætopterin, leaving the ether pure green; the reaction depends on the fact that chætopterin is more soluble in concentrated acid than in ether; while the derivative is, on the other hand, soluble in ether, but not in strong acid. The spectroscopic examination of the ether usually, however, shows a trace of a band at λ 661,—that is the strong band of chætopterin, in addition to the band at λ 641 characteristic of the derivative (see fig. 3). Another method is to treat the precipitate from the acid solution with methylated spirit, and add lead acetate to the solution so obtained. The chætopterin is precipitated, leaving the derivative in the solution.

The green derivative obtained in either of these ways forms in ether or alcohol a pure green solution without fluorescence, and giving a one-banded spectrum. On evaporation it is obtained in the form of green oily drops, which are readily soluble in dilute ammonia. It is not soluble in pure water. It was not obtained in large amount, and is apparently formed by the acid splitting chætopterin into a brown insoluble substance and the derivative: the derivative cannot be reconverted into chætopterin. In the absence of detailed chemical investigation this pigment will be simply called the acid derivative.

(3) Action of Alkalies.

(*a*) Ammonia.—The action of ammonia upon solutions of chætopterin is somewhat peculiar. If a few drops of ammonia be added to the alcoholic solution, there is, as noticed by Professor Lankester in the case of caustic soda, a colour-change to yellow-green without distinct change in the spectrum. If the alkaline solution be allowed to stand for some time, however, the colour becomes a deep pure green with strong fluores-

cence, and the spectrum shows, in addition to the four bands of chætopterin, a new band at λ 625 (see fig. 4); the first band also tends to shift slightly to the right. There is at the same time a slight precipitate of a green colour. On prolonged standing with ammonia the solution loses its fluorescence, and the bands, with the exception of two in the red, tend to disappear (see fig. 5). The addition of acid in excess to this solution does not produce a blue colour. A more speedy and certain method of attaining these results, however, is to boil solutions of chætopterin with ammonia for some time on the water-bath, then evaporate to dryness, and extract the residue with methylated spirit.

Only a part of the residue is soluble, a portion remaining undissolved, and being of a green colour. This is identical with the precipitate already described as being formed when ammonia is added to alcoholic solutions, and is apparently an ammonia compound; it is insoluble in alcohol and ether. After treatment with dilute acid it yields to methylated spirit a solution containing a mixture of chætopterin and its acid derivative. The reason for this will be considered under the action of salts on chætopterin; it is sufficient for the present purpose to note that chætopterin, like certain of the lipochromes, is at least in part precipitated by ammonia, the compound being insoluble in alcohol or ether.

While heating with ammonia converts in this way a portion of the chætopterin into an ammonia compound, another portion is converted into a derivative. This is present in the alcoholic extract of the residue after evaporation of the alkaline solution. There is usually present in addition some unaltered chætopterin, which can be precipitated by addition of lead acetate, when the derivative remains in solution. The derivative is characterised by its green colour, its peculiar spectrum consisting of two bands in the red, one about λ 650 and the other at λ 625 (see fig. 5), its want of fluorescence, and finally its solubility in water as well as in dilute alkali. It may be formed, as already indicated, by allowing alkaline solutions of chætopterin to stand for some time in the cold, but usually

the presence of the derivative in such solutions is only indicated by the appearance of a new band at λ 625, and sufficient unaltered chætopterin remains for the solution to retain its fluorescence and its original four bands in addition to the new one. The point is emphasised because it seems to have some bearing upon the characters of bonellin. When the ammonia derivative is formed by heating chætopterin with ammonia, the band at λ 625 is often difficult to demonstrate.

(*b*) Caustic Soda and Potash.—Very dilute solutions of these when added to the alcoholic solution produce the same effect as ammonia,—that is, there is a slight green insoluble precipitate, and the solution remains pure green, showing two bands in the red, one at λ 661 and one at λ 625, with more or less distinct traces of other bands, and yields a pigment which is readily soluble in water or dilute alkali. If a considerable amount of caustic soda or potash be added, however, the solution turns dull yellowish brown, and when shaken with ether, the ether removes a yellow pigment which gives a faint band at λ 661, the band of chætopterin. This pigment seems to me to be a product of decomposition, showing that chætopterin is destroyed by strong alkali; a mere trace of unaltered chætopterin would be sufficient to yield the band.

It thus appears that dilute alkalies have a double effect upon chætopterin. In the first place, the pigment forms with alkalies a compound of green colour which is insoluble in water, alcohol, and ether, and from which original chætopterin can be recovered by the action of acid. In the second place, alkalies give rise to an alkaline derivative of green colour, which is soluble in water as well as in dilute alkali and alcohol. Solutions of this in alcohol are not fluorescent, and show two bands, one at λ 650 and the other at λ 625.

(4) Action of Salts.

The addition of salts like lead acetate or copper acetate or sulphate to alcoholic solutions of chætopterin causes a precipitation of pigment. In the case of lead acetate the precipitated pigment is bright yellow-green in colour, and is insoluble

in ether or alcohol. If the precipitate is treated with a little dilute acid the colour changes, and, after washing with water, the pigment can be dissolved in ether or alcohol. The alcoholic solution is blue-green in colour, but the blue is not intensified by acid, and spectroscopic examination shows that the solution consists of a mixture of unaltered chætopterin and the green acid derivative already described. The colour and fluorescence vary according to the amount of the derivative present, and this depends upon the amount of acid employed to decompose the compound. If a considerable amount of the derivative is present the fluorescence becomes indistinct, and an appreciable amount of a brown insoluble residue remains behind when the acidified precipitate is treated with methylated spirit. These results show that precipitation with lead acetate cannot be readily employed as a means of purifying chætopterin, for when the insoluble lead compound is treated with acid a portion of chætopterin as it is set free is converted into the green acid derivative (cf. the effect of acid on the ammonia compound as noted above, p. 406); other salts give similar results.

The solution obtained in methylated spirit from the acidified precipitate after lead acetate is of a singularly pure and beautiful blue-green colour, and rarely gives more than one definite band, though the band in the yellow may be represented by a shading. The band at C has its apparent centre about λ 654 (cf. the first band of fig. 2), but it is of course really the result of the apposition of the band of chætopterin and that of the green derivative. Before the relation of chætopterin to acids was fully understood this solution was thought to contain a single pigment, and a considerable quantity of the dried pigment was tested for nitrogen by igniting with metallic sodium. The test showed the presence of nitrogen, but, as the pigment was a mixture of the acid derivative and original chætopterin, the result is only to show that chætopterin itself contains nitrogen. In this respect it resembles bonellin. It would be of interest to know whether, when chætopterin splits into the green acid derivative and the brown

insoluble residue, the nitrogen does or does not enter into the composition of the green pigment, or whether it is all contained in the brown substance.

Summary.

The pigment chætopterin dissolves readily in cold methylated spirit or in ether to form solutions, which are indefinite in colour, strongly fluorescent, and which give a spectrum consisting of four distinct bands and a shading.

When acid is added to the solution in methylated spirit, it becomes first blue and then green.

The blue solution is fluorescent, and distinguished by certain peculiarities of the spectrum, but the fact that it can be made to yield only original chætopterin and a single-banded green derivative seems to prove that its peculiarities are not in themselves diagnostic of the existence of an acid derivative comparable to Krukenberg's bonellidin, but are due to the combination of the two pigments present.

The green acid solution shows little fluorescence, and yields similarly a mixture of chætopterin and the one-banded acid derivative.

This acid derivative is characterised by its single faint band, its green colour, and the absence of fluorescence. It is apparently formed by the splitting of chætopterin into an insoluble brown residue and this derivative.

Dilute alkalies have a twofold action upon chætopterin. They in part precipitate it as a compound insoluble in alcohol, ether, and water, and in part convert it into a green derivative. This derivative is characterised by its colour, its spectrum consisting of two bands in the red, and its solubility in water.

Salts of the metals, such as lead acetate, precipitate chætopterin from its solutions, forming compounds which are insoluble in water, alcohol, or ether. By the action of dilute acid on these compounds chætopterin can be regenerated, but it is liable to be intermixed with the acid derivative.

Chætopterin, like bonellin, contains nitrogen.

The points which seem to me of special importance are that

while chætopterin itself is indefinite in colour and strongly fluorescent, and exhibits a complex spectrum, the action of reagents is to tend to produce pigments of bright definite tint and simple spectrum, which may be soluble in water and are without fluorescence. Certain points of resemblance to bonellin are also of much interest.

B. Enterochlorophyll.

(1) Previous Investigations.

The name enterochlorophyll was given by Dr. MacMunn (6) to a pigment, found in the digestive glands of Mollusca and other Invertebrates, which turns green on the addition of acid, and then gives a spectrum resembling that of acid chlorophyll. The same pigment is apparently denoted by Krukenberg's term hepatochrome, but Krukenberg did not isolate the pigment or define it clearly.

Dr. MacMunn's observations may be briefly summarised as follows:—He found that the epithelium lining the "liver" tubules in Mollusca contains pigmented oil drops and granules which dissolve in alcohol to form a greenish-yellow solution with strong red fluorescence. The solution gives a spectrum with three bands, one in the red, one to the left of D, and one to the left of E, and also strong absorption of the violet end. In some cases in dilute solution there may be one or two very faint bands in the violet, but these are always ill-defined as compared with the dominant three. The addition of strong acid turns the solution grass-green, shifts the bands slightly to the right, adds an additional band at the right of the D line, and diminishes the absorption at the violet end, so that one clear band to the left of F is now visible there. It is this five-banded spectrum which Dr. MacMunn compares to the spectrum of chlorophyll. In his first paper (1883) he compares it to the spectrum given by an acidified solution of chlorophyll,—that is, to the spectrum given by a mixture of chlorophyll and phyllocyanin; but in the second communication (7) he dwells upon its resemblance to the spectrum of pure chlorophyll. There is

thus a slight ambiguity in the use of the term enterochlorophyll, for it is not quite apparent whether it is to be used to designate the pigment contained in the yellowish extract of molluscan liver, which gives a three-banded spectrum, or to that in the green solution produced by the addition of acid to this solution, which has a five-banded spectrum.

In his second paper Dr. MacMunn applied the method of saponification to the pigment, and showed that solutions of his enterochlorophyll, like solutions of plant chlorophyll, contain a yellow lipochrome pigment in addition to a greenish constituent. In the case of plant chlorophyll it is now known that the association of "chlorophyll green" and the lipochrome, xanthophyle, is merely incidental, and the term chlorophyll is restricted to the former. It is not quite clear whether Dr. MacMunn regards "enterochlorophyll" as a combination of a lipochrome and a green constituent, or whether he regards them as associated pigments; but he was not able to obtain complete separation, and believes that "in enterochlorophyll there is probably a more intimate union between the constituents than in plant chlorophyll."

Krukenberg's (2) observations are much less detailed. He found in the "bile" of Mollusca what he regards as evidences of three pigments. One of these, he says, is a lipochrome, and gives two bands in the violet; another gives a strong band in the red and one in the green, and is a "hepatochrome:" a band at the beginning of the green appeared in some of his solutions and puzzled him greatly, but he believed that it belonged to a third unknown pigment. He evidently worked with very small amounts of pigment, and did not go beyond this point. Incomplete as the observations are, however, Krukenberg was right in every one of his inferences. The bands in the red and the green are two of the bands of "enterochlorophyll;" the occasional band in the beginning of the green is that band of enterochlorophyll which is only distinct in acidified solutions; the violet bands are due to the presence of an additional yellow pigment.

(2) Mode of Occurrence.

Dr. MacMunn describes enterochlorophyll in various Echinoderms as well as in the Mollusca; my own observations were made entirely on the Mollusca. As the pigment occurs in relatively small amount, it is necessary to choose a form which can be obtained in large quantity. The garden snail (Helix aspersa) and the common limpet (Patella vulgata) both fulfil this condition, but I soon found that the snail contains a very much smaller amount of pigment than the limpet, and in consequence, in spite of the greater difficulty in dissection, the latter was employed in all the later observations.

In the limpet the pigment has been described by Dr. MacMunn in the "liver" and in its secretion; I find it also in the cells of the gut, in the contents of the gut, and in very pure condition in the fæces. In all these situations it can be recognised by the microchemical reaction already described for chætopterin (p. 393)—the vivid green colour with hydrochloric acid.

In order to study the distribution of the pigment in the "liver" and intestine, portions of the visceral hump were hardened in formalin, and sections cut through both the digestive gland and the coils of the intestine. The sections of the gut show with low power a band of brownish-green pigment placed in the epithelial cells near their inner margin (see fig. 9). When examined under a higher power (fig. 10) the pigment is seen to occur in minute closely packed granules, brownish green in mass, green when viewed singly. They in all respects resemble the granules in the cells of the gut in Chætopterus, but the amount of pigment is much smaller. The sections of the tubules of the digestive gland (fig. 11) do not show cells having this peculiar granular appearance. The large cells near their inner surface contain several of the characteristic molluscan pigmented vesicles, usually of a brownish-yellow colour, while scattered through the proto-

plasm, as described by Dr. MacMunn, coloured oil-drops occur. Sections of the digestive gland are by no means easy to cut when, as in the present instance, the ordinary hardening agents are inadmissible. Many of the cells are ruptured in the process, and mingled with the débris there occur green oil-drops like those found mixed with the contents of the gut in Chætopterus. I am of opinion that these contain the pigment enterochlorophyll, while the brownish vesicles probably contain it intermixed with yellow pigment, which may also occur diffused through the protoplasm. The colour of the liver varies greatly in different specimens, the differences being apparently due to variations in the amount of enterochlorophyll present. The presence of the pigment in the cells of the gut and in the fæces seems to me of prime importance from a comparative point of view.

(3) Characters of Solutions.

In the earlier experiments great care was taken to diminish the risk of contamination of the solutions by plant chlorophyll from the gut. In the limpet portions of the liver were carefully dissected away from the coils of the gut and dropped into methylated spirit. As it takes some thirty or forty limpets to yield a very moderate amount of solution, the process is a somewhat tedious one. It was found later that the risk of an intermixture with plant chlorophyll is in reality very small if care be taken to remove the "manyplies" and its contents, for the digestive juices seem to very speedily destroy the chlorophyll, and it rarely occurs in the small intestine. After the discovery of the presence of the pigment in the fæces was made these were employed as sources of the pigment. The absence of lipochrome pigment and of fat in solutions obtained from them greatly simplifies further operations.

To obtain the pigment the parts employed, whether liver or fæces, should be dried and powdered, and the powder extracted with cold methylated spirit, in which the pigment is exceed-

ingly soluble. Solutions obtained from the digestive gland are yellowish in colour, from the fæces greenish brown; both have strong red fluorescence. The spectroscopic characters of the former solution have been already described by Dr. MacMunn; the latter differs chiefly in showing less absorption of the violet end, and a more or less distinct band in the neighbourhood of the F line: the differences are associated with the absence or diminished amount of lipochrome pigment in the solution from the fæces. The "liver" extract, as shown by Dr. MacMunn, turns green on the addition of acid; the extract of the fæces, on the other hand, turns first bluish, and then green on further addition of acid.

Relation to Chlorophyll.—The resemblances to chætopterin which have been incidentally noted in the above description tend to disprove the identity of "enterochlorophyll," and plant chlorophyll, but it may be well before proceeding further to state more clearly the difficulties which the suggestion has to encounter. "Enterochlorophyll," in the first place, differs from true chlorophyll in giving the peculiar green reaction already described. Further, the solutions are much more stable than those of chlorophyll. As is well known, the decomposition of a solution of chlorophyll in bright light is a matter of minutes, while even in obscurity the fading is rapid. Detailed observations on the effect of sunlight on enterochlorophyll were not made, but solutions did not show marked change when left to stand in the diffused light of the laboratory, and remained unaltered during months of standing in a cupboard, while an extract of green leaves standing in the same cupboard lost its green colour entirely. More striking is the fact that, although the colour and spectrum of "enterochlorophyll" solutions change on the addition of acid, the original spectrum can be restored by alkali, and the process repeated any number of times; this is impossible in the case of chlorophyll. The addition of aqueous or gaseous hydrochloric acid does not produce a precipitate, or only to a very slight extent, and if precipitated the pigment shows the character of the original enterochlorophyll. Gaseous hydrochloric acid when introduced

into solutions of chlorophyll produces a precipitate of phyllocyanin which differs markedly from chlorophyll, and cannot be reconverted into it. It is probably unnecessary to pursue these contrasts further; a comparison of the properties of "enterochlorophyll" as detailed in the present paper with Schunck's results (10) in the case of chlorophyll and phyllocyanin will show that enterochlorophyll is a much less complex pigment.

The apparent points of resemblance are the fluorescence, the association with a yellow lipochrome, and the spectrum. Of the fluorescence, a not uncommon character amongst certain classes of pigments, it is not necessary to say anything. The analogy of chætopterin, and the conditions seen in the fæces of Patella, show that the association of the lipochrome is an accidental character of no significance. With regard to the third point, the spectrum, there is more difficulty. In his first paper Dr. MacMunn compared the spectra of acidified solutions of chlorophyll and acidified alcoholic extracts of the "liver" of Ostrea, and found them almost identical. This is at first sight a very striking result, but the reflection that the first solution contained a mixture of chlorophyll and phyllocyanin, and the second a mixture of "enterochlorophyll" and an acid derivative, somewhat diminishes the force of the comparison. In his second paper Dr. MacMunn compares the spectra of unaltered chlorophyll and "enterochlorophyll" directly. The most striking difference is, then, the absence in the latter of a band to the right of D. Such a band appears in enterochlorophyll solutions on the addition of acid, which Dr. MacMunn regards as evidence of the existence of "enterochlorophyll" in the "reduced condition, or in the form of a chromogen." He found this band in the normal extract in some cases, cf. the observations of Krukenberg as quoted above (p. 411). The occasional appearance of this band in both cases I am inclined to regard as due to the presence in the solution of traces of acid, probably derived from the gut or its contents, or to the solution containing an unusually large amount of pigment (cf. chætopterin, where the band is present in very strong solutions, p. 394). The resemblance between the spectrum of

"enterochlorophyll" when this band is present, and the spectrum of true chlorophyll, I believe to be merely a striking coincidence, emphasising the danger of relying upon spectroscopic observations unsupported by chemical investigation, rather than indicating true affinity. In view of these facts Prof. Lankester suggests the emendation "enterochlor," or "enteroverdin," in place of the term "enterochlorophyll," but it is to be noticed that neither in the natural condition nor in solutions is the greenness of the pigment at all well marked, except after the addition of acid.

Characters of the Associated Lipochrome.—Although the yellow pigment found in company with enterochlorophyll was not subjected to a detailed examination, it may be useful to point out in what respects its presence modifies the reactions of enterochlorophyll. In the first place it modifies the spectroscopic characters, in that it produces marked absorption of the violet end, and so blurs the fourth band of enterochlorophyll, that is the band in the neighbourhood of the F line. Like other yellow lipochrome pigments, it is decolourised by the addition of hydrochloric or other acids to the alcoholic solution. The result is that the addition of acid makes the fourth band of enterochlorophyll distinct by diminishing the absorption of the violet. The yellow pigment also exercises a marked and often very puzzling effect on the colour of the solutions. When it is virtually absent solutions of enterochlorophyll, like those of chætopterin, turn blue on the addition of acid, though the tendency for the blue to pass into green is much stronger in the former than in the latter. When the yellow pigment is present acid turns the solution green without trace of blue. The reason for this is that though the yellow pigment is destroyed by acid, yet the amount of acid necessary to completely remove it is also sufficient to turn the enterochlorophyll solution green instead of blue (cf. chætopterin, p. 396). It is exceedingly difficult to remove the yellow pigment from solutions of enterochlorophyll, for although acid decolourises it, the colour returns on the addition of alkali, so that the pigment is liable to appear at very unexpected

points. Dr. MacMunn has shown that saponification with caustic soda or potash is ineffective, precipitation with acetate of lead is better, for the yellow pigment remains in the solution. Traces of it are, however, always liable to be carried down with the enterochlorophyll, and the difficulty of re-obtaining unaltered enterochlorophyll from the precipitate greatly diminishes the utility of the method. The most useful method is, perhaps, to add excess of acid, and shake with ether several times, then, rejecting the ether, add alkali to the acid solution in too small amount to neutralise the solution, and shake again with ether. The ether takes up almost pure enterochlorophyll, which after careful washing may be used for further experiments. The method is, of course, wasteful, and not entirely effective, and it is better, when possible, to obtain a solution from the fæces where the yellow pigment is virtually absent. The purification of enterochlorophyll from fats, and the other impurities with which it is associated, is a matter of great difficulty.

(4) Action of Acids.

Enterochlorophyll resemble chætopterin so closely that it is not necessary to do more than note its characters, referring to the description of chætopterin for details.

The bands shown by a solution of enterochlorophyll differ slightly from those of chætopterin, but the difference is not marked. A neutral solution shows the following bands:

I λ 667, II λ 604, III λ 539, IV λ 503 (fig. 6).

If acid is added to a solution containing little lipochrome a bluish colour develops, and the solution becomes five-banded with the bands as follows:

I λ 657, II λ 599, III λ 567, IV λ 534, V λ 500.

This is the "enterochlorophyll" spectrum so frequently figured by Dr. MacMunn. The fifth band, as in the case of chætopterin, tends to be indistinct. On further addition of acid the solution turns green, the right-hand bands tend to disappear, and the band in the red shifts back to its original position at about λ 667, just as occurs in the case of chæto-

pterin. The association between colour and spectrum is not, however, so close as in the case of chætopterin, for the solution even when only slightly acidified shows a strong tendency to become green.

When the acidified solution is diluted with water and shaken with ether, the ether extracts a considerable amount of pigment, more than is the case with chætopterin, enterochlorophyll being apparently somewhat less soluble in dilute acid than is chætopterin.

The ether is pale green in colour; it usually displays four bands, but the first band tends to be about λ 657 instead of λ 667, as in the original solution. That is, the spectrum is that of fig. 6, but the first band tends to be replaced by the first band of fig. 7. When the ether is evaporated and the residue dissolved in methylated spirit, a green solution is formed, which turns brown and not green with acid. If the ether before evaporation be placed in a separation funnel and concentrated hydrochloric acid poured in, the acid becomes bright deep green, and the ether remains yellowish green. When examined with the spectroscope it then shows two bands in the red, one at λ 667, and one at about λ 650 (fig. 8), which in a very thick layer overlap and produce the appearance of a broad band at λ 657.

It is thus obvious that acid has the same effect on solutions of enterochlorophyll as it has on those of chætopterin. That is, it produces small amounts of a one-banded acid derivative which is not fluorescent, and is readily soluble in ether and alcohol.

It may perhaps be well to notice, in regard to the action of acid on these pigments, that the statement that they differ from chlorophyll in that they can be converted into the acid form, and then reconverted to the normal by means of alkali any number of times, is not strictly speaking accurate, for acid acts on the pigment very slowly, and the "reconversion" is in the general case merely due to the removal of acid from the solution; in the case of the Patella pigment, indeed, the normal pigment often precipitates from a dilute acid solution

on standing, which can hardly be described as a "reconversion." When the pigment is actually converted into the one-banded acid derivative it cannot be reconverted into the normal pigment. The difference between chlorophyll and the pigments enterochlorophyll and chætopterin as to the action of acids, is more accurately expressed by saying that the latter are relatively insensitive to the action of acids, while the former is exceedingly sensitive.

Enterochlorophyll can be precipitated from acid solution by the addition of marble, just as chætopterin can. Solutions of the former, however, when obtained from the liver and intestine always contain fat and other impurities, which may more or less disguise the reactions.

(5) Other Reactions.

In its other reactions also "enterochlorophyll" shows a remarkable resemblance to chætopterin. The action of ammonia is to turn the pigment green, although the colour may be concealed by the presence of the yellow pigment; to alter its spectrum; and to render it soluble in water. As to the spectrum of this ammonia derivative, I have only been able to find one band at about λ 655, instead of the two of the corresponding chætopterin derivative; but as the second band is often difficult to demonstrate in the case of chætopterin, I am not disposed to lay much stress upon this fact. In regard to it and to some other trifling differences from chætopterin, it seems only necessary to point out that not only does enterochlorophyll occur in smaller amount than chætopterin, but also the solutions contain a large admixture of foreign substances, which are of course increased in relative amount with every increase in the strength of the solution. These intermixed substances greatly increase the difficulty of making observations, especially in regard to solubilities and so forth.

Enterochlorophyll is to some extent precipitated by ammonia just as chætopterin is. The addition of acetate of copper or lead to a solution of enterochlorophyll causes a precipitation

as in the case of chætopterin, the precipitate being a bright green colour. The precipitate is insoluble in alcohol, and after treatment with dilute acid dissolves in alcohol to form a solution which contains a mixture of enterochlorophyll and its one-banded acid derivative. Lead acetate affords a useful test for the presence of enterochlorophyll in a solution which contains so much lipochrome as to disguise the ordinary reactions. Such a solution may be pure yellow, but with lead acetate a green precipitate at once forms, the colour being in striking contrast to that of the solution.

(6) Relation to Chætopterin.

Enterochlorophyll is so closely related to chætopterin that the question at once arises whether or not it is identical with that pigment. While leaving that question open, I may point out one or two differences between the two.

First, as to the spectrum, the bands in enterochlorophyll are slightly nearer the red end than those of chætopterin, and the difference appears also in the derivatives. According to Dr. MacMunn, however, there is some variation in the spectrum of the pigment in different animals.

Second, as to colour, solutions of enterochlorophyll even when apparently free from lipochrome pigment, do not give so marked a blue on the addition of a little acid as do those of chætopterin, the colour always inclining towards green. Solutions of enterochlorophyll in concentrated acid are of a bright green colour, but the green inclines towards yellow, while that of chætopterin solutions inclines towards blue. The derivatives show analogous differences.

Finally, as to the solubility, enterochlorophyll seems to be distinctly less soluble in dilute acid than chætopterin. I am inclined to suspect, however, that this is in part due to the large amount of fat in most solutions of enterochlorophyll. The differences are thus not very well marked. In relation to acids, and in giving rise to a one-banded acid derivative; in the action of alkalics, and the production of a soluble alkaline derivative; in the action of salts; and in the general spectro-

scopic properties, the pigments show much resemblance to one another.

(7) Distribution of Enterochlorophyll.

I have not yet made observations on this subject, but it seems desirable to briefly consider the literature. Dr. MacMunn describes the pigment in the Mollusca and Echinoderma as well as in some other cases. In the two groups mentioned it seems exceedingly common, and usually present in considerable amount. Its presence in the latter is especially interesting because of the existence in the group of Moseley's (9) pentacrinin and antedonin. I am strongly of opinion that these two pigments are in some way related to enterochlorophyll, but I am not able to explain why they should present such striking differences in spectra and so forth. In Echinoderms "enterochlorophyll" is said to occur chiefly in the so-called digestive gland, but in sea-urchins it is said also to be found in the perivisceral fluid, although I have not succeeded in obtaining the full spectrum there.

As to the presence of enterochlorophyll in the great group of Arthropods there is much less certainty. Dr. MacMunn speaks of it as existing occasionally in the digestive gland in Crustacea, but he does not seem to have obtained the full spectrum, or to have isolated the pigment. I have examined the pigment of the digestive glands of a few Crustacea, and have not succeeded in obtaining the reactions of enterochlorophyll. If present it can only be in small amount, and the most abundant pigment of the "liver" is certainly not "enterochlorophyll" in the sense in which that name has been used here. The question whether the pigment does exist in Arthropods in considerable amount is one worthy of further investigation.

In the Cœlenterata there seems little reason to doubt that a pigment related to enterochlorophyll is widely distributed. Such a pigment probably exists, for example, in Anthea cereus, though it appears difficult to isolate, and presents certain peculiarities (see Krukenberg [4] and MacMunn [8]).

Moseley's polyperythrin (9), which is widely spread in certain corals and sea-anemones, is perhaps an allied pigment.

There is thus much reason to believe that pigments related to chætopterin and "enterochlorophyll" are widely spread in Invertebrates.

c. Bonellin.

(1) Comparison with Chætopterin.

The amount of bonellin at my disposal was so limited that I have not been able to make a full investigation of it. There are, however, certain points which seem to suggest a close relation to chætopterin, and are worthy of note. First, as to the spectra, I quote from Engelmann the following points of maximum absorption in neutral solutions of bonellin and chætopterin. It will be noted that, for the reasons already stated, the points in the case of chætopterin do not exactly correspond to the apparent centres of the bands as determined by an ordinary spectroscope.

Bonellin . I λ 635, II λ 585, III λ 520, IV λ 490.
Chætopterin I λ 655, II λ 600, III λ 535, IV λ 500.

When compared together the two sets show some curious analogies. Thus both pigments have four bands placed in similar parts of the spectrum, and, curiously enough, the distances between the bands are almost identical in the two cases, as a little calculation will show.

In addition to its four bands, chætopterin in strong solution shows also a shading, not yet described for neutral bonellin.

Then, as to the spectra of the acid solutions, I quote again from Engelmann:

Bonellin . I λ 613, II λ 570, III λ 545, IV λ 515.
Chætopterin I λ 650, II λ 597, III λ 560, IV λ 533, V λ 500?

On comparing these spectra, no such relation as that noted for the neutral solutions is observed, but there are several points which require notice. In the first place, it is obvious that the first band of acidified bonellin does not correspond to the apparent first band of acidified chætopterin. We have

already seen that the apparent single band of the latter is in reality formed by the apposition of two bands, of which the second has its centre about λ 641. It is, as I think, this band which corresponds to the first band of acid bonellin. In other respects there is, as already seen, considerable analogy between the two sets of spectra. Thus the band at λ 545 in acidified bonellin obviously corresponds to the band at λ 560 in acidified chætopterin; the disappearance of the violet band of neutral bonellin is paralleled by the dimness of the corresponding band in the case of chætopterin. On the fact that the yellow and green bands of chætopterin change little on the addition of acid, while those of bonellin show considerable movement, I am not inclined to lay any stress. My own observations showed considerable discrepancy from those of Engelmann on this subject, and I am strongly of opinion that the movement varies with the amount of acid in the solution.

The most apparent difference from chætopterin which bonellin shows is in the characters of its alkaline solution. Professor Lankester has made the exceedingly interesting discovery that normal bonellin is alkaline,—that is, that the pigment apparently occurs in the animal in the alkaline condition. This alkaline solution gives no less than six bands, of which four are those of neutral bonellin, while the other two have their maximum points of absorption at λ 614 and λ 551 respectively. The solution is bright pure green, with strong fluorescence. Now we have already seen that when chætopterin stands for some time with alkali it becomes pure rich green with undiminished fluorescence, and then shows a five-banded spectrum (fig. 4) with two bands in the red, the original one and a new one at λ 625; at the same time the bands in the yellow and green show a marked diminution. When one passes from the study of normal chætopterin to that of normal (alkaline) bonellin, the most striking differences are, in the latter case, the definiteness of the colour and the indistinctness of the bands, except that in the red and to a less degree that in the violet. In working at the action of alkalies on chætopterin these same characteristics, definiteness

of colour and indefiniteness of bands, reappear; when it is known that bonellin normally occurs in the alkaline state it is difficult not to regard this fact as an additional proof of affinity. As to the additional band at λ 550 in bonellin, it is to be recollected that it is very faint, so faint that it was missed by Sorby (11) entirely; I believe that it is the same band as that described at λ 545 in acidified solutions. It will be recollected that in solutions of chætopterin a similar band occurs, which is only distinct in acidified solutions, but is represented in normal ones by a faint shadow.

It may be well to summarise briefly these facts.

1. Neutral solutions of chætopterin and of bonellin resemble one another in their indefiniteness of tint, in their strong fluorescence, and in possessing a spectrum of four bands occupying similar but not identical positions. Chætopterin solutions show, in addition, a faint shadow in the green, not yet described in neutral bonellin solutions. A similar band, however, occurs in normal (alkaline) solutions of bonellin.

2. The addition of a little acid turns chætopterin solutions blue, bonellin solutions violet, without diminution of the fluorescence. The spectrum is considerably altered, but the alteration in the two cases is similar in so far as in each a new band appears or becomes distinct, and the fourth band of the original spectrum tends to grow faint or disappear. The most striking difference is seen in the fact that in chætopterin the position of the red band only alters slightly, in bonellin it alters much; but the difficulty is diminished by the fact that the acid chætopterin solution can be proved to possess a band analogous to that of acid bonellin, which is concealed by the presence of the original band in the red. Further addition of acid in both cases produces a further change of colour, and the reappearance of the original band in the red.

3. Normal (alkaline) bonellin and chætopterin which has stood with alkali resemble one another in their deep green colour and fluorescence, and in showing the four-banded spectrum of the neutral form, plus an additional faint band in the red. They differ from one another in that bonellin shows

in addition a faint band in the green, probably corresponding to the band in this position in acidified solutions. But a similar band is represented in the normal solution of chætopterin, and apparently is absent in the alkaline solution only because this is more dilute. The balance of evidence, therefore, seems to me to be in favour of an affinity between the two pigments.

In their distribution, however, the two pigments seem to differ markedly. Chætopterin occurs in the Annelid Chætopterus. "Enterochlorophyll," which is undoubtedly at least nearly related, occurs in Mollusca and Echinoderma—not to mention other doubtful cases. In these three sets of animals, certainly not nearly related, the pigment when carefully studied has been found to occur in endodermal tissues, in connection with the alimentary tract or its outgrowths. There is, I believe, much evidence that bonellin is related to chætopterin and enterochlorophyll, and yet we find it described as occurring in the epidermis and in "subepidermic cells apparently belonging to the connective tissue,"—that is, in ectodermal and mesodermal tissues. The latter position is that also described for Professor Herdman's (1) thalassemin—a pigment differing markedly from bonellin. In view of these facts the possibility suggests itself that in Bonellia a pigment similar to chætopterin occurs in the cells lining the gut; that, instead of being eliminated intact with the fæces, as is enterochlorophyll in Patella, the pigment undergoes modification, forming a green derivative which is deposited in granules in the epidermis and underlying tissues. When Bonellia is placed in alcohol both the original pigment and its derivative may dissolve out, resulting in the formation of the solution called alkaline bonellin. The suggestion seems to me to be supported by a comparison with Professor Herdman's thalassemin.

(2) Relation to Thalassemin.

The fact that although a green colour is common in the Echiuridæ, the peculiar pigment bonellin has only been

described in Bonellia, makes the characters of the green pigment of Thalassema a question of some interest. I do not propose to repeat here my notes on the subject (see Professor Herdman's paper), but wish merely to point to the special differences from bonellin. The pigment is greenish blue in tint, the blue being accentuated by acid—a point I did not at first notice, but which is of some interest. It is readily soluble in formalin or in water, and the residue after the evaporation of the formalin is soluble in alcohol, although Professor Herdman did not find that the worm itself yielded a coloured solution when placed in alcohol; this may, however, have been due to the small amount of pigment present. None of the solutions are fluorescent, and the spectrum shows a single band at about λ 617 (cf. the band at λ 614 in alkaline bonellin). If we compare these characters with those given previously, as tending to characterise the derivatives of chætopterin, we find that the solubility in water, the loss of fluorescence, the definiteness of colour, the simple spectrum, all reappear. It seems to me not improbable that thalassemin, which is apparently common among the Echiuridæ, is a derivative of a pigment allied to chætopterin, and that it quite possibly occurs in Bonellia itself, in addition to a chætopterin-like pigment.[1]

(3) Other Green Pigments.

In view of the simultaneous occurrence in the Echiuridæ of an apparently complex pigment like bonellin and a simple one like thalassemin, it is interesting to inquire whether similar simple pigments do not occur in association with enterochlorophyll and chætopterin. There is one marked case of this kind which does not appear to have been yet described.

The tortoiseshell limpet, Acmæa testudinalis, so common

[1] In connection with thalassemin it may be well to mention that I find that the green reaction with nitric acid described in my previous notes is an error. The reaction is the result of the action of impure nitric acid on alcohol, and has no connection with the pigment. (See Dastre et Floresco, 'C. R. Soc. d. Biol.,' x, 1898.)

on Scottish coasts, is remarkable in having the epithelium which covers the visceral hump coloured a bright vivid green, which passes into brown at the margin of the mantle skirt. The epithelial layer which is turned in to cover the dorsal surface of foot, is also coloured by the same pigment. The pigment occurs as minute granules in the epithelial cells, and in life varies from blue to green in colour. Sections of the mantle skirt show that it is the superficial cells only which are pigmented. At its margin the green pigment passes gradually into a brown one of similar distribution. The pigment of the shell, it will be remembered, is deep brown. The green pigment readily dissolves in sea water, in distilled water, or in formalin. It is also soluble in alcohol, but is then apt to be mingled with enterochlorophyll derived from the liver and gut. When a number of limpets are preserved in formalin, the formalin becomes clear greenish blue. The solution resembles that of thalassemin in its colour, in turning distinctly blue on the addition of acid, in being, at least in part, decolourised by alkalies, which tend to turn the pigment yellow. The pigment also, of course, resembles thalassemin in its solubility in water; it differs from it in that I have not succeeded in obtaining any band in its spectrum, and in that it is very unstable. In view of the effects of reagents on enterochlorophyll, it does not seem to me improbable that this pigment is a derivative of enterochlorophyll. There can be little doubt that the brown pigment of the margin of the mantle is derived from the green, and that it is identical with the pigment of the shell; this suggests the possibility that the enterochlorophyll of the gut and liver, instead of being entirely eliminated with the fæces, may give rise in the Mollusca to the bright pigments of shell and mantle. There seems little doubt that soluble green pigments, like those of Acmæa and Thalassema, are widely distributed in Invertebrates; whether they usually originate in the way suggested here must, of course, remain at present undetermined. Such a green pigment occurs, as I believe, in many Annelids, notably in Eulalia viridis, especially in the eggs, but is there not easy to extract.

Whether the suggestion as to the origin here made is correct or not, it is at least interesting to note that in many groups of Invertebrates, either in the same animal or in related forms, there may occur two different sets of green pigments, distinctly marked off from one another, but connected by the derivatives of the more complex series. Such are bonellin and thalassemin in the Echiuridæ, chætopterin and the pigment of Eulalia in the Chætopoda, enterochlorophyll and the pigment of Acmæa in the Mollusca; it is probable that there are many other similar cases.

Conclusion.

If the observation and deductions set forth in this paper are correct they go to prove that there exists in Invertebrates a widely spread group of pigments occurring primarily in connection with the alimentary tract or its outgrowths, and characterised by forming in alcohol fluorescent solutions of indefinite colour which exhibit a complex spectrum, consisting when fully developed of five bands. In the fluorescence and in the complex spectrum these pigments resemble chlorophyll, but the other characters, and especially the relation to acids and alkalies, show that this resemblance is entirely superficial. Of such pigments, chætopterin, and the pigment or pigments described as "enterochlorophyll," are typical examples. Whatever the primary function of the pigments—and of this I have nothing to say—they at least so far resemble the bile pigments of Vertebrates that they occur mingled with the contents of the gut, and at least in some cases are eliminated with the fæces. It seems desirable to have a general name to designate these pigments; and in view of our present ignorance of function, the position in which they occur, and the fact that they give rise to highly coloured derivatives, seem to be the only available characters from which a name can be based. Krukenberg's term hepatochrome is in many respects very suitable, but it has the great disadvantage that he himself used it for "liver" pigments giving banded spectra, without, so far as I can find, defining it clearly.

Perhaps the term "enterochrome" might be found more suitable. I should suggest it as a general term for a series of pigments occurring in connection with the alimentary tract and its outgrowths in Invertebrates, and characterised by solubility in cold alcohol to form strongly fluorescent solutions, usually of greenish tint, which give in neutral solution a four-banded spectrum with a trace of a fifth band which is rendered more distinct by the addition of acid. Acid also changes the colour of the solution, and produces other changes in the position of the bands. As further characters are to be noted the power of yielding derivatives which may be soluble in water, are of bright colour, exhibit simple spectra consisting of one or two bands, and are without fluorescence in alcoholic solution. It is probable that it will be found that such derivatives are numerous.

Further, while the enterochromes may be eliminated intact from the body, as in Patella, the fact that forms containing them not infrequently also exhibit bright green pigments, soluble in alcohol without fluorescence, and often soluble in water in addition, suggests the possibility that the enterochromes may in some cases, instead of being eliminated, give rise to brightly coloured derivatives, capable of being employed in surface coloration.

The peculiar pigment bonellin resembles the enterochromes in most of its characters, but is entirely absent from the alimentary canal of the form in which it occurs. The same is true of the pigments Pentacrinin and Antedonin, and many others. Hence perhaps any reference to the "enteron" in the group-name of these pigments is misleading, and such a term as "polychromes" would be preferable.

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EXPLANATION OF PLATES 30 & 31,

Illustrating Marion I. Newbigin's paper "On Certain Green (Chlorophylloid) Pigments in Invertebrates."

Fig. 1.—Absorption spectrum of normal chætopterin for comparison with its derivatives, showing four distinct bands and a trace of the band to the right of D.

Fig. 2.—Spectrum of a blue acid solution of chætopterin, showing the result of an intermixture of the green acid derivative and acidified Chætopterin.

