Bound 1948

QUARTERLY JOURNAL

OF

MICROSCOPICAL SCIENCE

EDITED BY

E. RAY LANKESTER, M.A., F.R.S., F.L.S.,

Fellow of Exeter College, Oxford, and Professor of Zoology and Comparative Anatomy in University College, London;

WITH THE CO-OPERATION OF

F. M. BALFOUR, L.L.D., F.R.S., F.L.S.,

Fellow and Lecturer of Trinity College, Cambridge;

W. T. THISELTON DYER, M.A., F.R.S., F.L.S.,

Assistant Director of the Royal Gardens, Kew;

AND

E. KLEIN, M.D., F.R.S.,

Lecturer on Histology in St. Bartholomew's Hospital Medical School, London.

VOLUME XXI.—New Series.

With Lithographic Plates and Engravings on Wood.

LONDON:

J. & A. CHURCHILL, NEW BURLINGTON STREET.

1881.

CONTENTS.

CONTENTS OF No. LXXXI, N.S., JANUARY, 1881.

MEMOIRS:

NOTES AND MEMORANDA:

CONTENTS OF No. LXXXII, N.S., APRIL, 1881.

MEMOIRS:

NOTES AND MEMORANDA:

CONTENTS OF No. LXXXIII, N.S., JULY, 1881.

MEMOIRS:

CONTENTS OF No. LXXXIV, N.S., OCTOBER, 1881.

MEMOIRS:

NOTES AND MEMORANDA:

MEMOIRS.

NOTES *on a* PECULIAR FORM *of* POLYZOA *closely* ALLIED *to* BUGULA (*Kinetoskias*, Kor. and Dan.). (With Plates I and II.) By GEORGE BUSK, F.R.S.

AT a meeting of the Scientific Society of Christiania on the 15th March, 1867, D. C. Danielssen gave a preliminary account of two new forms of Polyzoa found by him in Nordland and Finmark, and referred to a new genus *Kinetoskias*.[1]

Of these a fuller account has since been published by Koren and Danielssen, accompanied with figures;[2] and one of the two forms in question was enumerated by Sars, in a catalogue of deep-sea species, under the name of *Bugula Smittii*.

In May, 1867, Professor Smitt, apparently not aware that a new generic name had been already applied to it by Danielssen, described apparently one of the two forms noticed by that observer under the name of *Bugula umbella*.[3] Smitt's specimens were procured from Wijde Bay, Spitzbergen.

On the 30th January, 1873, on the "Challenger" Expedition, a specimen of large size, of a form very closely allied to if not identical with one of those described by Koren and Danielssen, was described and figured by Sir C. W. Thomson,[4] under the name of *Naresia cyathus*. This specimen was procured in the North Atlantic in lat. 36° 23′ N., long. 11° 18′ W., from a depth of 1525 fathoms. A second specimen of the same form, but of smaller size, was procured on the 2nd March, 1876, in lat. 36° 44′ S., and long. 46° 16′ W., from a depth of 2650 fathoms. Other specimens of a closely

---

[1] 'Forhandl. i Videnskabsselskabet i Christiania,' 1867, p. 23.

[2] 'Fauna littoralis Norvegiæ,' part iii, 1877, p. 104, pl. iii, figs. 12—14; pl. xii, figs. 4—14.

[3] "Kritisk förteckning öfver Skandin. Hafs-Bryozoer," in 'Öfversight af Kongl. Vetensk.-Akademiens Handlingar,' May, 1867, pp. 292 and 353, pl. xix, figs. 28—31.

[4] 'Nature,' i, p. 387.

allied but quite distinct form were obtained on the 10th September, 1875, in lat. 9° 5′—10′ S., long. 30° 49′—53′ W., from a depth of from 32 to 400 fathoms; and again, on the 14th December, 1875, in lat. 33° 31′ S., long. 74° 43′ W., from a depth of 2160 fathoms.

These different forms constitute certainly three and, as I am inclined to think, four distinct and well-characterised species; but they all agree in certain very peculiar characters, which would seem to be almost, if not quite, sufficient to render the group composed of them of generic value, or, at any rate, to rank as a distinct sub-genus of *Bugula.*

To this genus or sub-genus the appellation bestowed upon it by Koren and Danielssen obviously has priority over *Naresia.*

The species belonging to this group at present known are:

1. *Bugula* (*Kinetoskias*) *Smittii,* Dan.; *Kinetoskias Smittii,* Kor. and Dan., l. c.; *Bugula Smittii,* Sars.

2. *B.* (*Kinetoskias*) *arborescens,* Daniells.; *Kinetoskias arborescens,* Kor. and Dan., l. c.; *Bugula umbella,* Smitt, l. c.

3. *B.* (*Kinetoskias*) *cyathus,* W. T.; *Naresia cyathus,* C. W. T., l. c.; ? *Kinetoskias Smittii,* K. and D., l. c.

4. *B.* (*Kinetoskias*) *pocillum,* n. sp., mihi.

1. *K. Smittii* is thus characterised by Koren and Danielssen.

"Zoarium umbellate, with four strong main branches springing from a stem about 150 mm. (6 inches) in length, which is cylindrical, naked, and completely pellucid, and gradually thickened towards the lower end, from which numerous radical fibres proceed, by which the growth is affixed to small stones or sand. The branches are biserial, and divide several times dichotomously. The zoœcia are about 0·8 mm. (0·03 inches) long, with a breadth of 0·31 mm. above, and of 0·13 mm. below. In shape they are elongated and tapering below, truncate above. The posterior surface is striated transversely towards the lower part. A short spine is placed on the upper and outer angle. On the upper and anterior border of the *zoœcium* there is a blunt, solid, horny, conical process, which appears to serve as the point of insertion of a strong muscle, which expands on the anterior surface of the superjacent *zoœcium.* This muscle is attached to the conical process by a tendon."

"The avicularia are placed on the outer border of the *zoœcia,* a little above the middle. They are of an elongated form, with a rather long mandible curved at the point. The oœcium is sub-globular and affixed to the upper and outer border of the zoœcium, arching, as it were, over the aperture."

The anterior muscle above mentioned is described as lying between a fine exterior membrane and the proper ectocyst. This membrane, again, is said to pass down from one zoœcium to another, and as connecting the branches together, being finally continued into the stem.

Amongst other particulars, the authors state that the ovary is attached to the endocyst on the upper and anterior part of the surface of the zoœcium, consisting of an agglomeration of cells wherein the ova are developed. The testis, on the other hand, they state, is situated at the bottom of the zoœcium, and is formed of similar cells to those forming the ovary, but filled with spermatozoa.

Kinetoskias, therefore, is regarded by them as a complete hermaphrodite.

The incurvation of the branches of the *zoarium* towards their termination is ascribed to the action of the anterior muscles above noticed. There is, they observe, no common colonial muscular system.

2. *Kinetoskias arborescens* is described as having a flexible zoarium, supported on a very short stem, from which radiate four large main branches which sub-divide dichotomously. The zoœcia, which are biserial and alternate, are 0·58 mm. long by 0·31 broad above, and 0·18 mm. below. They are of a narrow form, and nearly pointed below, especially when viewed from behind; above they are broad and rounded, the upper border being oblique in a direction from within upwards and outwards, so that the upper and inner angle is rounded off, and the outer rendered more acute; there is no spine on this angle. From the middle of the upper convex border of the zoœcium nearer the anterior margin is a strong, horny, obtuse conical process or apophysis, which is said to give attachment to a muscle, which, as in *K. Smittii*, is described as spreading out over the anterior surface of the superjacent zoœcium.

The anterior surface is said to be covered with scattered calcareous granules, and the posterior surface, which is very convex, is strongly striated transversely. The striæ or rugæ are elevated and oblique from below upwards and inwards.

The avicularia are placed on the upper and outer angle of the zoœcia. They have the form of an eagle's head, and are supported on a short articulated stem. No oœcia were observed.

Professor Smitt, in his independent account of the latter of the two species described by Koren and Danielssen, remarks that the zoœcia are widely distinguished by their form from those of other species of *Bugula,* inasmuch as the lower tubular portion is entirely wanting, as viewed in front, the membranous aperture occupying the entire anterior face. The aperture is wider above than below, so that the zoœcium, he observes, has more or less of a boat shape, as in *Beania.* But the *Bugula type* is nevertheless evidenced in the incurvation of the inner border, whilst the lower border of the aperture is straight. The upper and inner angle is rounded off, and the outer more acute. The avicularia are placed high up on the upper and outer angle of the zoœcium, and assume the same position, he says, as in *B. avicularia,* though rather more pointed upwards.

On the dorsal aspect the zoœcia present a still greater peculiarity. In this aspect they are flatly convex, with the outer border acute and the inner more rounded.

The surface, as in *Ætea anguina,* is traversed by raised granular lines or ridges, which curve obliquely upwards and inwards.

The lowest portion of the zoœcium, which is in other species of *Bugula* more or less tubular, is in this case simply constricted, so as to constitute a laterally compressed peduncle, placed somewhat external to the middle line of the zoœcium.

Close to this constricted part, and near the inferior and outer angle of the zoœcium, the radical tubes arise, the somewhat dilated commencement of which completely fills up the space between the summit of the inferior or older zoœcium, and the base of the superior or younger, being wedged in, as it were, between the two. From this point a radical tube grows downwards, running along the outer border of the lower zoœcium, near the bottom of which it unites with a similar radical tube arising from that zoœcium.

Sometimes, however, he observes this conjunction does not take place, but the two (or more) tubes are continued side by side. But, generally speaking, as they descend all distinction between the tubes disappears, their *lumina* appearing to run together, so as to form a broad expansion, which fills up the angular space between two contiguous branches of the zoarium, stretching across from one to the other.

Professor Smitt also states that in the interior of this expansion numerous colonial nerve filaments may be seen, besides which are only some free nucleiform particles (fettkropskulor).

It is not clear, in Professor Smitt's description, whether the specimen of *Bugula umbella,* from which it was drawn, had or had not a simple peduncle. But, so far as my imperfect knowledge of Swedish enables me to say, I should gather that he thinks it possible that the growth may be more or less detached or free, the radical fibres only penetrating the loose muddy bottom without, as in most other cases in the Polyzoa, the fibres being attached to any fixed object.

The above brief summary comprises, I believe, the main points hitherto made known with regard to the structural peculiarities of *Kinetoskias.* My object in the present communication is to show how far the accounts given by the eminent and excellent observers above cited are in accord with what I conceive, from direct observation of two closely allied forms, to be the actual conditions.

The two forms that have come under my own observation from the "Challenger" collection are:

1. *Kinetoskias cyathus,* C. W. T., and
2. *Kinetoskias pocillum,* n. sp. (fig. 3).

1. *B. cyathus.*—The general aspect and dimensions of this remarkable and beautiful form are well shown in Sir C. W. Thomson's account of it,[1] and to which, so far as external characters go, I have but very little to add. The *zoarium* consists of an elegant infundibuliform, vase-like expansion, constituted of numerous, long, sparsely dichotomising, biserial branches, springing from an apical point at the bottom, and curving gently outwards till, towards the extremities, they are curled round upon themselves, the anterior aspect of the zoœcia looking outwards. This infundibuliform portion of the zoarium is supported on a point, a little to one side of the actual apex, upon the summit of a terete peduncle, about four or five inches in height, and from about half an inch in diameter at the bottom, tapering to a diameter of less than the one tenth of an inch at the summit. At the lower part of the vase-like cup the branches, to a height of about an inch, are united, like the ribs of an umbrella, by a delicate transparent membrane, stretching across from one to the other. This membranous cup is brought to a point at bottom, a little to one side of the spot from which the branches diverge, and it appears to be quite closed, a very tight constriction existing at its junction with the peduncle. The latter, though

---

[1] L. c.

flattened in the spirit specimen, is, in the natural condition, cylindrical, and probably, when distended, sufficiently stiff to support the upper portion in an upright position, whose weight, of course, must be very little in the water. The wall of the peduncle, though perhaps rather thicker than the web, is perfectly transparent, and, so far as I can make out, quite homogeneous; and in the interior, in the spirit specimen, nothing is to be seen except a few minute nuclei and slender branching threads, probably belonging to an extremely delicate endosarc, and which it is allowable to suppose may represent the so-called "colonial nervous system," seen by Smitt in the radical tubes of his *B. umbella.*

The zoœcia are about 0·045 inch long by 0·02 in width, which is tolerably uniform from top to bottom. The outer border, as in most species of *Bugula,* is hollowed on the external border and towards the lower end in most of the zoœcia, a sort of step is thrown out (Pl. I, fig. 1), upon which is articulated the *avicularium.* The inner border is evenly rounded, and the upper and inner angle is completely rounded off, whilst the external is produced and crowned with a short, pointed, spinous process.

Viewed behind, the zoœcium is convex and the surface perfectly smooth, without a vestige of any transverse ridges. The outline is much the same as in front, and the outer border is acute, the inner rounded. At bottom the zoœcium is seen to arise from the back of the subjacent one by a constricted neck, on the outer side of which there is a chitinous, thickened, ring-shaped process, which appears to represent the spot from which, in the lower part of the branches, the radical tubes spring; and the insertion of the zoœcium appears to be also surrounded with a rather thick, chitinous ring. The oœcia are of large size, attached to the middle of the summit of the zoœcium in front, and projecting forwards in the form of a wide shallow hood.

The avicularia are about 0·02 inch long and about 0·006 wide. The mandible is about 0·01 inch in length, and much curved; within it presents the usual arrangement of muscles, and a thickened, glandular, (?) digitiform sac or pouch.

Within the zoœcium a rather large polypide is lodged, of the usual conformation and muscular connections, and having about twenty-four or twenty-six tentacles. So far there is nothing very remarkable: but other peculiarities remain to be mentioned, possessed in common by this and the other species of *Kinetoskias.* These are—1. The existence of a distinct muscle, which, arising from the front of the base of the zoœcium passes obliquely backwards and upwards, expanding in a fan-shaped manner, to be inserted into its hinder wall to the height of about one third or one fourth of the zoœcium (fig. 1 *a*). The action of this muscle must be to draw the entire zoœcium downwards and forwards, or, in other words, to bend it on itself, and thus, by the concurrent action in many zoœcia, to curl the branches forwards ; an action that has, in fact, been noticed by Koren and Danielssen in the living condition. Besides this flexor muscle in *K. cyathus* there is an additional, smaller, fan-shaped bundle of fibres, for the purpose, apparently, of curving the step-like process of the wall of the zoœcium, upon which the avicularium is fixed, forwards, so as to cause the avicularium to come in front of the zoœcium (fig. 1 *c*), which appears in the spirit specimen to be its usual position. I have been unable to discern anything like ovary or testis within the zoœcia ; but many, if not most, of the oœcia are filled with an apparently vitelline mass or ovum of large size.

Koren and Danielssen are inclined to consider that their *Kinetoskias Smitii* is identical with *Naresia cyathus* of Wyville Thomson ; but, so far as I am able to judge, from their detailed description and figures, I do not see how this can well be. The form and size of the zoœcia and of the avicularium and oœcia undoubtedly appear to correspond with those of *B. cyathus* ; but the general aspect of the zoarium in the two cases is utterly dissimilar. In this particular, however, it must be remarked that the natural-size figure given in pl. iii[1] does not at all correspond with the description in the text. 2. In *K. cyathus* there are no transverse *rugæ* on the back of the *zoœcia*. 3. The avicularium is attached in *B. Smitii* above the middle of the outer border of the zoœcium, whilst in *K. cyathus* it arises from a distinct step-like process, quite at the bottom.

On these grounds there does not appear to me sufficient reason to regard *Kinetoskias Smittii* and *Naresia cyathus* as specifically the same.

2. *Kinetoskias pocillum.*—Though very much smaller than *K. cyathus*, the structure of the zoarium is exactly the same. Like that species it consists of a vasiform infundibuliform expansion composed of branches springing from a common point, and, as in that form, united at their base by a transparent membrane, which is connected with an equally transparent, terete, membranous stem, about one and a half to two inches high, which, as in *K. cyathus*, terminates inferiorly

---

[1] L. c.

in a thick tuft of very fine tubules or hollow fibres, each of which is individually affixed to an empty *globigerina* shell, or to some other Foraminifer. And there is the same complete constriction between the stem and the membranous expansion forming the bottom of the infundibular cup. The zoœcia differ from those of *K. cyathus* in having no spine on the upper and outer angle, and in having the avicularium attached by a distinctly articulated peduncle to the outer border rather above the middle and without any projecting process for its reception. Posteriorly, the *zoœcia* are irregularly oblong, the outer border being sharp and nearly straight, and the inner as it were gibbous. The surface, as in *K. cyathus*, is perfectly even and smooth, and very convex. In many of the zoœcia, more especially towards the lower end of the branches, a small tubercular projection rises from the upper border of the *zoœcium* (fig. 2) in the middle, which would seem to correspond to the "horny conical process" in the same situation noticed by Koren and Danielssen, and supposed by them to serve for the attachment of a muscle; but it is clearly nothing of the kind, and, as it seems to me, merely a rudimentary oœcium. In *K. pocillum* these organs are much smaller than in *K. cyathus*, but, as in that species, cucullate in form. They differ, however, very markedly in the direction in which the opening looks, which, in *K. cyathus*, is directly downwards, and the *K. pocillum* obliquely outwards and downwards. In the interior of the zoœcia the arrangements are of the usual kind, except in the presence of the additional fan- or rather brush-shaped flexor muscle, which, in this species, is of larger size or more developed than it is in *K. cyathus* (fig. 2.). The additional muscle connected with the insertion of the *avicularium* is absent in *K. pocillum*. Besides these parts, there may be also seen within the base of the zoœcium behind, an apparently chitinous process of irregular figure, and probably hollow. It springs apparently very close to the spot whence the radical tubes arise, and may have some connection with them. Koren and Danielssen notice a similar process in *K. Smittii*, and I have seen it occasionally of smaller size than in *K. pocillum* in *K. cyathus*. I am unable to define its function, but it most certainly does not serve as the point of attachment for a muscle as supposed by Koren and Danielssen.