Fig. 3.—Spectrum of the acid derivative obtained by dissolving the precipitate from an acid solution in ether, and treating the ether with concentrated acid. The band to the right of the C line is the band of the derivative, the other two are due to the presence of traces of normal chætopterin.

Fig. 4.—Spectrum of a solution of chætopterin which has stood for some time with ammonia; the band between those near C and D indicates the presence of the ammonia derivative, the other four are the bands of chætopterin.

Fig. 5.—Spectrum of the alkaline derivative obtained by allowing chætopterin to stand for a prolonged period with dilute alkali. The solution no longer contains unaltered chætopterin.

Fig. 6.—Spectrum of a solution of "enterochlorophyll" obtained from the fæces of Patella; the solution contains little or no lipochrome.

Fig. 7.—Spectrum of the same solution after the addition of hydrochloric acid; note especially the position of the band in the red.

Fig. 8.—Spectrum of the acid derivative of enterochlorophyll intermixed with a trace of the original pigment. The spectrum corresponds to that figured in 3 for chætopterin, and the solution was obtained in a similar way. The second band is that of the derivative. The solution was too dilute to show the band corresponding to the third band of Fig. 3.

Fig. 9.—Section of visceral hump of Patella, showing the epithelial cells of the intestine with their pigment granules, and sections of the liver tubules. *i.* Intestine. *l. t.* Liver tubules.

Fig. 10.—A few cells from the intestine, more highly magnified to show the pigment granules. *n.* Nucleus. *p.* Pigment granules.

Fig. 11.—Section of liver tubule more highly magnified, showing the pigmented vesicles, *v.*, and the numerous oil-drops scattered through the protoplasm. *n.* Nucleus.

Note on a (? Stomatopod) Metanauplius Larva.

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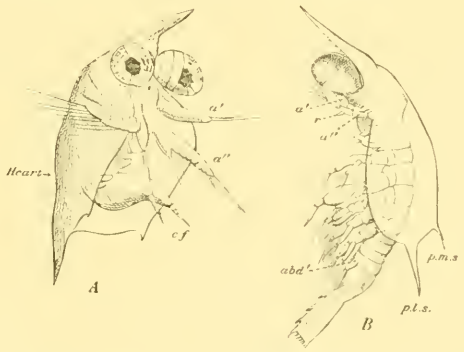
The larva to which I wish here to invite attention was caught in a tow-net out at sea, off the south coast of Tasmania,



A. The larva here described. (The right anterior antenna was omitted in the original drawing, and in order that the figure may be as far as possible a reproduction of this it is omitted here.)

B. The Erichthoidina stage of a Stomatopod. (From H. J. Hansen, Isopoden, Cumaceen, und Stomatapoden der Plankton Expedition, 'Ergebnisse der Plankton Expedition,' Bd. ii, G. c., pl. viii, fig. 14. *a'*, *a''*. First and second antennæ. *abd'*. First abdominal appendages. *c.f.* Caudal fork. *p.l.s.* Postero-lateral, and *p.m.s.*, postero-median spines (the latter is rather too large in *A*). *r*. Rostrum.

during daylight on December 25th, 1886. I had at that time very small acquaintance with larval forms of Crustacea, and

did not recognise the features of particular interest which this larva presented. Having made the drawing which is here reproduced (*A*) and a few notes, I paid no further attention to it. To my regret, I cannot now find the specimen. Although the evidence in my possession is thus very imperfect, I have, after some hesitation, decided to publish it, because it appears to throw some light, though far from a bright one, on an obscure corner of crustacean larval history.

As may be seen from the figure, the body is enclosed in a large transparent shield, produced anteriorly into a strong rostral spine, and posteriorly into a smaller median spine. On either side the lateral parts of the carapace fold round the body of the larva, and where the ventral and posterior borders meet, a small, backward pointing, postero-lateral spine is situated. A median eye is present beneath (in a ventral view) a low eminence, and two large globular compound eyes project on either side of the base of the rostral spine. Of the two pairs of antennæ, the first seems to have been simple, and the second is biramous, possessing a short endopodite, and a well-developed swimming exopodite, jointed and beset with long setæ. The body appears to have been unsegmented, and the posterior part is small, free from the dorsal shield, and, in the position drawn, strongly flexed ventrally. It terminates in a caudal fork, the divisions of which are articulated and setose. The dorsal region of the posterior part of the body was tinged with red. A note attached to the drawing calls attention to "rudimentary appendages" behind the second antennæ, and states that a heart was to be seen (in the position indicated) under the hinder part of the carapace. There is some indication in the drawing of an upper lip, between and a little behind the second antennæ, and the "rudimentary appendages" are shown to the number of perhaps three, between this and the flexed posterior part of the body.

It will, I think, be admitted that the larva is in the meta-nauplius stage. It seems improbable that the mandibles were really rudimentary, but the mandibular palp was at any rate inconspicuous, and two, perhaps, of the succeeding pairs of

limbs had already made their appearance. As far as the development of the limbs is concerned, the stage appears to correspond with that of Euphausia pellucida, represented by Metschnikoff in the 'Zeitschrift für Wiss. Zoologie,' Bd. xxi, plate 34, fig. 6. Further, it is clear from the character of the eyes that we have to do with a Thoracostracan form. It is, then, a Thoracostracan larva at about the metanauplius stage.

In the Cumacea the young leave the brood-pouch nearly in the form of the adult. The young of the Thysanopoda, among the Schizopods (as shown by Metschnikoff[1]), as well, probably, as those of the Decapod Peneus (Fritz Muller[2]) are hatched as nauplii, while those of Lucifer (Brooks[3]) appear as metanauplii. But in all these the paired eyes are absent in the metanauplius stage, and are not fully developed until a long and fully segmented abdomen has been formed. The carapace is without spines in the metanauplius stage, and though in Euphausia, and also in Lucifer, spines make their appearance in later stages, corresponding in position with those above described, their shape in Euphausia and the shape of the shield in both genera are markedly different.

The remaining Thoracostracan group is the Stomatopoda. In Squilla (Paul Mayer[4]) and Gonodactylus (Brooks and Herrick[5]) the eggs have been seen to hatch as Alima larvæ in a stage which has been compared with the Zoœa stage of Decapods. But, as was shown by Claus,[6] larvæ of Stomatopods also occur in another form, the Erichthus, of which stages are known prior to that at which the Alima larva is

[1] Loc. cit.

[2] "Die Verwandlung der Garneelen," Erster Beitrag, 'Arch. für Naturgeschichte,' Jahrg. 29, 1863.

[3] "Lucifer: a Study in Morphology," 'Phil. Trans.,' vol. clxxiii, 1882, p. 57.

[4] "Carcinologische Mittheilungen, IX," 'Mittheilungen aus dem Zool. Stat. zu Neapel,' vol. ii, p. 219.

[5] "Embryology and Metamorphosis of the Macroura," 'Memoirs of the Nat. Acad. of Sciences,' vol. v, p. 353.

[6] "Die Metamorphose der Squilliden," 'König. Gesell. d. Wissenschaften zu Göttingen,' Bd. xvi, 1871.

hatched. The youngest known member of this series of forms, the Erichthoidina, is represented in fig. *B*.

The carapace is furnished with spines resembling those of the larva shown in fig. *A*, and well-developed compound eyes are present. The first antennæ are obscurely biramous, while the second are uniramous. The thorax is distinctly segmented, and the five anterior segments bear biramous swimming feet, while the three following segments are without appendages. The first pair of abdominal feet are present, but behind the segment bearing them, the abdomen is unsegmented, and ends in a large truncated telson.

In succeeding stages the Erichthoidina larva changes into a Zoœa-like form comparable with the Alima larva of Squilla and Gonodactylus, but the stages which precede the Erichthoidina are unknown.

Now the development of the Erichthus larva differs from that of the Schizopod and Decapod larvæ with which we are acquainted, in one respect, among others, namely, that in a stage, the Erichthoidina, antecedent by many moults to the Zoœa stage, the paired eyes are already well developed. In this respect, as well as in the shape and large size of the carapace, the disposition and direction of its spines, and in the fact that it is a reduplication exclusively of the cephalic region, the larva under consideration resembles the Erichthoidina.

I would submit then, that it is rather probable that this larva is a Stomatopod larva at a stage prior to the Erichthoidina stage; that it is, in fact, a Stomatopod metanauplius.

The condition of the antennæ, the first apparently simple, and the posterior biramous, differs from that found in the Erichthoidina stage, but the difference is precisely that which from analogy with other nauplii we should expect to find in the metanauplius form. The first antenna of the stage figured by Hansen appears to be just acquiring its biramous character. The larva appears to be unique among those hitherto described in possessing well-developed compound eyes in the metanauplius stage. The articulated condition of the divisions of the caudal fork is also, so far as I know, peculiar

among the Malacostraca; and is interesting in view of the great prominence of these processes in that isolated and primitive Malacostracan form Nebalia, and in the Phyllopods Apus (in which again they are articulated) and Branchipus, with which Nebalia forms a connecting link.

On the Nephridia of the Polychæta.

Part II.—Glycera and Goniada.[1]

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With Plates 32—35.

In the following pages are described the nephridia and "ciliated organs" of the two closely allied genera, Glycera and Goniada. The observations on the first genus were begun on fresh and preserved material obtained at Naples in 1896 and 1898, where I examined the three common species, Glycera unicornis, Sav., Gl. siphonostoma, D. Ch., and Gl. convolutus, Keferst, continued on the coast of Normandy, where Gl. convolutus is fairly abundant, and completed here in Oxford. Unfortunately I have not been able to obtain living specimens of the genus Goniada, and the work on this form has been therefore entirely carried out on preserved specimens of the species G. emerita, Aud. and Edw., and G. maculata, Oerst.[2]

To Dr. Lo Bianco I am indebted for a fine specimen of the rare Goniada emerita, to Prof. Benham for a ripe specimen of G. maculata, and to Dr. Levinsen, of Copenhagen, Dr.

[1] Part I is published in vol. 40 of this Journal (1897), and contains an account of the nephridia of Nephthys.

[2] I am glad to have the opportunity of thanking Professor Dohrn for generously placing a table at my disposal last winter in the Stazione Zoologica, and to Professor Perrier for the kind way in which he received me in his laboratory near St. Vaast-la-Hougue last summer.

Appelöf, of Bergen, and Dr. Thiel, of Stockholm, for other specimens of the same species.

Glycera.

In this genus the "ciliated organ" is so closely connected with the true nephridium, although it does not actually open into its lumen, that the two structures have been confounded by Ehlers (2), the only observer who gives an account of the excretory organs of Glycera.

The nephridium, the ciliated organ, and a peculiar organ which I shall call the nephridial sac, are all united into a single structure which may for convenience be called the nephridial complex. Ehlers, in his well-known work on the Polychæta, described the position of this nephridial complex on the anterior surface of the septum accurately enough; but he failed to make out its real structure, partly no doubt owing to the fact that he studied it in spirit specimens only. What he describes as the duct of the segmental organ would appear to be, judging from his figure, the extended outer lip of the "ciliated organ;" and what he took for an internal opening may perhaps be another portion of the same. The nephridium itself escaped his observation; and, indeed, it is very difficult to make out in preserved specimens.

Before describing these organs in detail it is best to give a general account of their distribution, shape, and mutual relations.

On examining a fresh specimen of Glycera siphonostoma which has been opened up dorsally, and from which the gut has been removed, small round bodies can be seen on either side near the base of each parapodium, attached to the front face of the septum, and lying below and partially hidden by the bundle of chætæ with its muscles (fig. 1). One pair of these bodies occurs in every segment excepting the first few and the last one or two; in a full-grown specimen they appeared to be absent in the first twenty segments. Each body constitutes what I have called the nephridial complex, consisting of a hollow sac, the "nephridial sac," forming a flattened disc-like

organ, into which opens the "ciliated organ." The outer lip of the latter stretches out to the body-wall. The true nephridium is spread over the surface of the nephridial sac, and has no internal opening (cf. figs. 1, 3, and 30). The description applies to both sexes.

The Nephridium.—The structure of the nephridia in the three species of Glycera I have studied differs only in detail. The nephridium opens to the exterior ventrally by a minute pore, situated just outside the limit of the large bundles of ventral longitudinal muscles. The nephridiopore leads into a very slender canal, difficult to follow in sections, which passes through the body-wall into the septum above; then running inwards and piercing the septum, the canal reaches its anterior face, where it soon joins the nephridial sac. On arriving here the canal divides repeatedly, giving off branches which spread over the outer surface of the sac.

In Glycera convolutus, where the nephridium is small and the sac scarcely developed (figs. 13 and 21), the body of the nephridium forms a somewhat flattened pear-shaped mass applied to the ciliated organ. A fresh nephridium dissected out and examined in sea water appears to consist of a protoplasmic mass in which the lumen of the canal branches, forming a sort of sponge-work. Along the course of the canals are rounded diverticula or chambers, projecting towards the free cœlomic surface of the organ (figs. 13 and 12).

The wall of the lumen of the canal leading to the exterior, and of its main branches, is provided with long cilia (fig. 12); the smaller branches leading from the chambers are also ciliated, but to a less extent. By their action the cilia tend to drive a current from the chambers towards the external pore.

The protoplasmic walls of the nephridium are very granular, being more or less loaded with excretory matter in the form of granules or droplets, small and large, which often give the organ a yellowish colour. There are nuclei here and there, but I have seen no distinct cell outlines.

The outer wall or roof of the chambers projecting towards the cœlom is very thin, and arising from near the centre of

each is a " tube-bearing " flagellated cell, somewhat similar in structure to those I have described in Nephthys (4). Occasionally two or even three tubes may open into the same chamber (figs. 12, 14, and 21).

Each of these peculiar cells consists of a little rounded mass of finely granular colourless protoplasm, in which is placed the round nucleus, supported at the free end of a long conical tube. As in the case of Nephthys, so in Glycera, the nuclei of the tube-bearing cells have the property of staining very deeply and rapidly. The tube itself is formed of a thin layer of cuticular substance; it is flattened from side to side,[1] inserted by its narrow end into its cell, and by its broad end into the roof of the nephridial chamber. Thin longitudinal lines give it the appearance of being delicately fluted. A long flagellum attached to the cell at the apex of the tube works rapidly within the latter, reaching into the underlying nephridial cavity.

The tube-bearing cells rarely, if ever, stand alone. They are not ranged in rows as in Nephthys, but are dispersed over the whole surface of the nephridium in pairs, or in groups of three, four, or even five cells, resting against each other, no doubt for mutual support. In this position the cells form roundish masses without actually fusing, and project into the cœlomic fluid, standing on their tubes as on stilts. The tube is so delicate, and the entire apparatus so slender, that the mere action of the flagellum inside often makes the whole cell waggle backwards and forwards. In a teased preparation of a nephridium the tube-bearing cells may break away from the chambers, and move about in the fluid actuated by the long projecting flagellum: in this condition their resemblance to the collar-cells of sponges is most striking (fig. 5). For these peculiar nephridial cells bearing a tube and a flagellum, which I have hitherto designated by the cumbrous descriptive term " tube-bearing cell," both in the Nephthyidæ and in the Glyceridæ, I now propose the more convenient term solenocyte σωλήν, a pipe). As far as I have been able to make out

[1] The tube is shown in profile in fig. 14, at the left-hand lower corner.

there is no communication of the lumen of the nephridium with the cœlom, either directly or indirectly, through the nephridial sac, in this or any other species of Glycera.

Over the chamber-bearing surface of the actual nephridium there appears to be no regular layer of cœlomic epithelium. An occasional nucleus here and there may indicate its remains, but the nephridium seems to have made its way through the epithelium, as in the case of the nephrostome of an ordinary earthworm.

In the much larger species, Gl. siphonostoma, the nephridial complex is a structure of considerable size (figs. 3 and 30), visible even to the naked eye. Figs. 13 and 3 represent these organs in Gl. convolutus and siphonostoma respectively, drawn to the same scale. Here the nephridial sac is very much more developed, and the nephridium itself extends almost all over its surface, forming a sort of outer layer or shell (fig. 30). The system of branching canals is extremely complicated, and the number of chambers immense. Their structure is best studied in the very similarly developed species, Gl. unicornis.

The solenocytes (tube-bearing cells) in Gl. siphonostoma, though so much more numerous, closely resemble those just described in Gl. convolutus. Occasionally I have noticed little amœboid processes with thickened ends radiating from the cells (fig. 4); they remind one of the pointed protoplasmic processes originating from the tube-bearing cells of Nephthys (fig. 4), but appear to arise rather from the bases of the cells where these are applied to each other.

The third and last form which I shall describe, Gl. unicornis, is intermediate in size between the first two species. Here the sac is large, but the nephridium does not, as a rule, cover over its whole surface, being specially developed along the rim of the disc (figs. 2, 11, 16, and 26). There seems, however, to be considerable variation in the extent to which the nephridium spreads over the sac; in some specimens it forms a layer covering its whole surface, as in Gl. siphono-

stoma, whilst in others this is not the case. These different conditions may be dependent upon the state of expansion of the thin-walled underlying nephridial sac.

The structure of the nephridium can be very well studied in this species. The nephridial canal is divided into several branches which spread over the surface of the organ; the branches all converge towards and finally open into the canal leading to the exterior, and their lumen is provided with powerful long cilia (fig. 24).

Coming off from the inner surface of these branches are numerous secondary canals, which branch repeatedly, and form a network throughout the substance of the nephridium leading from one chamber to another (figs. 24, 29, 32, and fig. 15 of Gl. siphonostoma). The secondary canals appear not only to branch, but to anastomose; the canals coming from one main branch, however, do not seem to open into those coming from another. The system, except for the anastomosis, may perhaps be compared to a river, tributaries of which are separated by watersheds. Here and there the secondary canals lead up to the very numerous chambers into which open the tube-bearing cells.

In Gl. unicornis the solenocytes are generally distributed in pairs, never in groups, and are intermediate in structure between those of Nephthys and those of the species of Glycera described above. Instead of being entirely supported by the tubes, as in the latter, the cells are attached at their base to the wall of the nephridium (figs. 9 and 32) by a short stalk. The cell is more elongated, and a neck of considerable length bends round from the body of the cell to the top of the tube. Although in some specimens this neck is quite long, yet it is always much shorter than in Nephthys (fig. 10). The nucleus is large and oval.

Such is the structure of a typical tube-bearing cell in Gl. unicornis; but in many cases these cells seem to resemble those of Gl. siphonostoma (fig. 29). These apparent exceptions may possibly be due to the worms having been wrongly identified; I am inclined to believe, however, that

there is considerable variation in the structure of the nephridia, perhaps owing to the specimens being of different ages or in different stages of maturity.[1]

The Ciliated Organ.—The ciliated organ in Gl. unicornis and siphonostoma forms a considerable part of the nephridial complex. It is of essentially the same structure in both these species, and can be seen in a dissected specimen as a thick band running from the nephridial sac, in front of the septum, to the body-wall near the base of the parapodium (figs. 1 and 2).

This band is hollowed out on its upper and anterior surface by a groove which runs longitudinally along it, becoming deeper at its inner end, where it reaches the sac and passes into its interior (figs. 16 and 30).

Having entered the mouth of the sac, which aperture it entirely surrounds, the ciliated organ is bent back on itself so as to extend into the "cæcum," a region of the nephridial sac which will be described farther on.

Outside the sac the edges of the grooved ciliated organ are drawn out into two pointed flaps guarding the opening (fig. 3, *fl.*). This structure forms a sort of one-sided funnel, and from the free edge inwards extend slight ridges (fig. 3, *r.*), more conspicuous in living specimens, which probably represent in a very rudimentary condition the high ridges characteristic of the ciliated organs in so many forms, such as Nereis and Hesione (3 and 4).

The whole of the grooved surface of the organ is provided with a dense covering of cilia, by the action of which floating bodies are driven into the sac. The ciliated organ is formed on its inner surface of ordinary ciliated columnar epithelium, consisting of narrow cells with oval nuclei, which is continuous at the edges with the flat cœlomic epithelium, by means of which it is attached to the septum, muscles, and body-wall along its course (figs. 16, 17, 26, 27, and 28).

It should be noticed that the band-like grooved outer lip of

[1] I have found the typical fixed solenocytes in quite small and apparently young specimens of Gl. unicornis.

the ciliated organ varies considerably in development in different individuals. Not always does it reach the body-wall, and I am inclined to think, after examining a very large number of worms, that it is more developed in those animals which approach sexual maturity.

In the small species, Gl. convolutus, the ciliated organ is relatively little developed, without the pointed flaps on either side of the entrance into the nephridial sac, and never reaching the body-wall so far as I have been able to make out (figs. 13 and 21).

The Nephridial Sac.—As already mentioned, this interesting organ consists of a round flattened sac, over which the nephridium lies. It must be clearly understood that structurally, and no doubt also in its origin, it is quite distinct from the nephridium, and is only called "nephridial" because of its close connection with that organ.

It is, in fact, a hollow sac formed by a layer of flattened epithelium continuous with the epithelium of the ciliated organ, and communicating only with the cœlom by means of a single opening (figs. 2, 3, 16, and 30).

Just as the ciliated organ is to be considered as formed of differentiated cœlomic epithelium, so no doubt the sac is essentially a mere pouching of this same epithelium covering the nephridium at that region towards which the ciliary action of the ciliated organ converges—or, in other words, at the point where this organ accumulates the cells floating in the cœlom.

In Gl. convolutus the nephridial sac is quite a small chamber (figs. 13 and 21), into which the lip of the ciliated organ is produced, forming its wall on one side.

In connection with the quite anterior nephridia of Gl. unicornis and Gl. siphonostoma the sac is not much more developed than in the first species (fig. 11); but farther back it gets larger and larger, until it becomes a relatively huge and almost spherical structure (fig. 3).

Moreover these large nephridial sacs become differentiated into two regions—a main rounded sac which opens to the cœlom, and a diverticulum from the main sac, to the blind end

of which extends the lip of the ciliated organ (figs. 16, 17, and 30).

The latter division, which I shall call the cæcum, extends between the nephridial duct and the main limb of the ciliated organ. It is best developed in Gl. unicornis.

On examining this cæcum more closely its cavity is found to be subdivided by means of thin walls, formed apparently by folds of the epithelium, projecting inwards from the side opposite to that on which the ciliated organ is situated (figs. 19, 20, and 30). The chambers thus formed, resembling somewhat the cells of a honeycomb, are partially, but not entirely, cut off from the main cavity by roofing extensions of the walls (fig. 19).

At the blind extremity of the cæcum the chambers become small, and disappear near the ciliated epithelium. At the opposite open end they extend into the main cavity of the sac, becoming more and more shallow, and soon dying out (fig. 30).

The contents of the sac will be described below.

On the Functions of the Ciliated Organs, Nephridial Sac, and Nephridium, and on the Cœlomic Fluid of the Glyceridæ.—The functions of the organs forming the nephridial complex are no doubt connected with excretion. When the worm attains sexual maturity the ciliated organ probably acts as a genital duct; of this, however, I have no direct proof, and the discussion of the matter must be reserved for a future paper.

The nephridium itself is of course a kidney excreting waste matter. In freshly killed specimens the walls of this organ are found, as already mentioned, to be full of dark granules and paler droplets of varying size, which sometimes flow together, forming quite large drops embedded in the protoplasm. As might be inferred from the fact that the nephridium does not communicate with the cœlom, it seems to be entirely concerned in the excretion not of solid particles, but of substances dissolved in the cœlomic fluid. These appear to be stored up as granules and droplets, which are subsequently discharged into the lumen of the nephridial duct.

This view is supported by the following experiment:—If a living worm be injected with a mixture of Indian ink and carmine in sea water, and opened a few hours after, it will be found that of the mixture which has entered the cœlom the solid particles have been ingested by the amœboid cells, whilst the small quantity of carmine which was dissolved has been taken up by the nephridium. In such a specimen the nephridia are tinged a delicate pink colour, which can be distinctly seen with a lens.

The solenocytes do not appear to be in any way affected by the injection, and the pink colour is entirely due to the carmine having been deposited in globules occupying the same position in the nephridial cells as the yellow excretory matter in an uninjected specimen. Never have I found solid particles of carmine or Indian ink in these cells.

The nephridial sac, on the other hand, seems to be concerned with the elimination of solid waste products. Before discussing this question, however, I wish to make a digression on the subject of the cœlomic fluid of Glycera.

It is well known that in this genus there is no separate canalicular blood system, and that the cœlomic fluid contains numerous hæmatocytes, or round flattened nucleated cells, stained red with hæmoglobin.

In the cœlomic fluid of Gl. convolutus are found a large number of these round hæmatocytes deeply stained with hæmoglobin, a relatively small but yet considerable number of white amœboid cells, leucocytes, and a number of rather larger oval and flattened cells containing minute colourless granules (these cells are quite similar to those found in Gl. unicornis, shown in fig. 6).

On examining the cœlomic fluid of specimens which have been injected with carmine and Indian ink, it is found that the foreign granules have been taken up rapidly by the leucocytes, which soon become filled with them. No particles occur in the red or in the oval cells; these would appear, then, to be neither amœboid nor phagocytal.

In Gl. siphonostoma the majority of cœlomic cells are

faintly tinged with hæmoglobin and of very irregular shape, being generally covered with amœboid processes. Normal rounded hæmatocytes are occasionally present, especially in young specimens. The numerous processes on the hæmoglobinous cells give them a spiny appearance; but, as a matter of fact, the pseudopodia are not merely spine-like in shape, being really thickenings in thin expansions of protoplasm. When these cells are watched under the microscope the pseudopodia can be seen to begin as little rounded knobs, which gradually expand, spreading out in thin sheets, supported here and there by ribs or thickenings. It is these which, on a casual glance, have the appearance of freely outstanding processes (fig. 22). The granular oval cells occur also in small numbers, but the ordinary white amœboid corpuscles appear to be very rare or entirely absent in this species. On injecting a Glycera siphonostoma with carmine or Indian ink, we find that the granules are taken up in quantities by the hæmoglobinous cells, just as in the previous species they are absorbed by the leucocytes. The hæmatocytes are therefore both amœboid and phagocytal. This, so far as I am aware, is the first instance in which a free cell containing hæmoglobin has been shown to ingest foreign particles.

Gl. unicornis is, in respect to its cœlomic cells, intermediate between the two species described above. As a rule, in Gl. unicornis normal round hæmatocytes occur in the cœlom, together with a number of amœboid leucocytes and the usual granular oval cells (fig. 6). Such specimens, when injected, show that the particles of carmine or Indian ink are taken up exclusively by the leucocytes.

On the other hand, it may be frequently observed that the leucocytes are very rare, and that the hæmatocytes have a more or less pronounced tendency to produce pseudopodia. In accordance with this it is often found in injected worms that the particles have been ingested by the red hæmoglobinous cells.

I have not met with any evidence distinctly supporting the view that the hæmatocytes are modified leucocytes, or vice versâ; yet there seems to be no doubt that the functions of

ordinary leucocytes are assumed by the hæmatocytes in Gl. siphonostoma, and to a lesser extent in Gl. unicornis.

We can now return to the study of the function of the nephridial sac.

In the large species Gl. unicornis the nephridial sac is always more or less filled with a mass of cells (figs. 3, 8, 16, and 26), consisting of some hæmatocytes and a large number of amœboid cells. In Gl. siphonostoma the cells in the nephridial sac are almost all amœboid hæmatocytes. These cells are no doubt brought in by the action of the ciliated organ from the cœlom. They penetrate also into the cæcum, but in fewer numbers. In the sac are generally found masses of waste matter in the form of concretions, brown or yellow granules, and irregular aggregations, together with bits of chætæ and any other particles which may occur in the cœlom (figs. 7 and 25).

Similar but smaller aggregations of waste material may be found floating in the cœlom, especially towards the posterior end of the body; they are either actually within or surrounded by a number of amœboid cells,[1] which evidently move about as scavengers, and eventually find their way either singly or in masses into the nephridial sac.

The accumulation of such waste materials in the nephridial sacs gives these organs a brown or black colour in the posterior region of the body. The waste materials do not as a rule penetrate into the cæcum.[2]

Experimental proof of the account given above is afforded on examining specimens which have been injected with powdered

[1] I can confirm Cuénot's observation that in such cases the leucocytes secrete a chitinous substance round the foreign body, apparently as a protective measure. For instance, a broken piece of chæta will be found with the jagged ends covered over with concentric layers of secreted substance.

[2] The ciliated organ has already been described as producing a current from without inwards; occasionally, however, the action of the cilia appears to be reversed, leading from the cæcum outwards. The whole mass inside the sac is sometimes seen to rotate, and it is possible that this is the normal action of the cilia in the living worm. When the nephridia are dissected out and placed on a slide under a cover-slip they are naturally subject to pressure, which would be sufficient to impede the rotation.

carmine or Indian ink. In such worms the nephridial sacs are found to be crammed with cells loaded with particles of these substances. I have noticed that many of the loaded amœboid cells make their way into the cæcum.

There can be no doubt, then, that solid waste matters are accumulated in the nephridial sac. We may now ask what becomes of them when they have reached this cul-de-sac.

In connection with this question I may now describe another peculiar variety of cell which occurs in the nephridial sac and its cæcum. These cells are large, and generally of an irregularly oval flattened shape; they are distinguished by the possession of an immense number of minute colourless granules, giving the whole cell a characteristic greyish appearance (fig. 23). They are seen in numbers creeping over the inner surface of the nephridial sac (figs. 3, 18, and 19, *gr. c.*), and also in the secondary chambers of the sac and cæcum. In the latter position, indeed, they are always found, sometimes being flattened against the walls, and at other times piled up in rows (figs. 19 and 20, *gr. c.*). The nucleus of these finely granular cells is often of very remarkable appearance, being most irregular in form, like a hollow sphere, or very frequently bent round so as to form a horse-shoe shape (figs. 18 and 20).

Appearances have led me to believe that these cells originate in the cæcum, but I have never been able to find convincing proof of this supposition. They bear a great resemblance to the granular oval cells already described as occurring in the cœlom, and it is quite possible that they are really derived from this source.

Never are foreign particles ingested by them, and they do not appear to be at all of a phagocytal nature, although occasionally amœboid. The fine granules in them are evidently an endoplastic product, and I should suggest that the cells secrete a ferment which helps to dissolve the waste material in the sac. The matter in solution would then be carried through the wall of the sac to the nephridium to be excreted. However this may be, it is certain that the cells aggregated in the sac die and undergo degeneration; this can be clearly seen in sec-

tions through the organ, where cells and nuclei can be found in every stage of dissolution (figs. 26 and 25).

It would appear, then, that the ciliated organ and nephridial sac are concerned in the gathering up, through the agency of phagocytes, of the solid waste products found in the cœlom, whilst the function of the nephridium is to eliminate the soluble excretory material derived from the cœlomic fluid, and also perhaps from the sac. The function of the solenocytes or tube-bearing cells themselves is possibly analogous to that of the Malpighian capsules in the Vertebrate, namely, to excrete liquid, which presumably can pass by osmosis through the thin wall of the tube.

Goniada.

The Nephridium.—As in Nephthys and Glycera, so also in Goniada, the nephridium is an organ without opening into the cœlom, and with a branching termination provided with tube-bearing flagellated cells. The organ can best be studied in the large species G. emerita. It consists of a ciliated canal leading to the exterior, which, except near the internal end, is much wider than in Glycera. This canal passes through the septum and emerges on the anterior face, where it branches to form a lobed terminal organ almost exactly intermediate in structure between that of Nephthys and that of Glycera. For whereas in the latter this region spreads out to form a plate, the surface of which is evenly covered with nephridial chambers and scattered solenocytes, and in the former it divides into long branches with a regular row of solenocytes on either side facing each other, in Goniada emerita the terminal organ consists of massive short branches or lobes, on which the solenocytes are set in somewhat regular rows, generally, but perhaps not invariably, facing each other (figs. 33 and 35). These cells are also themselves of intermediate character: as in Nephthys, they are fixed at the base, have oval nuclei, long necks, and long tubes; but the tubes and necks are relatively short, resembling those described above in Glycera unicornis.

In G. maculata[1] the terminal organ of the nephridium is much smaller, and appears in section as little more than a bunch of solenocytes (fig. 39).

The Ciliated Organ.—It has been my good fortune to obtain a perfectly ripe male specimen of Goniada maculata, which proves, I think, beyond the possibility of doubt the fact that the ciliated organ acts as a genital duct or funnel,—in this species, at all events. This is all the more gratifying to me, since I suggested that this is its function when I first described this organ in the Lycoridea five years ago (3).

It will be remembered that Goniada is divided into two dissimilar regions, somewhat as the heteronereid phase of Nereis. Sections show that in the first few segments both nephridia and segmental organs are absent; that in the anterior region generally the nephridium is present, but the ciliated organ not at all, or scarcely, developed. It is only in the posterior region that this organ is fully formed in the adult. In these segments it takes the shape of a wide-mouthed funnel, a trumpet-shaped structure, the lips of which spread and gradually thin out on the septum. Here the thickened ciliated epithelium, of which the wall of the funnel is formed, flattens out and passes into the ordinary cœlomic epithelium lining the body-cavity (figs. 36, 39, and 31). Bunches of cilia may be seen on this epithelium some way up the septum (fig. 36).

The ciliated cells of the funnel are remarkably striated, and the cilia numerous and very powerful (fig. 40).

The funnel leads into a wide tube, which passes backwards to join the nephridial duct behind the septum (fig. 36).

The ciliated organ opens here into the lumen of the nephridial canal, the junction or grafting of the two organs being marked by quite a sudden change in the character of the tissues. Behind this point the nephridial duct widens out, and affords an easy outlet to the spermatozoa, which in

[1] The description applies to both sexes.

this specimen can be actually seen to pass out by its means (fig. 38).

In front of the communication between the two organs the nephridial canal passes forwards to the lip of the genital funnel, round which it runs inwards to the terminal nephridial organ beset with solenocytes (figs. 37 and 31). Nephridium and genital funnel are, therefore, really quite independent, except at the point of junction behind.

In other specimens, unripe males and females, the ciliated organ is much less developed, being represented by a scarcely opened funnel, the epithelium of which is composed of relatively small closely packed cells, often not yet provided with cilia. Posteriorly this rudimentary organ abuts on, but does not actually open into, the nephridial duct.

In the specimen of G. emerita, which shows no signs of maturity, the funnel is even less developed, being completely closed and entirely without cilia (figs. 33 and 34).

Owing to the unexpected discovery of nephridia with "tube-bearing cells" or solenocytes in the Phyllodocidæ, I have been obliged to postpone the general summary and theoretical conclusions of these researches to a third part, which I hope to publish in the near future. I may so far anticipate these conclusions as to point out that it has been shown in the foregoing pages that the "ciliated organ" is the morphological representative of the peritoneal or genital funnel of other Annelids, and probably of the Cœlomata in general.

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EXPLANATION OF PLATES 32—35,

Illustrating Mr. Edwin S. Goodrich's paper "On the Nephridia of the Polychæta."

List of Reference Letters.

ap. t. Aperture for the tube of the solenocyte. *b.* White amœboid corpuscle. *c.* White granular corpuscle. *ch.* Fragment of chæta. *cil. org.* Ciliated organ. *deg. nuclei.* Degenerating nuclei. *excr. gr.* Excretory granules. *fl.* Pointed flap of the ciliated organ. *gr. c.* Granular cell. *hæm.* Hæmoglobinous cœlomic cell. *int.* Intestine. *n.* Nucleus. *neph.* Nephridium. *r.* Ridge. *t. b. c.* Tube-bearing cell or solenocyte.

PLATE 32.

Fig. 1.—Inner view of a portion of Glycera siphonostoma opened along the mid-dorsal line and stretched out. The gut, part of the longitudinal dorsal muscles on one side, and one bundle of chætæ, have been removed to expose the nephridia.

Fig. 2.—View of the "nephridial complex" in two segments of a similarly dissected Gl. unicornis.

Fig. 3.—Nephridial complex of Gl. siphonostoma dissected out. The outer lip of the ciliated organ is torn off short. The contents of the nephridial sac are seen by transparency. Fresh. Cam. × 100.

Fig. 4.—Edge of the nephridium of a Gl. siphonostoma, showing the tube-bearing cells, or solenocytes, with small processes. Fresh.

Fig. 5.—Three solenocytes broken off free, from a teased nephridium of Gl. convolutus. Fresh. Cam. l. Oil im., oc. 3.

Fig. 6.—Corpuscles in the cœlomic fluid of Gl. unicornis. Fresh. Cam. × 400.

Fig. 7.—Optical section of the edge of the nephridial sac, and overlying nephridium. The "excretory mass" is composed of amœboid cells, degenerating cœlomic cells, and refuse; from a posterior nephridium of Gl. unicornis. Fresh. Cam. × 400.

Fig. 8.—Transverse section of Gl. unicornis. Cam. Z. aa, oc. 3.

Fig. 9.—A pair of solenocytes of Gl. unicornis. The flagella are seen by transparency in the tube and underlying chamber. Fresh.

Fig. 10.—Another variety of the same cells. Fresh. Cam. l. Oil im., oc. 3.

Fig. 11.—Nephridial complex of an anterior segment of Gl. unicornis. The nephridial chambers and canals are partially shown by transparency. Fresh. Cam. l. 4, oc. 3.

Fig. 12.—Portion of the nephridium of Gl. convolutus near the origin of the duct. Fresh. Cam. × 400.

Fig. 13.—Nephridial complex of Gl. convolutus. Fresh. Cam. × 100.

PLATE 33.

Fig. 14.—Surface of the nephridium of Gl. convolutus. Fresh. L. Oil im., oc. 3.

Fig. 15.—Diagrammatic reconstruction of a portion of the nephridium of Gl. siphonostoma, cut so as to show the course of the canals, &c. The inner surface would be applied to the wall of the nephridial sac.

Figs. 16 and 17.—Two sections through the same nephridial complex of Gl. unicornis (longitudinal through the animal). Cam. × 140.

Fig. 18.—Optical section of the edge of the nephridial sac (near the cæcum) of Gl. unicornis. Perenyi, paracarmine. Cam. l. Oil im., oc. 3.

Fig. 19.—Section taken almost transversely near the extremity of the cæcum of the nephridial sac of Gl. unicornis, showing the secondary chambers separated by thin partitions. Cam. l. Oil im., oc. 3.

Fig. 20.—Section along the cæcum of a similar specimen. Cam. × 400.

Fig. 21.—Section across the nephridial complex of Gl. convolutus. Cam. l. Oil im., oc. 3.

Fig. 22.—Two amœboid hæmoglobinous cœlomic cells of Gl. siphonostoma, fresh from a specimen eighteen hours after injection with Indian ink and carmine. Cam. × 500.

Fig. 23.—Three of the "granular cells" from the nephridial sac of Gl. siphonostoma. Fresh. Cam. × 400.

PLATE 34.

Fig. 24.—Portion of a preserved nephridium of Gl. unicornis seen from the outer surface. The tube-bearing cells are not drawn; the canals, &c., are seen by transparency. Cam. l. Oil im., oc. Z. 4 c.

Fig. 25.—Portion of a section through the nephridial sac of Gl. unicornis. Cam. Z. D, oc. 3.

Figs. 26, 27, and 28.—Three longitudinal sections of Gl. unicornis taken from within outwards, showing the nephridium, the nephridial sac, and the ciliated organ, extending on to the body-wall. Cam. × 200.

Fig. 29.—Portion of the nephridium from a section similar to that drawn

in Fig. 26, showing the canals, chambers, and solenocytes. An irregular cavity is left between the nephridium and the wall of the nephridial sac, *cav.* Cam.

Fig. 30.—Diagrammatic reconstruction of the nephridial complex of Gl. siphonostoma, showing the nephridial sac (represented as empty), the ciliated organ, and the nephridium (yellow).

Fig. 31.—Diagrammatic reconstruction of the nephridium (yellow) and genital funnel (ciliated organ) of a ripe Goniada maculata.

PLATE 35.

Fig. 32.—Portion of a section through the wall of the nephridial sac and overlying nephridium of Gl. unicornis. Cam. × 1600.

Fig. 33.—Longitudinal section through Goniada emerita (median region), showing the septa, nephridia, and undeveloped or rudimentary genital funnels. Cam. × 100.

Fig. 34.—More enlarged view of a portion of a similar section. Cam. × 400.

Fig. 35.—Section through the lobed inner extremity of the nephridium of G. emerita, showing the solenocytes on the surface. Cam. × 400.

Fig. 36.—Longitudinal section (lower half) of Goniada maculata, showing the genital funnels opening behind into the nephridial ducts. Cam. × 100.

Figs. 37 and 38.—Sections from the same series taken farther outwards, showing the course of the nephridium carrying the spermatozoa to the exterior. Cam. × 100.

Fig. 39.—Section from the same series taken farther inwards than Fig. 36, and showing the lip of the genital funnel and the inner extremity of the nephridium. Cam. × 100.

Fig. 40.—Enlarged view of the lip of the genital funnel and the underlying nephridial canal. Cam. × 400.

On Differences in the Histological Structure of Teeth occurring within a Single Family—the Gadidæ.

By

Charles S. Tomes, M.A., F.R.S.

With Plate 36.

Some years ago, whilst engaged upon an investigation which had a different object, I was struck by the fact that the teeth of various members of the Gadidæ presented marked differences in histological structure; but, partly from want of material and partly from other causes, I have only lately examined a sufficient number of the genera which are grouped together in this family to enable me to draw any conclusions about them.

It was, of course, only to be expected that fishes differing so much in respect of food, habits, size, and external form as the various Gadidæ should have dentitions which in naked-eye characters differ much from one another, and, as a matter of fact, the family shows examples of adaptive modifications of an interesting and very complete kind. And it is comparatively easy to see how these adaptive characters have arisen by gradual slight modifications of a type which runs through them all.

But it is not so easy to understand how the group of influences known as natural selection should have operated in the direction of producing differences of minute structure, for it would seem as though it mattered little what the histological structure of a tooth might be, as far as the exercise of its

functions goes, so long as it is sufficiently strong, sufficiently sharp, and of an appropriate shape. Hence it appeared to be a matter of interest to ascertain what the extent of this variation in structure is, and to see how far these differences are found to coincide with the lines of classification which have been adopted on general grounds.

As a preliminary to this inquiry I sought to ascertain how far the Gadidæ are to be regarded as a good natural family ; and being perfectly unable to myself form any opinion upon this point, I consulted Dr. Günther and Mr. Boulenger, who will be acknowledged to be in a position to speak with the highest authority.

They tell me that the Gadidæ are a well-marked and natural group, and therefore well adapted for the purpose of such an investigation ; to their kindness I am indebted for the opportunity of examining several genera not otherwise accessible to me.

The teeth of the Gadidæ all consist of that modification of dentine which is known as vasodentine proper, and, as there is still a little confusion in the application of this term by various authors, it seems desirable to very briefly define what the meaning of the term really is ; this I did some years ago at greater length (1).

Retzius (3) was the first to describe the tissue accurately, but he did not give to it a distinctive name. Owen (2), following in his footsteps, gave it the name of vasodentine, but at the same time included certain other forms of dentine which hardly come under the same category.

In 1878 (1) I endeavoured to carry further the existing descriptions, and to point out that the term vasodentine should be limited to those dentines in which, in the words of Retzius, the larger canals " formed, with others of the contiguous tubes, large loop-shaped anastomoses, and the outer extremities entered also into closed anastomoses, almost like the more minute blood-vessels in the villi of the abdominal canal."

I also pointed out that in the fresh state these canals contained capillary blood-vessels through which red blood circulated, so that a typical vasodentine tooth in the living

fish is quite red, and that, the canals being of just the size of the blood-vessels, they contained practically nothing else.

A typical vasodentine, therefore, consists of a matrix permeated by a rich plexus of blood-carrying channels, and contains no dentinal tubes whatever of the ordinary kind; the matrix is laminated, but is quite solid (4).

It would, however, be out of place to recapitulate here all the details which are, for the most part, to be found elsewhere, and it will suffice to describe those points only which are relevant to the title of the paper.

The teeth of the ling (Molva) may be taken as typical of that group in which the vascular system attains to its fullest development; they are long conical teeth, slightly curved and very sharp, and have large axial pulp chambers which are also elongated cones, and extend through the greater part of the length of the teeth.

From these pulp chambers the vascular canals run outwards, almost at right angles to the surface in the lower part of the teeth, whilst near to their apices they run obliquely upwards; they are disposed with the utmost regularity, and anastomose with one another to some extent.

They do not reach to the surface of the dentine, but all terminate in loops at exactly the same distance from the surface, the loops in which they terminate being flattened, so that the terminal canals lie parallel with the surface, and there is an appearance of a bounding channel parallel to the exterior of the tooth (figs. 1—3).

This disposition of the vascular canals is equally characteristic in the hake (Merlucius); in both fish the outer solid layer of the dentine shows a faint striation parallel with its surface, which appears to be due to slight lamination.

The apex of the tooth is kept sharp by a little spear-point of enamel, neatly fitted on to the dentine in such wise that it does not much increase its size, although itself of material thickness. This spear-point of enamel is possessed by all the family, and does not show much distinct structure, though in

favourable specimens fine lines may be seen to run through it more or less at right angles to its surface.

The ling (Molva) and hake (Merlucius) are the two genera in which the teeth are largest and most conspicuous ; they are also the genera in which the development of the vascular network is most complete. In the genus Gadus the arrangement of the vascular canals is somewhat different, the loops are more rounded, and the peripheral loops less flattened, so that there is no appearance of a circumferential vessel such as was seen in the ling and hake, and consequently the outer non-vascular layer of the dentine is less sharply marked off (figs. 5 and 6).

In the common cod (G. morrhua) the teeth are fairly large and are not very firmly fixed, having a certain degree of motion, though nothing approaching to the very definite hinge (4, p. 224) of Merlucius is to be found. The vascular network is rich, but it takes the form of isolated loops to a great extent (fig. 5) ; the lamination already spoken of exists, but is perhaps rather less marked than in the ling or in the hake, and there is the usual enamel tip to the teeth. The whiting pout (Gadus luscus) has (fig. 6) a similar structure in its teeth, as has also the poor cod (Gadus minutus), although in this latter the canals are less abundant (fig. 7).

But in the remaining species of the genus Gadus the reduction goes much further; thus the pollack (Gadus pollachinus) has dentine in which the vascular loops remain much more distinct from one another, and there are larger intervals of laminated matrix without any (fig. 8). In the haddock (Gadus æglefinus) the whole upper part of the tooth is often composed of finely laminated dentine without any vascular canals, while in the lower portion they do occur as sparse and isolated loops (fig. 9).

In the whiting (Gadus merlangus) the loops are present in about the same proportion as in the pollack, while in the coal-fish (Gadus virens) they are still more scanty, being confined to a few loops here and there (fig. 10).

Thus of the whole genus Gadus it may be said that in none do the vascular channels present the beautiful and complete

network characteristic of the ling and hake, and that within the limits of this single genus many stages in the reduction of the vascularity are exemplified, although in none has it wholly disappeared.

In the burbot (Lota vulgaris), the only fresh-water representative of the family, there is an interesting arrangement. This fish is generally regarded as closely allied to the ling, of which it is sometimes spoken of as the fresh-water representative. In it the vascular channels are few, and the upper part of the tooth is generally free from them, but where they do exist they present the flattened exteriors of the loops. They thus recall the circumferential canals of the ling, and differ from those teeth found in the genus Gadus, in which the canals are reduced to a similar extent. So far, then, as dentine structure goes, the relationship of Lota to the ling appears to be somewhat confirmed (fig. 11). A similar but richer disposition of vascular canals occurs in Brosmius (fig. 3).

In Lotella, usually regarded as allied to the hakes, the vascular canals have utterly disappeared, and the dentine presents no structure save a well-marked lamination (fig. 12).

To sum up the differences which have been so far described, they are of two kinds: the one the diminution in abundance of the vascular canals; the other a slight difference in their arrangement, consisting in the absence of the distinctly bounding canal which is formed in the cases of the hake and ling by great flattening of the exterior loops and their anastomoses with their neighbours. In each case, therefore, it is a difference of degree rather than of kind; nevertheless no one could possibly mistake the teeth of the hake or of the ling for those of the genus Gadus; and, as has been already pointed out, the difference in the form of the loops is still perceptible, even when they have become greatly reduced in number, as in the burbot (Lota).

The teeth which I have examined are those of the following genera:—Gadus, Merlucius, Molva, Lota, Lotella, Uraleptus, Phycis, Motella, Raniceps, and Brosmius; whilst of the genus

Gadus, the species æglefinus, luscus, minutus, tomcodus, merlangus, virens, and pollachius have been investigated.

These genera are usually arranged, with but little deviation on part of the various writers, in the following order (5):

1. Gadus.
2. Merlucius.
3. Lotella.
4. Uraleptus.
5. Phycis.
6. Lota.
7. Molva.
8. Motella.
9. Raniceps.
10. Brosmius.

Were we to attempt to classify them by their tooth structure alone we should arrive at something like the following arrangement:

2. Merlucius 7. Molva 10. Brosmius 6. Lota ? 4. Uraleptus	Highly developed systems of vascular canals, the outer non-vascular portion being sharply marked off by the "bounding canal:" in Brosmius the canal system is reduced somewhat, and in Lota it is disappearing.
9. Raniceps 1. Gadus 8. Motella	The vascular canals, abundant in G. morrhua, becoming less frequent in other species, but always retaining rounded external loops; in some species of Gadus they are sparse, and in Motella there are hardly any.
5. Phycis ? 4. Uraleptus 3. Lotella	The vascular loops are so reduced that little can be said as to their arrangement. In Lotella they are quite absent.

It will be noticed that the teeth of Brosmius (fig. 3, the torsk) partake pretty closely of the structural characters of those of the ling and the hake, whereas in the accepted classification it does not come near to them; and this is perhaps the most conspicuous instance of incompatibility between the tooth structure and the usually accepted affinities of the creature met with in the family.

For the dentines of Phycis and Motella (the rockling), figs. 15 and 16, which fish do not stand far apart from the ling and hake, have the vascularity so far reduced that its pattern can hardly be positively deciphered, though, so far as any evidence does exist, it appears to resemble that of the genus Gadus more closely than that of the ling.

It is, of course, fully recognised that no linear arrangement of genera and species can ever truly represent relationships, but still the classification by tooth structure departs somewhat more widely from that adopted upon general grounds for the deviation to be thus accounted for; whilst if we were to take into account simply the quantity of vascular canals present we should have still more discrepant results, for it would then come about that some species of the genus Gadus would be interspersed amongst other widely different genera, as is here shown.

2. Merlucius
7. Molva } (rich system of canals).
1. Gadus morrhua.
9. Raniceps.
10. Brosmius.
1. Gadus luscus.
1. Gadus minutus.
1. Gadus pollachius.
1. Gadus æglefinus.
6. Lota.
1. Gadus virens.
4. Uraleptus.
5. Phycis.
8. Motella.
3. Lotella (no vascular canals at all).

It is true that the genus Gadus has been sometimes divided into several genera, but even in that case they are very closely allied genera, and cannot possibly be regarded as interspersed amongst other genera with any propriety, so that the conclusion is irresistible that these differences of tooth structure only to a limited extent follow the lines of the general affinities of the

animals, and the question next presents itself, what lines do they follow?

The first thing that would suggest itself is that as the dentitions become less pronounced, and the individual teeth smaller, so does their intimate structure become simplified.

Some facts may be adduced in support of this view; thus the most elaborate tooth structure is found in the two genera which have the largest teeth, namely, the hake and the ling (figs. 1 and 2); while in the Lota (fig. 11), which is regarded as allied to the ling, the teeth are both much smaller and simplified in structure. Similarly, Gadus minutus, which has teeth large for the size of the fish, has a richer vascular network than many of the genus possess. But if this be a truth, it is only a partial truth, for, at all events in the same individual, the size of the tooth does not determine its structure.

For the hake is peculiar in having comparatively long and moveable gill-rakers upon its first gill arch, and these gill rakers are beset with tiny teeth; nevertheless these minute teeth are exactly, so far as space will allow, of the pattern of the large teeth which are twenty times their size, so that here at least reduction in size has not been accompanied by any simplification of structure (fig. 2*). Again Uraleptus (fig. 13) has teeth of quite considerable size, but they are almost devoid of vascularity, while teeth from its branchial arches exactly agree in structure with its large teeth (fig. 14).

A similar inference may be drawn from the teeth of the Chætodonts (4, p. 249) in which the teeth, although attenuated to an extreme degree, yet retain a full supply of vascular canals whenever there is room for them. Hence there seems to be some degree of fixity in the vascular pattern of dentine, and it does not seem to be quite lightly abandoned, as might at first be inferred from the fact that within the single genus Gadus so many grades of vascularity are met with. It seems possible that the fact that a large tooth like that of Uraleptus should present that absence of vascularity which pertains to the reduced dentitions, might be accounted for by the supposition that the ancestors of this genus had suffered

great reduction in their teeth, and that a more powerful dentition had again been evolved, without any recovery of the minute structure which belongs to the larger teeth of those which have never lost strong dentitions. The teeth round the margins of the mouth in Uraleptus are villiform, with large ones interspersed. However, these and similar speculations may be more safely indulged in by those who have a more intimate acquaintance with these fishes than I can pretend to, and it will suffice for me to merely point out the facts.

It may, perhaps, seem that in this communication a very small point has been laboured, but it appears to me that such facts as these are worthy of being recorded, for, so far as I know, very little has been done in the way of investigating the existence or non-existence of structural change in tissues under the influence of evolution. And the existence of these structural differences seems to me to have some bearings of a wide nature.

The teeth of the Gadidæ appear to furnish an argument against the adequacy of the purely mechanical theory of the evolution of tooth forms, so warmly advocated by Cope under the name of kinetogenesis, and adopted in its entirety by a large number of the American school of naturalists. For if the form of a tooth is the direct consequence of the direction of stimulation that it has received by use in successive generations, then a tooth which is subject to the very minimum of use, such as that of the gill-rakers of the hake, ought not to be so exact a copy of the teeth round the margins of the mouth. And if very considerable use be essential to the maintenance of elaborate structure, then we might expect, on the one hand, that the teeth in the gill-rakers of the hake should be of very simple structure, which is not the case, and, on the other hand, that the large teeth of Uraleptus, which must be held to be important in function, and so to receive the stimulus of use, should not have lost the structure typical for the family whilst retaining the size, and more, indeed, than the average size. Hence mechanical theories do not suffice to account for the structural degradation. For such it must be termed

when we get a dentine which neither presents a vascular canal system nor ordinary dentinal tubes, structures which a wider investigation and the observation of cases in which both co-exist, as in the teeth of Pleuronectidæ, seem to stand in a complementary relation to one another.

To sum up the results of these observations, true dentinal tubes are not met with in the Gadidæ. The spear-point enamel tip exists in all of the family, even upon the smallest teeth. Those members of the family which have the largest teeth, either fixed to the jaws by anchylosis (ling, some of the teeth of the hake) or by a highly elaborated hinge (the hinge-teeth of the hake), have the vascular canal system in its highest development. Those which have the teeth small in relation to the size of the animal, as happens in a large number of Gadidæ, and those in which the teeth are not very firmly attached, show also a simplification of the minute structure of the teeth. But mere smallness of size does not necessarily involve simplification of structure, as is exemplified by the tiny teeth of the gill-rakers of the hake. Nor does large size necessarily involve elaboration of structure, as is instanced by the large teeth of Uraleptus. On the other hand, large and small teeth in the same creature present identical structure; and whilst the differences in tooth structure in some instances follow the lines of the accepted classification of the genera, in others they do not.

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DESCRIPTION OF PLATE 36,

Illustrating Mr. Charles S. Tomes' paper "On Differences in the Histological Structure of Teeth occurring within a Single Family—the Gadidæ."

Fig. 1.—Apex of tooth of a ling (Molva) with its enamel cap. × 40.

Fig. 2.—Apex of tooth of a hake (Merlucius). × 40.

(Figs. 1 and 2 comprise about one fourth of the entire length of the teeth.)

Fig. 2*.—Portion of a moveable gill-raker from the first branchial arch of a hake. × 100.

Fig. 3.—Entire tooth of Brosmius. × 30.

Fig. 4.—Nearly entire tooth of Raniceps. × 50.

Fig. 5.—Portion of dentine of cod (G. morrhua). × 90.

Fig. 6.—Tooth of Gadus luscus. × 50.

Fig. 7.—Tooth of Gadus minutus. × 50.

Fig. 8.—Tooth of pollack (G. pollachius). × 50.

Fig. 9.—Tooth of haddock (G. æglefinus). × 60.

Fig. 10.—Tooth of coal-fish (G. virens). × 60.

Fig. 11.—Tooth of burbot (Lota vulgaris). × 80.

Fig. 12.—Tooth of Lotella. × 45.

Fig. 13.—Portion of tooth of Uraleptus. × 50.

Fig. 14.—Teeth from branchial arch of Uraleptus. × 80.

Fig. 15.—Tooth of Phycis. × 80.

Fig. 16.—Tooth of Motella. × 50.

A Description of Two New Species of Spongilla from Lake Tanganyika.

By

Richard Evans, B.A.

With Plates 37 and 38.

Contents.

I. Introduction.

The two species of Spongilla described in this paper were collected by Mr. J. E. S. Moore, of the Royal College of Science, during his visit to Lake Tanganyika, in the summer of 1896, and were taken at a depth of 350 fathoms.

Mr. Moore entrusted the material in question to Mr. E. A.

Minchin, Fellow of Merton College, Oxford, who in turn handed it over to me that I might study it. There were, besides four specimens preserved in alcohol and kept in separate bottles, a number of fragments which had been most carefully preserved, some in corrosive sublimate, and the rest in Flemming's fluid, and which were kept apart in separate tubes.

On examination all the material, with the exception of one small piece of that which had been preserved in corrosive sublimate, proved to belong to the same species. The small piece mentioned above must have been cut from a sponge which in its external appearance was almost exactly similar to the one to which the bulk of the material belonged, however different it may be in its skeletal characters, or else Mr. Moore would have noticed the difference. However great the external similarity, a single glance at a section suffices to distinguish them as belonging to two entirely different species.

I have given these species the names Spongilla moorei and Spongilla tanganyikæ respectively. The former species is represented by the bulk of the material, and is named in honour of its discoverer.

Of the latter I had but a small fragment, and have chosen its designation from its locality.

II. Description of Spongilla moorei.

(1) Habits of Growth and External Form.—Spongilla moorei grows on shells of various mollusca, and partially covers them as a crust. The upper surface is raised into lobes or mound-like elevations, which in no case are more than half an inch above the general surface, and which are usually no more than an eighth of an inch above the shallow depressions which separate them. The surface texture of the preserved sponge is somewhat woolly in appearance, though this is probably the result of the broken condition of the dermal membrane, for it has been observed that some of the fragments preserved in Flemming's fluid are smooth, and the spicules of

the skeleton, though supporting the dermal membrane, do not in the natural condition penetrate it.

An osculum is situated at the tip of each of the lobes or mound-like elevations of the surface of the sponge. This opening measures about an eighth of an inch in diameter, and underlying it there is a fairly large gastral cavity. The dermal pores are small, as usual, and are situated on the flanks of the lobes as well as in the intermediate depressions.

(2) The Skeleton.—In treating of the skeleton or the supporting part of the sponge, first, the spicules will be described; secondly, the arrangement of the spicules to form fibres, and of the fibres at large to form the skeleton; and thirdly, the spongin which binds the fibres together.

(A) The Spicules.—In order to facilitate description the spicules will be divided into three classes, the ordinary division into "megascleres" and "microscleres" being intentionally avoided, because it is—to say the least—doubtful whether the small smooth spicules are microscleres or young megascleres.

The three classes of spicules are—

(α) Diactinal monaxons which taper to a sharp point either gradually (amphioxea), or more rapidly (amphitornota), and are without swellings on their shaft. The former are always straight, the latter curved (Pl. 37, fig. 3).

(β) Similar straight amphioxea or curved amphitornota with distinct swellings on the shaft (Pl. 37, fig. 4).

(γ) Irregular systems formed by the fusion of spicules belonging to class α (Pl. 37, fig. 2).[1]

(α) The straight amphioxea taper gradually into a sharp-pointed end (Pl. 37, fig. 3, *a* and *b*), while the curved amphitornota, which are far more numerous, taper much more abruptly into a similar point (Pl. 37, fig. 3, *c*—*e*). Both the straight amphioxea and the curved amphitornota are highly variable in thickness, and exhibit all stages of development. The axial thread is of even thickness throughout its whole length in all these spicules.

(β) In addition to being slightly more slender than the

[1] Cf. Schulze and Lendenfeld's nomenclature (10).

spicules already described, the main feature of these spicules is the presence of a number of swellings, which varies from one to five. As a rule they are situated symmetrically with regard to the middle point of the spicule; that is, if there is only one swelling, it is situated at that point, but if there are two they are placed one on each side of that point, and at equal distance from it; and similarly the symmetry is retained when there are three, four, or five swellings. The absence of the symmetrical arrangement as seen in fig. 4, *d*, is very exceptional. The axial thread, in contrast to spicules of class *a*, present a dilatation corresponding to each swelling on the spicule.

(γ) The spicules in this class are of variable and irregular form, since the individual amphioxea or amphitornota which form them may fuse at any point, and at any angle (Pl. 37, fig. 2, *a—j*, especially fig. 2, *d*). As a rule, these compound systems are formed from spicules of class *a*, though occasionally a spicule of class β is found to take part in their formation.

With regard to their origin, two suppositions are possible; first, that they are the result of irregular growth, and branching of a single spicule derived entirely from a single scleroblast; secondly, that they arise by fusion of spicules primitively distinct, and formed each by its own scleroblast. Fig. 2, *e—j*, might be taken as evidence for the former view, but such forms as that represented in fig. 2, *d*, render such a supposition highly improbable, to say the least. The view that these spicular systems are of compound origin receives strong support from the way in which their axial threads cross one another instead of branching. If these irregularities arose as outgrowths from one spicule formed in one mother cell, it might well be expected that their axial threads should be also formed as outgrowths from that of the main spicule, and would therefore be continuous throughout the system, but this is certainly not the case in many spicules of our Spongilla, as can be seen from the figures. In another sponge, which is probably a monaxonid of the family Axinellidæ, viz. Tricentrium muricatum (Pallas, 1756), Ehlers, 1870 (=Plectronella

papillosa, Sollas, 1879) [11], there are branched spicules in which the axial threads are continuous throughout, a fact which may indicate that the spicules themselves owe their form to branching. It seems clear, therefore, that the irregular spicules of Spongilla moorei have in many cases been produced by fusion. Judgment must be suspended for the present with regard to those systems in which no discontinuity can be detected in the axial threads of the component spicule rays; such spicules may be simply branched. The question cannot be decided until the actual origin of the spicules has been studied, and the same may be said for Tricentrium. Since now it has been shown that the triradiates and quadradiates of the Ascons are formed by fusion, there is no inherent improbability in a similar process occurring in other cases [8].

Spicules of a similar character to the compound systems here described have been figured by many authors in various Spongillidæ (Spongilla aspinosa, Potts [9]; Lubomirskia intermedia, Dybowski [4]). All these authors regard them as abnormalities, but in moorei they are so frequent that they must be considered as a normal feature of the species. It is possible that in other Spongillidæ these systems have not received the attention they deserve.

In addition to the spicules described above, there are small masses of silica in Spongilla moorei, comparable with those found in Spongilla aspinosa (Pl. 37, fig. 5).

(b) The Arrangement of the Spicules to form Fibres, &c.—The spicules which form the polyspiculous fibres belong mainly to the first and third classes above described. Spicules of the first class form the greater part of the fibres, while others lie about in the sponge tissue, presenting for the most part an irregular method of arrangement, though many such spicules are placed so as to bridge over the spaces between the fibres in a perfectly definite way. Spicules of the second class, which are far less numerous than those of the first, seldom participate in the formation of the fibres, but, as a rule, lie scattered irregularly between the

fibres. The spicular systems of the third class are seldom found in any other position than in the fibres.

As a rule, the spicules are arranged in the fibres with their axes parallel to one another, and in the deeper parts of the sponge the connecting spicules are rather numerous, and more strongly developed than in the more superficial parts. The connecting spicules are usually the most strongly developed spicules in the whole sponge as regards size, differing, however, only in thickness from the smooth curved amphitornota which constitute the fibres (Pl. 37, fig. 3, *d—g*). Speaking generally, the largest spicules of the first class, together with a few of the second and all the third, form the fibres and the connecting links between them; while the smaller spicules belonging to the first, and nearly all those belonging to the second class, are scattered about irregularly in the meshes between the fibres. The smallest spicules of all appear to be absolutely independent of the skeletal meshwork, and this is the strongest argument that can be adduced in favour of the view that they are microscleres, and not young megascleres.

The arrangement of the skeleton at large is reticulate. The most prominent feature of the general conformation of the fibres is the way they pass from the surface of fixation of the sponge to the dermal membrane which they support. Along their course from one surface to the other they present a wavy appearance, often dividing and again reuniting, approaching the dermal membrane nearly always at right angles, and in many cases expanding into a kind of brush-like structure which supports that membrane (Pl. 38, fig. 6). In some of the largest lobes of the sponge the fibres nearest the centre pursue a straight course, while those furthest from that position curve outwards, so as to form supports to the dermal membrane which covers the flanks of these mound-like elevations. Owing to this arrangement a longitudinal radial section of one of these lobes presents an almost fan-like appearance as regards the skeletal fibres.

(c) The Spongin.—All the skeletal fibres of this sponge are enclosed in a distinct sheath of spongin, which is greatly

thickened at the points where the connecting spicules occur, these being either partially or completely surrounded by it (Pl. 38, figs. 6 and 7). Not only are the fibres and the connecting spicules enclosed in a sheath of spongin, but the surface of the sponge is covered by a thin layer or cuticle of the same substance, which dips down between the cells of the dermal membrane, and communicates with that which envelops the fibres (Pl. 38, fig. 6).

(3) The Canal System.—Owing to the fact that the material which had been preserved for histological study of the sponge had been shaken considerably in moving from place to place, a great number of cells had apparently become loose, and were found lying in the spaces of the canal system. In consequence it was impossible to make a complete and thorough study of that system, though individual cells were in many places nicely preserved; nor is Spongilla, for other reasons, a favourable object for the study of the canals in the Monaxonida.

The canal system in Spongilla moorei belongs to the type usually described as the third. The dermal pores, which are situated on the flanks of the mound-like elevations of the surface and in the intermediate depressions, are small, and open into the subdermal cavity, which is lined by flattened epithelium, and considerably reduced by the passing through of the skeletal fibres, which are enclosed in a sheath of spongin, which is covered by cells of the epithelial layer.

The inhalant canals which pass from the subdermal cavity into the chambers are narrow and difficult to make out. In some cases these canals are short, owing to the flagellated chambers being situated close to the floor of the subdermal cavity. Those canals which pass into the chambers which are situated more deeply in the sponge are long and narrow, following a winding course, and keeping nearly always between the chambers and the fibres of the skeleton. On their way down into the deeper parts of the sponge they give off branches which open into the chambers by way of prosopyles, which are so small that it is almost impossible to make them out. The apopyle was easily distinguishable as a wide opening, communicating

directly with the wide exhalant canals, and occupying nearly a fourth of the surface of the otherwise almost spherical flagellated chamber, which is lined by collar-cells with nuclei situated at their bases. The canals of the exhalant system are much wider than those of the inhalant, and, as a rule, occupy a central position between the fibres. As they pass down into the deeper parts of the sponge they converge and unite together, forming wider canals, which are few in number, and which open into the somewhat spacious gastral cavity, which communicates with the exterior by way of an osculum situated at the summit of each of the mound-like elevations of the surface.

(4) The Gemmule.—The gemmules, which are few in number and scattered about singly, are spherical in shape and small in size, measuring only ·35 mm. in diameter. They possess a thin coat, which is not surrounded by spicules specially characteristic of the gemmule, but by the ordinary skeleton spicules. Their cellular contents present the same characters as do those of the common species of Spongilla, and each individual cell is full of the two kinds of granules which are quite characteristic of the cells of Spongillid gemmules. It is just possible that had the material been preserved later in the year the gemmules would have been more numerous, though there would appear to be no absolute necessity for the production of gemmules, since the sponge lives at a depth of 300 fathoms, and cannot possibly be either dried or frozen.

III. The Affinities of Spongilla moorei.

The presence of the gemmule is the most important character tending to fix the position of Spongilla moorei among the Spongillidæ. Gemmules have been described in marine sponges, and this fact diminishes the importance of the existence of gemmules in a newly discovered sponge as a character supposed to be distinctive of the Spongillidæ (Topsent [12]). It appears that there is no special feature in the structure of the skeleton of Spongilla moorei that would cause it to be separated from the Chalinidæ had it been a marine sponge. It most decidedly

possesses more spongin than the Spongillidæ are usually supposed to have. As a matter of fact, it is difficult to make out what structural reasons there are for retaining the family Spongillidæ. It is not at all improbable that when they are more carefully studied they will be distributed among the several genera of the Homorrhaphidæ. But as our knowledge has not yet attained a stage which will enable us to do this, it is deemed advisable for the present to place this new species among the Spongillidæ, and to retain that assemblage of sponges as a family, however artificial it may be.

The characters of the gemmule of Spongilla moorei place this species among the sub-family Spongillinæ, and not among the Meyininæ or the Lubomirskinæ. They lack the amphidiscs which surround the gemmule of the Meyeninæ, while, on the other hand, the Lubomirskinæ is a sub-family which has been created for the purpose of including certain fresh-water sponges in which, up to the present, the gemmule has not been discovered.

Spongilla moorei appears to be more closely related to Spongilla aspinosa (Potts) than to any other species of the Spongillinæ. Both species agree in possessing spicules which are smooth, straight or curved, and for the most part rather abruptly pointed. Malformed spicules, as they are described by Potts [9], are found in both, but they appear to be more numerous and more complicated in Spongilla moorei than in Spongilla aspinosa. Further, both species produce gemmules which are small in size, spherical in shape, and supplied with a thin crust which is not protected by spicules characteristic of the gemmule, but by the ordinary skeleton spicules. Though the gemmules are few in Spongilla aspinosa, they are more numerous than in Spongilla moorei, a feature which may be explained either by the lesser importance and consequent scarcity of the gemmule in the latter species, or simply by the season at which the material was collected.

Spongilla aspinosa differs from Spongilla moorei in that it possesses small flesh spicules, which lie on the dermal membrane and among the smooth, slender skeleton spicules.

These small spicules are not found in Spongilla moorei, unless they are represented by those drawn in Pl. 37, fig. 3, *j—l*, and fig. 4, *j—n*, which is probably the case. However, it must be admitted, as has been done by Potts, that in both cases these small spicules may be young megascleres, and not microscleres. The only distinction obtaining between megascleres and microscleres, viz. that the former are bound up in the general skeleton of the sponge while the latter lie scattered about freely, is a functional rather than a morphological character, and seems to break down in the Spongillidæ, whose Homorrhaphid ancestors were probably without microscleres. The consequence of this is the impossibility of deciding definitely the true character of certain spicules. It seems, however, a safe conclusion that these small spicules are the same in Spongilla moorei as in Spongilla aspinosa, though in the former they are not found in the dermal membrane, their place being taken by the cuticular layer of spongin which covers the surface.

The form of growth of these two species appears to differ. Spongilla aspinosa is provided with long, slender, cylindrical branches which occasionally subdivide. These branches grow from a thick basal membrane. Spongilla aspinosa, however, at times forms merely a sheet which envelops the support on which it grows, while Spongilla moorei in all the specimens examined presented this appearance.

The spongin has not been described in Spongilla aspinosa, and therefore neither comparison nor contrast is possible.

The colour of Spongilla aspinosa is said to be green, a fact which is the result of the position in which it grows, for Spongilla lacustris and Ephydatia mülleri and fluviatilis may be either green or pale, according as they grow in direct sunlight or in the shade. Owing to the depth at which Spongilla moorei lives, the green colour of Spongilla aspinosa would appear to be wanting.

IV. Description of Spongilla tanganyikæ.

Owing to the fact that there was but a small piece of this sponge among the material presented to me for investigation, is impossible to make any statement with regard to its external form and habits of growth. However, it may be conjectured that in both respects it must have resembled Spongilla moorei, since Mr. Moore failed to detect it as a distinct form. Though the two species are probably similar to one another in their habits of growth and external characters, they are strikingly dissimilar in the characters of their individual spicules, though the general arrangement of the spicules in the fibres and of the fibres at large is strikingly alike.

The description of this species must, of necessity, be brief. The same plan will be followed as far as possible as in the case of the description given of Spongilla moorei.

(1) The skeleton will be described under the following heading:

(A) Spicules.
(B) Arrangement of spicules, &c.
(C) Spongin.

(A) Spicules.—It may be safely stated that there are megascleres and microscleres in this sponge. The megascleres consist of amphistrongyla and amphitornota, which are for the most part thickly covered with small spines. In addition to these there are a few smooth or sparsely spined amphioxea (Pl. 38, fig. 10, *a*, *f*, *g*, *h*—*l*, and *o*—*q*). A few irregularly shaped spicules, which appear to be the result of fusion, are present (Pl. 38, fig. 10, *m*). The microscleres are much slenderer than the megascleres, though they almost equal them in length. They are always smooth and slightly curved (Pl. 38, fig. 10, *n*).

(B) The General Arrangement.—The arrangement of the spicules does not differ materially from that already described in Spongilla moorei. The spiny amphistrongyla and amphitornota, together with a few smooth or sparsely spined amphioxea, are arranged with their axes parallel to one another

to form the skeletal fibres. These divide and again reunite, producing an arrangement which is usually described as being reticulate. The fibres are connected together in many places by spicules which bridge over the intermediate spaces. These spicules are the largest in the whole sponge, as a rule, as was found to be the case in Spongilla moorei. In addition to these there are many spicules, both spiny and smooth, which appear to lie about more or less freely in the tissues. The slender microscleres are nowhere connected with the fibres, but lie absolutely free in the tissues.

(c) The Spongin.—The spongin is not so highly developed in Spongilla tanganyikæ as in Spongilla moorei. The former, therefore, in this respect resembles more closely the ordinary species of the Spongillidæ than the latter appears to do. The spongin does not appear to extend to the surface, and the layer which covers the fibres is correspondingly thin. The greater development of spongin occurs at the points where the fibres branch or reunite, and at the places where the connecting spicules penetrate the fibres.

2. The Gemmule.—Though there was but a small piece of this sponge it happened to contain several gemmules. These are devoid of special spicules, but are surrounded by the ordinary skeletal spicules and the microscleres. They possess a thin coat as in Spongilla moorei, and are spherical and of small size. As regards their cellular contents they present the ordinary characters of the Spongillid gemmule.

V. The Affinities of Spongilla tanganyikæ.

This subject must be considered from two aspects. In the first place, the characters of the gemmule must be taken into consideration, since the grouping of the Spongillidæ into the three sub-families, Spongillinæ, Meyeninæ, and Lubomirskinæ, and the division of the sub-families into genera, usually adopted, depends on these characters. In the second place, the spicules are of great importance as presenting a close resemblance to the spicules of Lubomirskia intermedia var. *a* (Dybowski, cf. pl. iv, fig. 3, *b*, [4]), which belongs to the sub-family Lubomirskinæ.

(A) The Gemmule.—The gemmule of Spongilla tanganyikæ lacks the amphidiscs which surround the gemmule of the Meyeninæ. It therefore appears that this species cannot belong to that sub-family. But it equally lacks the small spicules which are usually found in close relation with the gemmule of the Spongillinæ. Potts, however, places Spongilla aspinosa among the Spongillinæ, in spite of the fact that its gemmules lack characteristic spicules. If this arrangement be followed, the absence of such spicules from Spongilla moorei and Spongilla tanganyikæ should not be considered as a barrier against including these species among the Spongillinæ. But the inclusion does away with the importance of the presence of special gemmule spicules as a sub-family character.

The thin coat of the gemmule resembles that found in Spongilla moorei, Spongilla aspinosa, and others of the Spongillinæ, and has no similarity to the thick coat of the gemmule of the Meyeninæ. The characters of the gemmule, therefore, as far as they go, point to this new African species discovered by Mr. Moore being one of the Spongillinæ.

(B) The Spicules.—It is generally stated that the skeletal spicules of the several species of the Spongillidæ have no characters of higher than specific value. It is difficult to make out from the literature of the family how far such a statement is justified. However, the spicules of Spongilla tanganyikæ possess such characters that it is almost impossible to believe that they have not a wider application. This sponge, considered from the point of view of the skeleton, seems to present a certain amount of affinity with a few species of the Spongillinæ on the one hand, and of the Lubomirskinæ on the other.

The megascleres of the greater number of species arrayed under the sub-family Spongillinæ are sharp-pointed,—that is, they are either amphioxea or amphitornota. There are, however, a few species which possess spicules with rounded ends, that is, amphistrongyla. The species in question are Spongilla nitens (Carter [3]), Spongilla böhmii (Hilgendorf [5], and Spongilla loricata (Weltner [13]), to which may be added Spongilla tanganyikæ, now described for the first

time. Spongilla tanganyikæ, therefore, seems to be more closely related to these species, so far as the characters of the skeleton are concerned, than to any other species of the Spongillinæ. Of the three species named above, it appears to present closer affinity with Spongilla böhmii than with either of the other two, for in Spongilla nitens and in Spongilla loricata the amphistrongyla are smooth, while in both Spongilla böhmii and Spongilla tanganyikæ they are spiny. In the former the spines are more thickly set at the end, which is a special feature of the megascleres of some species of the Lubomirskinæ, and which may point to a certain amount of affinity in that direction, while in the latter they are evenly distributed over the whole spicule. In Spongilla böhmii the megascleres are curved as in Spongilla nitens, Spongilla loricata, and most of the Lubomirskinæ, while in Spongilla tanganyikæ they are straight. However, there is among the Lubomirskinæ a variety of Lubomirskia intermedia, described by Dybowski as var. *a*, in which the spicules are spiny and almost straight. The spines are evenly distributed, and the ends of the spicules in many cases present the amphistrongylote character. Another feature of Lubomirskia intermedia agreeing with Spongilla tanganyikæ is that the microscleres are smooth, and almost equal the megascleres in length. In Lubomirskia baicalifera and Lubomirskia papyracea the spicules are Amphistrongylote, though in the former the spines are arranged at the ends of the spicules, in contrast with those of Spongilla tanganyikæ, but to a certain extent agreeing with those of Spongilla böhmii, while in the latter the spines are evenly distributed over the shaft of the spicule, in contrast with those of Spongilla böhmii, but similar to those of Spongilla tanganyikæ.

From these points of comparison it seems that Spongilla tanganyikæ as well as Spongilla böhmii must be closely related to the Lubomirskinæ. Had it not been for the presence of the gemmule in the small piece of Spongilla tanganyikæ at my disposal, I would certainly have placed it among the

Lubomirskinæ. On the other hand, were gemmules to be found in any species of the Lubomirskinæ, it would have to be removed from that sub-family as at present defined. Consequently I venture to suggest that the sub-family Lubomirskinæ should be abolished, and the species contained in it placed under the Spongillinæ, which then could be rearranged into a number of genera according to the characters of the megascleres.

Appendix on some Sponge Spicules found in the Mud of Lake Tanganyika.

Along with the sponge material which Mr. Moore entrusted to Mr. Minchin, there was a microscopical slide with some of the mud of Lake Tanganyika mounted on it. There were on the slide, among other things, some sponge spicules which in shape resemble those of the genera Uruguaya (Carter [3]) and Potamolepis (Marshall [7]). They vary from ·14 to ·31 mm. in length, and from ·013 to ·05 in breadth. A great number of intermediate stages between the two extremes are present. Some of the spicules seem to be "micropunctate." They are nearly always curved, though the amount of curvature varies considerably. The smallest spicules are of even thickness throughout, being amphistrongylote. The spicules of intermediate size in some cases present the same form as the small ones, but differ in other cases in that they are slightly swollen at the ends. The largest spicules are in all cases club-shaped. In passing from the smallest spicules to the largest there seems to be a gradual change from amphistrongyla to amphitylota (Pl. 38, fig. 11).

It is difficult even to suggest what these spicules are. From their characters they might well be the spicules of a species belonging to the genus Uruguaya. But as the species of this genus belong to the New World, and those of Potamolepis to the Old, their locality seems to favour the view that they are the spicules of some Potamolepid sponge. The variation in size increases our difficulty, for it is impossible to decide whether the smallest forms are young spicules, or a different class of spicules, belonging either to the same or to an entirely

different species. If, however, the largest spicules are looked upon as fully developed forms, and the smallest ones as immature forms, the size of the normal spicule, that is the fully developed form, agrees with those of Potamolepis leubnitziæ rather than with the spicules of any other Potamolepid sponge. Still there is a real difference in that these spicules are distinctly swollen at the ends, while those of Potamolepis leubnitziæ are not. It seems, though closely resembling the spicules of the species mentioned above, that the spicules now discussed may belong to another species. The matter, however, must be left open for the present, as the above feature, when considered alone, is not a sufficient reason for the formation of a new species.

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EXPLANATION OF PLATES 37 and 38,

Illustrating Mr. Richard Evans' paper "On Two New Species of Spongilla from Lake Tanganyika."

The figs. 1—8 on Plates 37 and 38 refer to Spongilla moorei, figs. 9 and 10 on Plate 38 to Spongilla tanganyikæ, and fig. 11 to Potamolepis from the mud of Lake Tanganyika.

PLATE 37.

Fig. 1.—(× 1.) Spongilla moorei growing on a molluscan shell.

Fig. 2.—(*a*—*c* and *c*—*m* × 300; *a'*, *b'*, *c'*, and *d* × 750.) A number of irregularly shaped spicules showing both the variety and the irregularity of form which they present. The spicules *a'*, *b'*, *c'* represent *a*, *b*, and *c* on a larger scale of magnification, and together with the spicule *d* show the relation which exists between the axial threads in these compound spicules.

Fig. 3.—(× 300.) Amphioxea and amphitornota without swelling. *a*, *b*, and *l*. Straight amphioxea. *c*—*k*. Curved amphitornota. *j*, *k*, and *l* are possibly microscleres, or they may be young megascleres.