In *K. pocillum* the *avicularium* is of larger size, and wider in proportion to its length than in *K. cyathus*, resembling in that respect the avicularium of *K. arborescens*, but otherwise they are alike, both containing, besides the usual muscles, a digitiform glandular sac, fine branching nucleated fibres, which may be nervous.

I have as yet scarcely adverted to the most remarkable feature of *Kinetoskias,* viz. the peduncle or stem, which appears to exist in all the species, though it is not shown in Professor Smitt's figure of his *K. umbella,* having probably, as I should imagine, become accidentally detached.

The mode of formation of this part of the zoarium, which is undoubtedly the homologue of the bundle of separate radical tubes so commonly met with among the Polyzoa, is extremely curious and interesting, and at the same time, in some points as yet, more or less obscure; as, in fact, may be said respecting the mode of formation and development of the more ordinary form of radical tubes.

In the more common form they are cylindrical, jointed, chitinous tubes, with rather thick walls, and with very scanty contents, beyond a few minute granular particles and irregular threads, representing, as it would seem, the remains of an endosarc, with which, in order that their progressive increase in length, and occasionally complicated branching, &c., may be effected, we must suppose the tube to be furnished. In fact, it is otherwise impossible, without assuming the presence of a germinal material to account for the fact, that even after the tubes have attained a considerable length the extremity, or a considerable part of the tube, may undergo great changes in form, as is seen in the production of hooks and other means of ensuring adhesion to foreign bodies; changes showing a most extraordinary adaptability to circumstances. Not the least remarkable of these adaptations is the division of the extremity of the tube into a multitude of very minute tubular filaments, each of which may be traced into independent connection with some small foreign body. In the deep oceanic forms these are most usually dead *globigerina* shells, or the skeletons of other foraminifera, so that having no more stable foundation than the soft globigerina ooze, which forms so extensively the bottom of the ocean, the delicate Polyzoan growths which inhabit those profound depths, are able to support themselves by the innumerable multitude of solid particles to which they are attached by the hair-like terminations of the radical fibres.

And this is well shown in the case of *Kinetoskias,* in which the dilated lower end of the peduncle breaks up into a thick and dense tuft of excessively fine filaments, at the end of each of which, when the tuft is slightly teased out, a *globigerina,* or other foraminiferous shell, is seen to be firmly attached. In many instances the filament may be seen entering the cavity of the empty shell and coiling about within it.[1]

It is difficult to imagine how this subdivision of the distal end of the tube or stem can take place, unless at that part there is active power of growth, as at the extremities of the root fibres in plants, though in a different and at present unknown way. And a still more remarkable fact is the power of adaptation to the environment that is possessed by these delicate filaments, which might almost lead to the conclusion that an active living power resides in even the ultimate fibrillæ.

In *Kinetoskias* the peduncle, as I have observed, represents a radical tube, or rather, it may be said, a coalesced bundle of tubes.

The wall of the peduncle, in the living or fresh condition, is described by Sir C. W. Thomson as being as clear as glass, and it retains this transparency scarcely impaired even after long immersion in alcohol. Unlike the radical tubes in all other Polyzoa that I have examined, the corresponding structures in *Kinetoskias* have no action on polarised light.[2] Though very thin the wall is extremely tough, and beyond an obscure appearance, in the contracted state, of a very delicate, longitudinal, irregular striation, no trace of structure can be observed in it.

Within, as I have stated, the remains of a very delicate endosarc or cyst may be observed, as represented by a few scattered, minute nuclear bodies, and irregular branching filamentous strings.

I have already cited Professor Smitt's account of the mode of formation of the radical tube or stem and sheathing membrane in *K. arborescens*, and this, in the main, is equally applicable to *K. cyathus* and *K. pocillum*.

In fig. 5 is represented the bifurcation of one of the branches in the latter species, just above the point at which the branches are connected by the sheathing, umbrella-like expansion.

In this figure are shown delicate, dilated, radical tubes, passing across from one branch to the other. These tubes arise from the constricted part of the *zoœcium* behind and immediately above its point of origin from the subjacent one. And they are apparently inserted into the corresponding point of the zoœcia in the opposite branch. The tubes

---

[1] This arrangement, however, is equally well shown in many other of the abyssal forms of Bugula, Bicellaria, and other genera.

[2] Which would probably indicate the absence of any calcareous element.

may be seen to arise with a small transparent protrusion (*a*, *a*, *a*, *a*), which gradually increases in length and diameter, but in the latter sense very irregularly. Whether those tubes, which are attached at both ends, *arise* at one end and are *inserted* by the other, or whether the continuous tube is formed by the coalescence of a separate one from each side, I am unable to determine, but am inclined, from the appearance the connecting tubes sometimes exhibit, to think that the latter supposition is the more probable.

The continuous web-like connecting membrane appears in like manner to be formed by the lateral fusion of similar connecting tubes. But how the peduncle itself below the constriction at which it is attached to the sheathing membrane is formed is very difficult of explanation. It seems to me not improbable that the stem, notwithstanding its size and length, actually represents merely a segment or internode of a largely dilated radical tube, extending from the point of constriction above to the lower expanded end, where it breaks up into the bundle of fibres and fibrillæ, all of which, it may be observed, are more or less distinctly divided into joints or internodes, as is the case almost universally, so far as I am aware, in all radical tubes among the Polyzoa.

In *Kinetoskias cyathus* I have not noticed any similar connecting tubes between contiguous branches, but the mode of formation of the web-like expansion is very plainly shown to arise from the coalescence of radical tubes.

In fig. 4 is shown the bifurcation of a branch at a point a little above and including a portion of the edge of the web, whilst on the two outer sides of the figure are shown adherent portions of the same. It will be seen, as described by Professor Smitt, that a radical tube arising from the hinder and inferior part of a zoœcium descends behind the outer border of the subjacent ones, and that the descending tubes on both sides becoming dilated, and gradually approaching each other, eventually coalesce to form the membranous expansion by which the branches are connected. It is to be remarked, however, that at the angle formed by the edge of the web (*b*), the contents exhibit an aggregation of minute germinal corpuscles, exactly like those which characterise the endosarc in cell-budding zoœcia. From this one might almost be tempted to suppose that the radical web possessed an innate power of growth or development like an ordinary zoœcium, and that it is not altogether beyond the bounds of probability that it may, in some cases, throw tubular prolongations *upwards* along the sides of the zoœcia above. This surmise is strengthened by the circumstance that occasionally the lateral tube may be seen terminating in a rounded extremity, not at the constricted base of the zoœcium as usual, but at about the middle of its length, being, as it were, simply applied, like ivy to a wall, and without entering the zoœcium. In most cases, however, the radical tubes may be seen entering or emerging from the usual point at the base of the *zoœcium.*

The inter-connection of the branches of a ramose zoarium by transverse tubes of the same nature as radical tubes is of common occurrence in more than one family of Polyzoa. As instances may be cited, among the Cabereidæ, the well-known *Canda arachnoides* with which I think the *Caberea* (or *Canda*) *reticulata* of Smitt[1] should be associated as a variety, whilst in the genus *Bugula* the occurrence of such a condition may be observed in several species. Of these I would more particularly notice two as yet undescribed forms in the Challenger collection, which I propose to name *Bugula reticulata* (fig. 7) and *Bugula unicornis* (fig. 8), in which this mode of connection is very well displayed; but in both these instances, as well as in *Canda arachnoides,* the connecting tube is distinctly seen to arise from a zoœcium in one of the branches, and to be attached to the other branch by means of clasping fibres, or by an expanded disc, obviously in this respect resembling a common condition in the radical tubes. In this respect, therefore, differing from the apparent condition of the transverse connecting tubes in *Kinetoskias pocillum.*

In a third species of *Bugula,* also, as yet unpublished, which I propose to name *Bugula mirabilis,* although there are no connecting tubes between the branches, the mode in which the radical tubes are collected into a long, rope-like peduncle, shows a complete analogy with, or approach to, the assumed mode of origin of the peduncle in *Kinetoskias.* In fig. 6 is represented the terminal portion of the zoarium of *B. mirabilis,* composed, as will be seen, of a bundle of tubes arising from the usual point in the lower zoœcia, and assembled into a close fasciculus, in which some of the tubes, in fact, may be seen in such intimate union as to render it uncertain whether their *lumina* are not confluent. The branching terminal portion of one of the radical tubes is shown subdividing into slender-jointed filaments, each of which, as in *Kinetoskias* and many other radicellate forms, is attached individually to a foreign body; and the figure also shows the segmented condition of the tube and filaments.

---

[1] 'Floridan Bryozoa,' p. xvi, pl. v, figs. 43—46.

In this particular species it will also be seen that the growth of the zoarium commences with an enormously elongated zoœcium, from the bottom of which two prolongations are continued, which at the upper part are slightly calcareous, but below become altogether chitinous or horny, and exactly like the other radical tubes. In fact, the branched termination shown in the figure belongs to one of these initial tubes as they may be termed.

That the radical and connecting tubes, like the avicularia and vibracula, represent modified zooids, is, I believe, generally admitted; nor can it be denied in this case that each successive joint or internode is a distinct zooid. In confirmation of this view I would take this opportunity of citing a very striking exemplification. This is afforded in a species of Carbasea (*C. ovoidea*, Bk.), in which, from the edge of the fronds, may frequently be seen numerous filamentous tubular processes, in all respects homologous with radical tubes, and like those destined to afford attachment to foreign bodies, or between the separate fronds themselves. A portion of the edge of a frond of this *Carbasea* is shown in fig. 9, in which it will be seen that cells of irregular form, and never containing a polypide or other structure, beyond the usual granular endosarc and branching fibres, lie along the border. And that from some of these aborted cells (for they cannot be termed zoœcia) tubular-jointed filaments arise, each of which may, in fact, be considered as representing one of the longitudinal series of zoœcia in the frond.

At *a* a short tubular process is seen from which two tubes arise, exactly in the same way that two zoœcia arise in the course of the longitudinal series of ordinary zoœcia; and what is very remarkable, as proving the homology of these aborted zoœcia with those of the ordinary kind, at *a* the first internode of one of the filaments is actually furnished with a semicircular lip, although there is not the faintest indication of muscles or polypide in the interior. The growing end of the tubular filament presents a granular substance in the interior (*b*), precisely like that with which all the young budding zoœcia are filled.

These marginal cells and their tubular prolongations appear to me to afford the clearest possible evidence of the true nature of the radical tubes and clasping organs of the Polyzoa.[1]

Note.—Since the above was written I have noticed in a

---

[1] In *Bicelluria* and in *Notamia* it may almost be said that the inhabited part of the zoœcia is simply a dilatation at one part of the internode of a radical tube, which is continued to the ultimate extremity of the branch.

species belonging to an abyssal genus, which I propose to name Angularia, a web-like expansion at the angle of most of the bifurcations, which is sometimes of considerable extent, and apparently homologous with that at the base of Kinetoskias. In this case, however, the web seems to be formed by an expansion and reduplication of a general epitheca, which is strongly developed in this and other of the abyssal forms.

On the GERMINATION *and* HISTOLOGY *of the* SEEDLING *of* WELWITSCHIA MIRABILIS. By F. ORPEN BOWER, B.A., Camb. With Plates III and IV.

The Mature Embryo.

THE development of the embryo of Welwitschia has been described by Sir J. D. Hooker, in his monograph on the plant ('Trans. Linn. Soc.,' vol. xxiv), and again by Strasburger ('Angiosp. und Gymnosp.,' p. 155). I find the structure of the mature embryo to correspond in the main with these descriptions, but the embryos, which I have had the opportunity of examining, are larger, and possibly better matured than those described by the latter writer; while a comparison of his fig. 92 with fig. 1 A, B, C, will show a difference of form, as well as of structure.

I find the mature embryo to consist of a straight radicle, with largely developed root cap. To the apex of the radicle adheres the suspensor, which, together with the embryonic tubes (embryonal-schlaüche), forms a mass of considerable size. Surrounding the body of the embryo may be seen a swelling or collar (fig. 1 X). Passing from this towards the apex of the embryo, there is a sudden diminution in thickness, and at a short distance above the collar are borne the two cotyledons. From a comparison of fig. 1 A and B, it will be seen that the hypo-cotyledonary part of the embryo is nearly cylindrical, the cotyledons only being compressed. The plane of their compression is that in which the whole seed is flattened. A longitudinal section (fig. 1 C) shows that between the cotyledons lies the apical cone of the plumule. This, as stated by Strasburger, remains undeveloped, as a simple papilla of tissue, up to the time of maturity of the embryo. From a longitudinal section it is seen that the radicle is short in comparison with the root cap, and that the latter extends back almost to the thickest part of the collar. The epidermis proper, which covers the cotyledons and the hypo-cotyledonary stem, loses its identity at the point where the root cap begins. From here onwards to the apex of the radicle, the external covering is made up of a succession of layers of the root cap. These merge imperceptibly into the cortical tissue. At the apex of the radicle the arrangement of tissues corresponds to the general type for the coniferæ. In Welwitschia, however, the tissues of the root cap are more diagrammatically arranged than is usual in the group, the regularity of the central series of cells being maintained up to the mass of suspensors and embryonic tubes, which cover the apex of the root cap.

Germination.

On being exposed to conditions favorable for germination the seed swells, and the embryo begins to increase in length. The root is the first to push its way out through the ruptured testa. The elongation which produces this result takes place chiefly in the tissues of the root itself, the hypo-cotyledonary stem increasing at first only slightly in length.[1] The point of perforation of the testa is near to the apex of the seed, but is variable according to the position of the seed during germination. It is on the side which happens to be undermost (figs. 4, 5). The root in its growth pushes aside the persistent remains of the nucleus, together with the suspensors, and the apical part of the largely developed root cap. These may be seen in the germinated seedling attached laterally to the exterior of the root[2] (figs. 3, 4).

Thus far the hypo-cotyledonary portion of the stem has extended only slightly. The cotyledons, however, and the upper part of the hypo-cotyledonary stem, begin now to increase in length, and the thickest part of the embryo is pushed out of the cavity of the endosperm. The room thus made is, however, filled by the growth of a peculiar excrescence from that side of the hypo-cotyledonary stem, which is made concave by the curvature of the root downwards.[3] The first stages of development of this organ I have unhappily been unable to follow from want of suitable materials. Long before the escape of the cotyledons from the seed it may be found lying side by side with them in the cavity of the endosperm (fig. 2 A). In form it is wedge-shaped, but with the edge of the wedge (*i. e.* the apex of the organ) rounded. It is compressed in a plane parallel to that of the cotyledons.

On searching for this lateral structure in the mature embryo before germination, no external trace of it was found, the swollen collar being, as described, uniform round the axis. Even on cutting sections of the hypo-cotyledonary

---

[1] According to Strasburger ('Conif.,' p. 319), the hypo-cotyledonary stem usually elongates rapidly during the first stages of germination of the Coniferæ.

[2] Cf., "Description of Germination in Ephedra," Strasburger, 'Conif.,' p. 321.

[3] It is natural for seeds shaped like those of Welwitschia to lie on their side during germination. The possible case of a seed sown so that the radicle of the embryo should point vertically downwards, I have never had the opportunity of observing.

stem of the mature embryo, there was not found in the arrangement of the tissues any indication of this organ, or apparent preparation for its development; the arrangement of epidermis and cortical tissue being uniform all round the stem.

Since my work depended upon a limited number of seeds only, I was not able to study the early stages of development of this organ as fully as I should have wished; nor could I obtain anything further than the following facts:

In a seed which had been sown three days no change was found in the embryo. In a seedling of twelve days, however, the organ was found almost fully formed (fig. 2). In this case the root had extended to a length of about one and three quarter inches. The cotyledons, though still enclosed in the seed, had grown to about one quarter inch in length. Side by side with these lay the lateral organ, which had already attained a length almost equal to that of the cotyledons. From these observations we see that the development of the organ is, like others of the first processes of germination, very rapid.

In all cases of seeds sown flat, the position of the lateral organs with relation to the cotyledons, was found to be as represented in fig. 2. In seeds sown on edge, as soon as room is allowed by the extrusion of the root and thicker part of the hypo-cotyledonary stem, the cotyledons suffer torsion in the cavity of the endosperm; so that the body of the embryo is rotated on its axis, and thus it assumes a position, relatively to the direction of gravity, similar to that in the case of the seed sown flat. The lateral organ is meanwhile developed in the same position, relatively to the rest of the embryo, as in the former case (fig. 3). It always appears on that side of the stem which is made concave by the curving of the root downwards. Hence it appears that the direction of gravity, relatively to the germinating seed, has an indirect determining influence upon its position. If we consider the mature embryo, we shall see that the lateral organ might be formed at either of two points (*i.e.* either of the points marked *x* in the fig. 1 B). It depends upon the position of the seed, and hence upon the direction of gravity relatively to it, at which of these two points the development shall take place. As to what happens when the plane of the seed or the axis of the embryo is *exactly vertical* I have no observations to offer. These cases would be particularly interesting for comparison with Ephedra.[1]

---

[1] Strasburger, 'Conif.,' p. 320.

During the development of this lateral organ, the cotyledons gradually increase in length, while the hypo-cotyledonary stem also extends. The result of this is the gradual arching of the upper part of the seedling (fig. 3) till it bursts through the testa. The lower limb of the arch extends more rapidly than the upper. The cotyledons are thus withdrawn from the endosperm. The point of perforation of the testa by the stem is variable; it is usually on the opposite side of the seed from the hole made by the root, and is often quite separate from this (fig. 4). In other cases the two perforations run together as a wide split of the testa (fig. 5).

When freed the hypo-cotyledonary stem straightens itself, and now by its further extension the cotyledons are raised above the surface of the soil, and expand as green assimilating leaves. The lateral organ, however, remains in close connection with the endosperm, and, growing further, completely fills the cavity vacated by the embryo.

That the lateral organ we have been describing is a means of transfer of nutrient materials from the endosperm to the seedling is proved by the following facts:—(1) That the endosperm still contains a considerable quantity of nutritious substances after the cotyledons free themselves from it. (2) That the cell walls of the outer cells of the organ are not cuticularised. (3) That it is not a permanently useful organ, since, when the endosperm is exhausted it also shrivels. To express this view of its function, and at the same time avoid any term bearing with it a definite morphological idea, it may be called the "Feeder."

We have seen that the plumule in the mature embryo consists of a simple papilla of tissue. When the cotyledons have expanded it appears, however, to have developed further. The time of this change I have not been able to ascertain accurately. In seedlings, such as those represented in figs. 4, 5, the plumule consists of a pair of lateral leaves, decussating with the cotyledons; between these may be seen the apical cone (fig. 9). I have not, in any of the seedlings grown at Kew, observed any further development of the plumule than that described. The size and form of the plumular leaves of the oldest seedlings now growing at Kew may be gathered from figs. 7 and 8, which represent plants of ten and a half weeks' growth. The cotyledons are, as stated, two in number. In one case (fig. 5) I observed three, but this may be explained by a splitting of one of the typical pair. Moreover, the position of the two smaller cotyledons in this case, with regard to the plumule, and the other cotyledon, makes this almost certain.

The form and size of the cotyledons may be gathered from the figures 7 and 8. While still enclosed in the seed they are yellowish orange; they maintain this colour some time after appearing above the soil. Finally, they become green, and in this condition they remain persistent for a considerable time; they are at all times glabrous and have entire margins. Each cotyledon has two main central bundles, which run parallel to one another, and two lateral ones parallel to these. Each of the four gives off smaller lateral branches, which anastomose freely. I have observed no axillary buds in the axils of the cotyledons as in Ephedra.

The hypo-cotyledonary stem is variable in length, from 1½ to 2½ inches. It is compressed in the plane of the cotyledons, and is slightly swollen just below the point of junction with them. The root, which is a direct elongation of the radicle, has, in the plants already grown, attained a length of 4 or 5 inches, without a single lateral branch, with the exception of one case where the apex of the root had been injured; here a lateral root had been formed.

The undoubted presence of a pair of plumular leaves in Welwitschia suggested a comparison of the young seedlings with the smallest specimens, preserved in the Kew museums. The result is the discovery of evident traces of the existence of leaves, previous to the large expanded pair, which are characteristic of the plant. Fig. 10 represents the apex of the youngest plant in the Kew collections, as seen from above. Here may be seen, protruding from the stem below the main pair of leaves, the ragged remains of fibro-vascular bundles, which run directly into the tissues of the stem. These, from their position and apparent course, point to the existence of a previous pair of leaves, which have decussated with the present pair, but which have rotted off. Traces of these may be found in even older specimens than the one figured, so that, not from one plant only, but from several may be deduced the conclusion, that the main leaves of Welwitschia are not persistent cotyledons, but leaves derived from the plumule. Hitherto we have only seen two plumular leaves formed; we may, therefore, reasonably conjecture that those plumular leaves are persistent as the typical pair of leaves of Welwitschia. The absolute proof of this will, we may hope, be afforded by the successful growth of the seedlings now living at Kew.[1]

---

[1] Concerning the morphological value of the structures seen between the leaves of the plant, represented in fig. 10, I am not at present in a position to make any statement.

Histology of the Seedling.

If transverse sections be cut of the hypo-cotyledonary stem of the seedling, the tissues will be found to be arranged in the following manner:—At the periphery of the section is an epidermal layer with cuticularised outer walls. Here and there are stomata, which demand no special notice beyond their guard cells being slightly depressed. Beneath the epidermis is a fairly regular cortical tissue, in which, after treatment with alcohol, are found large quantities of the sphere crystals of inulin. The outer layers of cells of the cortical tissue have, in some cases, cuticularised walls (figs. 11, 12). Scattered irregularly through the cortical tissue, but more especially towards the outside are sclerenchymatous cells. These occur singly, or in groups of two to five or six. This sclerenchyma is not very constant in quantity, and was in some cases found to be absent. Towards the centre of the section will be found four fibro-vascular bundles, arranged as in fig 11. They are peculiar in having the xylem portion turned towards the periphery, and the phloem towards the centre of the stem. Moreover, the xylem tails off laterally in a manner represented in fig. 11 by the lines marked *pr, xy*.

The structure of the individual bundle corresponds pretty closely with that of the leaf as described by De Bary,[1] although not so complicated.

On reference to fig. 13, which represents the fibro-vascular bundle of the young hypo-cotyledonary stem before extension takes place, it will be seen that the first developed xylem elements are drawn out into a long lateral series. Development begins at the end of this series, marked *pr, xy.* and progresses in the direction shown by the arrow. The further development of xylem occurs only opposite the later-formed elements, marked *xy*, fig. 13. A layer of cells (*cb*) have already begun to divide as a cambium layer; and the cells between this and the already formed xylem are beginning to thicken their walls, and develop into xylem elements. The group of cells, with very small cell cavity, on the central side of the cambium layer, are the protophloem (*pph*, fig. 13).

We shall now be in a position better to understand the mature bundle. Fig. 14 represents a section through a fibro-vascular bundle taken from a hypo-cotyledonary stem which is fully extended. Here the protoxylem elements will be seen to have been drawn out thin by the extension

---

[1] 'Vergleichende Anatomie,' p. 347.

of the stem; and their cell cavity has been almost completely obliterated, so that they only remain as occasional irregular masses of lignified wall between the parenchymatous cells. In longitudinal section the thickening is seen to be drawn out into a loose spiral, or sometimes almost into straight lines. The bearing of this arrangement of the protoxylem will be seen when the course of the fibro-vascular bundles at the apex of the stem has been traced.

The constituents of the xylem of the hypo-cotyledonary stem are the same as described for the leaf bundle.[1] It will be noticed that, as we pass from the xylem, through the cambium, to the phloem, the tissues are arranged in regular rows, pointing to a development from a cambium layer which, as was seen (fig. 13), makes its appearance very early. A group of sclerenchymatous fibres skirts the fibro-vascular bundle on the central side (*f*, fig. 14). Lying between these and the cambium is the soft bast. Here, as is found in the bundles of older leaves, the study of the tissues is rendered difficult by the tendency of the cell walls to swell. Nevertheless, in longitudinal sections treated with Schultz's solution, it was possible to trace a structure corresponding to that in the phloem of other gymnosperms.[2]

Particularly worthy of notice is a lateral extension of the cambium layer, though variable in its time of appearance, and extent, it occupies a definite position in the stem (fig. 11). In most cases observed, the layer is intermittent as in fig. 14. In no case were there found any vascular elements developed from this meristem. Under the present circumstances it is therefore impossible to say what relation this cambium may bear to the process of secondary thickening of the hypo-cotyledonary stem.

We may now pass to the plumule and cotyledons, and trace the course of the fibro-vascular bundles which proceed from them, till they merge into the four fibro-vascular bundles of the hypocotyledonary stem.

It has been previously stated that there are four main bundles in each cotyledon, to which all the other bundles attach themselves. From each of the cotyledons accordingly four bundles enter the stem. This may be seen in fig. 15 A. Towards the base of the cotyledon these bundles arrange themselves in pairs, and finally, after

---

[1] Cf. De Bary, 'Vergl. Anat.,' p. 348.

[2] Loc. cit., p. 188. Bertrand ("Anatomie des Gnétacées, &c.," 'Ann. d. Sci. Nat.,' Série v—xx) has also described "cellules grillagées" in the phloem.

entering the stem, these pairs fuse into single bundles (B, D). The xylem of these bundles is turned towards the centre of the axis. Each of the two leaves of the plumule, whose median planes are at right angles to those of the cotyledons, has two main bundles.[1] As these pursue their course downwards, at first they gradually approach the centre of the stem (A and B), then curving suddenly outwards, but still approaching one another, they meet and fuse (B). Before this sudden curving outwards, a weak and irregular branch is given off from each; and the branches thus given off from each of the four bundles form a ring, which puts the bundle systems of the two halves of the plant (right and left as the sections fig. 15 lie) in connection with one another. This ring is, however, very weakly developed, consisting in parts of only a single series of elements. It will probably in older plants be more strongly represented. After fusion, as described, the bundles from the plumular leaves descend for a short distance as two bundles, which soon again divide (B), and each of the four branches thus formed passes directly to one of the compound bundles, formed by union of two of the cotyledonary bundles; they attach themselves to these at their point of junction. (B) Immediately below this point, each compound bundle thus formed begins to rotate upon its axis. This may be traced on comparison of the successive sections of fig. 15; the direction of rotation being here indicated by arrows. The result is that the bundles of the hypo-cotyledonary stem assume the position previously described, the xylem being external to the phloem. The diagram (fig. 16) may serve to make the course of the bundles just described more intelligible. We shall now be able to realise the position of the protoxylem with regard to the bundle in the hypocotyledonary stem. It is, so to speak, a streamer, which lags behind in the rotation of the bundles on their axes.

As previously stated, the apical papilla, which lies between the two plumular leaves, was not observed in the case of any seedling to undergo a further development. The figure 17 represents the apical cone as seen from above, lying between the two plumular leaves. The arrangement of cells here shown presents no point of special interest. It corresponds on the whole with the apex of others of the coniferæ.[2]

---

[1] Traces are to be seen of a further pair of lateral bundles in each plumula leaf, but in none of the plants I have examined were these lateral bundles sufficiently developed to admit of their course being followed with accuracy.

[2] Cf. Strasburger, 'Coniferæ,' pl. xxiii.

In longitudinal sections the epidermis does not appear as a regular layer covering the apical cone. Whether this cone does develop further or not I have no evidence to prove. In the young plants preserved in Kew there are, however, to be seen two conical structures lying between the plumular leaves. It must for the present remain undecided what the morphological value of these is.

In the structure of the cotyledons there is little of importance to notice. The arrangement of tissues is nearly the same on both sides of the leaf. Both the epidermal layers of the upper and under surface have stomata; beneath the epidermis is on both sides a double layer of pallissade cells, and between these a spongy tissue. The main fibro-vascular bundles show a structure similar to those of older leaves, though less complicated. There are no spicular cells to be seen in the parenchyma of the cotyledons, but along their margins the cells are slightly thickened in a collenchymatous manner.

As they pass down the hypo-cotyledonary stem the arrangement of the bundles does not change; their course being strictly parallel till the point is reached, where the feeder extends laterally into the endosperm; here two of the bundles curve towards the organ.

It was seen that the feeder appeared externally, as a lateral protuberance on the hypo-cotyledonary stem. It remains to be decided what is its structure and morphological value.

A longitudinal section through a young seedling whose cotyledons have not yet been withdrawn from the seed, may be cut, so as to include the median plane of the feeder, as well as the axis of the embryo. Figure 18 represents such a section taken from a seed, which had been sown flat. The dark lines here indicate the course of the fibro-vascular bundles. The position of the feeder is seen to be opposite the swelling of the collar, and this marks the place, which it holds relatively to the mature embyro. From the hypo-cotyledonary stem the epidermal layer may be followed up to the apex of the feeder, which it covers; the epidermal cells, however, alter their form, being, as the apex is approached, more and more drawn out in the direction parallel to the axis of the feeder. A like change may be tracedin the parenchymatous cortical tissue. The cells of the latter are, in the hypo-cotyledonary stem, arranged in regular longitudinal rows; these may be followed, as they curve into the body of the feeder. Here, however, their regularity has been disturbed by divisions in directions both tangential and transverse.[1] Still the serial arrangement may often be followed, and even at the apex of the feeder may be seen traces of it, more especially towards the middle of the organ (fig. 19, the series are marked by letters). There is no fibro-vascular system belonging to the feeder itself. The bundles of the hypo-cotyledonary stem, curve outwards (fig. 18), but give off no branches. At the point of greatest curvature there are to be seen irregularities of the reticulated constituents, which seem also to have been drawn out laterally (fig. 20).

We see then that the feeder is a structure formed by the lateral extension of the tissues of the hypo-cotyledonary stem, accompanied by cell divisions. Its origin is deeper than the epidermis, but it has no bundle system of its own. It is also an adventitious structure. It is therefore to be considered morphologically as an emergence. There are no sclerenchymatous elements in the feeder. All its tissues, but more especially those at the apex, are thin walled, with transparent protoplasm and a fine nucleus. The quantity of starch increases as we recede from the apex, till close by the fibro-vascular bundle the cells are densely filled with it (compare figs. 19 and 20).

Almost immediately below the feeder the transition from the stem to the root type of structure occurs. As described by Strasburger for others of the group of the Gymnosperms,[2] the epidermis in the mature embryo covers the hypo-cotyledonary stem; but as we pass towards the apex of the root it ceases. The successive outer layers of the root cap are the outer covering of the body of the root. In Welwitschia, as in Ephedra, &c., there is no develoment of a pseudo-epidermis like that in Taxus. Accordingly, in the seedling, since the feeder is developed at the base of the hypo-cotyledonary stem, the epidermis is found to cease immediately below it; and it is even difficult to trace an epidermal layer on the under side of the feeder.

The first change of the arrangement of the fibro-vascular system, in passing from the stem to the root, consists in the extension of the phloem portion of the two bundles nearest one another. These finally meet and coalesce (fig. 15 G). Meanwhile they become separated from the xylem portions. These latter also pair off and coalesce to form the two xylem masses of the root (fig. 15 G, H). At a point where the phloem masses have already fused, but the xylem masses are still

---

[1] The terms are here used as referring to the axis of the plant.

[2] 'Coniferæ,' pp. 362, &c.

separate, the bundle sheath makes its appearance. In the young root the latter is cuticularised on its radial walls only (fig. 21). It is developed from a layer of cells removed from the fibro-vascular tissue by a pericambium of two or three layers of cells in thickness; these are the direct outcome from the proto-meristem, and appear not to be formed by subsequent division.

In a transverse section of a young root, at a point not far removed from that of transition, we find a structure as represented in fig. 21. On the outside will be seen a thick covering of tissue, whose cell walls are much swollen, and the cells often elongated and intertwined among one another. This is especially well seen on the outside of fig. 16. This covering may be found partial or entire. The older the root the more incomplete is the covering. In fig. 21 it may be seen that it is in the course of being thrown off from the underlying cortical tissue by a mucilaginous swelling of the cell walls. This tissue, thus thrown off, is the root cap. When the underlying cortical tissue is thus laid bare, the single cells of it grow out (fig. 21), and apparently form root hairs. I have, however, never succeeded in finding these in close attachment to the soil. The cell walls of the outer cells of the cortical tissue usually become cuticularised.

As the root grows older the cuticularisation of the walls of the bundle sheath extends from the radial to the tangential walls (fig. 22). The change happens first opposite the phloem masses. The result of this is the cutting off of the cortical tissue from a physiological connection with the central cylinder. Accordingly it begins to break down and separate from the latter; and, as in fig. 22, the process is first seen opposite the phloem masses. The final result is that the central cylinder is completely laid bare.

We may now turn to the consideration of the central cylinder. As before mentioned, the bundle sheath is separated from the vascular tissues by a pericambium, two or three layer of cells thick. Within this are arranged, according to the usual root type, the two xylem and two phloem masses. With respect to the constituents of these I have no remarks to offer, except that the phloem tissues are, from a very early stage, compressed, so that their cell walls are irregularly folded. There is at the centre a parenchymatous pith, and I have never observed the connection of the two xylem masses centrally, so as to form a single plate.[1]

[1] Cf. "Description of Bertrand," who has already investigated the lateral roots of Welwitschia, 'Ann. d. Sci. Nat,' Série v—xx.

In the parenchyma, which thus separates all the four vascular masses, arise two zones of cambium, one surrounding each of the xylem masses. The further development which may take place is unknown to me, since fig. 21 represents a section from the root of the oldest seedling as yet examined.

It will be noticed that the outer layers of the pericambium divide, and that the process begins opposite the phloem masses, though opposite the xylem they remain for some time undivided (fig. 22). This corresponds to the fact previously noticed, that the complete cuticularisation of the bundle sheath, and throwing off of the cortical tissue, are later opposite the xylem than at other points. By this division of the pericambium a secondary layer of protecting parenchyma will be afforded to the central cylinder.

It may here be observed that no development of lateral roots has been found in the young seedlings of Welwitschia, with the exception of the one abnormal case already mentioned. Immediately above the injured apex a lateral root had been formed; but I had not the opportunity of investigating the histology of this lateral root, or its mode of origin.

Conclusion.

The general result of these observations is to show that, in its early stages of development, Welwitschia corresponds with those plants to which its alliance has already been demonstrated. Especially striking is the similarity between the germination of Welwitschia, and that of *Ephedra campylopoda,* a similarity which may possibly be found to be even closer than as yet recognised, if opportunity be afforded for further experiment on the variations in the process of germination, induced by the position in which the seed is sown. Externally the case of Welwitschia, as seen in fig. 5, resembles that in *Ephedra campylopoda,* when the seed is sown flat. It would be interesting to know if there be in Ephedra, under any circumstances, a development analogous to the feeder of Welwitschia.