Fig. 4.—(× 300.) Amphioxea and amphitornota with swellings. *a*. Straight amphioxea with four swellings. *b*—*l*. Curved amphitornota with a variable number of swellings from one to five. *d* shows the swellings asymmetrically arranged. *h*—*n*. Either microscleres or young megascleres with one swelling.

Fig. 5.—(× 750.) Masses of silica found in Spongilla moorei, varying both in size and shape.

PLATE 38.

Fig. 6.—(× 200.) The skeleton near the surface as seen in section. The spongin is shown forming a thin layer at the surface and covering the fibres in the interior.

Fig. 7.—(× 800.) A portion of two fibres with a large connecting spicule and a much finer one with a swelling. The spongin is greatly thickened at the points where the connecting spicules enter the main fibres.

Fig. 8.—(× 45.) The skeleton as seen in a section passing from the base to the upper surface. A portion of the shell on which the sponge was growing is seen at the bottom of the figure.

Fig. 9.—(× 200). A portion of the skeleton of Spongilla tanganyikæ seen in section near the surface, showing the main fibres formed almost entirely of spiny spicules, while both spiny and smooth ones are found scattered about irregularly.

Fig. 10.—(× 400.) Spicules of Spongilla tanganyikæ. *a—f*. Straight spiny amphistrongyla. *g* and *j*. Straight, spiny amphitornota. *h*, *k*, and *l*. Straight, sparsely spined amphioxea. *o*, *p*, and *q*. Straight, smooth amphioxea. *m*. Irregularly shaped, spiny spicule. *n*. Microsclere.

Fig. 11.—Potamolepis spicules from the mud of Lake Tanganyika. *a* and *b*. Two large spicules with a curved shaft and swollen ends, i. e. curved amphitylota. *c*, *d*, and *e*. Three intermediate spicules with curved shaft and rounded ends, but not swollen, i. e. curved amphistrongyla. *f* and *g*. Two small spicules similar to *c*, *d*, and *e*, i. e. amphistrongyla.

On Tetracotyle petromyzontis, a Parasite of the Brain of Ammocœtes.

By

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With Plate 39.

Whilst I was working as a student in the Department of Comparative Anatomy at Oxford, in the Histology Class of Mr. G. C. Bourne, my attention was drawn to a very extraordinary appearance in some sections of the brain of Ammocœtes. Examination of sections of several different individuals revealed a constancy in the appearance at first sight suggestive of some highly complex abnormality, but a more thorough examination of series of sections resulted in the discovery that I had to deal with a parasitic form, which eventually proved to be a Holostomid trematode.

Now the occurrence of a Trematode in the brain cavities of a vertebrate is a unique phenomenon, occurring, so far as I can discover, in no other case, and it therefore seems desirable to publish some sketches and a description of the structure of the parasite.

At the outset I wish to tender my thanks to Mr. G. C. Bourne for his help during the early stages of the work. My best thanks are also due, and cordially given, to Professor Ray Lankester, in the stimulating atmosphere of whose laboratory the work was done. Professor W. B. Benham, recently lost to Oxford, gave me much assistance throughout my work, and I am especially grateful to him for drawing my

attention to some obscure references of great importance shortly after I had begun to work on this form.

In O. F. Müller's 'Vergleich. Anat. der Myxinoiden' a statement occurs to the effect that the fourth ventricle of the brain contains many Diplosomids, and this is the earliest statement yet found. My discovery therefore is not new.

George Gulliver, in papers dealing with the blood-corpuscles of the lamprey published nearly thirty years ago, noticed these forms, and stated their occurrence. He promised a further description, but, so far as I can discover, did nothing more than give them the rather fancy name of Neuronaia lampetræ. The genus Neuronaia (or Neuronaina[1]) was founded by Goodsir for a parasitic form from the nerves of a shark which he called Neuronaia munroi, and Gulliver's association of his species with this appears to have no justification beyond a distant similarity of habitat.

The name Neuronaia is in every way an undesirable one. The form here described is immature, and has therefore no right to be considered even a new species till its adult form has been determined. This I have, unfortunately, not been able to do, but it probably belongs to an already named Holostomid genus. In the second place, it is now the custom to call such immature forms by the generic name of Tetracotyle, and I propose therefore, in order to bring this form into harmony with present-day nomenclature, to call it Tetracotyle petromyzontis. The most confirmed devotee of the laws of priority can scarcely object to this, seeing that Gulliver gave the form a name without attempting to determine its real position. If every fancy name is to be retained, all hope of reducing our zoological nomenclature to a simple uniform system must be abandoned.

I may here point out the remarkable similarity in appearance between Tetracotyle petromyzontis and the form known as Diplostomum volvens.[2] Indeed, at first I was

[1] There appears to be some irregularity in the spelling of this word.

[2] Nordmann's figure of Diplostomum volvens is reproduced in the 'Cambridge Natural History,' vol. ii, p. 64, fig. 31.

almost inclined to regard it as the same. Seeing, however, that species of these Trematodes are determined a great deal by the structure and arrangement of the generative organs, it is, of course, possible that two forms might bear a close resemblance to one another in their early stages, and yet belong to different species. On the whole, a separate name seems the most desirable. A list and descriptions of these immature forms is very much needed as a supplement to G. Brandes' admirable work on the Holostomeæ.

Occurrence.—Tetracotyle petromyzontis is found in great numbers in the brain cavities of Ammocœtes. I have examined many specimens from the river near Oxford, and in only one case have found it absent. Last April I found a number of young lampreys in a stream in Sussex, but not a single parasite was present in them. Gulliver states that they are never absent from the brain either in the young or adult conditions, but he does not say whence he obtained his lampreys. Kupfer, Shipley, Gaskell, and others make no mention of any parasites in the brain, and it seems to me probable that Gaskell would have seen them if present. I believe a good many of his lampreys came from Surrey. For myself I have never found Tetracotyle petromyzontis in the adult lamprey nor in any lamprey of greater length than six to seven inches. Its distribution therefore does not seem to be as universal as Gulliver thought, and I can hardly believe that many competent observers would have overlooked it if present in the lampreys examined by them.

In many Ammocœtes the brain is bulged out completely by these tiny animals, and I have sometimes seen them squirted eighteen inches into the air when the roof of the brain is divided suddenly with a sharp knife. They are found mostly in the region of the fourth ventricle, and especially under the choroid plexuses between the foldings of the roof of the brain, whence they apparently draw their nourishment.

In spite of all this, the Ammocœtes appear to live on quite comfortably and to suffer no great inconvenience, although the choroid plexuses of the brain often have an inflamed

appearance when the parasite is present in large numbers. I have kept several Ammocœtes alive for nearly three months, during which time they have lived and grown quite normally. On killing and opening, the brain was found packed full of parasites.

Tetracotyle petromyzontis will live for some time outside the brain; Gulliver says for several days, but mine have never lasted more than three hours. Whilst alive they fix themselves by the ventral sucker, and, contracting rhythmically, undergo rapid changes of shape. Outline sketches to illustrate these changes are seen on Pl. 39, fig. 2.

External Characters. — Tetracotyle petromyzontis has an average length of about ·42 mm. It possesses a typical fluke-like form. At the posterior end of the body, which is slightly broader than the anterior, the rudiments of the tail, in which the adult generative organs are developed, are visible. The mouth is at the anterior end, and is surrounded by the oral sucker. On each side of it are two retractile ear-shaped projections with which numerous gland-cells are connected. About midway on the ventral surface is situated the ventral sucker, and just posterior to this the large glandular adhesive organ. At the posterior end of the body is the excretory pore.

The intestine, which is easily seen under a low power of the microscope, is Trematode in character. Some little distance behind the mouth it expands into a powerful pharynx which seems to perform a kind of pumping action during life. Behind the pharynx the intestine forks, each fork ending blindly just posterior to the glandular adhesive organ.

The body appears filled with a number of bright globular bodies. These are contained in the terminal vesicles of the excretory system.

Careful focussing shows the integument dotted all over the surface with minute black dots arranged in regular rows. Sections prove these to be minute canals piercing the cuticular layer (Pl. 39, fig. 3).

Internal Anatomy.—Below the ectoderm come the usual

layers of muscles. The longitudinal bands of muscle are quite easily visible in the animal without the aid of sections. They extend almost the entire length of the body, except in the region of mouth and lateral adhesive organs, where the musculature has a circular arrangement. The general mass of the body is filled with a highly vacuolated parenchymatous tissue, the ultimate structure of which is very difficult to make out. In this lie the main organs of the body.

The intestine does not call for special mention. The fork of the intestine is occupied by a mass of gland-cells which extend on each side, forming a zone across the animal. These cells stain very readily, and show up in strong contrast with the general parenchyma of the body. They are filled with material of a slightly more yellow tinge. The space between the intestinal fork and the ventral sucker is entirely filled with them.

These gland-cells communicate with the retractile ear-shaped structures on the sides of the oral region, and open there to the exterior by fine canals. The "ears" themselves are retracted by a series of longitudinal muscles shown in fig. 6, which appear to be specially differentiated longitudinal muscles.

I was able to trace out the fine tubes leading from the gland-cells in several sections. Their connection with the cells in the intestinal fork is not so easily seen, but I am convinced that they do communicate with part of them at least, both groups of cells being similar in histological characters. Both group of cells, then, are connected either wholly or in part with the antero-lateral retractile processes.

Excretory System.—This system of organs commands the most interest. Fraipont, in 'Archives de Biologie, I,' has given an account of the excretory system of Diplostomum volvens which agrees pretty closely with this. It consists of two parts—a coarser, and a finer and more dorsal portion.

The coarser part leads from the excretory pore in the form of two main trunks, whose terminal portions are dilated to

form the excretory bladder. These large trunks run the whole length of the animal. About halfway each gives off a branch vessel, which runs near the lateral surface of the body backward nearly to the excretory pore.

The two main branches run on to the anterior end of the animal, when they become transverse, and, meeting in the mid-line, form a vessel running backwards as far as the ventral sucker.

All these vessels give off at intervals, and finally break up into smaller and smaller branches, each of which ultimately ends in a dilated vesicle containing a rounded body called by Fraipont "calcareous body." The calcareous bodies appear to be in a semi-fluid condition, and contain a good deal of calcium carbonate. On treatment with acid they dissolve and disappear, and at the same time give off gas. They are non-crystalline, and occupy nearly the entire terminal vesicle. In the centre of these calcareous bodies may be seen one or two bright dots looking like granules. In some cases I have found the terminal vesicles quite empty.

In Diplostomum volvens Fraipont described a more dorsal network composed of very fine tubules ending in flame-cells. This finer network joined the main trunk of the coarser at about one third of the entire length from the anterior end of the animal. Now I have seen flame-cells throughout the entire body of Tetracotyle petromyzontis in the dorsal region, but I am quite unable to assert the existence of a definite network joining the coarser part at a given point. My endeavours to see such a network have ended fruitlessly, although I have made many attempts, for the tubes are excessively minute and seem capable of occlusion at times. Consequently my observations only enable me to assert the existence of flame-cells forming scattered portions of network, but whether these communicate with the rest of the system, as in Diplostomum volvens, or at many points near the terminal vesicles—as I have imagined them to do in several cases—I am unable to say definitely.

The flame-cells are best seen by staining with very weak methylene blue.

I may mention that terminal vesicles of the excretory system appeared surrounded in parts by subsidiary vesicles. As mentioned above, the addition of acid to the animal causes the calcareous bodies to disappear gradually with evolution of gas which comes off at the excretory pore. The solution of these bodies is far too gradual to allow of them being considered entirely calcareous. Probably they contain a certain amount of organic matter as well. As to their formation I have nothing to say.

The physiology of this excretory system seems to me well worth further study, in view of the existence of calcareous bodies in the parenchyma of other Platyhelminthes. Some light might be thrown on the origin of the latter, and possibly even the older view that they were derived from the excretory system be re-affirmed.

Tail Region.—Reference was made earlier in this paper to the tail region of the body which grows out of the posterior region, and in adult forms contains the reproductive organs. In the younger stage the excretory bladders seem to occupy a great deal of it.

The tail is not of the same length in all individuals examined by me. In many it is hardly perceptible, whilst in others it may be nearly one third the length of the animal. A study of sections of the posterior end of the animal makes manifest the great number of nuclei aggregated there. They are scattered about pretty uniformly in individuals with an inconspicuous tail, but in those with longer tails they are already becoming gathered into groups—some of them very defined—and these are probably rudiments of the future generative organs.

Part, at any rate, of the development of Tetracotyle petromyzontis takes place in the brain of Ammocœtes, but does not proceed very far.

I have been quite unable to trace any connection of Tetracotyle petromyzontis with an adult form. I do not quite know how to trace it till more knowledge of the animals

preying on Ammocœtes is obtainable. Most Holostomids have birds as final hosts, but in the places from which my Ammocœtes were brought there are not many birds to prey on them.

Yarrel, in his 'British Fishes,' states that eels prey on Ammocœtes. I obtained some large eels, starved them for a time, and then tried to feed them with Ammocœtes, but neither alive nor dead would they touch them.

The most puzzling question of all to decide is how the parasites get into the brain. Possibly they reach it by means of the blood-vessels, but this seems rather improbable in spite of the apparent ease with which Ammocœtes can stand injury to the brain. It seems more probable that they get in before communication between the brain cavities and the external world is interrupted. There seems, however, no possibility of them getting out again without killing the animal, and their ultimate fate is at present a mystery. Do they kill off the forms they infest after a certain time? To determine this it would be necessary to keep a large stock of Ammocœtes and examine carefully all who died off.

It is almost incredible that any vertebrate could live on, apparently without discomfort, whilst its brain is packed with hundreds of flukes. It is true they do not appear to damage the brain substance, but they must consume a great deal of nourishment intended for the brain itself, and their excretory products must surely interfere in some way with the proper brain functions.

I have thought it well to publish these brief notes on this interesting form, although my attempts to solve its life-history have thus far proved ineffectual. The existence of so highly organised a form as a Trematode in the brain cavity of a vertebrate is a unique phenomenon in many ways, and this must commend it to the interest of all naturalists. For one thing it shows that even the brain is not exempt from the attacks of Trematodes.

Gulliver's statement of the occurrence of this form did not attract the attention it deserved, and he only gave an outline

sketch of it. I hope, therefore, that the accompanying figures and this description may help to gain for Tetracotyle petromyzontis the interest it merits.

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EXPLANATION OF PLATE 39,

Illustrating Mr. A. W. Brown's paper, "On Tetracotyle petromyzontis."

Key to the Lettering.

at. Atrium of the glandular adhesive body. *ex. b.* Excretory bladder. *ex. p.* Excretory pore. *ex. v.* Main vessel of the excretory system. *gl. b.* Glandular adhesive body. *gl. c.* Gland-cells. *int.* Intestine. *l. s.* Lateral adhesive organ. *m.* Mouth. *musc.* Retractor muscles of the lateral adhesive organ. *n. c.* Nerve-cord. *œs.* Œsophagus. *or. s.* Oral sucker. *p. v.* Posterior lateral branch of main excretory vessel. *t.* Tail region of body. *tub.* Tubules leading from gland-cells to lateral adhesive organs. *t. v.* Terminal vesicle of the excretory system. *v. s.* Ventral sucker.

Fig. 1.—View from the ventral surface of the entire animal. Leitz, cc. 1, obj. 5.

Fig. 2.—Outline sketches, showing changes of shape at four consecutive short intervals.

Fig. 3.—(*a*) Surface view of integument, showing dots caused by ends of canals.

(*b*) Section of cuticle, showing minute canals.

Fig. 4.—Camera drawing of a horizontal section, to show tubules connecting gland-cells with the lateral adhesive organs.

Fig. 5.—Diagram to show arrangement of the gland-cells of the lateral adhesive organs.

Fig. 6.—Horizontal section, showing retractor muscles of the lateral adhesive organs.

Fig. 7.—Semi-diagrammatic view of the coarser ventral portion of the excretory system. Terminal vesicles and finer tubules shown only on the right side of the figure.

Fig. 8.—More highly magnified part of the excretory system, to show the terminal vesicles and calcareous bodies contained in them.

Fig. 9.—Four calcareous bodies.

Fig. 10.—Longitudinal vertical section, to show the greater number of nuclei in the posterior region of the body. Leitz, oc. 1, obj. 7.

Fig. 11.—Schematic plan—combined from several horizontal sections—of the chief organs of the bodies.

Studies on the Structure and Formation of the Calcareous Skeleton of the Anthozoa.

By

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With Plates 40—43.

Milne Edwards and Haime, and the older authors on coral structure, regarded the calcareous skeleton of the Madreporaria as a calcified mesoderm. In 1881 Dr. A. von Heider, of Graz (11), described a layer of rounded cells underlying the corallum of Cladocora, and named them calicoblasts, since he judged from their position that they must be the coral-forming elements. In the following year G. von Koch (17), in a memoir on the development of Astroides calycularis, confirmed the observation of von Heider, and further demonstrated the fact that the calicoblast layer is a product of the basal ectoderm.

Von Koch described the first elements of the corallum as making their appearance between the basal ectoderm and the surface to which the larva attaches itself, and he described the first formed calcareous particles as "crystalline spheroids," which increase in number and eventually fuse together. "Bei Astroides bildet sich aus dem Ektoderm der Fussscheibe zuerst eine dünne Kalkplatte, welche sich irgend einer Unterlage anlegt, und die Fussplatte des spätern Polypars darstellt. Sie ensteht aus krystallinischen sphæroiden Körperchen, welche

wie sich aus ihrer Lage ergibt, aus Ausscheidungen der Ektodermzellen hervorgehen."

After the discovery made almost simultaneously by von Heider and von Koch of the calicoblast layer, these cells were recognised by several other authors working on the same subject. In 1886 Fowler (7) described and gave somewhat diagrammatic figures of the calicoblasts of Flabellum patagonichum, and in the same year von Heider (12) published a careful memoir on the anatomy and histology of Astroides calycularis and Dendrophyllia ramea. The calicoblasts of the former species were not described, but special attention was paid to them in Dendrophyllia. Von Heider describes the calicoblasts in this species as being in many places inconspicuous, in some quite invisible, but easily discoverable in others. Where they occur they are of two kinds: the most numerous are polygonal or fusiform cells, one or two layers deep, with a well-defined nucleus and granular contents. In other places, and especially where the mesoderm of the body-wall turns inwards to form the mesenteries, von Heider found cells of more or less wedge shape, their pointed ends turned towards the mesoglœa. He says that usually these cells have no nucleus, and that their inner ends are filled with exceedingly fine striations. Some cells adjoining these had less conspicuous striations, more granular contents, and a well-defined nucleus, and these he regarded as being transitional between the first and second kind of calicoblast. Finally, he concluded that the striæ were spicules of calcium carbonate formed within the cell much as are the spicules of Alcyonaria. Von Heider does not say whether he examined his hypothetical spicules with a polariscope.

In the same year W. L. Sclater (26), in a paper on Stephanotrochus Mosleyanus, described a tissue or series of cells as occurring everywhere between the corallum and the mesoderm. These, he says, are very different from the calicoblasts figured by von Koch, being of irregular shape, separated from one another by intervals so as to seldom form a definite layer, and striated in an extraordinary way. Sclater's figures leave

no doubt that the structures in question are identical with von Heider's second kind of calicoblasts.

In 1888, in a paper on the anatomy of Mussa and Euphyllia (2), I described the presence of elongate columnar calicoblasts on the upper and peripheral parts of the septa of Mussa distans, and at the same time I criticised von Heider's interpretation of the striated wedge-shaped bodies as calicoblasts, pointing out that I had found precisely similar structures in Mussa, but never in the region of most active coral growth. On the contrary, they were always associated with the mesogloea of the mesenteries; and, moreover, the striæ could not be due to the presence of spicules of carbonate of lime, for they were unaltered after prolonged decalcification. At the same time Fowler (8) referred to the existence of similar wedge-shaped structures in Pocillopora brevicornis, and gave his opinion that they were rather connected with the attachment of the mesentery to the corallum, than with the secretion of carbonate of lime. In the same paper Fowler described and gave a diagrammatic figure of elongated columnar calicoblasts in Lophohelia prolifera. In a subsequent paper (9) Fowler described and gave careful drawings of the striated structures in Stephanophyllia formosissima and Flabellum alabastrum, and pronounced them to be nothing more than offshoots of the homogeneous mesogloea of the mesentery, possessing neither nucleus nor cell-wall. He held that their occurrence only in the neighbourhood of the lines of attachment of the mesenteries, their position, shape, and striation, indicated that their function was to provide an increased surface for fixation of the mesentery, and a firmer fulcrum for the action of the powerful retractor muscles. No further observations of importance were made on the subject till 1896, when Mrs. Gordon (formerly Miss Maria M. Ogilvie) published a long and minute account of the microscopic structure of various types of Madreporaria (25), and suggested an entirely new classification of the group founded upon the microscopic characters. Mrs. Gordon rejects Fowler's and my accounts of the striated structures, and says

(loc. cit., p. 102), "I find, however, that Koch, Fowler, and Bourne are wrong in their conception of the calicoblast. My investigation of the superficial layers of the skeleton have given the fullest confirmation of Heider's idea, that the calicoblasts were actually converted into calcareous groups of fibres. I hope to show, in the course of this paper, that the fibre-containing calicoblasts which lie next to the skeleton are shed off, so to speak, from the polyp, new cells taking their place in the ectoderm by cell division. The shed calicoblasts build up successive layers of calcified cells, which hang together at first by their cell-walls, and ultimately, as crystalline changes continue, form the individual laminæ of the skeletal structures. The whole Madreporarian skeleton is formed of such laminæ; any apparent variation in microscopic structure is accounted for by some difference in the shape and position, locally, of the ectoderm." The quotation shows that Mrs. Gordon attached great importance to the conception of the formation of the corallum from a number of calcified cells, and she made it the basis of a number of interesting speculations concerning the origin of the different types of coral structure which she described.

On p. 115 of the same work Mrs. Gordon gives an account of the isolated skeletal elements of the Madreporarian corallum. These she discovered to be scale-like structures entirely filled with a bush of minute fibres; others had granular contents, or partly fibrous, partly granular. The resemblance of these scales to the striated structures described as calicoblasts by von Heider led Mrs. Gordon to seek for proof of the identity of the two. I give Mrs. Gordon's proof in her own words. "It has been frequently mentioned by writers on corals that organic remnants after removal of the polyp may be found on the skeleton. Wherever on the skeleton I found such remnants they consisted of calicoblasts, which showed in shape, size, and contents the varieties already drawn by von Heider. The cells were round or obovate. The contents varied from yellowish organic-cell material to the inorganic fibrous condition. Comparison of my own observations on

several corals with the figures given by von Heider left no doubt that the isolated skeletal element was a calcified calicoblast cell. . . . We may look upon the superficial layers of the skeletal elements, and of incompletely calcified calicoblasts, as the outer layers of a many-layered ectoderm. The ectoderm of the Madreporarian polyp" (I presume Mrs. Gordon here means the calicoblast layer) "is figured by Koch, Fowler, and Bourne as a simple layer of cells. I have observed, on the contrary, that a section through soft and hard parts shows an ectoderm, sometimes composed of a simple cell layer, sometimes several cells deep. Heider's figures indicate a similar observation. The calicoblasts remain adherent to one another in dense groups, or may be uniformly distributed. And in this manner they are gradually left behind on the skeleton and completely calcify, while active cell division develops constantly new ectodermal cells. The calicoblasts adherent to the skeleton represent such as were already in the course of losing living continuity with the polyp, at the time when the polyp was removed from the skeleton. The above observations made on the skeletal surfaces will be seen to carry out fully the opinion of Heider, that the skeletal deposit forms, in the first place, within the calicoblast. I pointed out in the introduction that Koch's view of the extra-cellular deposition of the skeleton had been accepted by Fowler and Bourne. The figures given by the two last-named authorities are confined entirely to preparations made from completely decalcified specimens. My observations have been made partly on dry skeletons, partly on skeletons from which the soft parts have been freshly removed, organic tissues remaining here and there adherent, and partly on preparations showing the body-wall in position."

Accompanying this statement are figures of individual scales taken from the dissepiments of Galaxea, which Mrs. Gordon claims to represent calicoblasts in various stages of calcification. The scales as figured show a distinct striation, due to their being composed of a tuft of elongate radiating calcareous crystals, and several of them exhibit some dark blotches which

are doubtless traces of organic matter. The figures certainly do not show to demonstration that the scales are what Mrs. Gordon claims them to be, calcified cells. And though she identifies the scales with the striated structures called calicoblasts by von Heider, she does not bring forward any new evidence, either in drawings or in the text, to show that the striated structure of the scales of Galaxea, and of von Heider's calicoblasts, is due to one and the same cause, viz. the presence of spicules of carbonate of lime.

It is easy to make a preparation from the vesicular exotheca of Galaxea, showing the scale-like calcareous structures figured by Mrs. Gordon (loc. cit., figs. 8A, 8B), and it may be conceded that their resemblance to the striated structures identified by von Heider as calicoblasts is sufficiently striking. But we have no evidence that the striated structures are found in this position in the fresh polyp; we have no evidence that the striations of von Heider's calicoblasts are due to the formation of spicules within them; but, as I shall show directly, we have evidence to the contrary, and from the analogy of other corals there is every reason to believe that the striated structures are not present in the soft tissues covering the exotheca.

Nor can I entirely agree with Mrs. Gordon's description of the superficial emergences of the corallum in Galaxea and other corals as "scales."

It will appear in this paper that the surfaces of certain corals present an appearance like that described for Galaxea, but that the apparent scales are really due to the emergence of bundles of parallel crystalline fibres at the surface of the corallum. But before going further into this question I will give the results of my observations on the structure and formation of the spicules of Alcyonaria. Since we know that these spicules are entoplastic products, it seemed to me that a knowledge of the minute structure of the spicule might assist in the interpretation of the minute structure of the Madreporarian corallum. Throughout this paper the word "spicule" denotes an entoplastic product of a single cell or of a cœnocyte.

Kölliker, in his 'Icones Histologicæ,' p. 119, has given the results of a thorough investigation of the spicules of Alcyonarians. He described them as consisting of an organic and an inorganic part. The organic part consists, according to him, of a thin cuticular membrane, but he denied the existence of any central organic basis. "Wenn ich übrigens vorhin bemerkte dass das Innere der Kalkkörper durch Säuren sich auflose, so muss ich bemerken, dass es sehr schwer ist, in dieser Beziehung volkommen ins Reine zu kommen. Nach Anwendung verdünnter Säuren bleibt in der Regel noch ein körnig streifige, oft wie aus Fäserchen zusammengesetze Masse im Inneren, und brachte mich dies anfänglich zu der Meinung, dass auch Inneren ein organischer Rückstand bleibe. Setze ich dann aber eine concentrirte Säure in genügender Menge zu, so löste sich auch dieser Rückstand, und habe ich in eine Reihe von sorgfältig untersuchte Fällen mich davon überzengt, dass schliesslich nichts als die Cuticula sich erhält, in welcher Beziehung ich übrigens noch bemerken will dass Salzsäure viel entschiedener wirkt als Essigsäure. Aus diese Thatsache folgt übrigens nicht dass im inneren der Kalkkörper gar keine organische Substanz enthalten ist, und zeigen schon die mannichfachen Farbstoffe dieser Körper, dass sie nicht einzig und allein aus Erdsalzen bestehen. Auch der Zahmschmelz enthalt etwas organische Materie, obschon er in Säuren keinen nennenswerthen Rückstand lässt." Though this statement is cautious enough, he is more definite further on, where he says (p. 121), "Ein centraler organischer Faden, wie ihn viele Kieselnadeln von Spongien besitzen, mangelt den Kalkkörpern der Polypen ganz und gar."

The intimate structure of the spicule is described by Kölliker as crystalline. The most conspicuous feature in the spicules is a fine concentric striation parallel to the surface, due to the apposition of concentric lamellæ. There is also a fine striation and punctation in the longitudinal axis, from which one may conclude that there is a special structure, as also by the fact that after prolonged action of weak acids the inner part of the spicule breaks up into small crystalloid needles. Kölliker also

noticed and figured (loc. cit., pl. xvii, fig. 7) that in the spicules of certain Gorgonids the superficial warts and projections were continued through the substance of the spicule nearly to its centre, as it were by roots, and that these internal prolongations were often forked or branched.

Kölliker entered at great length into the forms of Alcyonarian spicules, of which he distinguished two main classes, the smooth and the warty spicules. The smooth spicules he divided into sphæroids, spindles, lenticular spicules, and cylinders; the warty spicules into (*a*) simple spicules, which include spindles, clubs, double clubs, and double sphæroids, double stars, double wheels, double spindles and scales, with several subdivisions of the first four kinds, and into (*b*) composite spicules, subdivided into doublets, triplets, quadruplets, &c. Kölliker also denied the intra-cellular development of Alcyonarian spicules, a mistake which was subsequently corrected by Kowalevsky and Marion and von Koch.

The spicules of recent Alcyonaria, with the single exception of Heliopora, are, as is well known from the work of Kowalevsky and Marion (**23**) and von Koch (**16**), true spicules. They are formed in certain scleroblastic cells which originate in the ectoderm, and either remain there (some species of Clavularia, Xenia) or migrate into the mesoglœa. I have been at some pains to verify previous statements on the subject, and to discover, if possible, the earliest stages of spicular formation, and to trace them upwards.

Whilst working at Roscoff, by the kind permission of M. H. de Lacaze-Duthiers, I was able to make very satisfactory preparations of Alcyonium digitatum and Gorgonia cavolinii illustrating this point. The specimens were killed in an expanded condition by rapid immersion in a ·5 per cent. solution of osmic acid in sea water, were thoroughly washed with distilled water, and stained for twenty minutes with Ranvier's picro-carmine. The expanded polyps were cut off close to their bases, placed in dilute glycerine, and laid open. The ectoderm and endoderm having been removed with a camel's-hair brush, very satisfactory flat preparations were obtained

illustrating the formation of the spicules in the lower moieties of the exserted portions of the polyps.

The scleroblasts of Alcyonium digitatum (Pl. 40, fig. 1) have the form of irregularly polygonal, ovate, or amœbiform cells, varying very much in size and shape, but resembling one another in the coarsely reticulated structure of the protoplasm, and in their dark granular appearance. Vacuoles of small size (not the ultimate vacuoles of Bütschli) are visible in the protoplasm, and not infrequently a vacuole contains a single microsome. The scleroblasts run in strands and patches through the mesogloea at the bases of the expanded polyps, and may be found, though they are not easily studied, in the thickened mesogloea of the cœnenchyme. They are always accompanied by two other kinds of cells whose function is obscure. The one kind, marked *gr.* in fig. 1, are rather smaller than, but of similar shape to, the scleroblasts, and are filled with minute highly refringent granules; their nucleus is rarely to be seen, being hidden by the granules. My studies on other Alcyonaria lead me to believe that their function is to secrete the gelatinoid substance of the mesogloea. The second kind of bodies I call provisionally the ovoid bodies. Each of these is about ·0075 mm. in length, is surrounded by a protoplasmic sheath, and has a relatively large nucleus on one side of it (fig. 1, *ov.*). These were noticed but not well figured by Hickson (13), and similar structures were observed by von Heider in Cladocora. Hickson suggests that they may possibly be parasitic sporozoa, but my observations do not lend any support to his suggestion. Their contents are clear and highly refringent, but are not calcareous. They are not affected by prolonged treatment with dilute acids; they are unaffected by treatment with acetate of potash (used with the view of precipitating lime if any was held in solution); they do not light up when viewed through the polariscope with crossed Nicols. They stain deeply with hæmatoxylin, but are unaffected by other dyes. I have met with similar ovoid bodies among the scleroblastic cells of all the Anthozoa which I have examined carefully, and I am unable to guess their

function. I have, however, observed in some Madreporaria traces of a coiled filament within the ovoid body, which leads me to the conclusion that they are degenerate nematocysts. I have not been able to discover any traces of a coiled filament in Alcyonium, Gorgonia, or Heliopora, but the ovoid bodies have in other respects the characters of developing nematocysts, and it is not unreasonable to conclude that the scleroblasts in the course of their migration from the ectoderm have carried with them some of the most characteristic constituents of that layer in the shape of nematocysts, which, being functionless, have undergone degeneration.

One of the smallest sclerites which I was able to discover is shown in fig. 1, *scl.* It consists of a single minute crystal, ·01 mm. long by ·0025 mm. broad, lying in a distinct vacuole in the centre of the scleroblast. Adjoining it is another scleroblast, in which there is a minute nodule, some ·005 mm. in its greatest diameter, in addition to a relatively large and already irregular spicule.

Figs. 2 and 3 represent larger sclerites enclosed in the cells which have given rise to them. Fig. 4 shows what are apparently two cells containing sclerites fusing together, but it is probable that this is the result of division of the nucleus of the scleroblast without corresponding division of the cytoplasm, and the two sclerites which are contained in the binuclear cell, after reaching a certain size and form, are becoming joined together. Fig. 5 shows the normal condition of a young spicule of Alcyonium; the sclerite has the characteristic shape, somewhat resembling a caudal vertebra, and is enclosed in a protoplasmic sheath containing two nuclei. In older and more complicated spicules I have counted three or four nuclei, and where two are present they are generally at the ends of the spicule, and not at the sides as in fig. 5. Though the existence of two, three, or four nuclei suggests the presence of as many cells, I do not think that more than one cell, i. e. more than one continuous body of cytoplasm, is concerned in the formation of a single spicule in Alcyonium digitatum. The scleroblasts, as is seen in fig. 1, are often cœnocytes con-

taining two, three, or more nuclei; where two sclerites are formed in a single scleroblast two nuclei are usually present, and if only one sclerite is formed the nucleus apparently divides when the sclerite has attained a certain size, and the division is repeated as growth continues, without any corresponding division of the cytoplasm.

So far as the formation of the spicules is concerned, all that is true of Alcyonium is also true of Gorgonia Cavolinii, and these are the only two Alcyonarians which I have been able to obtain in a living condition, and to preserve by appropriate methods.

The spicules which I have examined belong to the smooth lenticular, the spindle-shaped and scale-like warty kinds described by Kölliker.

The lenticular spicules were obtained from Clavularia cœrulea, Ehrh., and from Xenia and Heteroxenia. In Clavularia cœrulea these spicules are formed within ectodermic cells, which do not migrate into the mesoglœa. Each spicule is of flat ovate shape, measuring some 0·02 mm. in its longer and 0·01 mm. in its shorter diameter. These lenticular simple spicules are remarkable for the large amount of organic matter which they contain, an amount so great that, after slow decalcification, the organic basis retains the shape and size of the spicule, and can readily be stained by diffuse stains such as hæmatoxylin or acid fuchsin. The undecalcified spicule, when examined by the polariscope with crossed Nicols, grows fainter in four positions at 90° to one another, but is never wholly extinguished. This suggests that it is made up of a number of crystals, the majority of which are parallel to one another. In some few of the spicules I could detect signs of a dark cross when the prisms are crossed, but this is only an occasional phenomenon. In some specimens which had been treated for a short time with very dilute acetic acid, I could detect minute crystalline fibres which seemed to diverge fan-wise from the centre towards the two extremities of the oval spicule. But the structure is so minute that one cannot see anything distinctly, even with an immersion lens. I can

only say with certainty that the spicule is composed of a number of very minute fibro-crystals. The organic matrix left after decalcification consists of a close feltwork of exceedingly fine fibres, the whole enclosed in a distinct and apparently structureless spicule sheath. The similar spicules of Xenia and Heteroxenia, which I have described in another place (4), behave under polarised light in exactly the same way as those of Clavularia cœrulea, and their organic basis is also similar.

Secondly, there are the flat scale-like spicules which occur in Primnoa, Plumarella, and in many other Alcyonarians. Examination by polarised light shows that the scale-like spicule of Primnoa and Plumarella is made up of a number of crystals of calcium carbonate lying in nearly the same plane, and radiating from a common centre. Fig. 6 is a drawing of a single spicule of Plumarella delicatissima, Wright and Studer, and fig. 7 is a drawing on a smaller scale of the same spicule when viewed through the polariscope with the prisms uncrossed. The characteristic black cross leaves no doubt as to the crystalline arrangement of the spicule, and exactly the same effect is obtained with many inorganic crystalline deposits, e. g. with platino-cyanide of magnesium. So far as I have been able to determine, these scale-like spicules in Primnoa and Plumarella are formed by several cells, or at least by a comparatively large cœnocytial investment containing many nuclei. The material at my disposal is not sufficiently well preserved to allow me to speak with certainty on this point, but sections made through the zooids of both genera show that the scale-like spicules, which form opercula corresponding in number to the tentacles, and situated at the bases of these organs, are each enclosed in a cavity in the mesoglœa, which cavity is lined by a protoplasmic layer in which many nuclei are embedded. The specimens were too much macerated by the prolonged action of spirit to enable me to say definitely that no cell outlines are present in the protoplasmic lining, but at all events I was unable to discover any in sections stained by various methods and

examined with the highest powers. The distinction between the cœnocytial layer and the cell layer is, perhaps, of no great importance, but it is of more importance to note that the arrangement of the crystals composing the spicule bears little relation to the structure and arrangement of the cells from which the spicule is formed.

The actual crystalline structure is but slightly indicated in the fresh spicule, but it becomes evident after treatment with dilute acids. A spicule which has thus been partially decalcified or "etched" is seen to be composed of a number of minute calcareous fibres which radiate from the centre towards the periphery. Their arrangement, however, is not perfectly radial. They cross and overlap one another to some extent, and many of the fibres crop out on the surface of the sclerite, and do not reach its edge. Hence there is some amount of interference, and the dark cross shown in fig. 7 is not perfectly symmetrical, nor are all parts of its limbs equally opaque. Some of the crystalline fibres may be isolated at the edges of the etched sclerite. Each is a minute acicular crystal about 0·03 mm. long, and behaves under polarised light as a true uniaxial crystal.

I did not succeed in isolating the organic constituents of the spicules of Primnoa or Plumarella.

Thirdly, there is the spindle-shaped spicule in its many varieties, usually covered with spines, warts, and projections of different kinds. My observations on this form of spicule were almost entirely confined to Spongodes and Siphongorgia mirabilis, whose spicules are of comparatively simple form, and large enough to admit of my making transverse and longitudinal sections.

The spicules of Spongodes (sp. incert.) are shown in fig. 9. They are long fusiform structures, covered with warty projections and of very variable length. Some of the larger spicules, which serve as supports for the individual zooid heads, are as much as 3·5 mm. long; the smaller, which lie in the walls of the zooids, are about 0·2 mm. long. They are variously coloured, crimson, orange, or pale yellow, according

to the species and the place from which the spicule is taken in each species. The colour is deepest along an axial line, and is considerably lighter or altogether absent in the more peripheral parts of the spicule. Most of the spicules show traces of a dark core running along their axes.

The structure of such a spicule may be studied by transverse and longitudinal sections, by crushed and "etched" preparations. A transverse section is shown in fig. 9. Centrally there is a little cluster of dark pores, which comparison with a longitudinal section shows to be the expression of longitudinal canals running along the axis of the spicule. Around this are concentric striations, which become darker and more evident as they approach the periphery. In some of these minute spaces may be detected. The most characteristic feature in the section is the presence of a number of radial cords, some of which are simple, others forked. They originate in the very centre, starting apparently from the central axial canals, and they end peripherally in the warty projections at the surface of the spicule. On examining the section by polarised light one finds, when the Nicols are crossed, that the whole section is bright, but that the radial cords stand out brighter than the remainder when in certain positions. Fixing one's attention on a single cord and the tissue immediately contiguous to it, one finds that as the object is rotated the cord becomes alternately brighter and darker without ever being wholly obscured, whilst there is no great change in the surrounding substance, though ill-defined dark shadows sweep across it. In certain positions the radial cords, besides being bright, show brilliant iridescent colours, due to interference. I gather from this that the whole structure, cords and ground substance alike, is crystalline, and that the axes of the crystals composing the cords are situated in a different plane from those composing the ground substance. Examination of a longitudinal section (fig. 10) shows the structure of ground substance and cords even better than a transverse section. The axis of the spicule is occupied by a number of very dark lines diverging at very acute angles from the centre. The

rest of the spicule exhibits longitudinal striations, more conspicuous and closer packed together in some places than others, so that the whole section has a banded appearance. In the section figured one may distinguish (1) a medullary portion surrounding the axial diverging lines, which is very finely striated and of a deep pink colour. Outside this is (2) an intermediate portion of lighter substance, coloured a faint pink, and showing few but distinct longitudinal striations. It should be observed that the intermediate portion ends on the left-hand side of the figure in a blunt point, and that the dark axial lines diverge in a brush-like manner as they approach the point. Both the medullary part, and the intermediate part forming the core of the spicule, have the shape and characters of spicules enclosed within spicules, and are without doubt the expression of former stages of growth. (3) The cortical portion, as is shown by its regular longitudinal bands, has clearly been added in successive layers to the intermediate portion. It consists of alternately darker and lighter bands, the darker bands being due to the presence of more numerous dark longitudinal striæ. The complete correspondence between the longitudinal and transverse sections is evident, the concentric laminæ of the latter being represented by the longitudinal bands of the former.