As to the morphological value of the latter organ, it has been seen to be formed by the lateral extension of the cortical, and epidermal tissues of the hypo-cotyledonary stem; this extension being accompanied by divisions of the cells of the cortical tissue. Its morphological value is then that of an emergence. Its physiological function is plainly that of transferring nutritious substances from the endosperm to the seedling. In the higher plants the physiological function of transference of nutritive stuffs, when carried on by any specially modified organ, devolves either on reduced cotyledons (*e.g.* Palmæ, Gramineæ, Cycas, Ginkgo), or on haustoria, specially developed from the suspensor (Tropœolum).[1] If we turn, however, to the vascular Cryptogams, we shall be able to trace a parallelism. In Selaginella we have a case which is very similar to that of Welwitschia. There the hypo-cotyledonary stem grows out laterally to form the so-called foot of Pfeffer.[2]

Whatever may be the embryo-genetic relation of this structure to the other parts of the embryo, as compared with the case of the Filicineæ,[3] still the fact remains, that in Selaginella the so-called foot is produced by the lateral extension of the tissues of the hypo-cotyledonary stem, accompanied by cell divisions in the cortical tissue. Further, on examining the figures of Pfeffer, it will be seen that the series of cells, though slightly complicated by tangential divisions, may, as in Welwitschia, be traced curving outwards into the lateral organ; and since, as in Welwitschia, there is no fibro-vascular bundle peculiar to the organ, we see that the correspondence from the histological side is complete. The correspondence between the two organs is also perfect from the physiological side. The only difference then lies in the time of appearance of the two organs, and this only a natural result of the difference in position of the latent period in the cycle of life of the two plants. While, then, the feeder of Welwitschia, and the so-called foot of Selaginella, must be considered as the analogues of the foot of the Filicineæ, when regarded from the physiological point of view, still (holding as I do that the term foot implies an embryogenetic relation to the other parts of the embryo, and since the feeder does not bear that relation to them) I have avoided using the term foot, and supplied its place with a name, which implies no preconceived morphological idea.

Besides the so-called foot of Selaginella, there is, in the higher plants also, a parallel case to that of Welwitschia. But the correspondence is here only morphological. In the

---

[1] Dickson, "On Embryogeny of Tropœolum, &c.," 'Trans. Roy. Soc.' Edin., 1875. It must be noticed that here the nutritive substances are derived from the carpel.

[2] 'Bot. Abh.' Herausgegeben v. Hanstein. Heft i.

[3] Cf. Vines, 'Quart. Journ. Micr. Sci.,' vol. xviii, New Series, p. 57. The question of homology of the suspensor, there discussed, seems to depend upon the definition of the term foot. If it implies a structure designed to meet a certain physiological need, the feeder would fall under the definition; if it be used to imply a structure whose embryo-genetic relations are constant, I do not see how the organ in Selaginella or in Welwitschia can be included.

Cucurbitaceæ, there has been described, by M. Ch. Flahault,[1] a lateral structure, the account of which, given by him, shows that it is the morphological equivalent of the feeder of Welwitschia. M. Flahault ascribes to it simply the mechanical function of assisting the escape of the cotyledons from the testa. Since the seeds are exalbuminous this is not surprising.

A similar structure is also described by him in *Mirabilis jalapa*. Though in this case the seed is albuminous, still M. Flahault ascribes to the lateral organ a mechanical function only, as in the case of its homologue in the exalbuminous seeds of the Cucurbitaceæ. Darwin[2] gives further experiments, with figures, on this "heel or peg" mentioning other plants (*Mimosa pudica, Abronia umbellata*) in which similar structures are formed. In none of these cases, however, is a function ascribed to the peg, similar to that in Welwitschia. Regarding the case of Welwitschia from a mechanical point of view, it is evident that here the function of helping the cotyledons to liberate themselves from the testa is more perfectly performed than in the cases described by Darwin, since here the hold of the feeder on the endosperm and testa is much more firm.

We have now noticed a number of plants in which occur outgrowths of the hypo-cotyledonary stem, having the same morphological value. In the light, however, of the facts brought forward by Darwin (loc. cit., p. 104), regarding the inconstancy of the presence of the peg in the Cucurbitaceæ, and indeed of emergencies generally, it seems improbable that we have here a case of survival of an ancient form, but rather of individual adaptation.

The peculiarity in the arrangement of the fibro-vascular bundles of the hypo-cotyledonary stem is easily put in relation to the bundle system of other plants.

The transition from the stem type to the root type of bundle is effected in most plants by a double process, the two parts of which are, in most plants, nearly simultaneous. They are (1) a rotation of at least a part of each bundle on its axis; (2) a separation of the phloem from the xylem (accompanied or not by splittings and fusions). In Welwitschia the two parts of the change are distinct and separate. The first (*i. e.* rotation of the bundles on their axes) occurs immediately below the junction of the bundles from the plumule and cotyledons, while the latter (*i. e.* fusion of phloem and xylem) is effected approximately at the point where the true

---

[1] 'Bulletin Soc. Bot. de France,' vol. 24, p. 201, 1877.

[2] 'The Movements of Plants,' 1880, p. 102.

epidermis ceases, that is, immediately below the feeder. This state of things may be compared with that in the seedling of the Abietineæ;[1] in which case, however, a bundle sheath may be found surrounding the bundles. In the structure of the root itself will be seen a resemblance to that of those Gymnosperms which have a di-arch bundle system. The relation of the bundle system of the root of the young seedling to that of the older root remains still to be discovered.

With regard to the plumule, it will be seen that the development of the two plumular leaves, decussating with the cotyledons, is a fact which is in complete accordance with what takes place in a number of coniferæ and guetaceæ (*e. g.* Thuja, Cupressus,[2] Cephalotaxus). Coupling the presence of the plumular leaves with the remnants of the leaves found in the plants preserved in the Kew collections, we are led to relinquish the older, and conferredly provisional view, that the whole plant of Welwitschia is a seedling arrested in its embryonic form. We now regard it as extremely probable (though not directly proved as yet) that the cotyledons of Welwitschia wither, while the two first plumular leaves remain persistent, and are the only two leaves developed from the plumule. The further history of the latter, and the morphological value of the structures which appear between the plumular leaves, I have not yet been able to determine.

It will be noticed that there are still large gaps in our knowledge of the development of Welwitschia, while several morphological and histological questions of great interest remain unanswered. The study of some of these problems I hope to be able to undertake later, with the help of fresh materials.

In conclusion, I must acknowledge my thanks to the Director of the Royal Gardens at Kew, and to Mr. W. Thiselton Dyer, for the supply of materials, and use of the Jodrell Laboratory attached to the Gardens, as well as for the interest they have taken in my work, and for suggestions which they have made.

Postscript.—Since the above was put into print, there has appeared in the 'Gardeners' Chronicle' (Nov., 1880) a communication from Chr. D. J. de Nautet Monteiro, who claims to have observed, in the germination of Welwitschia, a process differing from that described by me in 'Nature' (October, 1880). He compares the case of Welwitschia with that of *Commeleyna cœlestis*.

---

[1] Cf. De Bary, 'Vergl. Anat.'

[2] 'Coniferen and Gnet.,' p. 322.

I think, however, that the two accounts may be harmonised by the supposition that Mr. Monteiro has made the very natural mistake of regarding the feeder of Welwitschia as the homologue of the cotyledon of Commeleyna. This supposition is, moreover, supported by the fact that he describes the "plumule" as developing "two germinal leaves," which from his further description are, without doubt, the structures which I have throughout called cotyledons. To ascribe to the true cotyledons a plumular origin is the natural result of regarding the feeder as a cotyledon.

NOTES *on some of the* RETICULARIAN RHIZOPODA *of the* "CHALLENGER" EXPEDITION. By HENRY B. BRADY, F.R.S.

PART III.

1. *Classification.*
2. *Further Notes on New Species.*
3. *Note on Biloculina Mud.*

IN two previous papers ('Quart. Journ. Micr. Sci.,' Jan. and April, 1879) I have endeavoured to indicate some of the more important results that have been obtained by the examination of the material obtained by dredge and tow-net during the "Challenger" expedition, in respect of the large and varied group of testaceous Rhizopoda, which it is the custom to speak of under the general term Foraminifera or Reticularia. These preliminary papers have been principally devoted to the description of types, previously unknown, or at any rate little understood, presenting features of interest from a morphological stand-point. I propose to devote the present contribution chiefly to the consideration of questions connected with the classification of the group, supplementing what I have to say on this point by brief descriptions of a further instalment of new species, and a note on certain deposits, now in course of formation, in which the porcellanous group of Rhizopoda plays the most important part.

1. *Classification.*

Of the primary subdivision of the Class RHIZOPODA it is no part of my present purpose to speak; I intend rather to confine my observations to the Order to which my attention has been of late more particularly directed, and the subordinate groups into which its constituent organisms may best be arranged.

The term *Foraminifères* was originally employed by d'Orbigny in his scheme for the classification of the Mollusca, at a time when the Foraminifera, or at least a considerable section of them, were regarded as microscopic Cephalopods. In his "Tableau Méthodique,"[1] the Class

---

[1] 'Annales des Sciences Naturelles,' 1826, vol. vii, p. 245.
"IIIe Ordre.—FORAMINIFÈRES, Nob.; *Asiphonoïdes*, de Haan.
"*Caract. essent.*—Un test polythalame totalement interne; dernière cloison terminale; point de siphon, mais seulement une ou plusieurs ouvertures donnant communication d'une loge à l'autre."

CÉPHALOPODES was divided into three orders,—I. *Cryptodibranches;* II. *Siphonifères;* III. *Foraminifères;* and of these the last two, which comprised Mollusca with chambered shells, were distinguished from each other by the *Siphonifères* having the septa traversed by a continuous tube, whilst in the *Foraminifères* the chambers communicated with each other by an aperture or foramen,[1] or by several such.

There is little in d'Orbigny's classification or in his definition of the Order that commends itself to the student of the present day, and it is even probable that the term "Foraminifera" is more commonly associated with the general perforation of the shell-wall, which is a conspicuous feature of a single group, than with the character it was originally designed to indicate; nevertheless, it is certain that no other name which has been suggested—whether *Rhizostomes, Simplectomères, Polypodes, Trematophores, Asiphonoïdes* or *Polythalamia*—has found the same acceptance amongst naturalists. The term *Polythalamia* has been adopted by Ehrenberg, in his voluminous treatises, and to some extent also by Max Schultze, but by others it has been seldom employed except as an alternative name; and it is open to the objection that, etymologically, it is scarcely applicable to an assemblage of organisms of which a not inconsiderable proportion are monothalamous.

It may be questioned whether our knowledge of the structure and life-history of the animals constituting the Order, as distinct from their tests, is sufficiently extensive for purposes of nomenclature—the number of arenaceous types, for example, concerning which we have any recorded observations on living specimens, is very small—but, so far as is known, the term "Rhizopoda Reticularia," suggested by Dr. Carpenter and accepted by Prof. F. E. Schulze and others, is perfectly appropriate.

At the same time we may remember that it is to d'Orbigny we owe the first recognition of the Foraminifera as a distinct zoological group, as well as the researches which gave the first impulse to their independent study, and, in absence of any weighty argument to the contrary, rule and custom alike suggest the acceptance of the name given by him and already generally adopted.

Prof. R. Hertwig, in the scheme of classification of the Rhizopoda, appended to his recent admirable memoir on the Radiolaria,[2] limits the application of the term Foraminifera,

---

[1] *Foramen* (Lat.), "an opening or hole produced by boring; an aperture."

[2] 'Der Organismus der Radiolarien,' by Dr. Richard Hertwig, 1879, p. 142.

so as to correspond with Dr. Carpenter's sub-order *Perforata;* but, as has been before stated, the term does not refer to general shell-perforation but to the existence of stoloniferous orifices, and in this sense it is equally true of all polythalamous forms whether otherwise "perforate" or "imperforate." Nor is there much violence to d'Orbigny's original idea in accepting the orifice of *Lagena,* or analogous types, as a "foramen," though, in the absence of a succession of chambers, it serves only for the passage of pseudopodia. On these grounds, therefore, either the term *Foraminifera,* derived from the shell or other investment, or *Reticularia,* suggested by the distinctive character of the sarcode-body, is a sufficiently accurate designation for the group.

The *Foraminifères,* treated as an Order of *Céphalopodes,* were divided in the 'Tableau Méthodique' (1826) into five families, based upon the mode in which the segments were combined to form the polythalamous shell. After the discovery of monothalamous forms like *Orbulina* and *Lagena,* and the recognition of cyclical types such as *Orbitolites,* two new families were constituted for their reception. In the meantime, however, the researches of Dujardin had made known the true nature of the organization of the Rhizopoda, and had necessitated the removal of the group to a lower position in the zoological scale. Hence in d'Orbigny's later works,[1] the *Foraminifères* constitute the 4th Class of ZOOPHYTES (2nd Division, "*Zoophytes Globuleux,*" placed between *Polypiers* and *Infusoires*), and are divided into seven orders with characters which may be briefly summarised as follows:

Order 1. MONOSTÈGUES.—Shell formed of a single chamber.

Order 2. CYCLOSTÈGUES.—Shell discoidal, composed of concentric lines of cells, simple or multiple; never spiral.

Order 3. STICHOSTÈGUES.—Shell formed of chambers joined end to end in a straight or curved line; never spiral.

Order 4. HELICOSTÈGUES.—Shell composed of chambers in a single series, spirally coiled.

Order 5. ENTOMOSTÈGUES.—Shell composed of chambers arranged in two alternating series and spirally coiled.

Order 6. ÉNALLOSTÈGUES.—Shell composed of chambers

---

[1] "Cours Élémentaire de Paléontologie et de Geologie Stratigraphiques,' vol. ii, p. 189. Paris, 1852.

arranged alternately in two or three distinct axes, but not on a spiral plan.

Order 7. AGATHISTÈGUES.—Shell composed of chambers wound round a common axis, each forming half the circumference; texture smooth and imperforate.

Whilst there are certain advantages to be derived from a purely artificial arrangement—as, for example, the Linnean classification of plants—it is seldom that such a method can be adopted without violence in one way or other to manifest natural affinities, and the lowest divisions of the animal and vegetable kingdoms are perhaps least of all suited for its introduction. The chief difficulties that beset the student of systematic zoology, when engaged upon these low types of animal life, arise from the wide range of morphological variation he is obliged to admit within the limits assigned to species; and although there is a great difference in different genera as to the degree of persistence in the distinctive characters of their subordinate forms, it may be fairly doubted whether "species," in the sense in which the word is rightly applied to animals of more complex organisation, can be said to exist amongst the lower Protozoa. It is only as we learn to recognise the fact that amongst the Rhizopoda the so-called "species" represent no more than terms of a series of which very frequently every intermediate link can be supplied, that we arrive at any just conception of their relationship. This being so, it is easy to see where a purely artificial classification must inevitably break down; and though the d'Orbignian scheme was a fair attempt to deal with a great mass of facts, collected by its author with infinite labour, it has now ceased to be of service, and has fallen into desuetude. Its defects are too obvious to need comment; it had none of that elasticity which gives to a system of classification the element of permanence, and which can only exist in proportion to the degree in which it is based upon natural affinity and the natural sequence of forms.

In the year 1854 Professor Max Schultze published his classical memoir, 'Ueber den Organismus der Polythalamien (Foraminiferen),' and with it an exposition of his views on the classification of the Rhizopoda. His conclusions, summarised in a convenient table at the end of the volume, are briefly as follows. The Rhizopoda are divided primarily into NUDA and TESTACEA, the former with the genus *Amœba* for its type, the latter embracing all the forms having an external shell or other investment. The TESTACEA are divided into two suborders—*Monothalamia* and *Polythalamia*—the one subdivided into three families, the other into seven, and the principal genera, perhaps all that were then known, are distributed amongst these. Professor Max Schultze's scheme is characterised by a somewhat wider grasp of the subject than its predecessor; but with our present knowledge there is little to be said in favour of a classification that places *Orbulina* and *Lagena* in one of its two primary divisions, and *Globigerina* and *Nodosaria* in the other, or wherein *Nodosaria* and *Cristellaria* are to be found in different families. There was, in fact, no practical advantage to be derived from it, and, so far as I am aware, it has been accepted by no subsequent writer.

In the years 1861-2, practically simultaneously, appeared the memoirs containing the outlines of the two systems of classification which have been adopted, one or other of them, by the present generation of Rhizopodists. That Prof. von Reuss,[1] working on the Continent almost exclusively upon fossil specimens, and Dr. Carpenter with Professors Parker and Rupert Jones,[2] in this country, from the broader lines of the comparative study of living and fossil types, should have arrived independently at conclusions identical in their more important particulars, appears strong *primâ facie* evidence that a reliable foundation, whatever the superstructure, had been at length reached. These papers are still the standpoint from which the discussion of the subject must be commenced, and it is therefore necessary at the outset to state the general features of the schemes they embody, and by comparison, side by side, to show how far they agree in their details, and wherein they differ.

The basis of the primary divisions of both systems is the minute structure of the shelly investment—a ground of distinction hardly recognised by previous authors. In general terms Foraminifera are divided into the same two classes—those with non-porous or imperforate, and those with porous or perforate tests. The former of these two divisions (*Imperforata*) is in both systems subdivided into two sections, one including the types which have composite tests, that is, built up of sand-grains or similar extraneous bodies more or less embedded in calcareous cement, the other having opaque, porcellanous shells of fine texture.