The radiating cords are well exposed in the longitudinal section. It should be noticed that none of them are forked; their bifurcation occurs only in the transverse plane, and cannot be seen in longitudinal section. Each cord starts from the dark axial lines, diverging but slightly from the longitudinal axis at first, but soon turning sharply outwards to run at right angles to the surface, on which it emerges as a warty prominence. A spicule which has been partly ground down and afterwards crushed is seen to be composed of a number of longitudinal fibrous strands, interrupted here and there by the radial cords which traverse them at right angles. Each strand is composed of numerous fibres, which run nearly parallel to one another, and parallel with the long axis of the spicule. Some of these are isolated in a crushed specimen, and each when

isolated behaves as a single crystal under the polariscope. The fibrous structure, however, is better shown in etched specimens.

A spicule of Spongodes, which has been carefully treated with very dilute acid, breaks up into bundles of exceedingly fine acicular crystals, arranged with their long axes nearly parallel to the long axis of the spicule; they are not quite parallel, but are fitted together and interwoven so that any one bundle of fibres gives brilliant interference colours when viewed through the polariscope. The radial cords are not easily studied in etched specimens, but it can be seen that the longitudinal feltwork of crystalline fibres is interwoven with the radial cords much as a fabric would be which had very few and coarse strands in the warp, and very numerous and fine strands in the woof.

When a longitudinal section of a spicule is examined by polarised light with the Nicols crossed, the radial cords are seen, in certain positions, to stand out brightly; whilst the ground substance is nearly dark, though lit up here and there by iridescent tints. As one rotates the section the radial cords become dark, whilst the ground substance, still retaining its iridescence, becomes lighter, and these alternations take place regularly for every 90° through which the section is rotated. It has been shown that the ground substance is composed of nearly parallel longitudinal crystalline fibres, and it may be inferred that the radial cords are composed of similar crystalline fibres, whose axes are at right angles to the fibres of the ground substance.

A single crystalline fibre measures some 0·03 mm. in length, and has the optical characters of a single crystal, being extinguished in four positions at right angles to one another when rotated between the crossed Nicols.

As none of my sections were thin enough to obtain a section of a radial cord with two plane surfaces, and as the cords are neither straight nor of uniform diameter (see figs. 9 and 10), but swollen in places so as to be almost moniliform, they exhibited interference phenomena under polarised light in every

position of the Nicols; these of course were due to the irregularity of the crystalline fibres of which the cords are composed. I have dwelt at some length on the structure of the inorganic part of the spicule of Spongodes, because I am able to show that all its details are moulded, as it were, upon an organic matrix.

Kölliker, as has been stated above, denied the existence of axial fibres in the spicules of Alcyonaria. Some fortunate preparations of the partition walls of the stem canals in Ammothea arborea and Siphonogorgia mirabilis revealed the presence of an organic filament in the spicules of the partition walls of these species. Following up this observation I was able to discover similar axial and also radial fibres in the spicules of Spongodes, but not without considerable trouble. Kölliker remarked that hydrochloric acid gave much more uncertain effects than acetic. I found that treatment with even very dilute solutions of hydrochloric or nitric acid removed all traces of the axial organic basis. Decalcification with picric or very weak picro-nitric acid had the same effect, but very careful decalcification with a ·5 per cent. solution of acetic acid and subsequent staining with aqueous safranin and picro-nigrosin brought out the structure shown in fig. 12. The spicules, which were removed with needles from the colony, always had a certain amount of mesoglœa adherent to them. This was stained blue by the picro-nigrosin, and thus differentiated from the spicular sheath, which was stained crimson by the safranin. The sheath retained more or less faithfully the shape of the spicule, and the warts or projections due to the emergence of the radial cords on the surface were clearly seen. I could detect no trace of fibrillar or other structure in the uninjured sheath, though it broke up readily into longitudinal fibrillæ when torn or otherwise injured. Within the spicule sheath is an arrangement of organic fibrillæ, represented as faithfully as possible in fig. 12. These fibrillæ are stained deep orange by safranin, and stand out conspicuously from the spicule sheath. The axis of the spicule is occupied by a dense feltwork of longitudinal fibrillæ,

whose general direction is parallel to the long axis of the spicule. There can be no doubt that these fibrillæ occupy the spaces represented by the axial dark lines or dots in longitudinal and transverse sections. From the central bundle fibrillæ diverge outwards, and after making several connections with other fibres, whose direction is approximately longitudinal, they run straight towards the surface of the spicule, meeting it at right angles and ending in the warty projections on the surface of the sheath. The whole structure will be best understood from an examination of fig. 12. It will be seen that what I have described as the medullary portion of the spicule is occupied by a strand of closely apposed fibres forming a network, whose meshes are greatly extended longitudinally; that what I have described as the intermediate portion is occupied by a much more open network of fibrillæ, and that the cortical portion is traversed by the radial fibrillæ, connected only here and there by a longitudinal fibril. The correspondence between the organic and inorganic elements is obvious, and it is clear that the crystalline structure of the whole spicule is dominated by the organic matrix of fibrillæ. The needles of calcium carbonate are arranged parallel to the organic fibrils, and this circumstance explains why the long axes of the crystalline fibres composing the radial cords are set at right angles to those composing the ground substance. Professor Sollas kindly determined the specific gravity of the Spongodes spicules for me, and found it to lie between 2·63 and 2·7. They appear, therefore, to consist of calcite (sp. gr. 2·7), and the lightness of some of the spicules was probably due to their still retaining traces of organic matter in spite of prolonged treatment with Eau de Javelle.

In the case of the large spicules of Spongodes, Ammothea, and Siphonogorgia I could find no trace of a cell or cells enclosing the spicule. The last named are deeply embedded in the mesoglœa, and no trace of cellular structure could be discovered in connection with them. There can, however, be no doubt that they were originally developed, like the spicules of Alcyonium, Gorgonia, and Clavularia, within cells, and

indeed I have observed the small spicules from the body-walls of the polyps of Spongodes to be enclosed in cells. All that has been stated of the spicules of Spongodes is true, mutatis mutandis, for the spicules of Siphonogorgia, and Kölliker's figures of the spicules of Eunicea leave no doubt that the same is true of this genus also.

It is of importance to my present argument that the spicules of the Alcyonaria show a definite and complex crystalline structure, the details of which are, indeed, moulded upon and dominated by an equally complex organic matrix, but are not the expression of any particular arrangement of the cells from which the spicules are formed. It cannot for a moment be suggested that the radial cords and their warty emergences, or the longitudinal bundles of crystalline fibres, are formations due to a particular arrangement of calcified cells. The spiculoblast forms an organic matrix, and secretes carbonate of lime. The latter seems to be crystallised out, pari passu with the growth of the former, and the crystalline growth conforms to the organic growth, but that is all.

I have elsewhere (4) given some details of the structure of the skeleton of Heliopora cœrulea. As this Alcyonarian differs from all its allies in having not a spicular but a so-called lamellar skeleton, it seemed to me that a renewed investigation of its structure might throw some light on the questions raised by Mrs. Gordon's paper. Nor have I been disappointed. The skeleton of Heliopora proves on closer examination to be remarkably instructive, and to throw considerable light on vexed questions regarding the Madreporarian skeleton. In examining Heliopora I had recourse to flat and macerated preparations of the growing tips of the corallum. These were always stained with picro-carmine. I also made sections of the growing tips after very careful decalcification with 1 per cent. acetic acid, staining my preparations with picro-carmine and picro-nigrosin or with Heidenhain's iron hæmatoxylin followed by acid fuchsin. I cut some sections of hard and soft parts together, the fragile and delicate character of the corallum at the growing tips enabling me to do this without

entire destruction of the soft tissues, and for the study of the corallum itself I made use of sections, minute transparent fragments, crushed and "etched" preparations.

Most careful examination with high powers (Zeiss, $\frac{1}{12}$ oil immersion, with comp. oculars 4 and 8) convinced me that there is no question of any "spicular" structure in Heliopora. The corallum is, as I have before stated, a secretion product of a definite layer of cells derived from the ectoderm, which I have called, like those of Madreporaria, calicoblasts.

So far as the derivation and general arrangement of the calicoblasts is concerned I have nothing to add to what is contained in my previous paper, but improved histological methods and the use of higher powers of the microscope enable me to add a good deal in the way of detail. The calicoblasts form a somewhat thin covering over the cœnenchymal tubes, and the proximal moieties of the zooids, and are everywhere present between the living tissues and the corallum. But they seldom are arranged in a single layer. In some places, it is true, only a single row of cubical or fusiform cells of coarsely vacuolated structure with numerous granules can be distinguished. In such cases the calicoblasts always appear to have an external limiting membrane, turned towards the corallum, and this membrane, like the mesogloea, stains bright blue with picro-nigrosin. But in all places where the tissues are well preserved, and show no indications of having been displaced by the action of bubbles formed during decalcification, a layer of flattened cells, fusiform in section, can be distinguished outside of the external limiting membrane, and therefore abutting on the corallum. These are shown in fig. 13, *ca. e.* They are found in this particular condition only in those places where coral secretion is least active. At the bottom of the superficial canals, where active centrifugal growth of the corallum is taking place, and at the lower blind ends of the cœnenchymal tubes and zooids (the regions where the tabulæ are formed), the calicoblasts form a layer several cells deep, as I have shown in my previous paper (loc. cit., pl. 10, fig. 4). In these regions the calicoblasts are grouped

together in a manner which will be best understood by reference to fig. 20. The cells are very irregular in shape, and are separated from one another by considerable spaces. Some of the cells nearest to the edges of a group appear to be undergoing disintegration. In these groups of calicoblasts two other kinds of elements may be distinguished. The first kind comprises the "ovoid bodies," which I have already described in Alcyonium digitatum. These bodies, which form a constant element among the calicoblasts of Heliopora, do not differ in any essential particulars from the similar bodies in Alcyonium, except that, as is shown in fig. 14, some of them show a distinct vacuolated structure. Though I have devoted much time to them, I have been unable to throw any light on their history or function; they may frequently be observed in close association with the mesoglœal processes which I am about to describe (vide fig. 18).

Amongst the calicoblastic groups structures such as are represented in fig. 15 constantly occur. They consist of a much vacuolated cell with a nucleus and central contents, stained blue with picro-nigrosin, and exhibiting a concentric striation which may be due to the presence of a feltwork of fibrillæ. These are the earliest stages of structures which are clearly homologous with the striated calicoblasts of von Heider. They are shown in a somewhat later stage of development in fig. 16, and in later stages in figs. 17, 18, and 19. In fig. 16 two such structures are shown: one is so placed that its nucleus is clearly visible; there is no longer any trace of the surrounding layer of vacuolated protoplasm, and it appears as an ovoid body with contents feebly stained by picro-nigrosin and traces of striation. The other has no nucleus—probably it was not included in the section,—but it has a clearly defined striation along its lower margin, the striæ in this case being stained by the picro-carmine. It is noticeable that both the structures lie outside of the layer of calicoblasts. Fig. 17, which is an optical section drawn from a flat preparation, shows a further stage of development of one of these structures, which I shall henceforth call desmocytes. The previously

ovoid body has become somewhat flattened, and a process of mesoglœa, apparently formed by the cells contiguous to it, stretches downward towards the mesoglœal lamina, to which, however, it is not yet united. A somewhat later stage is shown in fig. 18, in which there are several such mesoglœal processes, apparently in course of formation from the cells overlying them, and the final stage is shown in fig. 19. In the last-named figure a shallow cup with irregular margins and with striæ deeply stained with picro-carmine is seen to be attached to the mesoglœal lamina by a band-like process of the latter. To the edges of the cup is attached an external cuticular membrane, stained blue by picro-nigrosin. The desmocyte shows different staining reactions according as the section has been stained for a short or long time in picro-nigrosin. It always stains in picro-carmine: if it is left for a short time in picro-nigrosin, the carmine is not displaced; if for a long time the picro-nigrosin displaces the picro-carmine and the desmocyte and its striations are stained blue. The same is the case with the similar structures in Madreporaria.

I was long inclined to attribute to these structures the predominant share in the formation of the corallum, the more so because their earlier ovoid shape and their final cup shape suggested that they were similar to goblet-cells, the cup shape being the expression of a goblet whose contents had been voided. But further study convinced me that this was not the case. The desmocytes are most abundant and best developed in places where coral growth is least active; only their earlier stages are to be observed among the groups of calicoblasts in regions of active coral growth. They are scarce relatively to the granular calicoblasts, and in my flat preparations of hard and soft parts at the growing points of the corallum I could not detect any of them amongst the numerous calicoblasts clothing the obviously newly formed points of coral. Moreover I am of the opinion that the corallum is formed as a result of the disintegration of calicoblasts. This is suggested by fig. 20, and fig. 21 is a drawing made from a partially decalcified preparation stained with eosin and methylene blue. The calicoblasts are

seen to be full of eosinophilous granules lying in small vacuoles. At the bottom of the drawing is a sort of loose feltwork of unstained tissue, which is probably the organic matrix of the corallum. Minute crystals are adherent to this feltwork, and in places it is covered with minute black granules, which are invariably present on similar organic remnants in partially decalcified specimens. To the left are some bundles of acicular crystals of carbonate of lime, broken up by the action of the acid. It appears as if some of the calicoblasts in the figure were in the course of disintegration, and I have observed this breaking up of the cells not only in many other instances in Heliopora, but also in many different species of Madreporaria. In the latter case the disintegration is not a result of maceration, for it is conspicuous in preparations of Caryophyllia, freshly killed by various methods, in which the remaining tissues were excellently preserved. Though I have hunted through scores of preparations with the polariscope, I have never been able to find in Heliopora any trace of the formation of spicules or of crystals within cells, such as may be readily discovered in Alcyonium or Gorgonia. I have specially examined the desmocytes, for I was for a long time inclined to the opinion that von Heider and Mrs. Gordon must be right, and that I should find evidences of spicule formation within these structures. But my results were always negative. The calcareous skeleton of Heliopora is not formed from spicules developed within cells, but is a crystalline structure formed by crystallisation of carbonate of lime, probably in the form of aragonite, in an organic matrix produced by the disintegration of cells which I have described as calicoblasts.

This view is confirmed by a minute study of the corallum itself. In carefully made maceration preparations of hard and soft parts the most delicate growing tips of the corallum are preserved uninjured, and in close contiguity to the soft tissues from which they originated. Figs. 22 and 23 are careful drawings made with the camera lucida of the spinous projections at the tips of a growing frond of Heliopora. They are drawn under different magnifications; fig. 22 shows more

clearly how the component crystals are arranged along the edge of such a spine; fig. 23, which is a surface view, shows the arrangement of the crystalline components on a larger scale. Both drawings might be taken for representations of an ordinary inorganic crystalline deposit. In fig. 22 the crystalline elements are seen to diverge, more or less regularly, from a centre of growth, and this is the usual condition; but in fig. 23 the components are arranged pell-mell, the long axes of the crystals crossing one another at every angle: only at the right-hand edge is there an indication of a more regular divergent growth from a centre.

Although the structure of the corallum of Heliopora is much coarser than that of any madrepore which I have examined, it is difficult to isolate the crystals, so as to identify the crystal system to which they belong. On crushing, the corallum breaks up into fragments, some of the most regular of which are represented in fig. 24, *a*, *b*, and *c*. The smallest fragments, such as *b* and *c*, behave under polarised light like single crystals, being regularly extinguished in four positions 90° apart when rotated between the crossed Nicols. Treatment with dilute acids breaks up the crystals into needle-like fibres resembling those of Spongodes, and every such fibre behaves as a single crystal under the polariscope.

There is no evidence whatever that the crystalline elements are formed within cells. In my previous paper I gave a drawing of a section through the corallum at the tip of a growing frond of Heliopora. The arrangement of the crystalline fibres was only indicated by faint diverging lines. By preparing thinner sections, and examining them with higher powers and polarised light, I was able to make out the disposition of the crystalline elements sufficiently clearly. The coarser structure of Heliopora makes it a good object for study. Fig. 25 is a representation of a portion of a transverse section magnified 420 times. The first thing to be noticed is the absence of dark lines or "centres of calcification," such as occur in Madreporaria, and have been so well figured and

described by Mrs. Gordon. But centres of calcification are clearly present, though not represented by dark spots. The section clearly shows radiating systems of crystals diverging from centres *a*, *a*, the latter appearing white and translucent when viewed by transmitted light. From these centres crystalline fibres diverge upwards and outwards, spreading out to greater distances from the centre in three positions, which are approximately at 120° to one another. These extensions meet and dovetail with similar extensions from adjoining centres, and thus produce the characteristic skeleton of the cœnenchymal tubes. The section figured is not from the extreme growing point, but rather below it. At the extreme growing point it would be seen that each centre is really trifid, producing the arrangement which I have described before, and which I need not further particularise. A little below the extreme growing edge the separate centres unite in the angle formed between three adjacent cœnenchymal tubes, and there form a more or less Y-shaped centre of calcification, from which the crystalline fibres diverge in three directions, meeting in sutural junctions with similar diverging systems with the diverging fibres from adjacent systems. The whole structure is displayed in the annexed diagram. From this it is seen

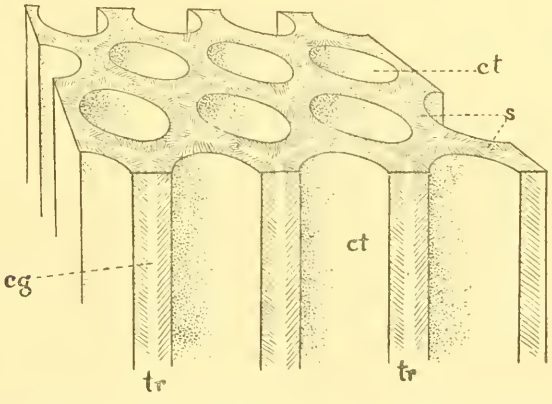

Diagram illustrating the arrangement of the crystalline fibres forming the corallum in Heliopora cœrulea. *ct*. Cœnenchymal tubes. *tr*. Trabeculæ. *s*. Sutures.

that at some little distance below the surface the cœnenchymal tubes are bounded by trabeculæ, which run at 90° to the surface of the colony. Each trabecula is triradiate in transverse section, and is seen in longitudinal section through its centre to be composed of crystalline fibres diverging upwards and outwards from a centre of calcification. In any given transverse section the fibres emerge at the surface of the section, and thus a series of concentric lines is formed around each centre of calcification, which are exactly similar to the "growth-lines" described by Mrs. Gordon in various Madreporarian sections. It is quite clear, from what has gone before, that these concentric striations in Heliopora are not the expression of layers of calcified cells which have been added to the thickness of the corallum. I shall hope to show that the same is the case in the Madreporaria.

Owing to the comparative coarseness of its component crystals, there is very little doubt as to the cause of the concentric markings in the Helioporan skeleton, and for the same reason the markings themselves are not so delicate, and betray their origin more clearly than do those of Madreporaria.

Professor Sollas kindly determined the specific gravity of the skeleton of Heliopora for me, and found it to be 2·82, calcite being 2·7 and aragonite 2·9. The skeleton of Heliopora therefore appears to be composed of aragonite, and not of calcite, as are the spicules of other Alcyonaria. The difference between the specific gravities of the Helioporan skeleton and aragonite is to be accounted for by the presence of a considerable amount of organic matter in the former. This organic matter, as I showed in my previous paper (4), is associated with the blue colouring matter characteristic of the species. The coralla of Euphyllia and Madrepora are, by Professor Sollas's determination of specific gravities, of 2·77 and 2·78 respectively, thus standing nearly halfway between calcite and aragonite. In this case, too, the difference between the coral fragments and aragonite is probably due to the fragments being full of organic matter, particularly of the filaments of a parasitic boring fungus, and a boring sponge of the genus Clione.

To sum up the characters of Heliopora :

1. The corallum is composed, like that of Madreporaria, of vertical or nearly vertical trabeculæ; but instead of the trabeculæ being in apposition by their lateral surfaces, as in Madreporaria, they are separated by cœnenchymal tubes. Each trabecula gives out arms at angles of 120°, and the arms unite suturally with similar arms from adjacent trabeculæ.

2. Each trabecula consists of crystalline fibres diverging outwards and upwards from a centre of calcification situated in the centre of the trabecula.

3. In transverse section the emergent crystalline fibres give rise to the appearance of striæ concentric with the centres of calcification. Such striæ are most conspicuous in the secondary deposits in the interiors of the calyces.

4. The separate crystals or bundles of crystalline fibres are not formed within cells, but are formed by crystallisation in connection with an organic basis produced by the disintegration of calicoblast cells.

5. Special structures, here called "desmocytes," are formed amongst the calicoblasts. They take their origin from certain cells in the calicoblast layer, and become secondarily attached to the mesoglœa by processes. Their function is to bind the soft tissues to the corallum.

Madreporaria.

As regards the shape and position of the calicoblasts and of the desmocytes there is no great amount of variety among the Madreporaria, though it will appear that my observations confirm previous statements by Fowler and myself as to the presence of unusually elongated calicoblasts in Mussa and Lophohelia. I have examined a considerable number of species, including Caryophyllia Smithii, Mussa distans, Lophohelia prolifera, Euphyllia (sp. incert.), Porites bulbosus, Madrepora subulata, rosacea, and hyacin-

thus. The results obtained were so nearly the same in all that I will not give details of all the species examined; to do so would involve a wearisome repetition.

In a previous paper (2) I described the calicoblasts of Mussa distans as being, in the regions of most active coral growth, long, narrow, and columnar, but elsewhere rounded or polygonal, with nuclei which stain faintly in borax carmine. At the same time I described structures which I identified with the striated calicoblasts of von Heider, but now with the desmocytes of Heliopora.

As it was very difficult to get good preparations of calicoblasts from decalcified specimens of Mussa, and as they could scarcely be studied in sections of hard and soft parts together, I had recourse to the following method :—Thick sections of hard and soft parts together were prepared in the usual manner by grinding. After examination of these sections by low powers suitable parts were cut out and the balsam was dissolved out by xylol. They were then placed in a very dilute solution of acetic acid, and the soft tissues were carefully removed as soon as decalcification had proceeded far enough to loosen their hold on the corallum. The tissues thus separated were either arranged as flat preparations, or were again embedded in paraffin and cut into very thin sections. For staining I used picro-carmine and nigrosin, or iron hæmatoxylin and acid fuchsin.

Fig. 27 is a drawing of part of a transverse section made as above. *Sep.* and *Dissep.* mark the positions occupied by a septum and a dissepiment respectively. *Mes.* are mesenteries. The calicoblasts (*ca.*) are seen to be tall vacuolated columnar cells, with distinct nuclei. At the places where the mesenteries join on to the tissues investing the corallum are seen groups of structures marked *dc.* They are the striated calicoblasts of von Heider and Mrs. Gordon. As I have no doubt of their homology with similar structures in Heliopora, I shall call them desmocytes. This section is characteristic in so far as the position of the desmocytes is concerned. They are always grouped in the greatest numbers about the insertions of the mesenteries, a fact which has already been recorded by von

Heider, Fowler, and myself. The fact is shown even better in a flat preparation of the edge-zone of Caryophyllia. The desmocytes appear as a number of thick bands, corresponding to the extra-thecal continuations of the mesenteries; the spaces between are occupied by the irregular vacuolated calicoblasts which will be described further on. I may here insist on the arguments which were put forward with great clearness by Fowler (9). The lines along which the desmocytes occur correspond neither to the septa nor to the costæ, but in corals in which there is a "pseudotheca" they correspond to the lines along which adjacent septa have met and fused. These are not lines of active growth, but contrariwise The regions of active growth, the septa and costæ, are not covered by desmocytes, but by a quite different kind of cells, whose characters vary according as the position is or is not one of active coral growth. The position of the desmocytes affords strong presumptive evidence against their being the active agents in coral secretion. Were this the case the corallum would grow most rapidly not along the edges of the septa or costæ, but along the lines between the septa and costæ where the mesenteries are attached to it. But these are exactly the places where its growth is least noticeable. A few desmocytes, it is true, may be observed abutting on the lateral surfaces of the septa. Such a one is shown in fig. 27, on the left-hand side of the drawing; but in such places the desmocytes never form a continuous layer, but are placed at intervals far apart from one another.

A surface view of a group of desmocytes is given in fig. 35, and a section made at right angles to their surfaces is represented in fig. 28. In the latter figure the striæ are seen to be dark branching fibres, which run with a wavy course from the base to the surface of the desmocyte. These striæ stain deeply with iron hæmatoxylin, eosin, Bismarck brown, picro-carmine, or picro-nigrosin. In fact, almost any diffuse stain will bring them out. Neither their shape nor their staining properties suggest their being spicular, as von Heider suggested, nor does a surface view lend any further support to the idea. Viewed from above, the desmocytes have a curious "machine turned"

appearance, due to the arrangement of the fibres, which will be best understood by reference to the figure. Both the surface view and section show that there is a small remnant of protoplasm with a nucleus in connection with each desmocyte. As a rule the desmocyte is cup-shaped, just as in Heliopora; and in such a case its centre appears dark when viewed from above. I have examined many hundreds of these desmocytes, freshly removed from the coral without decalcification, to see whether I could find any trace of crystalline structures in them with the aid of the polariscope. I have never found any either in Mussa, Euphyllia, Lophohelia, Madrepora, Astræa, Fungia (all of which were spirit specimens), or in the freshly removed tissues of a newly killed Caryophyllia. The striations, whatever they are, are not due to crystals of carbonate of lime.

It would be tedious if I were to give details of structure and position of these desmocytes in all the forms which I have examined. Fig. 27 shows their relation to the corallum in Mussa; fig. 33 shows their relation to the peripheral parts of the mesenteries in the edge-zone of Euphyllia; fig. 42 shows one of them in a radial connecting canal of Madrepora. Figs. 36 to 39 show various phases of their development in Caryophyllia Smithii. In the last-named species the calicoblasts, except at the extreme growing edges of the corallum, are small, highly vacuolated, and without definite cell outlines. At a spot where a desmocyte is about to be formed, one, two, or three nuclei become surrounded with a mass of darker, finely granular protoplasm. The next phase is the appearance of a band-shaped or ovoid body in the centre of the granular protoplasm, which already shows faint signs of striation, and stains readily with picro-nigrosin or acid fuchsin, the striæ being usually coloured by picro-carmine if this has been used. Usually one nucleus remains in close association with this body; the others (if more than one combine to form the granular protoplasmic mass) appear to be concerned in the formation of the mesoglœal process which will join the desmocyte to the mesoglœal lamina. The striations next become

more defined, and the desmocyte, which was at first separate from the mesoglœa, becomes attached to it by a process developed, as it seems, at the expense of neighbouring cells. In its final condition the desmocyte may be of various shapes—twisted as in fig. 39, or of a well-marked goblet shape. I have no doubt that Fowler (9) was right when he ascribed to the desmocytes the function of fastening the soft tissues to the corallum, though they do not appear to be mere processes of the mesoglœa, as he described, but structures sui generis, developed at the expense of cells otherwise indistinguishable from calicoblasts.

I claim, therefore, that not only their position, but also their microscopical structure, their development, and their behaviour under the polariscope, show that the desmocytes are not coral-secreting cells.

But if they are not, some further information is required as to how the corallum is formed, and as to what are the histological characters of the calicoblasts which form it. And the existence of the scale-like calcareous structures described and figured by Mrs. Gordon has to be accounted for, since it is evident from what has preceded that these cannot be calcified desmocytes.

In order to determine these questions I made a number of observations on Caryophyllia Smithii, this being the only coral which I could obtain living. If a Caryophyllia be killed in a five per cent. solution of osmic acid, stained for twenty minutes in picro-carmine, and then placed in glycerine, the soft tissues may, with the exercise of a little care, be readily removed from the exsert portions of the septa; but it is best to cut out the septa carefully with a stout pair of scissors, and to remove the tissues from the fragments of the septa with needles and a camel's-hair brush. The corallum is found to be everywhere covered by an exceedingly fine membrane, consisting on the one side of very flat endoderm cells, forming a sort of pavement epithelium. I noticed in these cells a peculiar structure, which is shown in fig. 26, and it may be described in passing. Each endoderm cell bears a rather long flagellum, and the flagellum may be traced to a minute prominence in the neigh-

bourhood of the nucleus. On focussing carefully the prominence exhibits radial striæ, as shown in the figure, surrounding a central clear space, and the flagellum seems to end in a minute granule in the midst of the clear space. The cytoplasm of the cells is much vacuolated, but usually there is a mass of denser protoplasm round the nucleus in which the star-shaped structure is situated. The invariable proximity of the star-shaped body to the nucleus suggests that the flagellum is connected with the centrosome. If this is so it has the same relations as the flagella of the spermatids of Elasmobranchs described by J. E. S. Moore. I was unable to find any example of these cells in course of division.

These endoderm cells are placed upon an exceedingly delicate lamina of mesoglœa, so delicate that it is easily overlooked, but it may be made evident by picro-nigrosin. On the other side of the mesoglœa—that is to say, next to the corallum—are a number of nuclei, round and clear in appearance, staining with great difficulty, and showing no chromatin network, but a single nucleolus.

Surrounding each nucleus is a thin layer of finely granular protoplasm, connected by fine processes with adjoining cells of the same character. This is all that represents the calicoblast layer over the greater part of the corallum, and so thin and delicate is it that it is hardly to be distinguished in transverse sections. But at the edge of a septum, where the sheet of tissue is folded over it, the calicoblast layer in the angle of the fold assumes more definite characters. It is still exceedingly thin, but the cytoplasm surrounding the nuclei increases in bulk, and exhibits a distinct vesicular structure in addition to the coarser vacuoles which are abundantly developed in it. The nuclei also show chromatin networks. Sections made through a Caryophyllia which has been killed in a mixture of 8 per cent. formol in sea water, to which is added about 3 per cent. of saturated solution of corrosive sublimate, and afterwards carefully decalcified in weak acetic acid, show the same results, and also that the calicoblast layer is more definite in the immediate neighbourhood of the mesenteries, both in the

edge zone and in the intra-calicular part of the polyp. (Not all specimens of Caryophyllia have an edge-zone, but it is well developed in some.)

In the latter situation the calicoblasts seem to serve chiefly for the formation of desmocytes, for they thin out to an almost unrecognisable layer in the areas between the mesenterics. But in the angles formed by the folds of tissue over the edges of the septa the larger granular cells may be distinguished just as in the macerated preparations. The remarkable thing about these calicoblasts is that they do not form a single or a regular layer of cells. There are, in fact, no separate cells, but there is a layer of very much vacuolated protoplasm containing nuclei—some I have observed undergoing division,—and the protoplasm seems to be "concentrated," i. e. to be more finely vacuolar and granular in certain places. In many places, as seen in surface view, there are hollows in the protoplasmic mass surrounded by ridges of the more vacuolar protoplasm, and the hollows are lined with finely granular cells, if one may apply the name to such a tissue as is shown in fig. 40.

Two other structural elements could be distinguished. The one consisted of the ovoid bodies with which I was familiar in Alcyonarians. In Caryophyllia these generally showed traces of a filament coiled up spirally within, and hence my conjecture that they are degraded nematocysts. The other structure is shown in fig. 30. It consists of a little eminence in the vacuolar protoplasm forming a ring or half-hoop, with a concavity in the centre, and the walls surrounding the concavity are striated as shown in the figure. These only occur at the sides of, and not actually on the edge of a septum, and they are probably rudimentary desmocytes, serving the same purpose of attaching the soft tissues to the corallum.

The soft tissues covering the pali in Caryophyllia correspond exactly with those covering the septa. Among the corals which formed a part of the collection of the late Mr. George Brook were some very well-preserved specimens of a species of Euphyllia. As I only have specimens embedded in paraffin or in balsam I have been unable to determine the species; a

portion of a section of this coral is represented in fig. 33. The calicoblasts have the same characters as in Caryophyllia, and where they form a deeper layer the same incoherent vacuolar structure is observable. Fig. 34 represents part of the tissues clothing the upper and inner edge of a septum in this species. The endoderm cells are well preserved, but the central mass of calicoblasts look as if they had undergone prolonged maceration. I do not, however, believe that this is the case, but that the loose vacuolar appearance of the protoplasm—one can hardly speak of cells, for no cell outlines are visible—really represents the structure of the calicoblast layer. For the same characters are present wherever the calicoblast layer attains any thickness, and the only places where it does attain any thickness are at the edges of the septa, and deep down in the angles between the septa where the edge-zone overlaps the edges of the theca. In all cases the surrounding tissues were very well preserved, and the state of their preservation may be judged from fig. 33.

In Mr. Brook's collection were also many sections of Madrepora. I am unable to determine the species for the same reasons as stated for Euphyllia. Fowler has spoken (7) of a "distinct" layer of calicoblasts in Madrepora Durvillei. The layer is distinct enough in my specimens so far as the presence of nuclei and surrounding cytoplasm goes, but there are no cell outlines, and the cytoplasm is vacuolated and irregular as shown in fig. 42.

I have noticed the same phenomena in several other corals, viz. extreme attenuation of the calicoblast layer in all but certain well-defined places, and in those a mass of vacuolar protoplasm of irregular outline with no cell boundaries. These characters, therefore, appear to be normal for a considerable number of corals.

On the other hand, there are the elongate cells of Mussa, and those described by Fowler (9) in Lophohelia prolifera. To satisfy myself as to the latter species I have made a series of preparations, but the tissues were too much injured by prolonged action of spirit to allow me to speak with certainty about details. It is sufficient to say that I found elongate

"cells" in the positions described by Fowler; and, as far as I could determine, the "cells" in question were not neat columnar cells with definite cell boundaries, as figured in Fowler's diagram, but resembled those of Mussa (vide fig. 29), —being, in fact, long amœboid, vacuolated, and partially fused masses of protoplasm containing nuclei. The cellular character of the calicoblasts is, however, more evident in Lophohelia and in Mussa than in any other forms which I have examined.

Fig. 29 is a representation of the elongated and quasi-columnar calicoblasts of Mussa distans, drawn under a high magnification. The section was stained by Heidenhain's iron hæmatoxylin method. Under a lower power (120 diameters) the calicoblasts appear to form a layer of simple columnar epithelium, but a higher power (1000 diameters) brings out the characters shown in the figure. The cells are confluent, both at their bases and at their free ends, especially the latter. The alveolar structure of the cytoplasm is well shown, and besides the minute alveoli there are a number of small vacuoles, each of which contains a microsome. The presence of vacuoles containing microsomata is characteristic of well-preserved calicoblasts. A comparison with fig. 10 shows that there is no essential difference between the calicoblasts of Mussa and Caryophyllia, though the elements are much smaller in the latter species. Fig. 29 does not show the presence of an external limiting membrane, but there are indications of it in fig. 27, which was drawn from the same slide. I have reason to believe that there is always an external limiting membrane to the calicoblasts in Madreporaria, and that when it is absent in sections it has been destroyed in the course of decalcification. As has already been stated, a similar membrane occurs in Heliopora. The limiting membrane is interposed between the calicoblasts and the corallum, and may be regarded as a sheath of the latter. It is clear that the carbonate of lime secreted by the calicoblasts must pass through the membrane, just as it has to pass through the spicule sheath in Alcyonarians. Throughout my researches I have looked most

carefully for evidence of the formation of spicules or crystals within cells; but no such evidence was to be found. Were spicules or crystals formed within cells I could hardly have failed to find them, either in Heliopora or the Madreporaria in the numerous preparations which I searched with the polariscope. I have found crystals in certain cases both in Heliopora and in Caryophyllia, and have nearly been deceived by them, but they occur in the endoderm and particularly in the zooxanthellæ, and I have no doubt that they are aleuron crystalloids, though I have not specially tested the point.

Moreover I am convinced that the structure of the Madreporarian skeleton does not admit of a theory of spicular formation. I can take no exception to Mrs. Gordon's drawings and descriptions of the numerous sections, and most of the surface views of Madreporaria which she has given. They are faithfully delineated, and constitute an important addition to our knowledge of corals. But I join issue with her on the subject of the ultimate skeletal unit. She has dismissed the "crystalline sphæroid" described by von Koch in almost contemptuous terms. "For what is this sphæroid? Is it a spicule? We can see and separate the spicule, then why not the sphæroid? It is noteworthy that the sphæroid has been presented from first to last by one or two transverse sections, no longitudinal section has demonstrated it." Mrs. Gordon's memory is at fault. The sphæroid was represented very clearly by von Koch between the base and the surface of attachment in an Astroides larva, and the section was a longitudinal one. Quite recently De Lacaze-Duthiers, in a beautifully illustrated memoir on the development of Caryophyllia Smithii and Balanophyllia regia (24), has completely rehabilitated the crystalline sphæroid or "sclerenchymatous nodule," if that name be preferred. Speaking of the larva of the former species, he says, "Le premier dépôt de la charpente calcaire se produit sous la forme d'un semis de globules très refringents." His illustration (loc. cit., pl. ix, fig. 16) leaves no doubt on the subject; the first

traces of the calcareous skeleton of Madreporaria are what von Koch described, sphæroids of calcium carbonate. I shall show presently that similar sphæroids may be observed on the septal surface of the adult Caryophyllia. At the same time the "calcareous scale" figured by Mrs. Gordon for Galaxea exists, and calls for explanation. I have said enough to show that the scales in question are certainly not calcified desmocytes. I have found these scales in preparations of the vesicular endotheca of Galaxea laperonsiana made for the purpose of verifying Mrs. Gordon's statements, but I have found them best developed in preparations of Madrepora subulata, M. rosacea, and M. hyacinthus, taken from the collection of the late Mr. Geo. Brook. In tolerably thick sections, longitudinal or transverse, of any of these species, the calcareous scales are very evident at the sides of the septa, cœnenchymal trabeculæ, and costal spines included in the section. The outer edges of the scales are commonly coloured bright crimson in specimens stained in borax carmine (see fig. 41), but this effect is not produced by aniline dyes, and after some study I became convinced that there is no staining of an organic residue, but a deposit of carmine particles on the edges of the scales. But the whole structure is so striking that it seems to afford positive proof of the statement that "the growth-lamellæ of Madrepora from first to last are composed of coalesced calcified calicoblasts, which once represented living ectoderm" (Mrs. Gordon, loc. cit., p. 213). One's belief is shaken, however, by the study of sections, both of hard and soft parts together and of decalcified specimens. The layer of continuous vacuolated protoplasm shown in fig. 42 lends no support to the conception of successive shedding off of calcified cells from a definite cell layer, nor does a side-by-side comparison of as much of the calicoblasts as can be seen in a ground-down section with the adjacent corallum. A portion of such a section, magnified 420 times, is shown in fig. 41. And here let me say that neither in this nor in any other sections of several species of Madrepora could I find any trace of a continuous layer of desmocytes. Here and

there was a desmocyte, such as is shown in fig. 42, conspicuous among the calicoblasts for its dark colour, but of tracts of desmocytes no trace whatever.

In the section under consideration the soft tissues have shrunk away from the corallum. At the edge of the latter the striated scales (Mrs. Gordon's calcified calicoblasts) are clearly seen, and adherent to the mesoglœa, and lying on the corallum where the latter is in contact with the soft tissues, are calicoblasts—irregularly-shaped rounded cells, and decidedly smaller than the calcareous scales. That the calicoblasts appear in the section as separate cells, and not as a continuous layer of protoplasm, is probably due to shrinkage. In other parts of the section, where the soft tissues have not shrunk away from the corallum, the calicoblast layer appears continuous. The rubbed-down sections are too thick for satisfactory examination with an immersion lens, and one cannot speak with certainty, but in decalcified sections from the same species the calicoblast layer always has the characters represented in fig. 42.

The other point noticeable in the section is the fact that the surface scales on the same level as the polished upper surface of the section appear to be emergences of tracts of fibres which diverge from a centre of calcification which is not included in the drawing. Mrs. Gordon's drawings of sections of Madrepora are represented on a much smaller scale than mine (loc. cit., figs. 60—63), but in her fig. 63 there is a distinct indication of the same fact. Now if her theory were true, viz. that each scale represented a calcified ectoderm cell, it would follow as a necessary corollary that the calcified cells were piled upon one another in perfectly regular series, cell fitting accurately on the top of cell, and in such a manner as to produce the regular diverging columns shown in my figure. This is extremely improbable, as anybody well acquainted with the arrangement and behaviour of cells will allow. And a comparison with the structure of the Spongodes spicule shows that such an assumption is unnecessary for the explanation of the structure. In the Spongodes spicule the ground substance is made up of regularly disposed concentric lamellæ, and each

lamella consists of strands of crystalline fibres which have a general direction parallel with the long axis of the spicule. I cannot say exactly how this arrangement was brought about, but it certainly is not due to the apposition of calcified cells, for the whole spicule was formed inside a cell or cœnocyte, and was covered in all stages of its growth by a spicular sheath of organic matter. In fact, the spicule was, from its early origin, separated from the protoplasm which elaborated the material necessary for its further growth by a layer of some cuticular material.

The case in the Madreporaria, and also in Heliopora, appears to be somewhat parallel. Fig. 19 shows that in Heliopora a cuticular external limiting membrane extends from the edges of the desmocyte over the adjoining calicoblasts. Figs. 27, 33, and 43 show the same phenomenon in Madreporaria. And there are numerous indications in flat preparations that the calicoblast layer both in Heliopora and Madreporaria is separated by an external limiting membrane from the corallum. I have frequently noticed at the edges of the septa of Caryophyllia that there was a space between the edge of the corallum and the overlying soft tissues, and that this space was occupied by a colloid substance in which minute particles could be detected. I was for a long time doubtful whether this appearance was deceptive or not, but studies of the growth of costal spines in sections through hard and soft parts of the different species of Madrepora named above have settled the question.