In the division comprising the perforate or porous-shelled forms the agreement is less complete, as might be expected

---

[1] "Entwurf einer systematischen Zusammenstellung der Foraminiferen," 'Sitzungsb. k. Akad. Wiss.,' vol. xliv, p. 355. (The volume for the year 1861, published, I suppose, in 1862.)

[2] 'Introduction to the Study of the Foraminifera,' London, 1862.

with the larger number of types to be accommodated and the greater diversity in their characters; but even in this the two classifications have very much in common.

Their general relationship will be readily understood by the following comparative table:

Von Reuss.	Carpenter, Parker, and Jones.
A. Foraminifera with non-porous tests.	Sub-order—**Imperforata.**
	Family—Gromida.
A. With arenaceous tests. 1. *Lituolidea.* 2. *Uvellidea.*	Family—Lituolida.
B. With compact, porcellanous, calcareous shells. 1. *Squamulinidea.* 2. *Miliolidea.* 3. *Peneroplidea.* 4. *Orbitulitidea.*	Family—Miliolida.
B. Foraminifera with porous shells.	Sub-order—**Perforata.**
A. With glassy, finely porous, calcareous shells. 1. *Spirillinidea.* 2. *Ovulitidea.* 3. *Rhabdoidea.* 4. *Cristellaridea.* 5. *Polymorphinidea.* 6. *Cryptostegia.* 7. *Textilaridea.* 8. *Cassidulinidea.*	Family—Lagenida.
B. With exceedingly porous, calcareous shells. 1. *Rotalidea.*	Family—Globigerinida.
C. With calcareous shells, traversed by a ramified canal-system. 1. *Polystomellidea.* 2. *Nummulitidea.*	Family—Nummulinida.

Professor von Reuss's classification, above quoted, is taken from the "Postscript" to the memoir referred to, in which the primary division into "Foraminifera Monomera" and "Foraminifera Polymera," adopted in the body of the paper, is abandoned. His family *Gromidea* also, which corresponded with the Gromida of the English observers, is omitted entirely in the revised scheme. In the correlation of the two classifications, the principal discrepancy occurs in the sub-order **Perforata.** The LAGENIDA and GLOBIGERINIDA together are almost exactly coextensive with Von Reuss's two sections **B**, *A* and *B*, but the (1) *Spirillinidea*, (2) *Ovulitidea*, (7) *Textilaridea*, and (8) *Cassidulinidea*, together with one or two genera from other groups, find place amongst the GLOBIGERINIDA of the British classification, and the family LAGENIDA is correspondingly reduced in extent. The family NUMMULINIDA corresponds exactly with Von Reuss's section **B**, *C*.

In his latest memoir[1] Von Reuss again somewhat modified his arrangement, making three primary groups of equal zoological value, and reversing the order originally adopted, thus:—**A. Kalkschalige Foraminiferen, B. Porenlose Foraminiferen, C. Kieselschalige Foraminiferen**; but its general features were left otherwise unchanged.

It will be seen at a glance that the "families" of the German arrangement are much smaller and more numerous than those adopted by the English naturalists, but this is counterbalanced by the more comprehensive "generic types" of the latter. The essential difference, not only between the two systems of classification, but in the entire methods of study and nomenclature, is to be found in the different values of their respective "genera" and "species."

Before proceeding to any further remarks upon these, we may notice briefly two other schemes of classification which have been suggested comparatively recently, one by Professor T. Rupert Jones,[2] the other by Professor Carl Zittel.[3] Their general features will in like manner be best understood from tabular summaries.

---

[1] 'Das Elbthalgebirge in Sachsen,' 2ter Theil, 1874.

[2] 'Monthly Microscopical Journal,' No. 86, Feb., 1876, pp. 89—92.

[3] 'Handbuch der Paläontologie,' vol. i, pt. 1, 1876, pp. 61—106.

RUPERT JONES.	ZITTEL.
Sub-order I. IMPERFORATA vel PORCELLANA.	Sub-order I. IMPERFORATA.
Family I. *Nubecularida.*	Family I. *Gromidæ.*
II. *Miliolida.*	II. *Cornuspiridæ.*
III. *Peneroplida.*	III. *Miliolidæ.*
IV. *Orbiculinida.*	IV. *Dactyloporidæ.*
V. *Dactyloporida.*	
Sub-order II. ARENACEA.	
Family I. *Parkeriada.*	
II. *Lituolida.*	
Sub-order III. PERFORATA vel HYALINA.	Sub-order II. PERFORATA.
Family I. *Lagenida.*	Family I. *Lagenidæ.*
II. *Polymorphinida.*	II. *Globigerinidæ.*
III. *Buliminida.*	(1) Globigerinæ.
IV. *Textularida.*	(2) Textularinæ.
V. *Globigerinida.*	(3) Rotalinæ.
(1) Globigerinina.	III. *Nummulinidæ.*
(2) Rotalina.	
(3) Polystomellina.	
(4) Nummulinina.	

The primary division of Foraminifera into *Perforata* and *Imperforata* would be very convenient if it could be employed in its original sense; but it is now well known that some, though perhaps not a large proportion, of the arenaceous types, have interstitial openings amongst the sand-grains of which the test is built, in addition to the general aperture: that others, like *Psammosphæra* and *Sorosphæra*, have no general aperture, but only interstitial orifices; whilst a few, *Thurammina*, for example, have numerous small mammillate orifices, irregularly disposed over the surface of the test, either with or without a general aperture. If the arenaceous group be removed from the *Imperforata* there remain but the chitinous and porcellanous forms. Rupert Jones follows Von Reuss in making a distinct sub-order of the *Arenacea*, and, as he entirely omits the chitinous group, his sub-order *Imperforata* is exactly co-extensive with Dr. Carpenter's family *Miliolida.* Professor Zittel, on the other hand, only admits arenaceous structure as a character of secondary importance, and distributes the sandy forms amongst the porcellanous, so that types as diverse as *Saccammina* and *Cornuspira*, or as *Miliola* and *Botellina*, are found in the same family.

That the *Porcellanea* are, under all circumstances, imperforate, I have convinced myself by observations extending over many years, and that a large proportion of the *Arenacea* are likewise imperforate I fully believe; but the exceptions in the latter case are so numerous and varied, that the arenaceous group cannot be included as a whole in a sub-order of which the distinctive character is the imperforate test, and if omitted the term becomes at once misleading; therefore it is manifestly better to abandon a primary subdivision based solely on the condition of the investment in respect to perforation.

The adoption of three sub-orders, instead of two, depending on shell-texture rather than on mere perforation, as latterly proposed by Von Reuss and adopted by Rupert Jones, meets the difficulty in part, but is still open to objection. On the one hand there is a considerable group of true *Miliolæ,* our knowledge of which is much extended by the "Challenger" collections, that have rough arenaceous tests; and on the other, the large and important family *Textularidæ* is practically unprovided for, inasmuch as it is sometimes truly arenaceous, sometimes hyaline and perforate, and sometimes externally sandy but with an inner perforate shell. Von Reuss meets this difficulty by dividing certain genera and placing the two halves in different sub-orders; thus *Textularia* appears as *Textularia* amongst the hyaline forms and as *Plecanium* amongst the arenaceous, and *Bulimina* in the same way as *Bulimina* and *Ataxophragmium;* but this is cutting the knot rather than untying it, and even were so summary a method practically convenient, the proposal to divide a natural group like that comprising the Textularian and Bulimine types in order to meet the exigencies of an artificial distinction, is not one to be lightly adopted.

Passing from sub-orders to families, even greater anomalies are apparent in Von Reuss's scheme, especially amongst the hyaline forms. For example, *Spirillina* is found at almost the opposite end of the scale to the Rotaline genera; *Nodosaria, Cristellaria,* and *Polymorphina* are placed in separate families, whereas, in point of fact, they form an absolutely continuous series; and *Textularia, Bulimina,* and *Cassidulina* are similarly separated. These appear to me fatal objections to the details of the classification.

In the collateral English arrangement there is no infringement of natural relationship apparent in the constitution of the families, except, perhaps, the association of *Textularia* and its immediate allies with *Globigerina* and the Rotaline genera. Apart from this, its chief drawback is that the divisions are too large to be zoologically convenient.

The families proposed by Rupert Jones in his redivision of the sub-orders, with a view to remedying this defect, hardly meet our present requirements. Of Professor Zittel's classification I have had occasion to speak elsewhere[1] at some length, and I have nothing to modify in the views expressed at the time of its publication as to its merits and shortcomings.

Every attempt to arrange in single series a class of organisms of which the constituent groups run sometimes in several parallel lines, or even form independent circlets, morphologically speaking, is of necessity open to objection at one point or other, and the aim of the systematist may be regarded as attained if the anomalies and inconsistencies are slight, and are confined to particulars of the smallest zoological importance. In the scheme which I now venture to propose I have endeavoured to embody the views already in part expressed. In one or two not unimportant points it differs from that originally devised by my friends Dr. Carpenter and Professors Parker and Rupert Jones, but in its essential elements there is little or nothing that is incompatible with the conclusions they have so ably expounded. It is put forward in outline, and with no pretence of completeness, in the hope that it may receive the criticism of naturalists whose pursuits qualify them to deal with the subject. The tabular summary needs, for the most part, but little explanation; the reasons for the course pursued, where it differs from that of other writers, are sufficiently obvious; but there are a few points in which the method of treatment has been dictated by the study of the "Challenger" collections, and the further exposition of these must be left until it can be made with the assistance of the plates of the memoir now in progress. The nature of the investment of the animal —in other words, the texture of the test—has been to a certain extent abandoned as a primary distinction, though it is still employed in a modified way. Under all circumstances it is an important character, and in some families is distinctive; but it will be seen that whilst there are certain families which are invariably arenaceous, and others which are invariably calcareous and perforate, there are some in which no uniform rule obtains.

A. *Test imperforate, chitinous.*

I. GROMIDÆ.—There has been much difference of opinion as to the zoological position of *Gromia* and its allies. So long as the animal of the Foraminifera was supposed to be

---

[1] 'Nature,' Sept. 21st, 1876, vol. xiv, p. 445.

mere undifferentiated protoplasm, *Gromia* might properly be regarded as pertaining to a higher type of organisation; but recent researches, notably those of Professor R. Hertwig and Professor F. E. Schulze, have shown that certain types of Foraminifera possess nuclei indistinguishable from analogous bodies in the chitinous fresh-water Rhizopods. Professor Leidy, in his elaborate work upon the 'Fresh-water Rhizopods of North America,' just published, practically confirms this view by treating *Gromia* as a genus of Foraminifera.

B. *Test imperforate; normally porcellanous, sometimes encrusted with sand; under starved conditions (e.g. in brackish water) becoming chitinous or chitino-arenaceous; at abyssal depths occasionally consisting of a thin homogeneous, imperforate, silicious film.*

II. MILIOLIDÆ.—Concerning the genus *Squamulina* there seems still considerable doubt. Judging from Max Schultze's description and figures it seems to be a monothalamous, adherent, porcellanous species, not far removed from the simpler varieties of *Nubecularia*.

Of the *Dactyloporinæ* there is little to be said at the present time inasmuch as they may have to be removed, either entirely or in part, to the calcareous *Algæ*; but as yet we have no authority for so important a step beyond the brief preliminary notice of M. Munier-Chalmas's researches, and it will be generally admitted that there are two sides to the question.

C. *Test invariably arenaceous.*

III. ASTRORHIZIDÆ.—Coarse sandy forms, usually of large size, and monothalamous; often branched or radiate, but never truly septate (*i. e.* as distinct from mere constriction). Polythalamous forms never symmetrical.

It is probably that this family may eventually require subdivision; as now proposed it includes all the deep-water arenaceous recent Rhizopods except those of the family *Lituolidæ*.

IV. LITUOLIDÆ. — Comprises sandy isomorphs of the simpler hyaline types (*Lagena*, *Nodosaria*, *Globigerina*, *Rotalia*, *Nonionina*, &c.), together with some adherent species. Septation of the polythalamous forms imperfect; chambers sometimes subdivided or labyrinthic.

V. PARKERIDÆ.—Large, spherical, lenticular, or fusiform fossils; constructed either on a spiral plan or in concentric layers; the chambers occupied to a great extent by labyrinthic or cancellated shelly growths.

For the present I know of no more appropriate place than this for *Parkeria, Loftusia,* and their immediate allies. It may be admitted that we have still a great deal to learn concerning them, and should the *Stromatoporidæ* be proved eventually to occupy an intermediate position, related on the one side to Rhizopods, on the other to Sponges, possibly these types may find their proper place as a further connecting link on the Rhizopod side.

D. *Tests of the larger species arenaceous, either with or without a perforate, calcareous basis; smaller forms hyaline and conspicuously perforated.*

VI. TEXTULARIDÆ.—I can see no advantage in the attempt to separate the arenaceous *Textulariæ* and *Buliminæ* from the clear-shelled species, but much the contrary; nor can I recognise any valid distinction, of more than secondary importance, between *Textularia* and *Valvulina.*[1]

E. *Test calcareous, finely perforate.*

VII. CHILOSTOMELLIDÆ.—With Reuss's genera *Chilostomella* and *Allomorphina,* which together form his family *Cryptostegia,* I have associated Seguenza's genus *Ellipsoidina.* The anomalous characters of the latter, viewed as a Nodosarian, I pointed out many years ago, and had I then known the genus *Chilostomella* by anything more than figures, I should have suggested the present position for it. The primary difference in the structure of these two types consists in the fact that in *Chilostomella* the segments alternate, the attachment to each other being at one side, and the aperture first at one end and then at the other, whilst in *Ellipsoidina* the attachment is at the base, the segments grow in the same direction, and consequently the aperture is always at the same end.

VIII. LAGENIDÆ.—This is exactly coextensive with the *Lagenida* of Messrs. Carpenter, Parker, and Jones.

F. *Test calcareous, generally very coarsely perforated, no trace of canal-system.*

---

[1] I arrived at this conclusion originally from the study of the Carboniferous types of Rhizopoda; meanwhile my friend Mr. Carter had come to the same result, from working on recent species. *Vide,* 'Ann. and Mag. Nat. Hist.,' ser. 4, vol. xix, p. 205. The close affinity of *Textularia* with *Valvulina* is fully recognised by Dr. Carpenter and his colleagues ('Introd.,' p. 192), though the two genera are widely separated in their scheme of classification.

IX. Globigerinidæ.—A compact group of essentially pelagic types. With the exception of a few species, generally of small size, all have been found living in open sea, at or near the surface.

G. *Test coarsely perforate, a few of the higher forms with double chamber-walls and interseptal canals.*

X. Rotalidæ.—Embraces the *Rotalinæ* of Messrs. Carpenter, Parker and Jones, with the addition of the genus *Spirillina*, which may be regarded as their non-septate modification, just as *Cornuspira* is a non-septate *Miliola* and *Ammodiscus* a non-septate *Trochammina*.

H. *Test very finely tubulated. All the higher types possessing a system of interseptal canals of greater or less complexity.*

XI. Nummulinidæ.—Coextensive with the *Nummulinida* of Carpenter, Parker and Jones. I have retained *Fusulina* in the place hitherto assigned to it, on the ground of its symmetrical contour, but, as Dr. Carpenter has shown, the genus has almost as much in common with the *Rotalidæ*.

Class—RHIZOPODA, Dujardin.

Order—Foraminifera, d'Orbigny—(Reticularia, Carpenter).

Family I. **GROMIDÆ.**—*Gromia*, Dujardin; *Lagynis*, Schultze; *Lieberkuhnia*, Claparéde; *Shepheardella*, Siddall.

Family II. **MILIOLIDÆ.**

- *α*. **Miliolininæ.**—*Bathysiphon*, G. O. Sars; *Squamulina*, Schultze; *Nubecularia*, Defrance; *Uni-*, *Bi-*, *Spiroloculina*, d'Orbigny; *Miliolina*, Williamson; *Cornuspira*, Schultze (*Ophthalmidium*, Kübler); *Hauerina*, d'Orbigny; *Vertebralina*, d'Orbigny (*Articulina*, d'Orbigny); *Fabularia*, Defrance.
- *β*. **Orbitolitinæ.**— *Peneroplis*, de Montfort; *Orbiculina*, Lamarck; *Orbitolites*, Lamarck; *Alveolina*, d'Orbigny.
- *γ*. (?) **Dactyloporinæ.**—*Ovulites*, Lamarck; *Dactylopora*, Lamarck, and sub-genera.

Family III. **ASTRORHIZIDÆ.**—*Psammosphæra*, Schulze; *Sorosphæra*, Brady; *Saccammina*, M. Sars; *Pilulina*, Carpenter; *Storthosphæra*, Schulze; *Technitella*, Norman; *Pelosina*, Brady; *Aschemonella*, Brady; *Astrorhiza*, Sandahl; *Dendrophrya*, Str. Wright; *Rhabdammina*, M. Sars; *Jaculella*, Brady; *Hyperammina*, Brady; *Psammatodendron*, Norman (Ms); *Sagenella*, Brady; *Botellina*, Carpenter; *Marsipella*, Norman; *Haliphysema*, Bowerbank; *Polyphragma*, Reuss.

FAMILY IV. **LITUOLIDÆ.**—*Lituola*, Lamarck (*Reophax*, de Montfort; *Haplophragmium*, Reuss; *Haplostiche*, Reuss; *Placopsilina*, d'Orbigny; *Bdelloidina*, Carter); *Trochammina*, Parker & Jones (*Hormosina*, Brady; *Ammodiscus*, Reuss; *Webbina*, d'Orbigny); *Nodosinella*, Brady; *Involutina*, Terquem; *Endothyra*, Phillips; *Stacheia*, Brady; *Thurammina*, Brady; *Hippocrepina*, Parker; *Cyclammina*, Brady.