Fig. 43 represents a costal spine with its adherent tissues in M. subulata. At the bottom of the figure is a dark "centre of calcification," from which lines, representing fibro-crystals, diverge to the surfaces of the spine. At the tip of the spine its contours become indefinite, and instead of the compact crystalline arrangement we have separate diverging fibres, and outside of these, minute particles which show a tendency to arrange themselves in lines. At the base of the spine calicoblasts may be seen lying close to the corallum. Towards the apex of the spine they no longer lie close to the calcareous

deposit, but are seen to be separated from it by a very delicate membrane, which is continued beyond the apex of the calcareous spine as an unquestionable sheath. Outside of the calicoblasts is the mesoglœa, and outside of this again the endoderm represented diagrammatically. Precisely the same features are shown in all the costal spines of this and several other sections, this particular spine being selected for representation because the sheath or membrane between the calicoblasts and the calcareous spine is very distinct. There can, I think, be only one interpretation of the phenomena. The spine grows by the addition of minute particles of carbonate of lime, which crystallise out of an organic liquid matrix secreted by the calicoblastic layer. These particles attach themselves to the crystalline structure already present, and become oriented conformably with it, so as to continue the pre-existent crystalline figure in the same mode and in the same direction. If anybody doubts the possibility of this, let him study the formation and growth of crystals forming in a concentrated solution of such a substance as potassium sulphate to which a small amount of some colloid substance, such as mucus or gelatine, has been added. A high power of the microscope should be used. As the solution dries upon the slide minute particles make their appearance: at first they do not behave as crystals under polarised light, nor do the particles in the apparently colloidal material lying between the calicoblasts and the corallum of Madreporaria. After a time the particles aggregate themselves at the growing point of a crystal already formed: they increase in number, and then slowly, imperceptibly, they orient themselves in the direction of the axis of the crystal and become a part of it, producing figures exactly like that shown in fig. 43. In the case of the inorganic crystal, the particles, when they are oriented but not yet attached to the crystal, show up with a pale lambent light when viewed through crossed Nicols, the rest of the crystal standing out brilliantly. Exactly the same phenomenon is observed in the growing tip of a costal spine of Madrepora.

The conclusion is irresistible, though I only arrived at it

after holding almost every other conceivable hypothesis on the subject. The corallum of the Madreporaria and of Heliopora, and the spicules of other Alcyonaria, are crystalline growths formed by the deposition of needles of carbonate of lime in a colloid matrix, and obeying the ordinary laws of crystalline growth in detail; but the general arrangement of the fasciculi of crystals is dominated, in some manner of which we are ignorant, by the living tissues which clothe the corallum.

We have seen that the characteristic structure of certain Alcyonarian spicules is moulded upon an organic pattern of fibres. I have looked for a similar organic pattern in Madreporaria, but without success. The corallum of Madrepores is invariably penetrated by the mycelium of the parasitic fungus which I have elsewhere described under the name given to it by W. B. Carpenter, Achyla penetrans. I believe, however, that it does not really belong to the genus Achyla. However, the presence of these fungus filaments have prevented my making any trustworthy observations on the organic matrix of the Madreporarian skeleton. I have slowly and carefully decalcified sections of coralla on a slide, and have invariably obtained a plexus of mycelial filaments, among which I could detect fine threads whose arrangement I could not determine owing to the presence of the fungus. I am of the opinion that there is an organic pattern, probably consisting of a fine network of threads analogous to those observed in the spicules of Spongodes, but I cannot at present say anything definite on the subject. Further, I suspect that the dark "centres of calcification" will be found to be the expression of a core of organic filaments, just as the central dark line in the Alcyonarian spicule is the expression of the central core of threads. In further support of the conclusions arrived at as to the formation of the Madreporarian skeleton, I must add that the "scales" which are so conspicuous in Galaxea, and in the genus Madrepora, as also in Mussa and some other genera, are by no means a constant feature in Madreporaria. I have failed to find them in Caryophyllia, Porites, Montipora, Lophohelia, and others. I have

examined Caryophyllia with special care. Fig. 31 is a representation of an optical section of a septal granulation as seen with the oil immersion. The apex of the granulation was, of course, out of focus, and is not represented, but its periphery is seen to be composed of a number of needle-like crystals radiating outwards. The crystals are grouped together in bunches, and all the crystals in a bunch diverge from an ideal centre towards the free surface, not only horizontally, but upwards and downwards in all directions where they can extend. Fig. 32 is a not very satisfactory drawing of the surface of a very thin palus of Caryophyllia near its extreme upper edge. The surface is very irregular, and studded with hemisphæroidal projections, each of which is composed of fibro-crystals, diverging in all directions where there is room for their growth. This is a much more common appearance, in my experience, than the flattish overlapping "scales." Similar sphæroids or hemisphæroids are formed in many inorganic solutions, and I opine that we find in these the "crystalline sphæroids" described by von Koch and derided by Mrs. Gordon. It is worth noticing that the extreme irregularity of the surface of the corallum of Caryophyllia corresponds with the incoherent and irregular arrangement of the calicoblasts which I have described for this species. The continued growth and aggregation of such sphæroids would give rise to what mineralogists term a botryoidal formation, and the Madreporarian skeleton may fairly be described as a complex botryoidal growth, whose character has been influenced profoundly by the living tissues which gave origin to it.

If my conclusions are correct the whole skeleton of a Madreporarian polyp has some sort of analogy to a single Alcyonarian spicule. Both are composed of fibro-crystals having an infinitely various, but for every species a definite and characteristic arrangement. As the spicule is enclosed in a sheath, and separate from the protoplasm of the cell or cœnocyte from which it is developed, so is the Madreporarian corallum covered with a membrane or corallum sheath, and separated from the calicoblastic layer which gave rise to it. If there is no diffi-

culty in admitting the fact that an Alcyonarian spicule can grow by deposition of successive crystalline layers within its sheath, there can be no difficulty in admitting the possibility that the Madreporarian corallum grows in the same way, by addition of new material elaborated by the calicoblasts and passed through the membrane which lies between them and the corallum. I do not wish to push the analogy further, but only to emphasise the fact that an explanation which is satisfactory in the case of such complex bodies as Alcyonarian spicules, with their bewildering variety of form and their complex arrangements of warts and spines, cannot be held to be unsatisfactory in the case of Madreporarian coralla, whose fundamental plan is not strikingly variable, and whose detail is scarcely more complicated than that of the spicules.

Of course there is no real explanation in either case. We are as ignorant of the laws which govern the formation of these organic crystalline growths as we are of the molecular laws which determine why a given mineral solution shall crystallise out according to a given system. But I enter my protest against the discovery of a Deus ex machinâ in the form of calcified cells.

I have been mainly occupied in the course of this paper in criticising a particular statement made by Mrs. Gordon, and I may appear to have been unappreciative of her excellent memoir on the structure of Madreporarian corals. I must conclude by saying that I do not conceive that her most important conclusions are affected by the error which she has made in the matter of the calicoblasts. The acceptance of my views on the formation of the corallum would not detract in the least from the importance and truthfulness of her observations on the different types of microscopical and macroscopical structure in recent and fossil Madreporaria, nor would it make them less useful as a basis for an amended classification of the group. Saving this question of calcified ectoderm, and some remarks on the anatomy of Fungia with which I shall have to deal on another occasion, I have found her descriptions of fact

to be remarkably accurate in the numerous cases in which I have taken the trouble to verify them.

In conclusion, I must express my thanks firstly to Professor Ray Lankester, in whose laboratory my investigations were conducted; next, to Professor De Lacaze-Duthiers for permission to work in the Marine Laboratory at Roscoff, where I was abundantly supplied with living specimens of Alcyonium, Gorgonia, and Caryophyllia: to Mrs. Geo. Brook, to whose kindness I am indebted for the valuable collection of specimens and preparations of Madreporaria, without which I could hardly have completed my work; to Professors Miers and Sollas, who have spent much of their valuable time in overcoming my profound ignorance on the subject of crystallography; and lastly, to my friend Mr. E. A. Minchin, whose studies on sponge spicules, made in the same room in which I have been working, have greatly influenced my work on the Anthozoa.

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EXPLANATION OF PLATES 40—43.

Illustrating Mr. G. C. Bourne's paper on "The Structure and Formation of the Calcareous Skeleton of the Anthozoa."

PLATE 40.

Figs. 1—5.—Alcyonium digitatum. Zeiss, $\frac{1}{12}$ hom. imm., c. oc. 8.

Fig. 1. A group of sclerоblastic cells from the base of a polyp. *scl.* A small sclerite in course of formation enclosed in a vacuole in the sclerоblast. *ov.* Ovoid bodies. *gr.* Finely granular cells which probably form mesoglœa.

Fig. 2. A sclerоblast enclosing a larger sclerite; the cytoplasm is still abundant, and contains granules and vacuoles.

Fig. 3. A larger sclerite enclosed in a sclerоblast.

Fig. 4. Two twin-sclerites enclosed in a twin cell with two nuclei.

Fig. 5. A characteristic spicule enclosed in a thin protoplasmic envelope with two nuclei.

Fig. 6.—A single scale-like spicule of Plumarella delicatissima, Wright and Studer. Zeiss B, oc. 4.

Fig. 7.—The same spicule viewed through the polariscope, showing the dark cross due to the extinction of light by those component crystals whose optic axes lie in four positions at right angles to one another. Zeiss B, oc. 2.

Figs. 8—12.—Spongodes, sp.?

Fig. 8. A single spicule from the stem, showing the warty projections and the dark axial core, *ax.*

Fig. 9. A transverse section of a similar spicule, showing *ax.*, the axial core; *ra.*, the radial cords, many of which are bifurcated; *c. g.*, the concentric lines of growth.

Fig. 10. A longitudinal section of a similar spicule, showing *me.*, the medullary portion; *in. p.*, the intermediate portion; and *co. p.*, the cortical portion: the other lettering as in Fig. 9.

Fig. 11. Part of a longitudinal section through a spicule which has been crushed after grinding. The strands of fibro-crystals are clearly seen, traversed by the radial cords, *ra.*

Fig. 12. Part of a spicule which has been carefully decalcified with weak acetic acid. *mg.* Remains of mesoglœa. *sh.* Spicular sheath. *or. c.* Core composed of interlacing organic threads. *or. ra.* Radial organic threads corresponding to the radial cords in Figs. 9 and 10.

PLATE 41.

FIGS. 13—25.—Heliopora cœrulea, Pallas. *ca.* Calicoblast. *ca. i.* Internal layer of calicoblasts. *ca. e.* External layer of calicoblasts. *mg.* Mesoglœa. *en.* Endoderm. *dc.* Desmocyte. *ov.* Ovoid bodies. *zo.* Zooxanthellæ.

Fig. 13. Portion of a section through the growing point of Heliopora, showing the external and internal calicoblast layers. *m. l.* Limiting membrane formed outside the internal calicoblast layer. Zeiss, $\frac{1}{12}$ hom. imm., c. oc. 4.

Fig. 14. Ovoid bodies, showing nuclei and vacuoles.

Fig. 15. Early stages in the formation of desmocytes. A striated body, stained blue by picro-nigrosin, is contained within a much vacuolated cell.

Fig. 16. Further stages in the development of desmocytes.

Fig. 17. A desmocyte is becoming attached to the mesoglœa by a process, *p.* *m. l.* Limiting membrane.

Fig. 18. Desmocyte in course of formation, attached by several bands to the mesoglœa.

Fig. 19. A fully formed desmocyte, showing the characteristic cupped shape. *m. l.* External limiting membrane.

Fig. 20. A group of calicoblasts, some of which are undergoing disintegration.

Fig. 21. Calicoblasts stained with eosin, showing the eosinophilous granules, *gr.* *crys. f.* Groups of crystalline fibres adherent to a meshwork of organic filaments. Zeiss, $\frac{1}{12}$ hom. imm., c. oc. 4.

Fig. 22. Growing edge of a cœnenchymal spine, showing the manner in which it is built up of numerous crystals. Zeiss D, oc. 4.

Fig. 23. Surface view of part of a partition wall of a cœnenchymal tubule from the growing edge of Heliopora. The component crystals are arranged pell-mell in no definite order. Zeiss D, oc. 4.

Fig. 24. Isolated crystalline elements from the same preparation as Fig. 23. Zeiss, $\frac{1}{12}$ hom. imm., c. oc. 4.

Fig. 25. Portion of a transverse section taken about 2 mm. below the growing edge of a frond of Heliopora. *a. a.* Centres of calcification, from which crystalline fibres diverge to meet those of adjacent systems at the sutures *b. b.* *cl.* Cœnenchymal tubes.

FIG. 26.—Surface view of the flattened endoderm cells forming part of the investment of a septum of Caryophyllia Smithii. *fl.* Flagella, each arising from a clear space closely contiguous to the nucleus. The clear spaces are in the centres of small asters, and appear from their position to be related to the centrosome. The astral rays were stained as figured by safranin, but the flagella are represented in red only for clearness' sake.

PLATE 42.

Fig. 27.—Portion of a transverse section through Mussa distans. *Mes.* Mesenteries. *Sep.* space occupied by a septum. *Dissep.* Space occupied by a dissepiment. *ca.* Calicoblasts. *dc.* Desmocytes. *en.* Endoderm. *m. l.* Limiting membrane. Zeiss B, oc. 4.

Fig. 28.—Four desmocytes of Mussa distans, magnified 1000. *n.* Nuclei with surrounding cytoplasm of the parent cells of the desmocytes. *mg.* Mesogloea.

Fig. 29.—Calicoblasts of Mussa distans, magnified 1000. *vac.* Vacuoles containing microsomata. *mg.* Mesogloea. *en.* Endoderm.

Fig. 30.—Surface view of a portion of the calicoblast layer from the lateral surface of a septum of Caryophyllia Smithii, showing a rudimentary desmocyte, *dm.* The vacuolated and pitted character of the protoplasm is indicated. Zeiss, $\frac{1}{12}$ hom. imm., c. oc. 4.

Fig. 31.—Optical section of a septal granulation of Caryophyllia, showing that it is made up of a number of bunches of radiating fibro-crystals. Zeiss, $\frac{1}{12}$ hom. imm., c. oc. 4.

Fig. 32.—Surface view near the growing edge of a palus of Caryophyllia, showing the hemisphæroidal bunches of fibro-crystals of which it is composed.

PLATE 43.

Fig. 33.—Part of a transverse section through the edge-zone of Euphyllia. *ec.* Ectoderm with two kinds of gland-cells and nematocysts. *dc.* Desmocytes. *en.* Endoderm. *mes.* Extra-thecal portion of a mesentery. *mg.* Mesogloea. *ca.* Calicoblasts.

Fig. 34.—Part of a section of the same species, showing the fold of the soft tissues over the inner and upper edge of a septum. *en.* Endoderm. *mg.* Mesogloea. *ca.* Calicoblast layer consisting of nuclei lying in a layer of vacuolated protoplasm.

Fig. 35.—Surface view of a group of desmocytes of Caryophyllia Smithii. *Cor.* Fragments of corallum adherent to the desmocytes. *p. c.* Parent cells of desmocytes.

Figs. 36—39.—Stages in the development of desmocytes of Caryophyllia. Lettering as in the previous figures. *gl. c.* in Fig. 39 is a gland-cell filled with highly refringent globules. Such gland-cells are often very abundant in the endoderm immediately overlying active calicoblasts.

Fig. 40.—Part of a transverse section of Caryophyllia taken at the point where the tissues turn over the edge of the theca between two septa to form the edge-zone. *en.* Endoderm of the intra-thecal portion of the polyp. *en'.* Endoderm of the edge-zone. *mg.* Mesogloea. *ca.* Calicoblasts.

FIG. 41.—Portion of a transverse section through the hard and soft parts of Madrepora subulata, showing *sc.* The scale-like emergences of bundles of fibro-crystals on the free surface of the corallum. *b.* The bundles of fibro-crystals as seen in section, lying parallel to one another and emerging at the free surface to form the "scales." *Csp.* is a costal spine. *ca.* Calicoblasts. *mg.* Mesoglœa. *en.* Endoderm.

FIG. 42.—Portion of a section through a radial cœnenchymal canal of the same species, showing a desmocyte, *dc.*; calicoblast, *ca.*; and endoderm, *en.*

FIG. 43.—A transverse section through a costal spine and the adjacent soft tissues of Madrepora rosacea. *Sp.* Costal spine. *Crys.* Deposit of crystalline fibres on its growing point. *m. l.* Limiting membrane. *ca.* Calicoblasts. *en.* Endoderm. *mg.* Mesoglœa.

The Structure and Development of the Hairs of Monotremes and Marsupials.

Part I.—Monotremes.

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With Plates 44—46.

In his paper dealing with the structure of the bill and hairs of Ornithorhynchus,[1] Mr. Poulton has described the structure of the hair in an embryo of Ornithorhynchus measuring 8·3 cm. long, in which the larger hairs had appeared above the surface of the skin. Thanks to the kindness of Professor J. T. Wilson, of Sydney, we have been able to study much earlier stages as developed in an embryo measuring 40 mm. in length, the same embryo upon which he and Dr. Martin have already worked when studying the structure of the bill. To Mr. Dudley le Souef we are indebted for an embryo measuring 77 cm., in which the hairs have not yet appeared above the surface, and to Dr. Gregg Wilson for pieces of skin of an embryo of the same age. In the case of Echidna we have been able to study the structure of an embryo measuring 55 mm. in length in which the tips of the larger hairs, which subsequently become modified into spines, have just appeared

[1] 'Quart. Journ. Micr. Sci.,' vol. xxxvi, pt. 2, p. 143.

above the surface, and we are again indebted to Dr. Gregg Wilson for pieces of skin of an embryo of the same age. Owing to the fact that in different parts of the body the hairs are not all developed to the same extent, we have been able to study various stages—in the youngest one of which the follicle is only just formed, while in the oldest the largest hairs are well developed.

We are indebted to Professor J. T. Wilson and Mr. J. P. Hill for the opportunity of consulting literature which was unavailable to us in Melbourne.

Whilst as the result of a considerably greater supply of material, which is also probably in a better state of preservation for histological work than was that available to Mr. Poulton, we have been led to conclusions different from those to which he came, especially in regard to the question of the development of the hair in an open and not a closed tube—conclusions to which, moreover, we feel sure that Mr. Poulton would have come had he been able to study earlier stages—we desire to express our indebtedness to the assistance which we have received from his work, especially combined as it was with a valuable résumé of previous work compiled by Dr. Benham, certain of the memoirs dealt with by the latter being unavailable to us. We are at the present time engaged upon an investigation of the development of hairs in Marsupials, but have thought it better to divide our work into two parts, and the following deals simply with the structure and development of hairs in Monotremes.

To Professor G. B. Howes we are much indebted for his kindness on this, as on other occasions, in revising the proof.

General Structure and Arrangement of the Hairs.

The presence of large and small hairs and their arrangement has been dealt with by various authors, Leydig being the first to point out that the small hairs are arranged in bundles with a common follicular neck.

Leydig[1] described in Ornithorhynchus the presence of four

[1] "Ueber die äusseren bedeckungen der Säugethiere," 'Arch. für Anat. Phys. und Wiss. Med.,' 1859, pp. 677—747.

or five hairs in each bundle; Welcker gives as many as fifteen to thirty; Poulton (p. 159) gives the number as varying between seven and eleven, including both young and old hairs, the greatest number of old hairs being ten, and, referring to his figures, states that they "prove that, at any rate in the dorsal region, Leydig's estimate of the number of hairs in a bundle is too small, while that of Welcker is far too large."

There is evidently a considerable amount of variation, possibly between different individuals but more probably at different times of the year, and this variation affects not only the number of small hairs in each bundle but also the number of bundles. On the dorsal area Poulton gives the latter as constantly four. In the same part we have found the number to be usually the same, but occasionally five and more rarely even six bundles may be present. In his work dealing with the arrangement of the hairs in various mammals, Meijere[1] figures, on the back, two groups with five bundles each.

With regard to the number of hairs in each bundle, the following table represents an average series in a fully-grown male examined by us; in the case of successional hairs the latter are represented with the sign + in front of them.

Group 1.	Group 2.	Group 3.	Group 4.	Group 5.
Bundle—	Bundle—	Bundle—	Bundle—	Bundle—
1—16	1— 8	1—13	1—16	1—16
2—12	2—12	2—13	2—18	2— 7+6
3—14	3— 7+5	3—12	3—13	3—12
4—12	4—10+3	4—16	4—14	4—13
	5—17+1		5—15	

These show the number of small hairs in each bundle to vary generally from twelve to eighteen, less than twelve being very rarely met with.

Successional hairs are rarely met with,—there is, as Poulton says, no difficulty in distinguishing them when they are present,—and the few which are developing have not yet reached the level of the common follicular opening of the bundle.

[1] 'Morph. Jahrbuch,' Bd. xxi, 1894, p. 312.

In regard to the large hairs, Poulton (p. 158) says "The protective large hairs are evidently subject to much wear and tear, and succeed each other very rapidly; the new successional hair, which is to be met with in nearly every section, emerging from the same follicular mouth, in front of and therefore overlapping the base of the old one." In another part (p. 167) Poulton says that "the appearance of two hairs in one follicle is spoken of as an occasional appearance in other mammals. In the large hairs of Ornithorhynchus it is the invariable rule." A glance at the transverse sections figured by us (figs. 1, 2) will show that no successional hair, such as is figured by Poulton, is present; nor, though we have examined both transverse and longitudinal sections of the skin of three adult animals, have we been able to find any successional hairs. The same author, as quoted, says that "the protective large hairs are evidently subject to much wear and tear, and succeed each other very rapidly." From the method of life of Ornithorhynchus we think that the reverse is the case, and that they are not subject to much wear and tear; and further, that the difference in regard to the successional hairs as described by Poulton and ourselves is to be accounted for on the supposition that there is not a constant but a periodic shedding and replacement of hairs. Poulton's sections are on this supposition taken from an animal in which the shedding and replacement was taking place, while ours are from an animal in which replacement had already taken place and in which the hairs were in the maximum state of development.

With regard to the size of the hairs, that of the larger ones (fig. 3) varies, of course, according to the part of the hair which is cut through. In our sections the shaft—always round in section—is cut through, the diameter varying from ·0245 mm. to ·035 mm.; the smaller hairs vary from ·0071 mm. to ·0105 mm. The latter measurement agrees closely with that given by Meijere and Welcker, viz. ·008 mm.; the size of the large hairs given by Welcker is ·048 mm., and by Meijere ·045 mm.

In Echidna the hairs are arranged in groups as in Ornitho-

rhynchus, one (or very rarely two) large hairs usually emerging from a common follicular opening together with a relatively small number of small hairs (figs. 4, 5). The exact arrangement varies, however, in different parts of the body. On the back, where the spines are strongly developed, each of these issues from its own follicle and is not associated with small hairs. Amongst these spines is, however, a strong development of small distinctly wavy hairs arranged in groups of from eight to ten in number. There may sometimes, especially on the dorso-lateral aspects of the body, be a large hair associated with these groups, but most often there is not. In the mammary region also the large hair is apparently wanting (fig. 6). The following table gives the number of large and small hairs present in the bundles from different parts of the body of a large adult female Echidna from Tasmania.

Under Chin.	Pre-axial Surface of Fore-limb.
Bundle 1.—8 small, 1 large.	Bundle 1.—2 small, 2 large.
„ 2.—8 „ 1 „	„ 2.—2 „ 1 „
„ 3.—7 „ 1 „	„ 3.—2 „ 1 „
„ 4.—6 „ 1 „	„ 4.—2 „ 1 „
„ 5.—7 „ 1 „	„ 5.—2 „ 1 „
„ 6.—6 „ 1 „	„ 6.—2 „ 1 „
Centre of Mammary Area.	**Side of Body.**
Bundle 1.—4 small, no large.	Bundle 1.—6 small, 1 large.
„ 2.—5 „ „ „	„ 2.—6 „ „ „
„ 3.—5 „ „ „	„ 3.—6 „ „ „
„ 4.—5 „ „ „	„ 4.—7 „ „ „
„ 5.—5 „ „ „	„ 5.—5 „ „ „
„ 6.—4 „ „ „	„ 6.—7 „ „ „

In connection with the numbers now given it must be remembered that (1) there is very great variation in the hairs of various specimens from different localities such as Tasmania, Victoria, Queensland, and Central Australia, and (2) it is very likely indeed that both in different individuals and at different

times of the year in the case of the same individual the numbers will vary.

The large hairs are easily distinguishable from the spines, and this feature is most marked in the case of Central Australian specimens, owing to the fact that whereas the spines are always circular in section, the large hairs are flattened (figs. 7, 8), though there is not the distinct division of the hair into haft, shield, and tip as in the case of Ornithorhynchus.

The distinct wavy nature of the small hairs, especially of those of the dorsal region, is a marked feature of Echidna.

On the ventral surface of the body the small hairs vary in diameter from ·0357 mm. to ·054 mm.; the diameter of the large hairs varies from ·0612 mm. to ·108 mm. On the dorsal surface the small hairs average ·126 mm., while the large spines vary from 3·5 mm. to 4·5 mm., those of the large size being scattered about amongst a larger number of the smaller ones, though they are more thickly developed over two areas, one on the dorso-lateral surface on each side of the body about half-way back from the head.

For further details of the adult hairs, reference may be made to the descriptions of the figures.

Muscles of the Hairs.

In both Ornithorhynchus and Echidna a bundle of muscle fibres is associated with each group of hairs. In the case of Ornithorhynchus the fibres are, as usual, unstriated, but in the case of Echidna the fibres are striated. The bundle, as usual, passes from the superficial part of the corium obliquely downwards, and is attached to the special modification of the dermic layer which envelops the group of hair follicles; or, in the case of the large hairs which develop into spines and are not accompanied by small hairs, the muscle is attached near the bottom of the follicle, there being no special development of the outer root-sheath to form a swelling at this part.

From their disposition these striated fibres are evidently the equivalents of those forming the arrector pili in other mammals, and the only other instance known to us of the

latter being formed of striated fibres is that of the muscles moving vibrissæ.[1]

Development of the Hairs.

So far as essential points are concerned, the development of large and small hairs alike agrees in both Ornithorhynchus and Echidna. As in all mammals, so in the Monotremata the hair is developed in a solid downgrowth of the epidermis. It is possible that owing to the remarkable development of the inner root-sheath an indefinite central cavity may be formed at a somewhat earlier stage than that in which the follicle becomes tubular in other mammals, but a prolonged investigation of a large series of sections comprising various stages has led us to the conclusion that it is incorrect to say that the hairs of either Ornithorhynchus or Echidna are developed in tubes open to the exterior, "in" to use Mr. Poulton's words, "a tubular and not a solid downgrowth from the exterior." The downgrowth is at first a solid one exactly as it is in other mammals, and it is only at some considerable time after the hair has been formed that there is developed any lumen opening to the exterior. Exactly as in all other mammals so in the Monotremata the developing hair has to push its way up through the centre of a solid follicle.

For the sake of convenience we have taken eight stages, the structure of the hair and its follicle in each one of these being represented in the figures.

Stage 1 (fig. 9).—We have not, unfortunately, been able to secure a young enough embryo to demonstrate the earliest appearance of the follicular downgrowth. The section of the earliest stage which we have so far been able to secure, and which is figured, is, however, sufficiently early to indicate without any doubt that, in Monotremes, the commencement of the development of the hair and its follicle is fundamentally identical with that of all other mammals. There is nothing

[1] Owen, 'Comp. Anat.,' vol. iii, p. 621. See also Heneage Gibbes, 'Quart. Journ. Mic. Sci.,' vol. xxiv, p. 193.

which gives any colour to the idea that the earliest trace of the hair is formed on the surface and subsequently sinks. In fig. 9, the nuclei of the outer layer of the follicle, which are continuous with those of the Malpighian layer, are seen to be somewhat elongated, and the central part of the follicle is occupied by a mass derived from, and continuous with, the middle part of the epidermis, and in which the nuclei are evidently undergoing rapid proliferation. The stratum corneum extends continuously over the external surface, and there is not the faintest trace of a tubular downgrowth; the follicle at this very early stage is, in fact, precisely similar to that of all other mammals.

Stage 2.—The difference between this and the preceding stage consists in a slightly greater elongation of the follicle and in an aggregation of the dermic cells at the base of the follicle affording the earliest indication of the formation of the future dermic papilla. Not infrequently, as shown in fig. 10, there is a trace of a blood-vessel running up towards the base of the follicle. There is still no indication of anything like a tube, the stratum corneum running continuously across above the follicle. It will be noticed that in these early stages there is no outline of cells to be distinguished except to a certain extent in the stratum corneum; elsewhere, and until later stages, there are simply series of nuclei, those of the Malpighian layer being distinguished as usual by their more elongate form and definite arrangement.

Stage 3 (figs. 11, 12).—Poulton was, we believe, the first to draw attention to the fact that in regard to the arrangement of the hairs in Ornithorhynchus there is a distinct bilateral symmetry; this he also showed to obtain in respect to the structure of the large hair. Whilst undoubtedly the latter statement is true if the hair be examined at a relatively late stage of development,—a stage such, for example, as Poulton's figures refer to,—we think, as will be shown later on, that this bilateral symmetry is only of a secondary nature and is not of any importance from a phylogenetic point of view. There is, however, at this early stage an indication of an original

bilateral symmetry, which in its turn soon becomes lost and has no influence upon the form of the hair; in fact, this bilateral symmetry completely disappears before there is any trace of the hair proper. To show the close agreement of Ornithorhynchus and Echidna in this respect we have drawn sections which pass longitudinally through the follicle of a future large hair in each animal at this stage. The earliest indication of any modification of the end of the follicle to give rise to a bulb, takes the form of a flattening from above downwards of the end of the follicle, attended by a slight pitting in of the plate-like structure thus formed. On this plate (separated from it by a slight space, evidently an artefact in the section) lies a mass of dermic cells, the early rudiment of the dermic papilla. In both cases, and especially in that of Ornithorhynchus, the nuclei of the plate are especially elongated. The points *a* and *b* indicate, as seen in section, the future lowest parts or rim of the bulb, the point *a* gradually growing downwards; while at the same time the dermic papilla becomes more firmly established, the bulb becoming, as it were, moulded upon the papilla. The follicle is still quite solid, and there are indications that the nuclei in the central part are beginning to arrange themselves so that their long axes are parallel to the length of the follicle. This is especially seen in the case of the section of Platypus (fig. 11). It will be seen that in each follicle we can distinguish four parts: (1) that which lies nearest to the epidermis, and in connection with which there can be seen in the section of Platypus a small swelling, the earliest indication of the sebaceous gland; (2) a slightly swollen part which indicates the position in which the hair when developed is most tightly enveloped by the wall of the follicle, and where, as will be shown subsequently, there is a special modification in the inner root-sheath; (3) a somewhat narrower part, which, as the hair grows, increases greatly in length in proportion to the other parts; and (4) the rudiment of the bulb.

Stage 4 (fig. 13).—The section figured is taken from an Echidna, and shows the definite form of the papilla round which

the bulb becomes moulded, though there is still an indication of the rim on the upper side not having grown down to quite the level of the lower side. The nuclei are beginning to have a definite arrangement; those continuous with the stratum Malpighii are set with their axes at right angles to the wall of the follicle, except on the outer surface of the latter, where, as in all later stages, they have become apparently subject to stress, resulting in their lying with their long axes parallel to the length of the bulb; on the inner surface of the latter, where they lie upon the papilla, they are placed with their long axes at right angles to the length of the latter. The central layer of nuclei in the follicle are distinctly arranged with their long axes parallel to the length of the follicle, while the outer layers, on the contrary (as can be seen in transverse section), show a tendency to elongation in the opposite direction. In the bulb there is the earliest indication of the formation of the hair itself. The nuclei of the outermost layer, that is those lying on the dermic papilla, are continuous with a series which lies in the central line of the hair rudiment, and around this central series others are beginning to be arranged in somewhat definite lines, giving the appearance of the hair rudiment just beginning to grow up through the solid follicle. As in the one which is figured, the follicle is often bent at a distinct angle to the surface at the part where the swelling occurs, to which reference has been made. It will be seen that this swelling is particularly prominent in this follicle, and that considerable growth relative to the other parts has taken place in the section of the follicle above the bulb.

Stage 5 (fig. 14).—This is an important stage, as in it the hair rudiment, though unmistakably discernible in the fourth stage, is now clearly marked out. Perhaps the most striking point at the first glance is the development of pigment in the layer next to the papilla, which has still more clearly begun, as it were, to grow upwards in the centre of the follicle, the nuclei becoming elongate and also taking the stain more deeply than those surrounding them in the follicle. Running up within the bulb, more or less definite lines can be

seen separating the nuclei into series which converge towards the point of the growing hair. These lines of nuclei become more definitely outlined as development proceeds, and are associated with certain definite parts of the hair and root-sheaths, to which reference will be made later. The gland at this stage is very clearly marked, and its nuclei are all small, round, and take the stain deeply. The outline of cells can be seen in parts of the gland (though not as yet in the hair follicle); and though there is no opening to the exterior there are traces towards the base of the gland of an internal cavity. Though the follicle is quite solid there is in both the stratum lucidum and stratum corneum an indication of the formation of a lumen. The nuclei begin to dip down towards the central line of the follicle, as indicated in the figure; this is most strongly marked in the case of the stratum lucidum, the outer layers of the stratum corneum still running continuously over the spot where will subsequently be the opening of the lumen. A transverse section across the bulb just above the level of the tip of the papilla is shown in fig. 15. In the centre lie the nuclei with pigment round them, continuous with those lining the bulb on its inner surface. It will be seen that there is no continuation upwards of the cells of the dermic papilla. This part of the hair probably corresponds to both the medulla and the cortex. Then follows a circle of six nuclei of considerable size, which represent, we believe, the nuclei of the layer forming the cuticle of the hair; outside these is an irregular circle of smaller and more deeply staining nuclei, which, judging by sections of later stages, represent those of the so-called cuticle of the inner root-sheath; outside these are one or more layers of larger nuclei irregularly arranged and belonging to the inner root-sheath, while the outer layer of nuclei represent the outer root-sheath. The whole is enclosed in dermic tissue.

A transverse section at a level just below the origin of the sebaceous gland is represented in fig. 16. In the centre lies the follicle of the large hair, and at either side is the early indication of the follicles in which the two first formed small hairs will be developed. These, as shown in the next stage,

are formed as outgrowths from the side of the large follicle, and include, in the centre, a growth from the stratum lucidum surrounded externally by a growth of the stratum Malpighii. In the large follicle the central nuclei appear to be smaller than those surrounding them, which latter are arranged in roughly concentric circles, owing to the fact that they are cut across their short axes. There is the earliest appearance in the central part indicative of the formation of a network, which becomes more definitely, in fact strongly developed in later stages, as described by various observers in the case of different mammals. This section shows also the bilateral arrangement of the large and small hairs. As yet only the rudiment of one small hair is shown on either side, but from this will subsequently be budded off (1) a second follicle giving two on each side; and from each of these, which remain proximally in connection with one another and with the large follicle, are budded off (2) the remaining follicles in which the small hairs are developed, with the result that the large and small hairs all have a common follicular opening.

Stage 6 (figs. 17—20).—The most important feature of this stage is the strong development of the inner root-sheath, which is, as described already by Poulton, a striking feature of the developing hair in Ornithorhynchus. It is equally strongly developed in Echidna. The section figured is taken from Ornithorhynchus.

As compared with the last stage it will be seen that important changes have taken place in the follicle. The most important one is that, except in the region of the bulb, the inner part of the follicle now forms a network of corneous material in which the now only faintly staining nuclei are embedded. This network, which is in the case of Echidna and Ornithorhynchus formed directly out of the central part of the follicle itself, gives rise to the inner root-sheath. The meshes of the network lie close together, and the strands have at once, as regards the follicle, a longitudinal and a concentric arrangement—that is, as seen in longitudinal section they appear to run irregularly along the length of the follicle,

while in transverse section they are seen to form more or less roughly concentric bands, which in the lower part tightly enclose the hair. If the corneous layer be traced downwards towards the bulb it will be noticed that the corneous nature gradually disappears, and that the network is really directly continuous with the softer, undifferentiated, nucleated layers of the inner part of the bulb, that is the part lying between the outer root-sheath and the layer of columnar cells next to the dermic papilla. On the other hand, at the opposite end of the follicle the network is clearly continuous with the layer lying immediately beneath the stratum corneum, which layers are in their turn becoming gradually corneous. Owing to the way in which the network closely envelops the hair which is growing up through it, it is usually difficult at this stage to detect exactly how far the hair has grown up. The central part of the network, which lies in close contact with the hair, as well as the tip of the latter, takes the stain very deeply, so that at times this central part almost appears to be distinct from the rest; but anything like close examination at once shows that it is only a special part of the general network which is most deeply stained, but which is at the same time in direct continuation with the latter.

It is at this stage that the lumen of the follicle really makes its appearance, though as yet it is ill-defined, except at the surface, though even here it has the form of a somewhat indefinite tubular cavity crossed by a meshwork of cornified cells, the substance of which is gradually breaking down to form a cavity. So far as the follicle is concerned, the structure can be best understood from the study of a series of transverse sections. In figs. 18 to 20 we have represented three such sections taken at different levels. Fig. 18 is taken through the bulb in such a way that the tip of the papilla is just cut through. The nuclei of the layers, which are, when traced upwards, found to be continuous with the medulla and cortex of the growing hair, are surrounded and almost hidden from view by the pigment, which is now strongly developed. Outside these layers is a clearly marked series of nuclei, the layer

in which they lie being marked off by a more or less definite line from those lying to the outside. This layer, which is a more or less clearly marked feature in all stages from the present onwards, represents the cuticle of the hair. Both in this section and elsewhere along the course of the hair the pigment passes continuously round the structure and is never confined to the under side, as Poulton found it to be in his preparations and as it is in the adult hair; nor after long searching over many hundreds of sections have we been able to find up to this stage any thickening of the cuticle on the upper surface such as is figured by Poulton in his figs. 19 and 25, and such as again exists in the adult hair (fig. 3). The cuticle is a clearly marked layer with large nuclei, and is a striking feature in sections, both transverse and longitudinal, from this stage onwards.

Immediately outside the cuticle layer lies a series of flattened nuclei which stain darkly and correspond to those which, in the previous stage, we described as representing the cuticle of the inner root-sheath. Outside this layer lies a series of layers arranged concentrically with regard to the central papilla and with nuclei which follow the trend of the layers, and as a general rule showing chromatin material in contact with the outer membrane, the inner part of the nucleus being generally devoid of stained material. These layers are, when traced upwards, seen to be directly continuous with the corneous inner root-sheath. There is not at this level, or indeed at this stage of development, any distinction of the inner root-sheath into an outer and an inner part corresponding respectively to Henle's and Huxley's layers, though, as Poulton has already clearly indicated in his descriptions, such a distinction can be easily seen at a later stage and in a part of the inner root-sheath close to the bulb. We are quite of Poulton's opinion when he says (p. 167) with regard to this differentiation that "it does not, in Ornithorhynchus at least, imply any differentiation of the sheath into layers, and when we consider the immense development of the structure in this animal it seems possible that the distinction can hardly be sustained through

mammals generally." We may add that, in respect to this point, Echidna agrees with Ornithorhynchus. The outermost layers of nuclei, which are large and deeply stained, represent the outer root-sheath, which, as usual at this level, is very thin and at most two layers thick, the inner of the two layers being represented probably by a very few small nuclei (fig. 18).

Fig. 19 represents a section cut across the follicle close to the top of the hair, that is about halfway along the length of the follicle. In the centre is seen the hair, circular in outline; in fact there is as yet no flattening of the hair to be seen, though when oblique sections are cut—and it is not always easy to cut true transverse sections—the appearance of a slight flattening is produced. The hair is enclosed in the meshwork of the inner root-sheath, which here has the form of a corneous network containing nuclei which stain in the way already described. The cuticle of the hair is well developed, and no nuclei can now be seen in it at this level; whilst the nuclei which at a lower level represent the cuticle of the inner root-sheath, cannot here be recognised. The outer root-sheath contains apparently a single layer of deeply-stained nuclei, and is well marked off from the inner root-sheath, owing to the fact that it is not corneous. The meshwork of the inner root-sheath is rendered distinct, even at this stage, and still more so at later stages, by the way in which it stains with indigo or picric acid.