FAMILY V. **PARKERIDÆ.**—*Parkeria*, Carpenter; *Loftusia*, Brady.

FAMILY VI. **TEXTULARIDÆ.**

a. **Textularinæ.**—*Textularia*, Defrance (*Bigenerina*, d'Orbigny; *Pavonina*, d'Orbigny; *Spiroplecta*, Ehrenberg; *Cuneolina*, d'Orbigny); *Verneuilina*, d'Orbigny (*Gaudrynia*, d'Orbigny; *Chrysalidina*, d'Orbigny; *Tritaxia*, Reuss); *Valvulina*, d'Orbigny (*Clavulina*, d'Orbigny).

β. **Bulimininæ.**—*Bulimina*, d'Orbigny (*Virgulina*, d'Orbigny; *Bolivina*, d'Orbigny; *Pleurostomella*, Reuss).

γ. **Cassidulininæ.**—*Cassidulina*, d'Orbigny; *Ehrenbergina*, Reuss.

FAMILY VII. **CHILOSTOMELLIDÆ.**—*Chilostomella*, Reuss; *Allomorphina*, Reuss; *Ellipsoidina*, Seguenza.

FAMILY VIII. **LAGENIDÆ.**

a. **Lageninæ.**—*Lagena*, Walker & Jacob; *Ramulina*, Jones; *Nodosaria*, Lamarck (*Lingulina*, d'Orbigny); *Frondicularia*, Defrance (*Flabellina*, d'Orbigny); *Vaginulina*, d'Orbigny (*Rimulina*, d'Orbigny; *Rhabdogonium*, Reuss); *Marginulina*, d'Orbigny; *Cristellaria*, Lamarck.

β.—**Polymorphininæ.**—*Polymorphina*, d'Orbigny (*Dimorphina*, d'Orbigny); *Uvigerina*, d'Orbigny (*Sagrina*, d'Orbigny).

FAMILY IX. **GLOBIGERINIDÆ.**—*Globigerina*, d'Orbigny (*Orbulina*, d'Orbigny); *Hastigerina*, Wy. Thomson; *Pullenia*, Parker & Jones; *Sphæroidina*, d'Orbigny; *Candeina*, d'Orbigny.

FAMILY X. **ROTALIDÆ.**—*Spirillina*, Ehrenberg; *Patellina*, Williamson; *Discorbina*, Parker & Jones, *Planorbulina*, d'Orbigny (*Truncatulina*, d'Orbigny; *Anomalina*, d'Orbigny); *Rupertia*, Wallich; *Carpenteria*, Gray; *Polytrema*, Risso; *Tinoporus*, de Montfort (*Gypsina*, Carter); *Cymbalopora*, v. Hagenow; *Pulvinulina*, Parker & Jones; *Rotalia*, Lamarck; *Calcarina*, d'Orbigny.

FAMILY XI. **NUMMULINIDÆ.**

a. **Polystomellinæ.**—*Nonionina*, d'Orbigny; *Polystomella*, Lamarck.

β. **Nummulitinæ.**—*Archædiscus*, Brady; *Amphistegina*, d'Orbigny; *Fusulina*, Fischer; *Eozöon*, (?) Dawson; *Orbitoides*, d'Orbigny; *Cycloclypeus*, Carpenter; *Heterostegina*, d'Orbigny; *Operculina*, d'Orbigny; *Nummulites*, Lamarck.

2. *Further notes on New Species.*

It is at all times difficult to devise concise zoological descriptions that shall be intelligible without the aid of figures, hence in the selection of a third instalment of new forms for publication, those with peculiarities most readily indicated by verbal characters have been preferred. As a matter of fact, figures of all the forms about to be enumerated have long since been drawn, and most of them are included in plates already lithographed by Mr. Hollick; but unfortunately the time required for the completion of so large a series of illustrations still delays the progress of the work for which they are intended. The object of the following notes is to furnish brief distinctive characters, sufficient for the recognition of the various species, without entering upon any detailed account of their structure or distribution.

Biloculina, d'Orbigny.

Biloculina comata, nov.—General characters similar to those of the typical *B. ringens*, but having a surface ornamentation of close, regular, longitudinal, raised striæ. Specimens often attain a very large size, especially in the North Atlantic.

Miliolina, Williamson.

Miliolina insignis, nov.—Has the same morphological characters as *M. trigonula*, but the surface is covered with delicate longitudinal costæ. Specimens often of large size.

M. cultrata, nov.—Test Triloculine, depressed; segments long, narrow, biconvex; superior end of the final chamber projecting far beyond the base of the penultimate; peripheral margin furnished with a continuous narrow keel or wing. Length, $\frac{1}{30}$ inch (0·8 mm.).

M. transversestriata, nov.—A minute, elongate, angular, Triloculine variety, with the peripheral margins of the chambers sharp or subcarinate, and the surface marked by regular, parallel, transverse or diagonal riblets or striæ. Length, $\frac{1}{50}$ inch (0·5 mm.).

M. separans, nov.—Test irregular in form, angular, outspread; consisting of several long, slightly inflated, strongly costate, Milioline segments; the earlier segments arranged on the normal plan, the later ones centrifugally, that is to say, at irregular angles, as though in process of uncoiling. Length, $\frac{1}{10}$ inch (2·5 mm.) or sometimes more.

M. Rupertiana, nov.—Test elongate, biconvex and Triloculine in the young condition, Quinqueloculine and depressed in the adult; peripheral margin rounded, in very old specimens thin or carinate. Final segment terminating in an oval collar, either continuous and regular, or with a deep notch at each end (bilabiate), or with four equidistant notches forming a sort of cruciate aperture. Surface, striatopunctate, *i.e.* with minute pits or depressions in close, regular, parallel lines from one end of the test to the other. The test nevertheless is imperforate, for the pitted depressions penetrate only about half the thickness of the shell-wall. Length, $\frac{1}{13}$ inch (1·9 mm.).

M. Parkeri, nov.—This form is figured by Professor W. K. Parker in one of his earliest papers,[1] where it is simply characterised as a "*Quinqueloculina* with oblique ridges," but without distinctive name. The test is elongate and subtriangular; the peripheral margins of the segments sharp, with a tendency to become carinate; their surfaces traversed by somewhat oblique transverse ridges or crenulations. Length, $\frac{1}{25}$ inch (1· mm.).

M. incrassata, nov.—A thick subglobular form of *M. agglutinans*, very coarsely arenaceous in texture. Segments embracing, with a tendency to become Biloculine (as in *B. contraria*), septation obscure. Aperture crescentic, situate in a short delicate neck rising from the superior extremity of the final segment. Length, $\frac{1}{33}$ inch (0·76 mm.).

Hauerina, d'Orbigny.

Hauerina borealis, nov.—Test planospiral, orbicular, compressed, biconvex; margin thick and rounded, very little hollowed at the sutures. Composed of several convolutions, the latter ones consisting of from three to five long narrow chambers. Aperture, simple crescentiform, situate on the face of the terminal segment a little removed from the line of junction with the previous convolution by a small erect lip. Diameter, $\frac{1}{20}$ inch (1·3 mm.).

This thick lenticular form of *Hauerina* appears to be limited in its distribution to the North Atlantic, and under the name of *H. compressa* it has been recorded

---

[1] "On the Miliolitidæ (*Agathistègues*, d'Orbigny) of the East Indian Seas," part 1, "Miliola," 'Trans. Micros. Soc.,' London, 1858, vol. vi, N.S., p. 53, pl. 5, fig. 10.

from the west coast of Scotland.[1] At times it is not easily distinguished from *Biloculina contraria*, for the internal structure is obscured by the thickness and opacity of the test; generally, however, there are slight constrictions or depressions at the periphery, marking the sutures.

H. circinata, nov.—Test nautiloid, thin, complanate; composed of two or three convolutions, the last, consisting of six or seven segments, completely enclosing the earlier ones. Segments arched, rounded at their peripheral margins; sutural lines marked by external constrictions. Colour milky white, sufficiently translucent to show the outline of the inner whorl of chambers. Aperture consisting of a number of perforations distributed irregularly over the front of the terminal segment. Diameter $\frac{1}{25}$ inch (1· mm.).

ORBITOLITES, Lamarck.

Orbitolites laciniatus, nov.—A variety of *Orbitolites* of the complex type, figured by Carpenter ('Phil. Trans.,' 1856, pl. 5, figs. 2, 3), and by Butschli (in Bronn's 'Klassen und Odrnungen des Thier-Reichs,' 1880, vol. i, pl. 5, fig. 4), but in both cases without name. It is little more than a local variety of *Orbitolites complanatus*, abundant on the coral reefs of the Friendly Islands and Fiji; but as it represents the best development of the structural modifications induced by redundant growth, it is convenient that it should be distinguished by name.

The centre of the disc is constructed on the normal plan, but near the margin it becomes strongly sinuate or plicate, at the same time splitting so as to form a double periphery, the two edges of which approximate at intervals, but otherwise are separated by deep irregular furrows. Specimens from the localities above-named are not unfrequently an inch (25· mm.) or more in diameter.

ASTRORHIZA, Sandahl.

Astrorhiza crassatina, nov.—Test (typically) elongate, subcylindrical, seldom of uniform breadth, often constricted near the middle, ends rounded; consisting of a tube of greater or less length open at both ends. Walls very

---

[1] "Rhizopodal Fauna of the Hebrides," 'Report Brit. Ass.,' 1866 (Trans. Sections), p. 69.

thick, composed of fine sand with but little cement. Cavity tubular, never of uniform diameter, but swollen at one or more points so as to form spurious chambers. Length about $\frac{4}{10}$ inch (10· mm.).

This form is nearly allied to *Astrorhiza granulosa* (*Marsipella granulosa*, 'Quart. J. Micr. Sci.,' vol. xix, n.s., p. 36, pl. 3, figs. 8, 9), and is perhaps its North Atlantic representative; but *A. granulosa* is of smaller dimensions, and the chamber-cavity consists of a narrow tube of even diameter throughout.

A. angulosa, nov.—Test triangular (rarely quadrangular), depressed, biconvex, with rounded margin; consists of a central chamber with radiating tubes, one passing to each corner, the open ends of which serve as apertures. Walls relatively very thick, and composed of loosely cemented fine sand. Diameter, $\frac{1}{7}$ inch (3·6 mm.).

A. angulosa appears to be a short, three-mouthed variety of *A. granulosa*, with which species it is found associated. In both of these, as also in *A. crassatina*, the orifices are often partially blocked with sand-grains, and not unfrequently are stained reddish brown.

RHABDAMMINA, Sars.

Rhabdammina discreta, nov.—Test cylindrical, open at both ends; consisting of a straight or nearly straight tube of indefinite length, spuriously segmented by slight constrictions at irregular intervals. Walls thin, composed of angular sand-grains firmly cemented; interior smooth. Specimens nearly an inch (25· mm.) in length are not uncommon.

BOTELLINA, Carpenter.

Botellina labyrinthica, nov.—Test arenaceous, cylindrical (probably growing attached by one end), straight, or slightly curved, somewhat irregular in external contour; one end round and more or less inflated, the other never hitherto found entire. The wall is of firm consistence and compactly built, except at the rounded extremity where it becomes a thin incomplete layer of sand grains with many interstitial openings. The interior, except near the rounded end, is subdivided irregularly by a labyrinth of coarse, sandy, spurious septa. The rounded terminal cavity forms an undivided chamber. Pieces hitherto obtained have seldom measured more than an inch (20· to 25· mm.), but it is impossible to say what length complete specimens may attain. The diameter is about $\frac{1}{10}$ inch (2·5 mm.).

REOPHAX, de Montfort.

Of the monothalamous and moniliform (*Lagena*-like and *Nodosaria*-like) *Lituolæ* there are half a dozen unrecorded modifications of sufficient interest to deserve preliminary notice.

Reophax ampullacea, nov., is monothalamous and compressed. It bears very much the same relation to *R. difflugiformis* that *Lagena marginata* bears to *L. globosa*. Length, $\frac{1}{30}$ inch (0·85 mm.).

R. bacillaris, nov., is a long, regularly tapering, slightly arcuate variety, with very numerous, short segments. The earlier segments are cylindrical, and have flush sutures not distinguishable on the exterior, the later ones sub-spherical. Colour very dark. Length sometimes nearly $\frac{1}{5}$ inch (4·7 mm.).

R. rudis, nov.—The largest species of the subgenus hitherto met with. Shape long, cylindrical, slightly tapering; sides even and unconstricted; extremities rounded. The walls thicker than those of its congeners and of looser texture; composed of fine grey sand. A longitudinal section reveals about six segments, each tapering at the summit to a stoloniferous tube, the mouth of which, as well as the external orifice, is tinted reddish brown. Length, $\frac{4}{10}$ inch (10· mm.) or more.

The Rev. A. M. Norman has placed in my hands some specimens of a form which, though certainly distinct, is very difficult to separate from this by any positive characters. The specimens are of smaller size and relatively long and slender, darker in colour, and more compactly built; but neither they nor those of the larger species show any sutural constriction or other external mark of segmentation.

R. dentaliniformis, nov.—A small, delicate variety of *R. scorpiurus*, but more slender and regular in contour; segments five or six in number, elongate, and but slightly ventricose. Length, $\frac{1}{14}$ inch (1·85 mm.).

R. guttifera, nov., has pyriform segments, broadest near the base, and tapering to a narrow stoloniferous tube at the point of union with the succeeding chamber. In small specimens the base of the segments is often truncate or even somewhat concave. Number of segments very variable. Length, seldom exceeding $\frac{1}{16}$ inch (1·5 mm.).

R. distans, nov.—A thin-shelled, dark-coloured form, never found entire. Segments distinct, fusiform, tapering nearly equally at the two ends into stoloniferous tubes, which are long and slender in proportion to the bulk of the chambers they unite. Specimens with three chambers, which are the largest hitherto found, have a length of nearly $\frac{1}{5}$ inch (4·8 mm.).

Haplophragmium, Reuss.

Of the section embracing the nautiloid, crozier-shaped and rotaliform *Lituolæ*, there are amongst the "Challenger" collection five species distinct from any hitherto described, in addition to several that may be left for the present as more or less doubtful.

Haplophragmium foliaceum, nov., has a very beautiful and delicate, crozier-shaped test, flat on both sides, and so thin as to be almost transparent. The segments are numerous, short and broad, and the peripheral margin is slightly constricted at the sutures. Length, $\frac{1}{20}$ inch (1·3 mm.).

H. rotulatum, nov.—A sandy isomorph of *Anomalina coronata*, nautiloid in contour and biconcave; the umbilicus is deeply sunk on both faces, and the periphery broad and square, often somewhat oblique. Diameter, $\frac{1}{40}$ inch (0·6 mm.) or less.

H. scitulum, nov.—Test nautiloid, excavated at the umbilicus, rounded at the periphery; composed of two to three convolutions, the outermost consisting of from eight to eleven segments only partially enclosing the earlier ones. Segments compactly fitted, with little or no depression at the sutural lines. Shell-wall finely arenaceous, nearly smooth externally, and of clear yellow-brown colour. Diameter, $\frac{1}{30}$ inch (0·8 mm.).

H. turbinatum, nov.—Test rotaliform, subglobular, depressed at the umbilicus; consisting of less than two convolutions. Segments somewhat ventricose; numbering about six in the peripheral whorl. Diameter, $\frac{1}{35}$ inch (0·75 mm.).

This form resembles *H. subglobosum* in size and texture, but differs from it in being rotaliform and unsymmetrical, not nautiloid.

H. nanum, nov.—Test miuute, rotaliform, depressed; superior face somewhat convex; inferior, plane, more or less excavated at the umbilicus; margin rounded, lobulate. Consists of about two revolutions, each composed of about six inflated segments, often irregular in shape and disposition. Shell texture thin, resembling that of *H. canariense.* Diameter, $\frac{1}{75}$ inch (0·34 mm.).

PLACOPSILINA, d'Orbigny.

There is, in addition, one simple adherent Lituoline species resembling in form the *Squamulina* of Max Schultze, but arenaceous instead of porcellanous in texture.

Placopsilina bulla, nov.—Test adherent; highly convex or approximately hemispherical but slightly longer in one diameter than the other; with a simple, rounded, pouting aperture at each end, close to the base. Walls thick, somewhat loosely sandy. Diameter, $\frac{1}{35}$ inch (0·75 mm.).

AMMODISCUS, Reuss.

Of the free non-septate *Trochamminæ* only two forms require present notice; both are somewhat remarkable for their size.

Ammodiscus tenuis, nov., is a large, thin, planospiral variety, consisting of a few broad, somewhat overlapping, convolutions. It bears the same relation to *A. incerta* that *Cornuspira foliacea* bears to *C. involvens.* Diameter, sometimes $\frac{1}{8}$ inch (3· mm.).

A. spectabilis, nov.—By far the largest species of the non-septate group, is composed of a tube wound upon itself, not regularly and symmetrically so as to retain a rectilinear shape (like *A. Shoneanus*), but in curved or twisted fashion, so as to form an arcuate or subhelicoid test. The shell-wall is very thin, the exterior somewhat rougher than usual amongst the *Trochamminæ,* the interior smooth and polished. Diameter, $\frac{1}{5}$ to $\frac{1}{4}$ inch (5· to 6· mm.).

HORMOSINA, Brady.

The following are representatives of the uniserial or moniliform section of the genus:

Hormosina Carpenteri, nov.—A fine species figured by Dr. Carpenter in his treatise on the 'Microscope' (5th ed., 1875, p. 531, fig. *f*), under the general name "Moniliform *Lituola,*" pretty common in deep water in the North Atlantic and elsewhere. It consists of numerous, elongate, pyriform segments, increasing but slightly in size as they succeed each other; connected end to end in a curved or crooked, never (as a rule) in a straight line.

The shell-wall is finely arenaceous, compactly cemented, and nearly smooth on both its inner and outer surface, except when irregularity of the exterior is produced by sponge spicules only partially incorporated. Length, $\frac{1}{2}$ inch (13· mm.) or even more.

H. monile, nov.—A variety with similar general characters to that last described, but differing in its comparatively small dimensions and in the form of its segments. The segments are short, subspherical, and uniform in size. The length of the longest specimen hitherto obtained is about $\frac{1}{4}$ inch (6· mm.).

H. Normani, nov., has an irregularly constructed test composed of few spherical segments, of which the earlier ones are relatively small, the final one usually very large. The orifice is seldom at the apex of the chambers, but often at some point of the periphery, very near to the entrance of the last stoloniferous tube, so that the new segment is sometimes put on obliquely, sometimes at right angles to the previous one, or even, as is not unfrequently the case, directed backwards, as a result of which, the test assumes a great variety of irregular forms. Length, $\frac{1}{3}$ inch (8·5 mm).

TROCHAMMINA, Parker & Jones.

Of the nautiloid and rotaliform *Trochamminæ* only two minute species require notice, in addition to those described in a former paper.

Trochammina galeata, nov.—Test nautiloid and symmetrical, subglobular or compressed, showing only three segments externally, of which the final one constitutes much more than half the visible shell. Aperture situated on the peripheral face of the final segment, near its junction with the antepenultimate; simple, often immediately below a projection of the shell-wall. Diameter, $\frac{1}{50}$ inch (0·5 mm.).

This species resembles *Tr. ringens*, described in a former paper ('Quart. Journ. Micr. Sci.,' vol. xix, N. S., p. 57, Pl. 5, fig. 12 *a, b*), in many particulars, but it is scarcely so large, and is relatively thicker; its few segments, and the disproportionate size and embracing contour of the final chamber, are sufficiently distinctive.

Tr. nitida, nov. — Test regular, rotaliform, compressed, superior face nearly flat; inferior convex, somewhat excavated at the umbilicus; margin rounded, slightly depressed at the sutures. Consists of two to three convolutions, of which the final one has about nine segments. Diameter, $\frac{1}{50}$ inch (0·5 mm.).

CYCLAMMINA, Brady.

The genus *Cyclammina* is of considerable importance, inasmuch as it presents the best development amongst living Foraminifera, of finely tubular cancellated growths of shell substance filling the chamber cavities—a sort of structure differing widely from the mere subdivision of the chambers by the building in of large sand-grains, which is not uncommon amongst arenaceous types. There are two interesting modifications of *C. cancellata* amongst the "Challenger" gatherings, which, though perhaps only local varieties, differ sufficiently from the typical form to deserve distinctive names.

Cyclammina orbicularis, nov.—A subglobular variety, bearing about the same morphological relation to the type that *Nonionina pompilioides* does to *N. depressula*, only that it is of much smaller size.

C. pusilla, nov., has a minute, biconvex test, depressed at the umbilici, and with thin, sharp, slightly lobulate periphery. A horizontal section shows that it consists of about three complete convolutions, the last of which has about fifteen segments. The cancellated structure is but little developed, there being only sufficient to form a superficial reticulation over the inner surface of the chamber walls. Diameter, $\frac{1}{25}$ inch (1· mm.).

TEXTULARIA, Defrance.

Textularia siphonifera, nov.—Test subcylindrical, nearly round in transverse section, tapering and pointed at the primordial end ; each of the two opposing series of chambers furnished with from two to four rows of tubulated fistulose openings, arranged with more or less regularity. Length, $\frac{1}{16}$ inch (1·5 mm.).

BIGENERINA, d'Orbigny.

Bigenerina robusta, nov.—Test elongate, compressed in its earlier (biserial) portion, cylindrical in its later (uniserial) growth. Uniserial segments numerous, short, somewhat irregular, often ventricose at their periphery. Aperture simple and Textularian in the biserial segments, becoming multiple and porous in the uniserial portion ; the pores either arranged in a ring or irregularly distributed in the central part of the exposed face of the terminal chamber. Interior non-labyrinthic. Length, about $\frac{1}{5}$ inch (4·8 mm.).

This species is of much interest in its bearing upon a group of Carboniferous Foraminifera which have been a source of difficulty to palæontologists. The fossils alluded to were described by myself some years ago under the provisional generic name *Climacammina*,[1] and since that time similar specimens from the Russian Carboniferous beds have been figured by Prof. von Möller with the fresh generic term *Cribrostomum*.[2] The characters of most, if not of all the fossil specimens, have been a good deal obscured by external agencies, such as pressure and the process of mineralisation, but they are easily recognised in the presence of the recent examples which we now have for comparison; indeed, it is not altogether easy to find positive features whereby to distinguish the palæozoic from the living species. Throughout the whole genus *Textularia* the aperture is one of the most variable features, and as the only conspicuous point in which the dimorphous forms under consideration differ from the typical *Bigenerina* is in the fact that their later segments have a porous instead of the usual simple aperture, I can see nothing to be gained by employing a distinctive generic or subgeneric name for them.

CHRYSALIDINA, d'Orbigny.

Chrysalidina dimorpha, nov.—Test elongate, triangular, tapering; the three sides nearly equal, the angles subcarinate; inferior extremity pointed, superior broad and convex. Test composed of many segments, the earlier ones triserial, the later uniserial. Aperture consisting of numerous minute perforations on the superior face of the terminal segment. Texture hyaline. Length, $\frac{1}{50}$ inch (0·5 mm.).

CLAVULINA, d'Orbigny.

Clavulina caperata, nov.—Test elongate, subcylindrical or fusiform, broadest below the middle; transverse section nearly circular throughout; triserial portion relatively very large. Inferior extremity tapering to a point, superior narrow, rounded, or truncate. Segments very numerous, irregular in form and arrangement, the

---

[1] 'Monograph of Carboniferous and Permian Foraminifera' (1876), p. 67.

[2] 'Mém. Acad. Sci., St. Petersburg,' ser. 7, vol. xxvii (1879), p. 39.

sutures marked by external limbate lines; chamber cavities much subdivided. Aperture central, terminal, with raised valvular lip. Length, $\frac{1}{10}$ inch (2·5 mm.).

C. indiscreta, nov.—Test elongate, three-sided, broad near the middle, and tapering towards both ends; edges rounded, except near the inferior end, where they are acute, and terminate in a point. Segments few, septition obscure externally. Texture subarenaceous, compact; surface smooth. Aperture a neat, round terminal orifice. Length, $\frac{1}{16}$ inch (1·6 mm.).

Tritaxia, Reuss.

Tritaxia lepida, nov.—Test triquetrous, broadest near the middle, tapering towards the ends; the three sides nearly equal, the angles sharp or subcarinate. Superior end rounded and terminating in a short neck; inferior, tapering to a sharp point. Texture hyaline. Length $\frac{1}{80}$ inch (0·3 mm.).

Bulimina, d'Orbigny.

Bulimina subteres, nov.—This name has been given to a small Bulimine form frequent in northern seas, but which hitherto has had no well-defined position. In my paper on North Polar Rhizopoda ('Ann. and Mag. Nat. Hist.,' ser. 5, vol. i, p. 436, pl. 21, fig. 12) it was provisionally assigned to *B. elegantissima*, d'Orb., with the remark that the specimens were "not of the precise contour by which the species was usually recognised;" and that though "the segments were similarly arranged, they were relatively shorter, and there were fewer in each convolution." It might have been added that the aperture is usually inserted further from the apex of the shell. In point of fact the species is almost equally related to *B. elegantissima*, d'Orb., and *B.* (*Robertina*) *arctica*, d'Orb., but it has larger, broader segments than either, and is altogether less elegantly made. Specimens from the North Atlantic are commonly from $\frac{1}{60}$ to $\frac{1}{40}$ inch (0·4 mm. to 0·6 mm.) in length, broad and rounded at the superior end, and tapering to a point; the sides are convex and but slightly excavated at the sutural lines.

Messrs. Parker and Jones ('Phil. Trans.,' vol. clv, p. 375) show that the two d'Orbignian forms above mentioned are in near relationship, but I cannot follow them so far as to include both under the same name; indeed, I should prefer to assign some of the specimens they figure (*op. cit.* pl. 15, figs. 13—17) to the species now described rather than to *B. elegantissima.*

B. subcylindrica, nov., is another form belonging to the same group as *B subteres.* The test is elongate, subcylindrical (not tapering), the two ends equally rounded, and the surface but little excavated at the sutures. The segments are few, and their spiral arrangement is very obscure: the aperture takes the form of a narrow, nearly erect slit, near the base of the final segment. Length, $\frac{1}{50}$ inch (0·5 mm.).

B. Williamsoniana, nov.—Test elongate, cylindrical, more or less sinuate in contour, circular in transverse section; composed of a spiral band of long narrow, nearly erect segments. Inferior extremity slightly tapering and rounded, superior obliquely truncate. Surface traversed from end to end by a series of somewhat sinuate and diagonal parallel costæ, which entirely obscure the internal structure. Aperture simple, situate in a depression at the centre of the oblique superior face, bordered by radiating lines. Length, $\frac{1}{40}$ inch (0·64 mm.) or less.

Bolivina, d'Orbigny.

Excepting the genus *Lagena,* there is no group of hyaline Foraminifera the knowledge of the varietal modifications of which has received larger accessions from the study of the "Challenger" material, than that comprising the aberrant forms of *Bulimina,* included under the subgeneric terms *Virgulina* and *Bolivina.* Both diverge from the typical plan of structure in their tendency to become more or less regularly biserial, instead of spiral, in the arrangement of their chambers, whilst they usually retain the characteristic Bulimine aperture. It is impossible to separate these two subgenera one from another by any well-defined or permanent peculiarity; all that can be said to distinguish them is that *Virgulina* is more Bulimine and less Textularian in the disposition of its segments, and that *Bolivina* is more Textularian and less Bulimine. Whilst, therefore, it is comparatively easy to associate *Virgulina* with its type, *Bolivina* often only betrays its affinity by the aperture, which is either comma-shaped, twisted, toothed, unsymmetrically oval, or of some other form within the range of variation to be found in *Bulimina* itself. In the varieties of *Virgulina* we find all the links connecting *Bolivina* with the typical *Bulimina.* Two or three undescribed species of *Virgulina* may be omitted from the present notice, as descriptions in few words would be scarcely intelligible without figures; of *Bolivina* the following new forms may be placed on record.

Bolivina porrecta, nov.—Test elongate, straight, slightly tapering, finger-shaped, somewhat compressed; margin and ends rounded. Segments about as broad as long, the earlier ones arranged on the normal Textularian plan, the later ones taking a nearly triangular form, each extending the entire width of the test, the sutures forming a zigzag line from side to side. Walls thin and clear, very finely perforated; sutural depressions very slight. Aperture large, terminal, oblique. Length, $\frac{1}{26}$ inch (0·9 mm.).

B. limbata, nov.—Test elongate, tapering, compressed, more or less twisted; margin angular or only slightly rounded, sinuate. Sutures irregularly curved, limbate, especially near the points of contact of the two series of segments on both faces of the shell. Length, $\frac{1}{35}$ inch, (0·75 mm.).

B. tenuis, nov.—Test thin, outspread, broadly elliptical, slightly convex on both sides; margin acute. Segments few, each with a sort of supplementary lobe, the lobes collectively presenting the appearance of a series of chamberlets down the median line. Aperture on the oblique face of the terminal chamber surrounded by radiating lines. Dimensions, $\frac{1}{80}$ by $\frac{1}{100}$ inch (0·3 by 0·26 mm.).

B. lævigata, nov.—Test elongate, thin, complanate, broadest at the centre, tapering and rounded towards the ends. Segments few in number, Textularian in arrangement, broad, flattened on both faces, bordered both at sutures and periphery by a narrow band of clear shell-substance. Sutures flush; aperture large, irregularly oval, oblique. Length, $\frac{1}{60}$ inch (0·43 mm.).

B. tortuosa, nov.—Test elongate, tapering, broadest near the top; the sides bent obliquely towards the median line, so as to give the whole shell a twisted contour; margin thin, sharp, lobulate. Segments numerous, long, narrow, projecting and rounded at the free ends. Shell conspicuously perforated. Length, $\frac{1}{55}$ inch (0·45 mm.).

B. pygmæa, nov.—Test short, broad, biconvex, widest near the top, and tapering to a point at the base. Segments numerous, somewhat inflated, the peripheral ends extended into sharp points directed obliquely or horizontally. Length, $\frac{1}{100}$ inch (0·25 mm.).

B. robusta, nov.—Test elongate, compressed, broad and rounded at the superior extremity, tapering to a point, and frequently terminating in a long, stout spine at the inferior end. Test thickest on the median line, and sloping away symmetrically to the sides; margin subacute. Segments numerous, about ten in each series; long, curved, obliquely set. Shell stoutly built, sutures thickened, usually limbate and somewhat crenulate externally. Length, $\frac{1}{45}$ inch (0·6 mm.).

B. decussata, nov.—Test elongate, compressed; broad and obliquely truncate at the superior extremity, and tapering to a rounded point at the inferior; margin thick, square, or slightly rounded, lobulate. Surface beset with low prominences or bosses, rounded or subangular in outline, arranged with some regularity in oblique rows, about four in each row, and entirely concealing the septation. Length, $\frac{1}{50}$ inch (0·5mm.).

B. Hantkeniana, nov.—Test depressed, equally convex on the two faces; varying in contour, from a relatively long form tapering to a point at the base, to broadly oval one with rounded ends. Composed of numerous, rounded, inflated segments, in two more or less regular alternating series, surrounded by a delicate keel of varying width and completeness. Surface often traversed by short, delicate, longitudinal costæ. The long narrow specimens seldom have a continuous wing or keel, and they attain a length of about $\frac{1}{28}$ inch (0·9 mm.), whilst those of wider proportions with the broader more regular wing are less than $\frac{1}{40}$ inch long (0·6 mm.) and about $\frac{1}{50}$ inch (0·5 mm.) broad.

B. Karreriana, nov.—Test elongate, tapering, broadest near the top, somewhat depressed; inferior extremity pointed, often mucronate; margin thick and rounded, lobulate. Surface of the test ornamented with numerous delicate, often branching, or otherwise irregular, longitudinal ribs. Segments inflated; aperture large, oblique. Length, $\frac{1}{40}$ inch (0·63 mm.).

B. lobata, nov.—Test elongate, depressed, digitate; superior extremity obliquely truncate or rounded, inferior obtuse. Segments inflated, especially the later ones, their peripheral margins subangular. Surface of the later chambers more or less granulated. Sutures thickened, deeply sunk. Aperture a long oval slit contracted at the middle; nearly central. Length $\frac{1}{60}$ inch (0·4 mm.).

B. Schwageriana, nov.—Test oblong, biconvex, broadest near the middle, tapering to a blunt point at the inferior extremity; margin carinate. Keel widest near the middle of the shell, absent at the inferior end.

Sutures limbate, the limbation taking the form of raised beads or irregular lines of shell-substance on both sides of the test, chiefly near the points of contact of the two opposing series of segments. Surface otherwise smooth. Aperture large, with an oblique projecting tooth near the superior end. Length, $\frac{1}{45}$ inch (0·56 mm.); breadth near the middle of the test only slightly less.

B. amygdalæformis, nov.—Test oval, compressed, almond-shaped; ends obtuse or rounded, periphery rounded. Segments few; septation obscured by a surface ornamentation of stout, branching, longitudinal costæ. Terminal chamber nearly smooth and conspicuously perforated; aperture central, of long oval form, slightly constricted at the middle. Length, $\frac{1}{36}$ inch (0·72 mm.).

B. subangularis, nov.—Test oblong, tapering, stoutly built, more or less angular, somewhat concave or excavated on both sides; inferior extremity obtusely pointed. The angular contour is determined by the prominence of superficial costæ, the principal of which, six in number, are placed, one down each lateral margin and two down each face of the test. Aperture comma-shaped. Length, $\frac{1}{55}$ inch (0·45 mm.).

CASSIDULINA, d'Orbigny.

Cassidulina Parkeriana, nov.—Test crosier-shaped; spiral portion short, somewhat compressed, composed of few segments arranged as in *C. crassa;* linear portion straight or arcuate, cylindrical, biserial, the ends of the segments overlapping alternately; chambers short, ventricose. Aperture comma-shaped, situated on one of the lateral faces of the terminal segment, near its apex. Length, $\frac{1}{45}$ inch (0·57 mm.).

The Rev. A. M. Norman has a crosier-shaped species in some respects similar to this, with the manuscript name *Cassidulina Bradyi*, but the segments are long and oblique, and the whole shell is compressed and *Vaginulina*-like.

C. Jonesiana, nov.—Test oblong or ovate; external aspect of the superior surface like that of a very thick Rotalian, with slightly inflated chambers and rounded margin. On the inferior face the umbilical ends of the chambers fall short of the centre, leaving a deep cavity or depression, from which the aperture proceeds, taking the form of a curved, nearly erect slit, on the inferior face of the large terminal chamber. Diameter, $\frac{1}{36}$ inch (0·7 mm.).

C. subglobosa, nov.—A large, thick, few-chambered, subglobular shell; the dorsal margin gibbous and rounded, the ventral less convex; aperture in the form of an obliquely-set loop on the ventral face of the terminal segment. Diameter, $\frac{1}{36}$ inch (0·7 mm.).

Ehrenbergina, Reuss.