Fig. 20 represents a slightly oblique section across the upper end of the follicle, cutting at one side through the level at which one of the first formed small hairs is given off. In the centre lies the corneous network, and outside this is the outer root-sheath. Two points of importance may be noticed. The first is that there is no sharp line of demarcation between inner and outer root-sheath; in fact, in the upper part of the follicle the two always merge to a certain extent into one another, while in the lower part they tend to become, as development goes on, more and more strongly marked off from one another. The second is a fact of greater importance, viz. that there is in this part of the follicle above the hair no distinct lumen, much less any structure which could be described as an

open tube. At a higher level, that is right in the epidermis, there is a more clearly outlined tubular space; but here, in the part immediately above the developing hair, there is merely a more or less loose corneous network up through which the hair pushes its way. This feature in the development of the hair is true of both Echidna and Ornithorhynchus, and can be seen in sections taken from any part of the body. We have seen it in sections taken from the following parts: the top of the head, the shoulder, the middle of the back, the thigh, under the tail, the chin. It is quite true that in the epidermis itself, and leading down into the very uppermost part of the follicle, there is a tubular space present, but this is only formed when, and not until, the hair has developed to a very considerable extent, usually for about three quarters of the length of the follicle; until it reaches the level of the very top of the follicle the hair simply, as described, pushes its way up through the corneous network which forms the inner root-sheath, and only reaches the more open tubular part when its tip lies a short way beneath the surface.

Stage 7 (fig. 21).—In this stage the hair itself can be readily distinguished, with the pigment extending in an unbroken way almost to the very tip. There is no distinction to be drawn between medulla and cortical substance; the cuticle towards the tip has the characteristic serrate outline. The inner root-sheath in the lower part of the follicle through which the hair has passed has become more compact, owing doubtless in part to the pressure of the hair, while just around and above the tip of the hair the network is clearly seen, and here it takes the stain more deeply than elsewhere. In the somewhat swollen part near to the upper end of the follicle the network is very clearly seen; and within the incompletely formed cavity is a certain amount of granular material, produced apparently by the breaking down of certain of the cells. Above this the tubular lumen, now for the first time freely open, leads to the surface. In the lower part of the follicle and in the bulb region we begin to see a more clearly marked differentiation of layers than in the last stage,

and corresponding to those which will be more fully described in the next stage. The nuclei of the outer root-sheath are seen in parts to be proliferating, so as to give rise to the more strongly developed and definite outer root-sheath of the more highly developed hair.

Stage 8 (figs. 22—26). — The figures represent the structure of the large hairs, the tips of which have just appeared above the surface, and are drawn from sections taken from the back of an Echidna measuring 5·5 cm. in length. They may be regarded as representing the structure of the hairs when the various parts are well developed; and before, owing to the great growth of the hair and extreme cornification and subsequent absorption of the softer parts, the structure and relationship of the different layers is more difficult to determine.

In fig. 22 we have represented a longitudinal section which, apart from the special modifications which take place subsequently to form, on the one hand the flattened hairs of both Ornithorhynchus and Echidna, and on the other the strongly developed spines of Echidna, may be regarded as representing the details of the typical structure of the hairs, large and small alike, of both animals. It will be noticed, in the first place, that the dermic papilla appears at this stage to lead up very distinctly in the direction of the medulla, the nuclei of the cells at the apex being elongated in the direction of the length of the hair. Whether there be at this stage any direct continuation of the dermic cells into the medulla of the hair, we are unable to state with certainty; and, despite the suggestive appearance of the section figured, which is only one of very many in which the same appearance is presented, we are strongly of opinion that there is no such continuity. In early development the medulla—which is very difficult to distinguish as a definite structure at this stage, though it becomes more prominent in the adult hair—is without any doubt whatever formed entirely from the structure of the bulb, the dermic papilla taking no part whatever in its formation. With the subsequent breaking down of the central cells of the hair and the formation of an open space, an upward growth of the dermic papilla may take

place, but in early stages there is no indication of any dermic structure to be seen in the medullary region immediately above the papilla.

Poulton says (p. 153), in speaking of the hair of Ornithorhynchus, "From the tip of the papilla, at any rate in the larger hairs, an axial rod of soft protoplasmic cells, deeply staining in reagents, is continued. This, when dry and shrivelled, admits the hair and forms the characteristic medulla;" and again, "the great length of the papilla projecting through the bulb into the lower part of the area is also very significant, suggesting a previous development like that of a scale or feather from the surface of the epidermic covering of a papillary core traversing the structures from base to apex."

In the first place, we have been quite unable in either animal to detect during development any such axial rod of deeply-staining cells in the medulla, and certainly during the early stages of development no such structure is present; nor, despite the suggestive appearance of the papilla, as seen in longitudinal section, can any upward prolongation of it be seen in transverse sections of later stages, of which a typical one is represented in fig. 26. Until a certain stage in development has been reached there is no such upward prolongation of the dermis in either Ornithorhynchus or Echidna, and we are inclined to draw from this fact the conclusion that, if there does take place, as perhaps there may, though we have failed to convince ourselves of its existence, any such extension of the dermic papilla into the medulla, this is to be regarded as a secondary feature associated with the special modification of the large hairs, and has no phylogenetic significance.

However, to return to the structure of the follicle and bulb. By means of the method adopted by Norris and Shakespeare, and used also by Mertsching, which consists in staining with a mixture of Mayer's hæmalum, indigo and carmine, the various layers become well differentiated, though we have not been able to obtain the strong carmine stain indicated in Mertsching's beautiful figures, all the nuclei in our preparations being stained with hæmalum. As a further differentia-

tion we have found it useful after over-staining to reduce with picric acid, the result of which is to render still more clear the variations in the amount of cornification undergone by the various layers. The most corneous layer, that is the stratum corneum, and the side of the inner root-sheath in contact with the hair, stain a brilliant green which shades off into a yellow tint as the cornification becomes less in the deeper (morphologically) lying parts (figs. 24—26). In this way it is clearly seen that no modification has taken place in the very base or rim of the bulb, which is formed of an outer layer with elongate nuclei and an inner part with rounded nuclei. In this part we have been unable to obtain a clear demarcation of cell outlines such as Mertseling figures,[1] though this may perhaps be due to the fact that our specimens were preserved merely in strong alcohol. As, however, we pass up from the base of the bulb into the region of the cuticle and inner root-sheath, there is clearly seen in parts an indication of cell outlines around the nuclei.

The layers which in life rest upon the dermic papilla are directly continuous upwards with the medulla and cortex of the hair, and are marked by a strong deposit of pigment. Immediately outside these there is a most clearly marked layer in which the nuclei are larger and more round than elsewhere. Passing upwards this layer becomes resolved into a single series of very distinctly marked cells set at an angle to the surface of the cortex of the hair and developed equally all round; in the lower parts specks of chromatin can be seen in the nuclei, then at a higher level the latter become flattened and more darkly stained all over, and gradually above the level of the top of the papilla the cell outlines and nuclei disappear and the layer passes up into the strong cuticle with its well-marked serrations. Just above the tip of the papilla there is a definite contraction of the hair, followed by a slight expansion, and then above this it tapers gradually away to the tip, which has just protruded beyond the surface. Outside of and in contact with the cuticle there can be detected three distinct layers in the inner root-sheath; next to the cuticle of the hair

[1] 'Arch. f. Mikr. Anat.,' Bd. xxxi, p. 32, Taf. 4 and 5.

there lies a single layer of flattened, deeply-stained nuclei, which, by the way in which they take the stain, can always be distinguished from those lying to the outside. These nuclei become more rounded as they are traced lower down in the bulb, and in this part the layer in which they lie can sometimes, as shown in the figure, be seen to be marked off by a more or less clear line from those of the inner root-sheath. In the region of the bulb the inner root-sheath is clearly distinguishable into an inner and an outer part, which, however, merge into one another at the level at which the hair is constricted. The outer layer has undergone cornification, while the inner one stains more deeply with the hæmalum; its nuclei are clearly visible, and below it melts into the more or less undifferentiated part which forms the rim of the bulb. Above the level at which the hair is constricted, that is slightly above that of the tip of the papilla, there is no differentiation of the inner root-sheath into the equivalents of Huxley's and Henle's layers. Outside the inner root-sheath, and in this part sharply marked off from it (fig. 24) lies the outer root-sheath, in which, in comparison with earlier stages, a considerable proliferation of nuclei has taken place, the outer layer being set with their long axes at right angles to that of the follicle. A transverse section (fig. 26) shows clearly the relative size of the various layers as they are seen when the hair at this stage is cut across in its follicle. The whole hair is filled with pigment, there is no clearly marked medulla, and the groundwork of the whole structure is evidently, from the way in which the stain is taken, of a corneous nature. The cuticle is sharply outlined, and is closely invested by the thick inner root-sheath, which has the form of a network, the nuclei having by this stage completely disappeared, the part next to the cuticle being more cornified than that on the outside. In somewhat younger stages in which the hair is not so large (fig. 23) the gradual cornification of the root-sheath can be well seen. Here, next to the cuticle, the nuclei have disappeared, while outside this they are evidently undergoing change prior to complete degeneration.

For the purpose of showing the relationship of the inner root-sheath we have represented in figs. 24 and 25, drawn under the camera lucida, (1) two entire follicles as seen in a longitudinal section of the skin of Echidna; (2) a portion of one of these on a larger scale. The exact relationship of the inner root-sheath to other parts of hair and its method of formation are matters of fundamental importance in connection with the consideration of the relationship which may exist between hairs and other structures.

The one point of fundamental importance is that the inner root-sheath is a differentiation of the inner layers of the original epidermic follicle, and that as shown by various authors, and most clearly perhaps by Mertsching (pl. iv, fig. 2) in a section through the hair of a guinea-pig, the sheath is directly continuous with the layers of the epidermis intermediate between the stratum corneum on the outside and the stratum Malpighii on the inner side. This direct continuity is very clearly brought out by adopting the method of staining already indicated, and the appearance always presented in the case of both large and small hairs of Ornithorhynchus and Echidna is well seen in fig. 24. It will be seen that, in regard to this point, our observations are at variance with those of Poulton, who (p. 165) says "The inner root-sheath is always present in the developing hair, and is a structure of great importance, throwing much light upon the corresponding sheath as it is described in other mammals. As in the latter, the inner root-sheath surrounds that part of the hair which is enclosed in the follicle, but growing less rapidly it does not extend to the neck through which the hair protrudes; hence we do not find it at all in sections of the upper part of the follicle (figs. 17 and 18)." On reference to the figures indicated by Mr. Poulton, we think that there is in them traces of the inner root-sheath to be seen; in fig. 17 the wavy lines surrounding the old hair, and evidently also passing round the successional hair, are very suggestive in this respect, as are also the wavy lines surrounding the old hair in fig. 18. In fact, judging by our own sections, we cannot but think that the structures drawn, but not referred

to, by Poulton are in reality associated with the inner root-sheath. Our material is certainly in a very fair state of preservation, and in all the stages examined in which the root-sheath is developed it can be seen most clearly that the latter extends throughout the whole length of the follicle, and at the open end is directly continuous with the middle layers of the epidermis. Possibly the difference in this respect in the various accounts may be due to the fact that at one particular part of the follicle there is a special modification of the sheath, which can be seen by reference to fig. 25. This represents on a large scale the part of the follicle which is slightly swollen and lies near to the open end. On the upper side of the follicle the swelling is more pronounced than on the lower side, and the nuclei of the outer root-sheath, to the special development of which the swelling is due, are arranged as shown in the figure in a somewhat radiating manner. The most important feature, however, is concerned with a special modification of the inner root-sheath; the lumen of the follicle becomes somewhat contracted in this part, and the hair is tightly grasped by the sheath, which itself is somewhat more solid in appearance than elsewhere, and is also less sharply marked off from the outer root-sheath than at a lower level. In addition to this, the lower part of the inner root-sheath as shown at *x* (fig. 25) is slightly produced into a kind of collar arrangement, so that on casual observation it might even be thought that the inner root-sheath only extended as far as this point. Anything like a close examination of sections in a good state of preservation shows clearly that there is merely a local differentiation in the sheath, which is again to be associated with the fact that at this spot the hair is tightly grasped in the follicle, and that we are, in reality, as can be seen from an inspection of the figures, dealing with a continuous structure.

The whole face of the inner root-sheath is clearly marked at this stage by the downwardly directed serrations which represent the cuticle of the sheath and fit into the upwardly directed ones on the surface of the cuticle. This thin serrated layer of the inner root-sheath is morphologically continuous with the

outermost layer of the epidermis, into which, at the mouth of the cuticle, it merges. For the greater part of the length of the follicle this cuticle of the inner root-sheath, though its serrations render it very easily distinguishable, shows, in common with the rest of the sheath, no cellular structure; indeed Poulton (p. 166) says, "I gained the impression that it is not a distinct and definite layer, but merely the condensation as it were of the innermost part of the inner root-sheath upon the exterior of the hair and the moulding of its surface by contact with the cuticle of the latter." If, however, the layer be followed down to the bulb, then, where the cellular nature of the sheath becomes evident, it is seen that the serrated cuticle is directly continuous (fig. 22) with a special line of nuclei which are distinct from those of the remainder of the sheath and from those of the hair cuticle, next to which they lie, by their very distinctly flattened appearance and dark staining. Traced still further down into the bulb the flattened nuclei become more rounded until they reach the lowest point at which the nuclei of the hair cuticle can be distinguished as a distinct layer, and at this point the two layers become continuous with one another (*A*, fig. 22).

General Considerations.

The figures and descriptions of Mertsching may be taken as representing the relationship of the layers of the hair and sheaths as most generally accepted. He shows the cuticle of the inner root-sheath as running down into the bulb where it turns back again in continuity with Huxley's layer. The cuticle of the hair is directly continuous with Henle's layer. Of the two layers into which the outer root-sheath resolves itself in the lower part of the bulb, the outer one is continuous with the medulla and the inner one becomes much expanded as it passes upwards through the bulb and becomes continuous with the cortical substance of the hair.

In Ornithorhynchus and Echidna, on the other hand, while we have been unable to distinguish such complete and definitely outlined cellular layers as Mertsching figures, the

arrangement is seen to be as follows:—The line of nuclei representing the cuticle of the hair is directly continuous with that of the inner root-sheath; of this fact we feel satisfied, after long examination of a very large number of well-preserved sections, and it may be pointed out that if, phylogenetically, the hair be regarded as a process from the surface of the epidermis, which was developed subsequently in a tube, and still later in a solid follicle, then this relationship is exactly what would normally obtain; for the cavity of the hair follicle being regarded, ex hypothesi, as formed originally as a depression of the surface at the base of which the hair arises, it is perfectly natural for the cuticular layer which lines the depression to be directly continuous on the one hand with that on the hair which arises in the depression, and on the other with the cuticle on the general surface of the body; indeed, any other relationship seems to be difficult to understand.

In connection with this it may be noticed that the outer root-sheath is in connection partly with the medulla and partly with the cortex. During the earlier stages of development the outer sheath is formed of a single layer of cells continuous with the layer which forms the stratum Malpighii of the epidermis. The layers which lie morphologically to the outside are modified in the follicle to form the inner root-sheath, which becomes strongly corneous, except in the region of the bulb, where it, as well as the elements corresponding to the stratum Malpighii, is in close relationship to the dermic papilla,—the source of nutriment,—and here they retain their soft protoplasmic nature. The stratum lucidum is, during life, constantly replenished, as the outer part of the epidermis is worn off, by proliferation of deeper-lying elements, and at a certain stage of development there takes place a similar proliferation of the elements of the outer root-sheath, and hence it is only natural that, in the region of the bulb, these newly formed elements should give rise to a portion of the layer corresponding to the one to which they would have belonged in the surface epidermis.

The continuity of the cuticle of the inner root-sheath and that of the hair is a matter of great importance, as it implies

that between the hair and the fenestrated inner root-sheath there lies a definite cuticular layer. In his discussion with regard to the homology of the various parts of the hair Poulton has suggested (p. 189) and has argued in favour of the theory that "the hair represents the axial, its inner root-sheath the appendicular part of a feather; and thus an intelligible morphological significance is given to the mysterious inner root-sheath—a true part of the hair itself, and with it a rising from the bulb, but which, owing to the mode of development, is buried deeply beneath the surface."

A general résumé of the various theories held with regard to the homologies and origin of mammalian hair has been given by Benham and in the second part of this work, when dealing with the development and structure of the hair in Marsupials, we shall have more to say upon this point; meanwhile the following will serve our present purpose. The two most important views with regard to the development of the various parts associated with the hair, so far as their origin from the follicle is concerned, may be taken as those expressed respectively by Gegenbaur and Klein. Gegenbaur[1] says "the shaft of the hair is differentiated from the invaginated epidermis by cornification of its cells, while other cellular parts of the follicle form the root-sheaths." Klein,[2] on the other hand, states that "henceforth the multiplication of the cells at the bulb naturally leads to the new offspring being pushed up in the axis of the hair rudiment towards the surface, and becoming elongated constitute the elements of the hair substance, its cuticle and inner root-sheath; the cells of the primary solid cylinder represent the rudiment of the cells of the outer root-sheath only."

Schafer,[3] in his figure of the longitudinal section of a hair, very distinctly represents the inner root-sheath as directly continuous with the outer more cornified layer of the epidermis.

[1] 'Comparative Anatomy,' English trans., p. 420.
[2] 'Atlas of Histology,' p. 325.
[3] 'Essentials of Histology,' 1885, p. 110, fig. 133.

Our observations upon the Monotremes point very clearly to the fact that in the most primitive mammals known to us the inner as well as the outer root-sheath is a direct transformation of certain parts of the follicle, and that during development the hair formed on and from the bulb forces its way up through the network, into which the inner root-sheath becomes transformed.

Giovannini,[1] in dealing with the successional growth of the hair in man, describes the inner root-sheath as formed first above the tip of the developing hair, which subsequently pierces the sheath in its upward growth. His figures are quite compatible with the idea that when the downgrowth from the old bulb to give rise to the new one is formed the inner part of the new short follicle thus formed gives rise to the inner root-sheath, which then occupies, exactly as it does in Monotremes, the central part of the tube through which the growing hair has to push its way upwards.

The relationship of the various layers as indicated by Mertsching and other workers on the one hand, and by ourselves on the other, can be represented by the following diagrams (pp. 575, 576). For the sake of clearness we have in each case represented the hair as if it were slightly separate from the walls of the follicle.

In both diagrams the cuticle of the hair, and the layer with which it is regarded as continuous, is indicated by the black line, and if it be granted that the external surface of the hair is to be regarded as morphologically equivalent in position to the outside of the epidermic layer of the general body surface, then it will hardly be denied that the diagram of the structure as represented by ourselves, and as actually exists in Monotremes, is, a priori, what might be expected to obtain. When the hair has reached nearly to the summit of the follicle, and the inner root-sheath is well cornified, there is, for the greater part of its length, no distinct cuticle, the latter being represented merely by the clearly serrated edge of the sheath, but near to the bulb this can be distinctly traced into connection

[1] 'Arch. f. mikr. Anat.,' 1890, Bd. xxxvi, p. 528, pls. xxxv—xxxviii.

with a special and well-marked layer of nuclei continuous with those in the layer, which traced upwards above the bulb is seen to give rise to the hair cuticle. Further still, at the open end of the follicle the cuticle of the root-sheath is directly

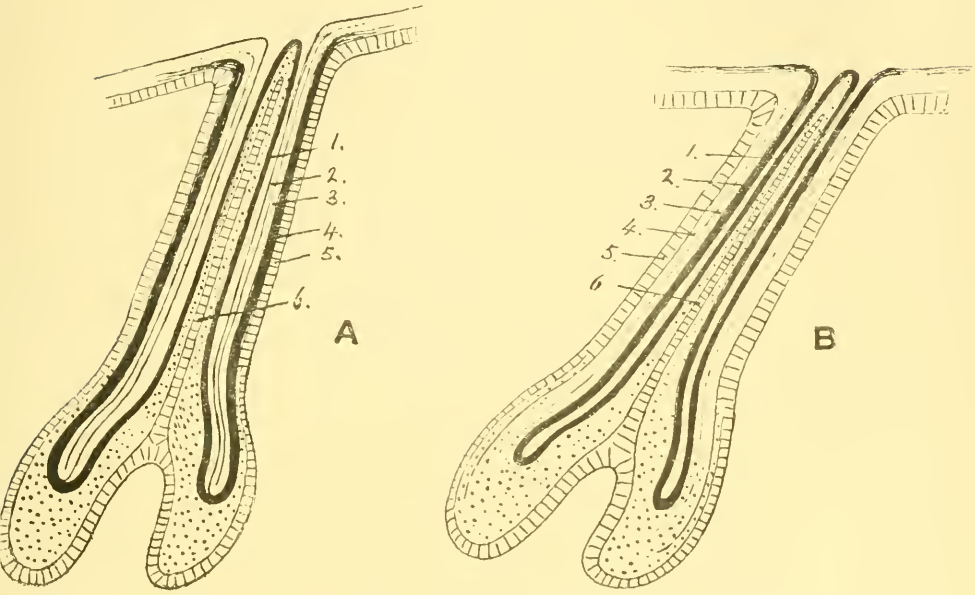

Fig. A.—Longitudinal section through the hair, showing the relationship of the layers as indicated by Mertsching.

Fig. B.—Longitudinal section through the hair, showing the relationship of the layers in Monotremes.

1. Cuticle of hair. 2. Cuticle of inner root-sheath. 3. Huxley's layer. 4. Henle's layer. 5. Outer root-sheath.

continuous with the outermost layer of the epidermis, and takes the stain, in its course along the follicle, in precisely the same way (figs. 24 and 25).

If now we represent diagrammatically the relationship of the various parts in the developing hair of a Monotreme, this can only be done as represented in the accompanying Fig. C, which, though diagrammatic, represents what is actually the relationship of the hair and inner root-sheath. If we suppose the hair

to have been originally developed on the surface, then the relationship of the parts can be represented in Fig. D.

The difference between ourselves and Mr. Poulton lies in the fact that he regards the inner root-sheath as "a true part of the hair itself, and with it arising from the bulb;" whilst, with the advantage of a larger series of stages than Mr. Poulton was able to study, and with probably better preserved material, we have been led to the conclusion that the inner root-sheath is not a true part of the hair itself, and is not developed from the

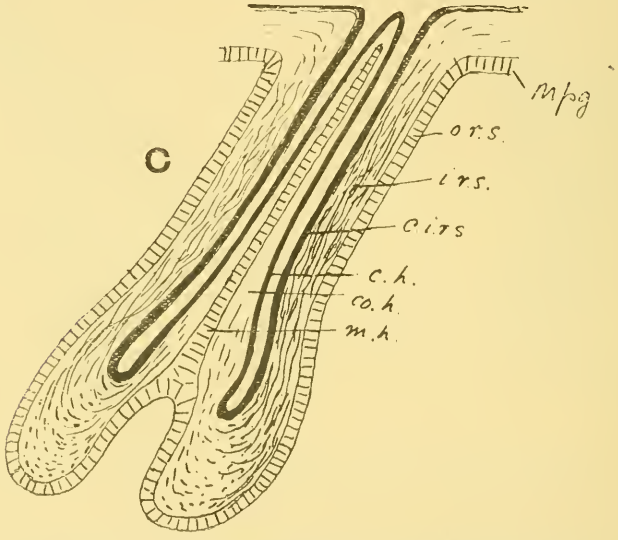

Fig. C.—Longitudinal section through the hair of a Monotreme lying in its follicle, showing the relationship of the layers.

bulb, a conclusion which our figures—all drawn under the camera lucida—will, we think, serve to demonstrate. It appears to us that the relationship now described renders untenable the suggested homology between the inner root-sheath and the appendicular parts of a feather.

We must, however, now return to a consideration of the early stages of the development of the hair. Mr. Poulton (p. 183) says, "It is, indeed, by no means improbable that the first and earliest trace of the hair is formed at the surface in

Ornithorhynchus, and subsequently sinks with the deepening tube. Material by which this suggestion could be tested is unfortunately wanting. But the open tube is not by any means the only, although it is the most important point about the development of the hair of Ornithorhynchus. The great length of the papilla projecting through the bulb into the lower part of the hair is also very significant, suggesting a previous development like that of a scale or feather from the surface of the epidermic covering of a papillary core, traversing

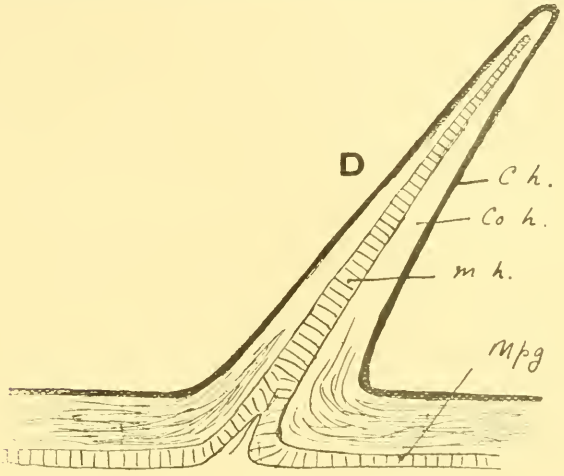

Fig. D.—Longitudinal section through a hair which is supposed to have developed on the surface and not in a follicle. The part corresponding to the inner root-sheath is marked by wavy lines.

m.h. Medulla of hair. *c.h.* Cuticle of hair. *co.h.* Cortex of hair. *c.i.r.s.* Cuticle of inner root-sheath. *i.r.s.* Inner root-sheath. *o.r.s.* Outer root-sheath. *Mpg.* Malpighian layer.

the structure from base to apex. Further confirmation is afforded by the axial rod of soft protoplasmic cells forming the medulla of hair; for a shortening papillary core, surrounded by cells of the rete mucosum superficially undergoing cornification, would tend to leave just such an indication of its former presence."

First of all as to the supposed open tube. We have already pointed out that neither the large nor the small hair of either

Ornithorhynchus or Echidna develops in what can be correctly called an open tube. In the earlier stages—indeed, until the hair is well formed and has reached a considerable distance up the follicle—development takes place in a solid follicle. Possibly the latter may open to the surface at a slightly earlier stage than it does in some other mammals, but the one important point in this connection is that the hair-follicle has the form of a solid downgrowth, and that the early stages during which the hair is laid down are absolutely indistinguishable from those of other mammals. Figures which represent the condition in one of the earliest stages of the follicle are precisely similar to those of corresponding stages in other mammals, and, with the elongate nuclei at the base of the follicle, correspond so closely to as to be in fact indistinguishable from those of Marsupials, such as Macropus and Perameles. The resemblance between the two is so complete, that we think there can be very little doubt but that in Monotremes, just as in Marsupials, in which our observations (to be published later) to a large extent confirm those of Maurer, the very earliest indication of the hair will be found to take the form of an elongation of the lowest epidermic cells. At all events, the figures now given show (1) that the hair cannot be described as developing in an open tube; and (2) that the earliest trace is not formed on the surface, and then sinks with the deepening tube.

The next point which we desire to lay emphasis upon is that in these lowest mammals, and indeed in all mammals, the hair is a radially symmetrical structure; by which we mean to imply not that hairs may not possibly be derived from structures which originally possessed a bilateral symmetry, but that, from the earliest moment at which we get the first rudiment of the hair itself laid down, the structure takes on a radial symmetry, and that any bilateral symmetry, such as may be found in the well-developed hairs of both Ornithorhynchus and Echidna, is a secondary and not a primary feature.[1]

[1] As we shall show later, the flattened bristles of such a Marsupial as Perameles are, in their early development, radially symmetrical, the bilateral symmetry, just as in Ornithorhynchus, being a secondary feature.

If we trace the development of the hair in Ornithorhynchus we find that at an early stage, before there is any appearance of a hair in the follicle, the base of the latter is somewhat flattened into a plate-like structure, indicating possibly the original development of the hair rudiment out of a bilaterally symmetrical structure; but it is not until the radially symmetrical bulb with its dermic papilla has been formed that we can see any trace of the hair itself, and the latter has at first, and until it is well established, a radial symmetry with a perfectly circular outline in transverse section. Subsequently, and as a secondary modification, there arises a flattening which produces in the scale-like part of the hair a bilateral symmetry, but this is in no sense a primitive feature of the hair. So far as we have any evidence, every hair is primarily a radially symmetrical structure,—that is, we never find the hair commencing to grow until the bulb of the follicle from which it grows has acquired a radial symmetry. In Echidna the radial symmetry is emphasised in some of the large hairs, which become modified into spines, and lost in others which become flattened, the latter feature being especially emphasised in Ornithorhynchus; but both of these are very clearly secondary developments, and cannot in any way be regarded as representative of the structure of the primitive mammalian hair.

In the same way the remarkably developed inner root-sheath of the large hairs of Monotremes is not to be regarded as a primitive feature; it is simply a secondary feature of no phylogenetic importance, and is to be associated with the strong development of all the various parts of the follicle. The modification of the large hair requires, as it were, that all the various parts of the walls of the follicle should be strengthened and stiffened, and that at the same time the root of the hair should be tightly held. These requirements are met by the cornification of the inner root-sheath, that is of the walls of the follicle, and also by the way in which it grasps the hair, and has its surface strongly imbricated so as to oppose the pulling out of the latter, which might otherwise be easily separated from the soft structures of the bulb.

We fail to see any relationship between the specially modified inner root-sheath of Monotremes and the appendicular part of a feather. The sheath is formed perfectly independently of the hair rudiment in the wall of the follicle, which, with the development of the hair, becomes transformed into a pit. A comparison of a transverse section of a developing hair and feather will serve to show there is no real relationship between the inner root-sheath of the one and the appendicular part of the other.

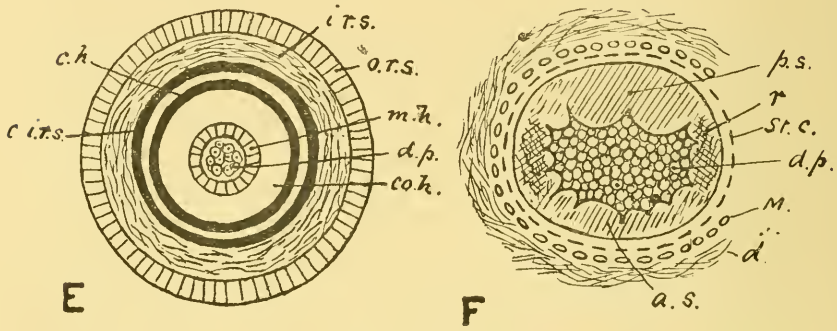

Fig. E. Transverse section across a Monotreme hair lying in its follicle, at the level of the top of the dermic papilla.

Fig. F.—Transverse section across a developing feather (modified from Newton and Gadow), to show the relative positions of the developing main shaft, after-shaft, and appendicular parts.

d.p. Dermic papilla. *m.h.* Medulla of hair. *co.h.* Cortex of hair. *c.h.* Cuticle of hair. *c.i.r.s.* Cuticle of inner root-sheath. *i.r.s.* Inner root-sheath. *o.r.s.* Outer root-sheath. *p.s.* Primary shaft of feather. *r.* Rami. *st.c.* Layer continuous with stratum corneum (= cuticle of inner root-sheath of hair). *m.* Malpighian cells of follicle. *a.s.* After-shaft. *d.* Dermis.

We have already stated that the bilateral symmetry in the large hair of Ornithorhynchus is not a primary, but a secondary feature, and the same remark holds true in the case of the large size of the bulb and papilla. The early development of these is precisely similar, so far as relative size is concerned, to that of other mammals, and it is only with the secondary modification of the hair to form in the one case a flattened

structure, and in the other a spine, that the papilla begins to increase in size, and to extend any distance up the shaft. Poulton (p. 183) says, "The great length of the papilla projecting through the bulb into the lower part of the hair is also very significant, suggesting a previous development like that of a scale or feather from the surface of the epidermic covering of a papillary core traversing the structure from base to apex."

Various authors, and especially Maurer, have insisted upon the fact that the dermic papilla is always developed at a slightly later time than the epidermic forecast. In the case of Marsupials, such as Perameles, Macropus, Sminthopsis, and Dasyuroides, we can confirm the conclusions of Maurer. The earliest indication of the hair forecast in these animals has the form of a lengthening and definite arrangement of the elements of the Malpighian layer, the dermic layer at first taking no part whatever in the formation of the structure. In the typical mammalian hair the dermic papilla is never of very large size, and it is only in those cases in which we get special modifications to form spines, &c., that we find, not as a primary but as a secondary feature, that the papilla increases in size and extends some distance up the shaft. The very fact that this development is only found in what is clearly a secondary modification of the hair, which up to the period at which the modication begins to show itself develops in the ordinary way, is sufficient to indicate the fact that in the case of the Monotremes the special development in question has no phylogenetic signification of any kind. All that it signifies is simply the fact that, as might be expected to be the case, the larger the hair (or its modification, a spine) becomes, the larger is the dermic papilla.

It seems probable that the connection between feathers and hairs, if any such thing really exists, is a very remote one, and can only be traced back to some simple form of epidermic, and perhaps scale-like structure, of the former existence of which common ancestral structure we have in hairs a possible indication in the flat plate, which at a very early stage is developed at the base of the follicle in Monotremes (figs. 11, 12, 12 *a*).

It must also be remembered that in the case of any comparison between the two we must compare not the hair and the highly developed quill or contour feather, but the former and the primitive nestling feather, or "Neossoptile," with its short calamus and terminal bunch of "spikes." In development even this, the earliest form of feather, shows traces of bilateral symmetry, while in the case of the hair we have a strongly marked radial symmetry; in the former the primitive symmetry of a possibly simple ancestral scale is retained, while in the latter it is lost before the rudiment of the hair is actually laid down. It must also be remembered that we have no evidence whatever as yet of any development of hair on the external surface of the epidermis. From the lowest to the highest mammals the development of the hair is practically identical, the Monotremes even showing us nothing which can be regarded as primitive in the development of the actual hair. It may, however, be worth while to draw attention to the fact that in the Monotremes, and probably also in certain of the Marsupials, the original follicle may give rise not only to the large hair, but to small hairs which are developed in follicles budded off from the large one, and that thus we may, perhaps, have a homoplastic relationship between, on the one hand, the axial part of a feather and the large hair, and, on the other hand, between the appendicular part of a feather and the small hairs. It appears to us that in the feather there is no representative of the inner root-sheath in the hair which is to be regarded as a special structure intimately associated with the growth in a follicle, while in the hair there is no representative of the feather sheath which is shed, and which is, it seems to us, correctly compared by Maurer to the moulted cuticle of reptiles.

We shall return subsequently, when dealing with the development of hairs in the Marsupialia, to the question of the relationship of the hair to other epidermic structures; meanwhile our conclusions with regard to the development of hair in Monotremes may be summarised as follows:

(1) The early development of the follicle is precisely similar

to that which takes place in other mammals, and is in the form of a solid epidermic downgrowth.

(2) The dermic layer takes at first no share in the development.

(3) The lower end of the follicle forms at first a flat obliquely slanting plate, indicating possibly a primitive bilateral symmetry in the structure from which the hair is originally derived.

(4) The plate, by ingrowth of the dermic layer, is transformed into a radially symmetrical bulb moulded on the dermic papilla, this radially symmetrical bulb being formed before the development of the hair itself takes place.

(5) The hair is formed as an upgrowth from the bulb within the solid follicle, up which it pushes its way just as in other mammals, and it is not developed in a tube open to the exterior.

(6) The inner root-sheath is developed as a modification of the walls of the follicle, and subsequently becomes transformed into a corneous network surrounding the growing hair. The development of the inner root-sheath is fundamentally similar in large and small hairs.

(7) There is no relationship between the inner root-sheath of a hair and the appendicular part of a feather.

(8) The medulla of the hair is formed primarily as a solid upgrowth of the cells which are continuous with those of the stratum Malpighii of the epidermis.

(9) The cuticle of the inner root-sheath is directly continuous with the cuticle of the hair.

(10) There is no real distinction of the inner root-sheath into Huxley's and Henle's layer.

(11) The larger size of the dermic bulb in the large hairs of Ornithorhynchus, and in the spines of Echidna, is a secondary feature of no phylogenetic importance.

(12) In all essential respects the development of the hairs in Monotremes is precisely similar to that of other mammals.

EXPLANATION OF PLATES 44—46,

Illustrating Mr. Baldwin Spencer's and Miss Georgina Sweet's paper on "The Structure and Development of the Hairs of Monotremes and Marsupials."

List of Reference Letters.

A. Point at which the cuticle of the hair is continuous with that of the inner root-sheath. *b. v.* Blood-vessel. *c. h.* Cuticle of hair. *co. h.* Cortex of hair. *d.* Dermis. *d'.* Special modification of dermis in Echidna to form an enclosure for the group of follicles. *d. p.* Dermic papilla. *f. p.* Plate-like structure at the base of the solid follicle. *h.* Hair. h_1. Tip of developing hair in follicle. *i. r. s.* Inner root-sheath. *i. r.* s_1. Equivalent of Henle's, and *i. r.* s_2, of Huxley's layer. *l. h.* Large hair. *m. p. g.* Stratum Malpighii. *o. r. s.* Outer root-sheath. *s. g.* Sebaceous gland. *s. h.* Small hair. *st. c.* Stratum corneum. *st. l.* Stratum lucidum.

The outlines of all the figures are drawn under the camera lucida.

Fig. 1.—Transverse section across a group of hairs from the back of an adult Platypus, showing four groups of small hairs. The left side lies at a deeper level than the right side, where in one group all the root-sheaths have coalesced to form a common follicle. In the other groups the sheaths of the different hairs are distinct. There is no successional large hair. Zeiss, apert. 0·95, oc. 1.

Fig. 2.—Transverse section across a group of hairs from the back of an adult Platypus, showing six groups of small hairs. The root-sheaths of all the hairs are distinct. Zeiss D, oc. 1.

Fig. 3.—Transverse section across the shield part of an adult large hair of Platypus. The distinct flattening is seen, and also the dorsal thickening of the cuticle, and the bilateral arrangement of the pigment in the ventral part of the cortex, nearer to which side lies the medulla.

Fig. 4.—Transverse section across a group of hairs lying to the side of the mammary area in an adult female Echidna. The large hair lies anteriorly. Each hair has its distinct outer and inner root-sheath, and the follicles are bound together into a group by a special deeply staining modification of the dermis.

Fig. 5.—Transverse section across a group of hairs lying on the ventral surface just behind the bill of an adult Echidna. A large flattened hair and six small hairs are present. The inner root-sheath is especially well developed. There is no distinct medulla to be seen.

Fig. 6.—Transverse section across a group of hairs from the mammary area of an adult female Echidna. No large hair is present. The section lies close to the surface, and shows the root-sheaths coalescing. At a slightly higher

level, i.e. closer to the surface, they will coalesce completely to form a common follicular opening through which the hairs emerge.

Fig. 7.—Transverse section across a large flattened hair of an adult Echidna. There is no dorsal thickening of the cuticle, or restriction of pigment to the lower surface. The hair is from the dorso-lateral aspect.

Fig. 8.—Transverse section across a large flattened hair of an adult Echidna, from the ventral surface just behind the bill. The anterior face is distinctly concave. This form of hair is especially developed in specimens of Echidna from Central Australia, and has not been noticed on specimens from Tasmania, Victoria, or Queensland.

Fig. 9.—Longitudinal section through the follicle of an embryo Ornithorhynchus on the chest region. The nuclei at the base of the follicle are slightly elongate. There is no trace of a dermic papilla. Zeiss, apert. 0·95, oc. 4.

Fig. 10.—Longitudinal section through a follicle at a slightly later stage from the same embryo of Ornithorhynchus in the chest region. At this stage there is the earliest appearance of a modification in the dermis (*d. p.*) to form a dermic papilla, up towards which a small blood-vessel runs (*b. v.*). Zeiss, apert. 0·95, oc. 2.

Fig. 11.—Longitudinal section through a follicle of the same embryo of Ornithorhynchus from the back just in front of the tail. The slight swelling indicating the future sebaceous gland (*s. g.*) is seen; below this the follicle is slightly swollen, and at the base a flat plate-like structure (*f. p.*) is formed, which has been slightly pushed in by the developing dermic papilla. *a* and *b* indicate the future lowest part or rim of the bulb. In the centre of the follicle the nuclei are arranged with their long axes roughly parallel to that of the follicle. Zeiss, apert. 0·95, oc. 1.

Fig. 11*a*.—Transverse section across the base of the follicle at the same stage as Fig. 11, to show the flat plate with its elongate nuclei.

Fig. 12.—Longitudinal section through the same stage in an Echidna embryo, to show the flat plate at the base of the follicle. There is no appearance as yet of the development of the sebaceous gland, the follicle being probably at a slightly earlier stage of development than the one represented in Fig. 11. Zeiss D, oc. 1.

Fig. 13.—Longitudinal section through the follicle of an embryo of Echidna, showing the early formation of the bulb and the upgrowths of the dermic papilla. At the external end of the follicle are two swellings (*s. h.?*) which may possibly indicate outgrowths to form the follicles of small hairs. The nuclei within the follicle are arranged in lines following roughly the length of the follicle. At the bulb end the nuclei are more definitely arranged, and there is the earliest indication of the upgrowths to form the hair rudiment itself, but as yet there is no pigment present and no cornification. Zeiss, apert. 0·95, oc. 2.

Fig. 14.—Longitudinal section through the follicle of an embryo Ornithorhynchus. The rudiment of the hair can be clearly seen arising from the bulb, faint but distinct lines being present, which converge towards the apex of the hair rudiment and separate series of nuclei from one another. Pigment has appeared, and stretches up towards the medulla. The follicle is solid, but at the external end the nuclei are beginning to dip in towards the centre. The gland is well developed, and reaches as far down as the level of the tip of the dermic papilla, which is not shown in the drawing. The stratum corneum is continuous over the top of the follicle. Zeiss apert. 0·95, oc. 1.

Fig. 15.—Transverse section just above the level of the tip of the dermic papilla of a follicle at a slightly later stage than that represented in Fig. 14. The layers of nuclei indicating the cuticle of the hair (*c. h.*) and of the inner root-sheath (*c. i. r. s.*) can be seen. Outside these lie the inner root-sheath, which is commencing to be corneous (*i. r. s.*), and the outer root-sheath (*o. r. s.*). Zeiss F, oc. 1.

Fig. 16.—Transverse section through the same follicle as the one represented in Fig. 15, at a higher level. In the centre lies the solid follicle in which the large hair is being developed. Anteriorly is the gland, and on either side a follicle, budded off from the central one in which the first formed small hairs will be developed. Zeiss F, oc. 1.

Fig. 17.—Longitudinal section through the follicle of an Ornithorhynchus from the dorsal surface of an embryo at a later stage than that represented in Fig. 14. The hair has grown up, through the corneous network into which the inner cells of the follicle have been modified, as far as the point h_1. The cornified inner cells form the inner root-sheath, which tightly encloses the growing hair and stains deeply. At the upper end an irregular lumen is developed leading to the exterior. The inner root-sheath is directly continuous with the stratum lucidum of the epidermis. In the bulb region the layer of nuclei continuous with the cuticle of the hair (*c. h.*), and with the cuticle of the inner root-sheath, can be distinguished (*c. i. r. s.*). Only the proximal part of the now well-developed sebaceous gland (*s. g.*) is indicated. Zeiss, apert. 0·95, oc. 1.

Figs. 18—20.—Transverse sections across the follicle of the same embryo as represented in Fig. 17.

Fig. 18.—Section at the level of the tip of the dermic papilla. The cells filled with pigment, and representing the medulla and cortex, lie next to the dermic papilla. The cuticle of the hair (*c. h.*) and of the inner root-sheath (*c. i. r. s.*) are clearly seen. Outside the latter lie the nucleated layers of the inner root-sheath, and outside this the outer root-sheath (*o. r. s.*), which is very thin in this part and consists of two layers of cells, the nuclei of the outer layer being large and deeply stained, and those of the inner layer very few in number and smaller. Zeiss F, oc. 4.

Fig. 19.—Section close to the tip of the developing hair. The follicle is seen to be solid; the cornified network which forms the inner root-sheath (*i. r. s.*) tightly envelops the hair. Zeiss F, oc. 4.

Fig. 20.—Section close to the external end of the follicle, showing the corneous and more open network of the inner root-sheath, the large outer root-sheath, and an outgrowth from the main follicle to form the follicle of a small hair. The main follicle is still solid, though the central open network indicates the position of the future lumen. Zeiss 0·95, oc. 4.

Fig. 21.—Longitudinal section through the follicle of an embryo of Echidna, showing the hair more highly developed. The inner root-sheath is more definitely established; the nuclei of the layers forming the cuticle of the hair (*c. h.*) and of the inner root-sheath (*c. i. r. s.*) can be clearly seen in the lower part of the follicle. The lumen is distinctly formed in the epidermis, and below this the central part is occupied by disintegrating material. Immediately above the hair there is still the definite network formed by the inner root-sheath. Zeiss, apert. 0·95, oc. 4.

Fig. 22.—Longitudinal section through the lower part of the follicle of an embryo of Echidna, in which the tip of the large hair has just appeared above the surface. The outer root-sheath is well developed. The inner root-sheath in the bulb region can, owing to the greater cornification of the outer part, be distinguished into two layers, one ($i. r. s._1$) presumably the equivalent of Henle's, and the other of Huxley's layer ($i. r. s._2$). The cuticle of the hair fits closely on to the surface of the inner root-sheath, the serrations being clearly marked. Traced down towards the bulb the outermost layer of the inner root-sheath is directly continuous with a series of nuclei (*c. i. r. s.*) which are flattened and stained deeply, and separated off in the lower part, where they become more rounded, by a distinct line from the rest of the inner root-sheath. At the point *A* this layer turns round and is continuous with the layer which passes upwards into the well-developed cuticle of the hair (*c. h.*). The cells of the latter are large and clearly outlined, the nuclei gradually fading away as the layer is traced upwards into the strongly cornified part. The medullary region has the appearance of opening up to admit possibly of a secondary upward prolongation of the dermic papilla. Immediately above the bulb is a constriction followed by a slight swelling of the hair. The cuticle is equally developed on both sides. Zeiss C, oc. 4.

Fig. 23.—Transverse section across a group of hairs of an embryo Echidna. The follicle is at a slightly earlier stage of development than that represented in Fig. 22, and the section is at some little distance below the epidermis. In the central follicle the large hair is seen surrounded by the inner root-sheath, the outer layers of which are not yet completely cornified. Four follicles in which small hairs will be formed are cut through; the one most to the right hand is budding off a secondary follicle. Zeiss E, oc. 4.

FIGS. 24—26.—Sections through the follicle and hair of an embryo of Echidna of the same age as that represented in Fig. 22. They have been stained with hæmalum, carmine and indigo, and subsequently treated with picric acid to show the cornification of the different structures and the continuity of the inner root-sheath with the stratum lucidum of the surface epidermis.

Fig. 24.—Longitudinal section through two follicles; in the one on the right side the hair has been torn away from the bulb. The inner root-sheath is seen to run continuously along the whole length of the follicle. At its lower end in the bulb region it can be divided into an outer (*i. r. s.*$_1$) and an inner part (*i. r. s.*$_2$), the latter being less cornified than the former. At *x* is a special collar arrangement where the hair is most tightly grasped. The cuticle (*c. i. r. s.*) of the sheath passes upwards, and is continuous with the stratum corneum.

Fig. 25.—More highly magnified part of the follicle shown in Fig. 24, to show the special modification in the inner root-sheath to form a collar. The face of the inner root-sheath on the side of the follicle is seen in part.

Fig. 26.—Transverse section across the follicle. The strongly pigmented medullary and cortical parts (*m.* and *co. h.*) are seen, the medulla not being distinct from the cortex. The network of the inner root-sheath is seen more cornified on its face next to the hair than on that next to the well-marked outer root-sheath.

Trophoblast and Serosa. A Contribution to the Morphology of the Embryonic Membranes of Insects.

By

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The substance of the following remarks formed the subject of a paper [1] read by me at the last meeting of the British Association in Bristol.

It is matter of common knowledge that, during the early stages of development, the embryos of insects are protected by two membranes,—an inner, the amnion, and an outer, the serosa,—which resemble in principle the homonymous fœtal membranes of the higher vertebrates.

Both in the embryos of the Vertebrata Amniota and in those of insects, the amnion and serosa are derivatives of the extra-embryonic blastoderm (abstraction being made of the adventitious mesodermal elements which accompany the membranes in vertebrates), and in both it may be stated in general terms that the amnion is subsidiary to the serosa. In insects the secondary character of the amnion, as compared with the serosa, is particularly clearly marked, and it has been recognised that the serosa, being a direct derivative of the blastoderm, is the older structure (Heymons, 4).

The peripheral, extra-embryonic, non-formative epiblast or blastoderm of the mammalian blastodermic vesicle has been

[1] Entitled "Considerations bearing upon the Phylogeny of the Arthropod Amnion."

defined as the trophoblast by Hubrecht (5), since its principal function is to provide for the nutrition of the embryo.

More recently (6) Hubrecht has published a very remarkable and what I venture to predict will become a classical theory of the mammalian trophoblast, in which he seeks to demonstrate a phyletic relationship between the latter and the superficial ectoderm ("embryonic epidermis," Balfour; "Deckschicht," Goette) of the embryos of Amphibia.

Hubrecht points out that the vertebrate amnion is a derivative of the trophoblast, which is a structure sui generis.

If the vertebrate amnion, in its capacity of derivative of the trophoblast, has a profound phylogenetic significance, one would be inclined to suppose that an analogous significance would attach to the embryonic membranes of insects.

Hitherto the prevailing impression seems to have been that the latter were of merely cenogenetic importance, and all serious attempts to account for them morphologically have been more or less coloured by this assumption.

The embryos of a species of Peripatus which I found in New Britain last year (1897), of which I have recently published a full description (14), seem to me to point the way out of this somewhat barren and unsatisfactory position, and to endow the embryonic membranes of insects with a singular interest. Because, if it could be shown that a common principle governs the theories applied to the explanation of the embryonic membranes of insects and of those of the higher vertebrates, the one theory would constitute an important complement of the other.

On this occasion I am only dealing with the embryonic membranes of insects, because they are the best known, and my own observations only bear upon these structures. There can be little doubt that the embryonic membranes of scorpions are capable of being explained in an analogous manner, only the data are not sufficient in this case.

My own idea is that three theories are necessary for the explanation of the embryonic membranes of scorpions, insects, and vertebrates, and that a common principle underlies the

whole. For the vertebrates, Hubrecht has provided the requisite theory. The present paper is written for the purpose of providing an analogous theory for the insects, while the scorpions must be handed over to posterity.

My chief object is to demonstrate the applicability of the conception of the trophoblast to invertebrate animals; in fact, to show that the serosa of the insect embryo can be traced back to a primitive trophoblast.

I believe, in short, that the trophoblast, as it is preserved to us in the embryos of Peripatus novæ-britanniæ, arose in adaptation to a viviparous habit acquired by the terrestrial descendant of an aquatic ancestor; and that it became transformed, whether directly or by substitution, into the serosa, in correlation with the secondary deposition of yolk-laden eggs.

In the case of the vertebrates, Hubrecht starts with a protective layer (Deckschicht), which becomes transformed into a nutritive layer (trophoblast). For the insects, I commence with a nutritive layer, which becomes changed into a merely protective layer, the serosa. This example will suffice to show that the two theories are quite distinct in their treatment of the respective problems, only they have the principle in common which is expressed in Hubrecht's admirable conception of the trophoblast.

Just as, throughout the series of the Amniota, the formation of the amnion by no means takes place according to the stereotyped plan with which we are familiar in the chick, so in insects there is a distinct gradation in the mode and extent of development of the amnion. It attains its completest development in the highest order of insects, namely the Hymenoptera, while its most nascent condition is exhibited in the most primitive insect in which an amnion has been found to occur, namely the Thysanurid, Lepisma saccharina, L. (Heymons, 4). The observations of Heymons on the development of Lepisma are particularly noteworthy. Not

only is the amniotic cavity of greater relative cubic capacity in Lepisma than in any other Hexapod, but it never completely closes, remaining permanently open by a minute orifice, the amniotic pore. Thus, in its most primitive condition, the amniotic cavity of insects is an open sac. This is in strong contrast with the vertebrate amniotic cavity, as exemplified in the embryo of Erinaceus. Two quotations from Hubrecht's memoir will suffice to call to mind the point of view adopted by him with regard to the vertebrate amnion.

He says (6, p. 24): "Ich denke mir das Amnion in seiner allerersten Entstehung gleich als geschlossene Blase;[1] ich betrachte somit die Entwickelungsweise durch sich entgegenwachsende Faltenränder als ein bedeutend cenogenetisch modificirten Entwickelungsprozess."

Again, on p. 25 he says: "Meiner Ansicht nach muss man nicht bei Sauropsiden, sondern bei den Säugern nach Andeutungen der primitiveren, der mehr ursprünglichen Amnionentwickelung suchen."

In Peripatus novæ-britanniæ the egg is without yolk and the embryo develops on the surface of a relatively enormous blastodermic vesicle, whose wall consists of an internal layer of endoderm and an external layer of ectoderm. The ectoderm of this trophic vesicle does not consist of flattened and passive cells, as does the serosa of insects, but it is a mucous epithelium, the cells having vacuolar contents and serving for the absorption of nutrient fluids from the uterine wall and their transference to the trophic cavity, whence they are available for the nutrition of the embryo. The trophic ectoderm is therefore the trophoblast of the embryos of this species of Peripatus. The chorionic membrane always intervenes between the trophoblast and the uterine mucosa.

At first the embryonic tract lies at the posterior ventral extremity of the trophic or blastodermic vesicle, and at this stage the entire embryo bears a remarkable resemblance to an

[1] The posterior amniotic canal of Chelonians (Mitsukuri) and Hatteria (Dendy) requires special explanation.

insect egg with the embryonic tract lying upon the yolk (Figs. 1, A and B).

At a later stage the trophic vesicle develops a caudal extension, so that the embryo attains a more central position, although the cephalic portion of the vesicle is, as a rule, considerably larger than the posterior portion (Fig. 2, A).

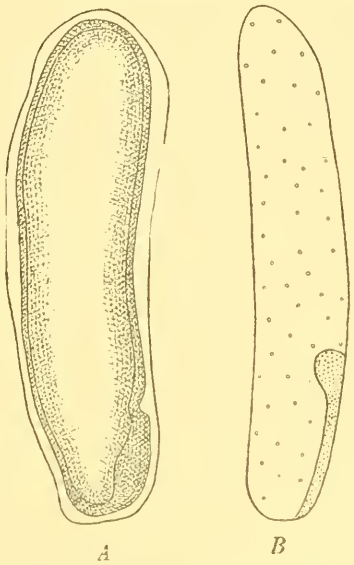

FIG. 1.—A. Embryo of Peripatus novæ-britanniæ, with embryonic tract at posterior ventral extremity of trophic or blastodermic vesicle (original).

B. Egg of Gryllus, with embryonic tract at the posterior ventral surface of the vitellus. (After Heymons.)

The young embryo rests upon the trophic vesicle as on a cushion, and there can be little doubt that in the fresh condition, when the cavity of the vessel is filled to repletion with its nutrient fluid contents, the embryo lies in a sort of lap or bay or depression, bounded by turgid lips, produced by the inflation of the surrounding thin wall of the vesicle. I cannot state this as a definite fact as I did not observe the living embryos. Everything, however, points to such a state of things.

As the embryo advances in development, the relative dimensions of the trophic organ decrease pari passu with the growth of the embryo. The posterior portion of the vesicle is the first to disappear, being used up in the formation of the dorsal body-wall of the embryo. When there is no longer any caudal extension of the vesicle there may still be observed a

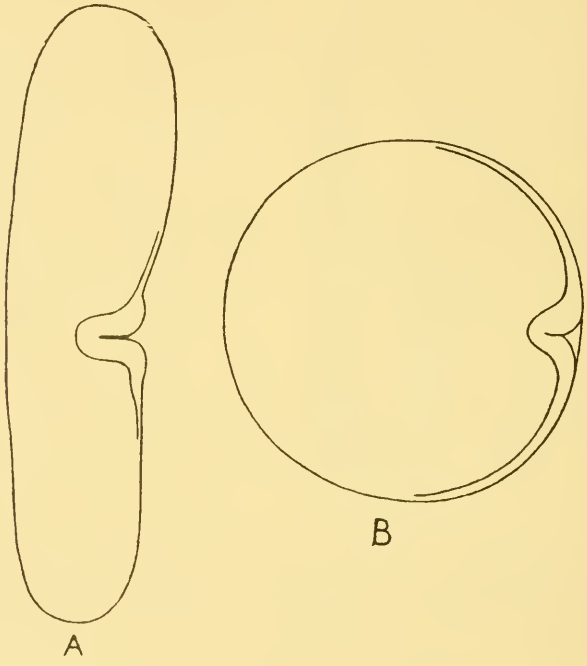

Fig. 2.—A. Embryo of Peripatus novæ-britanniæ, showing posterior extension of trophic vesicle and primary ventral flexure of embryonic tract. (Original.)

B. Egg of Strongylosoma guerinii, Gerv. (a Diplopod), showing primary ventral flexure of embryonic tract. (After Metschnikoff.)

large head-fold, which, in consequence of the cephalic flexure subsequently undergone by the embryo, is reflected like a cap over the ventral surface of the embryo (**14**, pl. iii, fig. 35).

This head-fold or cephalic portion of the trophic vesicle is attached to the embryo in the nuchal region; in other words, the point at which the final resorption of the trophic organ of these embryos takes place, lies in the nuchal region (cf. Fig. 3, A). This fact is of capital importance when taken in comparison with the remarkable phenomena attendant upon the involution of the serosa of insect embryos.

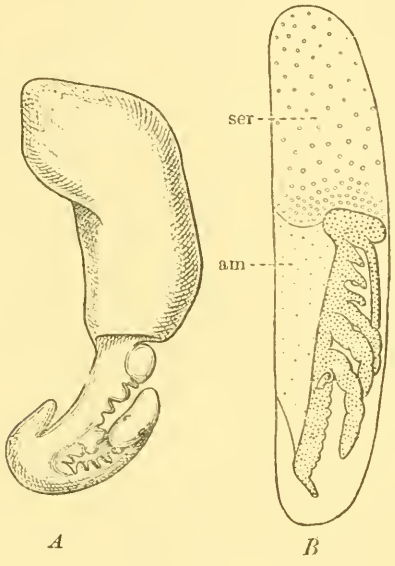

FIG. 3.—A. Embryo of Peripatus novæ-britanniæ, in which the posterior extension of trophic vesicle has almost entirely disappeared. The anterior portion of the vesicle is present as a large lobe attached to the head. The figure also illustrates the caudal flexure in addition to the ventral flexure of the embryo. (After Willey, 'Zoological Results,' Cambridge, 1898.)

B. Egg of Gryllus, at stage after the eversion of the embryo. *am.* Amnion reflected over dorsum of embryo. *ser.* Serosa withdrawn to anterior portion of vitellus and attached to head of embryo, like the trophic vesicle in A. A slight caudal flexure is present, but no ventral flexure. (After Heymons.)

After the amnion of the Insecta Pterygota is formed by the fusion of tail-fold, lateral folds, and head-folds, the amniotic

cavity is present as a closed sac, and the amnion itself has completely separated from the rest of the blastoderm, which has, ipso facto, become converted into the serosa.

At a later stage the amnion enters for a second time, i.e. secondarily, into relations with the serosa, fusing with it near the head end of the embryo. The fused area becomes perforated and the embryo undergoes a process of eversion (revolu-

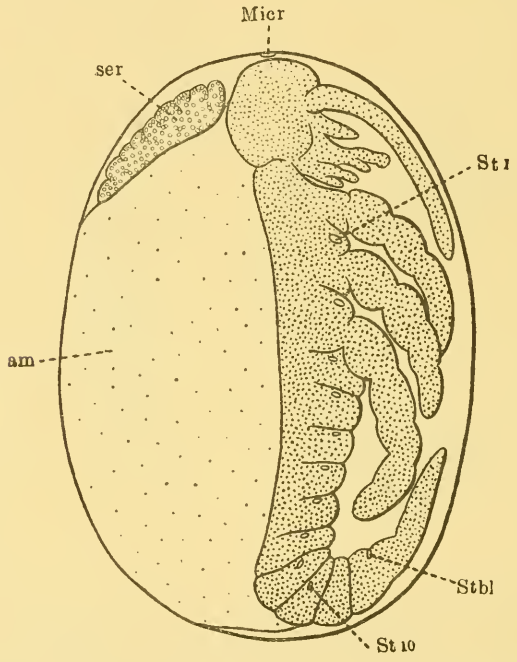

Fig. 4.—Egg of Forficula after eversion of embryo, with caudal flexure. *Micr.* Micropyle. *am.* Amnion reflected over dorsum. *ser.* Dorsal organ (remains of serosa). St_1.—St_{10}. Stigmata. *Stbl.* Stink-gland. (After Heymons.)

tion of Wheeler, Umrollung of Heymons and others), everting itself through the opening thus produced.

The secondary fusion of the amnion with the serosa is the initial stage in the involution or retrogressive development of the serosa. By the eversion of the embryo, the amnion

becomes reflected over the surface of the yolk, while the serosa shrinks away towards the anterior end of the egg (Fig. 3, B). Finally the serosa shrivels up until it constitutes the dorsal organ of the insect embryo, which in primitive insects is situated in the nuchal region, e.g. Lepisma, Gryllus, Forficula (cf. Fig. 4). The dorsal organ is subsequently withdrawn into the yolk, where it undergoes disintegration and absorption.

In the more primitive insects, therefore, the point at which the absorption of the serosa into the yolk takes place, lies in the nuchal region.

The resemblance between the trophic vesicle of the embryo of Peripatus novæ-britanniæ shown in Fig. 3, A, with the serosa of the embryo of Gryllus copied from Heymons in Fig. 3, B, is too striking to need further comment. If this were all, I should almost regard the suggested homology between the trophoblast of my Peripatus embryos and the serosa of insect embryos as a fait accompli. But it is not all.

The indusium of the Locustidæ described by Wheeler (12), and the dorsal organ ("micropyle," "micropylar organ") of the Poduridæ have to be taken into consideration. They can only be accounted for by invoking the aid of hypothesis; but then every explanation of any structure or phenomenon is more or less hypothetical.

Since the embryos of Peripatus novæ-britanniæ have enabled us for the first time, so far as the Invertebrata are concerned, to seize upon the most precious conception of the trophoblast, I think we are not only justified but absolutely called upon to face any difficulties which may obscure the application of this conception in all its phylogenetic ramifications.

To account for the dorsal organ of the Poduridæ and the indusium of the Locustidæ, we must call to our aid the principle of substitution. Without doubt Kleinenberg has left us a noble legacy in his principle of substitution, applicable as it is both to ontogenetic and phylogenetic changes.

I assume that the transformation of the trophoblast into the

serosa took place by substitution, and my meaning can best be illustrated by an observation of my own with regard to the endoderm in the embryos of P. novæ-britanniæ. The trophic cavity of the embryo becomes the gastral cavity of the adult, but the exceedingly thin layer of endoderm lining the trophic cavity does not become the definitive endoderm by simple continuous growth. On the contrary, the embryonic endoderm undergoes histolytic changes, the cells losing their mutual contiguity, rounding up and migrating as free trophocytes into the trophic cavity. After this remarkable histolysis, the endoderm reconstitutes itself, secretes a basal membrane, and produces the high columnar epithelium of the gut.

Thus, while the gastral endoderm of the adult is derived from the trophic endoderm of the embryo, it is not exactly the same as the latter since histolytic changes intervene.

The three principal phases in the development of the endoderm in the embryos of P. novæ-britanniæ may be summarised as follows:

I.	II.	III.
Trophic endoderm.	Trophocytes.	Gastral endoderm.

I regard this method of transformation of trophic endoderm into gastral endoderm as a special case of substitution, and well adapted to elucidate the significance of the assumption that the transformation of the trophoblast into the serosa has taken place by substitution.

Many other still clearer cases of epithelial substitution could be adduced. In insects, after the eversion of the embryo and consequent reflection of the amnion over the yolk, the amnion forms the provisional dorsal wall of the embryo. But it becomes replaced later by true ectoderm which grows out from the pleural edges of the embryo, while the amnion itself undergoes disintegration and absorption (Wheeler, **11**, **12**; Heymons, **2**). The origin of the definitive hypodermis of holometabolous insects from the imaginal discs is another case in point.

The above are actual instances of ontogenetic substitution. Instances of phylogenetic substitution can, in the nature of things, only be hypothetical.

In the embryos of the Poduridæ there is no amnion (Uljanin [10], Wheeler [12]), and therefore no serosa. The blastoderm, however, is not entirely employed in forming the dorsal body wall of the embryo, since there is a dorsal organ which is sometimes spoken of under the name micropylar organ (Wheeler) and spherical organ (Uljanin). This dorsal organ is a local thickening of the blastoderm lying in front of the embryonic tract; but at a later stage, when the true topographical relations are established, it is found to lie in the nuchal region (cf. Lemoine's figures [8]). It is destined to be absorbed like the dorsal organ of higher insects, which is the product of the involution of the serosa.

Appearances are in favour of the Poduridæ never having had an amnion, but the embryos present the remarkable peculiarity that the blastoderm outgrows the vitellus to such an extent that it is thrown into numerous complicated folds at the surface of the egg. "La superficie de la couche blastodermique," says Uljanin (**10**, p. xvii), "devient de plus en plus inégale; cette superficie, beaucoup plus agrandie à cause des inégalités de la couche blastodermique, sécrète une membrane cuticulaire, cuticule blastodermique, que l'on voit à travers le chorion de l'œuf fortement plissée et suivant toutes les inégalités du blastoderme épaissi." Lemoine (**8**) figures eggs with such a folded blastoderm.

The fact that the blastoderm of Poduridæ outgrows the vitellus, involuntarily suggests an atavistic repetition of a state in which the size of the egg bore no relation whatever to the dimensions of the blastodermic vesicle.

The indusium of Locustid embryos is a remarkable structure discovered by Wheeler (**12**). It arises as a small circular local thickening of the serosa immediately in front of the head of the embryo, i. e. in a position corresponding to the nuchal

region of the embryo at a later stage. According to Wheeler's perfectly clear account (12), the indusial thickening becomes overgrown by the surrounding serosa, sinks below the surface, becoming entirely separated from the serosa of which it is a derivative, and gradually spreads over the entire egg until its growing edges meet on the dorsal side of the vitellus and fuse together. The indusium then forms a complete envelope round the egg below the serosa, and it completely usurps the functions of the latter. Why this substitution of an indusium for the serosa should take place in Locustid embryos is a question not easily answered. At present all that can be said is that it happens so and there's an end.

In the course of its development, Wheeler found that the

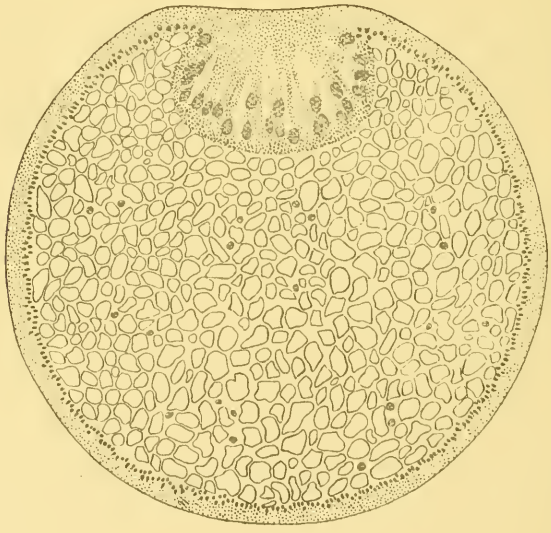

Fig. 5.—Median section of egg of Anurida maritima, showing dorsal organ with vacuolar cell contents, as a thickening of the blastoderm. (After Wheeler.)

indusium was subject to great time variations, growth variations, and even numerical variations. In two young embryos

he found that each possessed two separate indusial thickenings of the serosa or blastoderm.

In its first appearance as a local thickening of the serosa, the indusium has essentially the same topographical relations with regard to the nuchal region as the dorsal organ of the Poduridæ.

Wheeler says (12, p. 56): "Although much simpler in its structure, I do not hesitate to homologise this 'micropylar' organ in Anurida and the Poduridæ in general with the indusium of Xiphidium."

Again, with reference to the indusium Wheeler says (p. 58): "That the organ is rudimental is shown by its tendency to vary, especially during the earlier stages of its development; that it still performs some function is indicated by its somewhat complicated later development, and by its survival in but very few forms [Locustidæ] out of the vast group of Pterygotous insects. This seeming paradox may be explained if we suppose that the indusium was on the verge of disappearing, being the last rudiment of some very ancient structure. As such a rudiment it no longer fell under the influence of natural selection, and for this reason began to vary considerably like other rudimental organs. Some of these fortuitous variations may have come to be advantageous to the embryo, and were perhaps again seized upon by natural selection, the nearly extinct organ being thus resuscitated and again forced to take an active part in the processes of development."

Eventually the indusium undergoes retrogressive development like the serosa of other forms, and produces an indusial dorsal organ, which, however, is not absorbed but shed (Wheeler [12]).

Accepting Wheeler's view of the homology between the dorsal organ of Poduridæ and the indusium of Locustidæ, and admitting that these structures are not the same as the serosal dorsal organ of other insects, we are now in a position to discuss the bearing of these various observations. A certain amount of repetition is unavoidable if there is to be any

approach to clearness. In the following considerations, facts and hypotheses are given indifferently.

1. The trophoblast of the embryos of Peripatus novæ-britanniæ is a mucous membrane in virtue of its close proximity to the uterine mucosa; the chorionic membrane, however, always intervening between the trophoblast and the mucosa. This is a special case of Driesch's principle of the dependence of function on position—"Die Zelle ist Funktion der Lage."

2. The eggs of P. novæ-britanniæ have retained the primitive alecithality which must have characterised the eggs of the aquatic ancestor of Peripatus, which presumably spawned in the water. In connection with the neotropical species of Peripatus, Kennel was also of the opinion that their lack of yolk was a primary deficiency, and not a secondary loss.

If this postulate as to the primary character of the alecithality of the eggs of my Peripatus be not conceded, my theory would be thereby practically rendered nugatory.

3. The viviparity of Peripatus is to be regarded as a direct result of the transition from an aquatic to a terrestrial life. This was also Kennel's opinion.

4. In adaptation to the requirements of the intra-uterine nutrition of the embryo, various provisional embryonic trophic organs were evolved, e.g. dorsal (ectodermal) hump of P. capensis (Sedgwick), trophic vesicle of P. novæ-britanniæ, trophic sac of P. edwardsii, yolk of P. novæ-zealandiæ. It is neither easy nor necessary to decide which of these came first, or whether they were evolved more or less independently. Future investigation of the development of other species, such as P. tholloni, Bouvier, from the Gaboon district, may throw light on this matter.[1]

[1] For facts indicating the relatively primitive character of P. novæ-britanniæ the reader may be referred to my paper (14). According to the descriptions of Kennel and Sclater, the trophic organ of the embryos of the neotropical Peripatus has the form of a spheroidal vesicle, into which the embryo is inverted and to the wall of which it is united by an at first solid stalk. The vitelline membrane undergoes early resorption (Kennel), so that the wall of the sac comes into direct contiguity with the uterine mucosa. This inversion

5. We may, however, be quite certain that yolk is not the most primitive kind of trophic organ for the intra-uterine nutrition of the embryo, for the simple reason that the accumulation of yolk, such as occurs in the eggs of the Australian and New Zealand species of Peripatus, is a step towards secondary oviposition on terra firma, a condition which is actually realised in the case of the Victorian species, P. oviparus Dendy.

6. The lecithality and deposition of the eggs of insects are both secondary.

7. With oviparity the trophoblast necessarily ceased to act as an absorbent mucous membrane.

8. The trophoblast therefore became transformed into the blastoderm and its derivative the serosa, by substitution, this being apparently the prevailing method by which an epithelial transformation or metamorphosis is effected.

9. The serosal dorsal organ of insects is the product of the ontogenetic involution of the serosa.

10. The phylogenetic involution of the trophoblast has also to be accounted for, it being understood that the serosa was not directly derived from a primitive trophoblast, but by substitution. Although phyletically related, the trophoblast and serosa are therefore not identical structures. Accordingly we may well expect to find some vestiges of the true primitive trophoblast cropping up here and there.

11. I see in the dorsal organ of the Poduridæ and in the indusium of the Locustidæ, vestiges of the true trophoblast, both of these structures presenting, in the earlier stages of their development, many of the characters of a mucous membrane. This interpretation of facts at least accounts for the otherwise meaningless dorsal organ of the Poduridæ (Fig. 5).

12. The dorsal organs of Crustacean embryos and the primitive cumulus of Arachnids must require special explanation,

of the embryo shows that this is a special case, and not one from which phylogenetic conclusions may safely be drawn. An inverted development must necessarily be secondary, as compared with a development which is not inverted.

my theory assuming the diphyletic origin of the Tracheata and the monophyletic origin of the Hexapoda.[1]

[1] I am aware that the assumption as to the monophyletic origin of the Hexapoda is a very grave one to make, and I only make it in the most general way. It would take one much too far afield to attempt to justify and explain such an assumption in detail. At least two authors, Pocock and Kingsley, have made an onslaught against the group of the Myriapoda; and Pocock (R. I. Pocock, "On the Classification of Tracheate Arthropoda," 'Zool. Anz.,' xvi, 1893, p. 271) has gone so far as to suggest a scheme of classification, in which the group of the Myriapoda finds no place. Taking the position of the generative apertures as his criterion, he divides the Tracheata (apart from Arachnida) into two large groups, the Progoneata (Diplopoda and Pauropoda) and Opisthogoneata (Chilopoda, Symphyla, Hexapoda). The group of the Symphyla (Scolopendrella) is one of the utmost importance in phylogenetic speculations, and there seems to have been some misunderstanding about it, which however was subsequently corrected (see 'Nature,' vol. xlix, p. 124). Grassi discovered in 1886 that the unpaired genital pore of Scolopendrella lies on the fourth body-segment, and the same statement is contained in an important memoir by Kenyon (F. C. Kenyon, "The Morphology and Classification of the Pauropoda, with Notes on the Morphology of the Diplopoda," 'Tuft's College Studies,' No. iv, 1895, see p. 136). The Symphyla would seem to be related to the Chilopoda in an analogous manner to that in which the Pauropoda are related to the Diplopoda. It would, in fact, appear that the Symphyla are distinctly intermediate between the Chilopoda and Diplopoda (Chilognatha), having Chilopod affinities in respect of their ambulatory appendages, and Diplopod affinities in respect of their manducatory appendages and generative organs (consult table at end of Kenyon's memoir). Even if the two principal sub-divisions of the Myriapoda were sharply divergent, I think the loss of the name Myriapoda would outweigh the profit of a classification which omitted all mention of the name. As a matter of fact, although well-marked, the Chilopoda and Diplopoda are not irretrievably divergent.

Nevertheless, it seems not impossible that the Hexapoda have differential or heterogeneous relations to the Myriapoda. The Insecta Apterygota are divisible into two well-defined sub-orders, as shown by Stummer-Traunfels, namely the Apterygota entognatha (Campodeidæ, Japygidæ, and Collembola) and the Apterygota ectognatha (Machilidæ, Lepismidæ). (Rudolf Ritter v. Stummer-Traunfels, "Vergleichende Untersuchungen über die Mundwerkzeuge der Thysanuren und Collembolen," 'S. B. Ak. Wien,' Bd. c, Abth. 1, April, 1891.)

The Apterygota entognatha (e. g. Campodea) show strong external resemblance to Scolopendrella, and the embryonic development (Collembola)

Such are the considerations by which I seek to establish a phyletic relationship between the trophoblast, as it is presented to us in the embryos of Peripatus novæ-britanniæ, and the serosa of insect embryos, and for my part I am disposed to be content with this demonstration. Nevertheless a few words on the origin of the insect amnion may not be out of place. In accounting for the serosa we have not, eo ipso, accounted for the amnion, notwithstanding that they are both derivatives, and inseparable derivatives, of the blastoderm. The amniotic cavity requires special treatment.

Until Heymons discovered the amnion and serosa of Lepisma, he supposed that the embryonic membranes were to be regarded as a new acquisition of the Insecta Pterygota, and that there was no basis upon which to frame any hypothesis as to their phylogenetic history (2).

For statements of their own views and references to the views of others, the reader would find it well worth while to consult the well-known memoirs of Wheeler and Heymons. Suffice it to say here that Heymons (3) has conclusively shown that the superficial or ectoblastic type of embryonic tract as exemplified in Orthoptera is more primitive than the immersed or entoblastic type as exemplified in Libellulidæ and Ephemeridæ.

So far as I have been able to form an opinion from the data which are to hand, I think that the amniotic cavity of insect embryos was originally a product of invagination, and that this invagination was primitively derived from and associated with a ventral flexure of the embryo.

As already mentioned, Heymons has in fact found that the amniotic cavity of Lepisma is formed by invagination, which is brought about by the ventral flexure of the embryo, the orifice

strikingly calls to mind that of the Chilopoda, in that a dorsal flexure of the embryo precedes the ventral flexure.

The Apterygota ectognatha, as exemplified in Lepisma, recall in their embryonic development that of the Diplopoda in respect of the primary ventral flexure of the embryo.

of invagination narrowing down to a small pore which persists as the amniotic pore. My views, however, differ from those of Heymons, in that I regard the ventral flexure as primary, while Heymons regards the dorsal flexure of young embryos of Chilopoda and Poduridæ as primary, and he thinks that this dorsal flexure has been lost by the embryo of Lepisma. Knowing what takes place in the embryos of P. novæ-britanniæ, I am quite satisfied that the early ventral flexure of the Chilognatha (Diplopoda) is primitive, and that the provisional dorsal flexure of the embryos of Chilopoda and Poduridæ is a transitory, cenogenetic, intercalated phase of development.

The ventral flexure of the embryo of Lepisma is therefore to be regarded as comparable with the primitive ventral flexure which occurs in Chilognatha and in Peripatus (see below).

Heymons says (4, p. 620):—"Sucht man bei höheren Insecten nach einem Anklang an die ausgesprochen ventrale Krümmung von Lepisma, so ist dieser wohl zweifellos in der von mir Caudalkrümmung genannten Umbiegung des hinteren

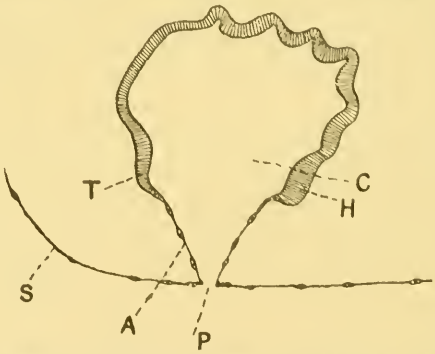

Fig. 6.—Sagittal section through embryo of Lepisma saccharina, to show ventral flexure, amniotic cavity, and amniotic pore. Mesoderm and yolk omitted. *a*. Amnion. *c*. Amniotic cavity. *h*. Head end of embryo. *p*. Amniotic pore. *s*. Serosa. *t*. Tail end of embryo. (Simplified after Heymons.)

Körperendes vieler Insectenembryonen gegeben. Dieselbe zeigt sich bei den Orthopteren, den Odonaten, Ephemeriden

fast in allen bisher bekannt gewordenen Fällen, und sie gelangt selbst dann zum Ausdruck, wenn der Keimstreifen im Übrigen vollkommen dorsal gekrümmt ist."

I cannot follow Heymons in the above comparison between the ventral flexure of his Lepisma embryos and the caudal flexure of other insect embryos.[1]

The embryos of P. novæ-britanniæ undergo three absolutely distinct ventral flexures, namely, (1) the primitive ventral flexure, (2) the caudal flexure, and (3) the cephalic flexure; the last-named flexure not being constant. It is very important to note that there is no connection whatever between the primitive ventral flexure and the caudal flexure (see Fig. 3, A).

The primitive ventral flexure serves the immediate purpose of releasing the primitive streak, i. e. the principal growing point of the embryo, from the rest of the embryonic tract, so as to enable it to continue its growth independently of the trophic vesicle; and it also serves the essential purpose of keeping the growing embryo, during its early stages, in as small a space as possible, in order to admit of the trophoblast coming into contact with the uterine mucosa (the chorion intervening) over as large an area as possible. The flexure of the embryo is sufficiently accounted for on such physiological grounds as these.

The occurrence of three ventral flexures in the embryos of P. novæ-britanniæ cannot be too strongly emphasised.

In the Myriapods the ventral flexure of the embryo is an essential and characteristic feature of the development, and is to be considered in connection with the absence of an amnion. It takes place at a very early stage in the Chilognatha (Fig. 2, B), while in the Chilopoda it is preceded by a dorsal flexure. The ventral flexure of the Myriapods (with special reference to the Chilognatha) is quite obviously to be iden-

[1] It is, however, quite possible that, in certain cases, the caudal flexure and the primary ventral flexure might coincide.

tified with the primitive ventral flexure of the embryo of my Peripatus (cf. Fig. 2, A and B), with the difference that in the Myriapods the flexure goes much deeper and is much more pronounced than in Peripatus (cf. Metschnikoff [9], and Korschelt and Heider [7]).[1]

Thus in the Myriapods (as in Peripatus) the ventral flexure of the embryo may be said to be a product of invagination, but the orifice of invagination does not narrow down at all, remaining freely open on all sides.

In Lepisma, as shown by the observations of Heymons, we have a primary ventral flexure of the embryo, strictly comparable with the ventral flexure of the Myriapod embryo, and the invagination which produces the ventral flexure is accompanied by a narrowing of the orifice of invagination, thus giving rise to an amniotic cavity opening by an amniotic pore.

The preceding remarks may be resumed in tabular form as follows :

P. novæ-britanniæ.	Myriapoda.	Lepisma.
Shallow ventral flexure.[2]	Deep ventral flexure.	Amniotic cavity and Amniotic pore.

Thus although the amnion itself first appears within the group of the Hexapoda, it does not owe its origin to purely mechanical causes, as has been so often supposed, but can be traced back, through the link supplied by Lepisma, to the primitive ventral flexure of ancestral forms.

I think I have now said enough to explain my theory of the embryonic membranes of insects, and I give it for what it is worth. I have endeavoured to trace the serosa back to a primitive trophoblast, and the amniotic cavity back to a primitive ventral flexure.

New Museums, Cambridge;
September 22nd, 1898.

[1] In the embryos of P. novæ-britanniæ the primary ventral flexure does not involve the trophic vesicle; whereas in Myriapods it does involve the vitellus.

[2] That is to say, shallow in its primary relations to the trophic vesicle.

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