Ehrenbergina hystrix, nov.—Shell somewhat ovate in general form, the superior end broad and rounded. Segments few, regular and alternate on the dorsal face, confused on the ventral, their free ends terminating in lateral spines. The sutural lines on the dorsal side marked by rows of spines, sometimes fused into a fringe-like projection from the shell-wall; the ventral surface of the earlier segments also beset with stout spines or tubercles. Aperture large, curved, situated in a depression on the inflated face of the terminal segment, which is ornamented externally with radiating lines. Length, $\frac{1}{33}$ inch (0·75 mm.).

Lagena, Walker & Jacob.

Whilst the simplicity of the typical structure of *Lagena* limits the range of variation in general form, it appears to favour the production of an endless diversity of surface ornamentation. It is impossible to recognise as "species," or by any word of similar significance, the successive terms of a series where every intermediate link may easily be found; nor is it easy under such circumstances to select the points where the chain may best be broken to form groups which have any approach to true specific value. In appending names, therefore, to some of the more striking and more easily defined modifications of the genus, it is to be understood that they are no more than varietal or subvarietal distinctions. Under these circumstances it has not been thought necessary, at the moment, to do more than indicate characteristic peculiarities, whether of contour or ornament.

Lagena botelliformis, nov.—Test unornamented; long, cylindrical, of even diameter, arcuate, ends rounded; entosolenian.

L. quinquelatera, nov.—A five-sided modification of *L. lævis*; angles sharp or carinate; surface unornamented or very faintly striate; ectosolenian.

L. stelligera, nov.—Pyriform, ento- or ecto-solenian, with a circular rim or collar at the base, one third the diameter of the shell, and a number of short ribs (8—12) radiating from it.

L. longispina, nov.—A variety of *L. globosa*, either globular or somewhat compressed, armed with long, stout spines at the base.

L. unguiculata, nov.—Pyriform, compressed; inferior end broad and tapering to a thin edge, which is furnished with a number of curved teeth set symmetrically.

L. samara, nov.—Test elongate, compressed, leaf-shaped, tapering to a point at both ends; consists of a central, circular, bi-convex chamber, with a large peripheral wing, narrow at the sides, but much developed at base and apex.

L. tubulifera, nov.—Chamber oval or pyriform, biconvex, with long ectosolenian neck; periphery furnished with a broad laminar wing traversed by parallel or radiating tubuli.

L. tubulifera, var. *tenuistriata*, nov.—A subvariety of the last-named, the body of the shell ornamented with delicate longitudinal striæ.

L. fimbriata, nov.—Compressed, broadest at the base, tapering upwards; ento- or ecto-solenian; furnished with a deep perpendicular wing or fringe running round the oval base; the wing traversed by parallel tubuli.

L. auriculata, nov. (*typica*).—Pyriform, compressed bilaterally, usually entosolenian; on each side, near the base, a loop-like wing encloses a portion of the peripheral margin; or sometimes the whole periphery is bordered by a wing which divides near the base, on each side, so as to form a sort of loop. When the wing forming the loop is deep it is usually tubulated.

L. auriculata, var. *substriata*, nov., has, in addition, indications of riblets near the base and apex of the test.

L. auriculata, var. *costata*, nov., has the body of the shell strongly costate, and is frequently armed at the base with short spines.

L. squamoso-alata, nov.—Body of the test like *L. squamoso-marginata*, P. & J., but with a further ornamentation consisting of a reticulated border and a broad tapering wing with radiate marginal markings.

L. variata, nov.—Shape unsymmetrical, subglobular, gibbous, somewhat compressed, with an entosolenian aperture at each end. Surface-ornament consisting of irregular, slightly raised, rounded, longitudinal riblets.

L. exsculpta, nov.—Shaped like *L. globosa*, or somewhat compressed; entosolenian. Surface-ornament consisting of an excavated star radiating from the centre of the inferior end. Radii fluted, broad, and rounded at the upper extremity, extending nearly half-way up the test.

L. Wrightiana, nov.—Test oval flattened, with a thin peripheral border, surmounted by a stout sessile phialine lip; aperture entosolenian. Surface-ornament consisting of a number of longitudinal, parallel, excavated grooves covering the two sides, except the central portions which are smooth.

L. favoso-punctata, nov.—Shape variable; surface-ornament consisting of a raised reticulation, with an orifice or perforation in the middle of each depression.

L. Schulzeana, nov.—Test oval, compressed, sub-carinate; sides flat; neck wide and very short, finished with a rounded lip. Surface-ornament consisting of transverse bars, horizontal in the middle and bent downwards at an angle, near the periphery.

L. trigono-ornato, nov.—General form similar to *L. trigono-marginata*, P. & J. The peripheral angles are limbate, reticulated externally, and much perforated.

L. plumigera, nov.—Flask-shaped, with long slender neck; surface-ornament consisting of ten to twelve longitudinal costæ, developed (especially at their lower ends) into wide tubulated wings.

L. quadralata, nov.—Flask-shaped, ectosolenian; furnished with four equi-distant, broad, tubulated wings, reaching from near the extremity of the neck to the base of the shell; the body of the test having an additional surface-ornament of fine longitudinal striæ.

L. torquata, nov.—Test flask-shaped with tapering neck. Surface-ornament consisting of broad longitudinal costæ with depressions or perforations at regular intervals down the centre of each; alternating with these are narrower non-perforate ribs, and the whole are united by secondary or less elevated crossbars.

L. Hertwigiana, nov.—Pyriform, with delicate ectosolenian neck rising abruptly from the apex. Surface finely reticulated, each angular mesh with a conspicuous perforation in the centre. Sections show that the shell-wall is double, that the intermediate space is divided into cells or chamberlets by perpendicular walls, of which the external areolation marks the position, and that the larger perforations open into the centre of the cells. Length, $\frac{1}{75}$ inch (0·34 mm.).

This is a particularly interesting species in its bearing upon recently expressed views on the *Dactyloporidæ*. Here, at least, is an undoubted hyaline Foraminifer with a general aperture in a delicate transparent ectosolenian neck, and a cellular shell-wall like *Ovulites*, each chamberlet provided with an external orifice.

NODOSARIA, Lamarck.

Nodosaria intercellularis, nov.—Test arcuate (Dentaline), inferior extremity usually mucronate, composed of about six segments, the earlier of which are sub-cylindrical, or only slightly inflated, the later ones elliptical or pyriform. Surface-ornament of the earlier segments consisting of longitudinal costæ; the later chambers marked by lines of closely set perforations which communicate with chamberlets formed in the furrows between the ribs. The structure of the later segments closely resembles that of *Lagena Hertwigiana*. Neck long, with annular or spiral raised ornament and phialine or cleft lip. Length, $\frac{1}{15}$ inch (1·6 mm.).

N. abyssorum, nov.—Test stout, thick-shelled, nearly straight, often irregularly built. Segments about five in number, subglobose, somewhat irregular in shape and size; primordial chamber, which is usually the largest, furnished at its base with a number of short stout spines; neck short and broad, with large phialine lip. Length, $\frac{1}{9}$ inch (2·8 mm.).

VAGINULINA, d'Orbigny.

Vaginulina spinigera, nov.—General form that of short, broad, somewhat tapering specimens of *V. legumen*, but furnished at the base with two (rarely three or more) long stout spines, one of which is usually continuous with the main axis of the shell, the others radiating at various angles. Length of the body of the shell, $\frac{1}{7}$ inch (3·5 mm.), the spines often two thirds as much, or even occasionally as long as the shell itself.

Mr. Whiteaves has accurately described this form,[1] but beyond alluding to it as a species of *Marginulina*, has not given it a name. In one of the dredging lists published by the late Dr. M. Sars, the name *Marginulina spinosa* occurs,[2] but without any description or other indication of characters, and it is difficult now to say what was intended. So far as the distinction between *Vaginulina* and *Marginulina* is of any value, the species appears to belong to the former rather than the latter genus; and as the want of a recognised name for it has been a source of some inconvenience,

---

[1] 'Report Brit. Assoc.,' 1872; 'Trans. Sections,' p. 144.

[2] 'Vidensk.-Selsk. Forhandlinger' for 1868, p. 248.

it seems best to take this opportunity to supply the deficiency.

Cristellaria, Lamarck.

Cristellaria Siddalliana, nov.—Test spiral, explanate, with a tendency to become centrifugal or crosier-shaped; extremely thin, usually surrounded by a broad, delicate wing, except the septal or ventral face of the terminal segment; the wing often extending between and separating the last two convolutions of the discoidal portion. Segments numerous, very slightly inflated, forming two or more convolutions, the whole of which are visible on both sides of the shell. Longer diameter, $\frac{1}{20}$ inch (1·26 mm.) or more.

C. gemmata, nov.—Test broad, oblong, compressed (Planularian); earlier chambers spiral and embracing, later ones broad and arcuate; each segment ornamented with a row of exogenous heads either upon the sutural lines or parallel to them. Length, $\frac{1}{20}$ inch (1·26 mm.).

Polymorphina, d'Orbigny.

Polymorphina longicollis, nov.—Test long-ovate, subcylindrical or fusiform; segments few, erect, slightly ventricose, the final one hispid externally, and terminating in a long neck with phialine lip. Length, $\frac{1}{40}$ inch (0·6 mm.).

An interesting intermediate link; the general characters are those of *Polymorphina*, the neck and lip essentially those of *Uvigerina*.

Uvigerina, d'Orbigny.

Uvigerina spinipes, nov.—Test elongate, subcylindrical, slightly compressed on three sides; tapering to a point at the inferior end, and armed with numerous spines directed downwards. Segments inflated, distinct, somewhat irregularly combined. Length. $\frac{1}{33}$ inch (·77 mm.).

Sagrina, d'Orbigny.

Sagrina columellaris, nov.—Test long, nearly straight, cylindrical, slightly tapering; inferior extremity round or bluntly angular; superior, broad and convex. Uvigerine chambers few, distinct; uniserial segments numerous, short, very little constricted at the sutural lines. Aperture large, simple, with sessile phialine lip. Length, $\frac{1}{23}$ inch (1·1 mm.).

S. bifrons, nov.—Test elongate, compressed, both sides slightly concave along the median line; margin thick and rounded. Uvigerine chambers few, distinct; those of the linear series numerous, short, not inflated. Sutures flush externally; septa thickened by deposit of clear shell substance. Aperture large, oval, surrounded by a sessile lip. Length $\frac{1}{30}$ inch (0·84 mm.).

DISCORBINA, Parker & Jones.

Discorbina tabernacularis, nov.—Test conical or tent-shaped, sides somewhat arched, inferior surface concave. Segments long, oblique, arranged in about three convolutions; septal lines externally limbate in small specimens, in larger ones hidden by the general thickening of the shell-wall. Inferior surface ornamented with radiating striæ or crenulations; superior with striæ or irregular costæ radiating from the apex. Diameter, $\frac{1}{100}$ inch (0·25 mm.).

In some localities specimens of *D. tabernacularis* are met with in pairs, that is to say, two shells firmly attached by their bases. The same condition is not unfrequent in *Discorbina pileolus*, d'Orb., and *D. Parisiensis*, d'Orb.

TRUNCATULINA d'Orbigny.

Truncatulina rostrata, nov.—Test biconvex, subnautiloid, slightly unsymmetrical; periphery thin, subcarinate. Chambers equitant; about ten in the final convolution, which completely encloses the penultimate. Sutures limbate, especially near the centre; marked by indentations at the periphery. The true aperture is an arched, labiate opening, placed transversely on the face of the terminal segment, close to the margin of the previous convolution; but there is usually, in addition, a second or spurious orifice, in the form of a vertical slit in the beak-like projection of the peripheral angle of the same. Diameter, $\frac{1}{30}$ inch (0·84 mm.).

Tr. Robertsoniana, nov.—Shell spiral, lenticular; superior surface slightly convex; inferior convex, somewhat depressed at the umbilicus; consists of four or more convolutions, of which the whole are visible on the superior face, whilst on the inferior the last whorl conceals all preceding it, except a small area in the centre. Segments very numerous, 13 or 14 in the final convolution. Periphery angular, even, not constricted at the sutures. Colour, rich brown, deepest near the centre and at the sutural lines. Diameter, $\frac{1}{35}$ inch (0·7 mm.).

Tr. margaritifera, nov.—Shell spiral; slightly convex or nearly flat on its superior surface, convex on the inferior; margin sharp, subcarinate, lobulate. Chambers very numerous, all visible on the superior face, the last convolution only on the inferior. Sutural lines on both sides marked by rows of exogenous beads of clear shell-substance, largest near the centre of the test. Diameter, $\frac{1}{20}$ inch (1·26 mm.).

Tr. soluta, nov.—Shell elongate, compressed; composed of a line of inequilateral segments, arranged spirally, the earlier ones embracing, the later ones free. Periphery sharp, furnished with a tubulated fringe or keel, and the surface of the shell otherwise more or less ornamented with tubercles. Aperture a curved slit in the line of the periphery at the extremity of the last chamber, furnished with a phialine lip. Length, $\frac{1}{70}$ inch (0·36 mm.).

PULVINULINA, Parker & Jones.

P. procera, nov.—Shell spiral; superior surface forming an elevated cone with rounded apex; inferior, flat or truncate. Chambers numerous, about six in the last convolution, oblique; segmentation usually obscure, except on the inferior aspect, where the sutures and periphery are more or less limbate. Aperture, an arched slit on the inferior side of the last segment, near the umbilicus. Diameter, $\frac{1}{22}$ inch (1·1 mm.).

POLYSTOMELLA, Lamarck.

Polystomella imperatrix, nov.—Test spiral, symmetrically discoidal, complanate; peripheral margin rounded or subangular, furnished with several (three to six) stout spines. Septal ridges only slightly limbate, marked with pitted depressions; retral bars very numerous, delicate, irregular, sometimes branching. Diameter, $\frac{1}{15}$ inch (1·7 mm.).

P. verriculata, nov.—Test spiral, much depressed; sides flattened; margin angular or slightly rounded. Septal ridges and retral bars forming a coarse, more or less regular, raised network, covering the surface of the shell. Diameter, $\frac{1}{50}$ inch (0·5 mm.).

CYCLOCLYPEUS, Carpenter.

Cycloclypeus Guembeliana, nov.—A single specimen, nearly complete, of a discoidal foraminifer referable to Carpenter's genus *Cycloclypeus*, and a fragment of a second

> of the same species, were found in material dredged in 210 fathoms off Kandavu, one of the Fiji Islands. Their structure is of much simpler type than that of the gigantic discs dredged by Sir E. Belcher on the coast of Borneo, which formed the basis of Dr. Carpenter's description of the genus. The better specimen is a thin disc about $\frac{1}{16}$ inch (1·5 mm.) in diameter, somewhat biconvex; the convexity is chiefly in a limited area near the centre of the test, the remainder being thin, and tapering to a sharp edge at the periphery. The texture is distinctly hyaline.
>
> This little shell appears to represent the "central chambered plane" of the large forms, without the thickened shelly plates on the upper and lower surface. The chambers form a single layer, disposed in tolerably regular annuli; in shape they are nearly square, not elongate in the direction of the radii as in the larger species, and the septal lines are slightly raised externally.

I would suggest, for the sake of distinction, that the large type, which, I believe, has never received a specific name, should be called *Cycloclypeus Carpenteri;* that now described I propose to name after Professor Gümbel, of Munich, who has worked with so much success on the allied genus *Orbitoides.*

3. *Note on "Biloculina-mud."*

In the second paper of this series,[1] some remarks were offered upon the Foraminifera collected at or near the surface of the ocean by means of the tow-net. A list was given of the free-swimming species, so far as they were known, and the question whether all the varieties of *Globigerina* and the three or four pelagic species of *Pulvinulina,* live exclusively at the surface of the open sea, was discussed. The recorded facts bearing upon the subject were summarised, as well as the results of my own observations, not with the view of announcing any conclusions in the matter, but chiefly in the hope of eliciting further contributions to the knowledge of a subject, concerning which there was still much to be learnt. The question, from a zoological stand-point, is now a comparatively narrow one. It is not whether Foraminifera do live at the bottom of the sea,

---

[1] 'Quart. Journ. Mic. Sci.,' vol. xix, N.S., p. 78, "Notes on Pelagic Foraminifera."

down to its greatest depths, for of that there can be no reasonable doubt; it does not even affect the greater number of types which are found in *Globigerina*-ooze, for of the forty or fifty species or more which *Globigerina*-mud often contains, those to which it refers may not exceed half a dozen, the remainder being recognised on all hands as living their whole life at the bottom. The point still in debate is, as already indicated, whether a certain limited number of forms live *only* at the surface, or also at various depths down to the floor of the ocean; and it derives its chief interest and importance from the fact that individuals of these few species occur in such enormous numbers that in many areas they constitute the mass of the calcareous deposit. The cruise of the "Knight Errant" during the past summer has in part removed one of the minor difficulties which were put forward as negative evidence, by furnishing us with surface gatherings of small non-spinous *Globigerinæ* from an area in which they had not previously been collected, and this is satisfactory as far as it goes: on the other hand, the comparison of the surface and bottom specimens, though not yet completed, appears to supply an argument in the opposite direction. I do not propose at the present moment to enter again upon the discussion of this subject, though I hope to revert to it at a future time; my object is rather to offer a few notes upon the fauna of the sea-bottom over an area in which the porcellanous Foraminifera (*Miliolidæ*), which are known to be exclusively bottom-living species, not only furnish the most characteristic feature of the deposit, but form by far the most important and bulky constituent.

Professor G. O. Sars, of Christiania, in his official report on the Norwegian Sea-fisheries for the year 1876,[1] gives a short account of the biological conditions of the northern "deep-water cold area," which occupies a considerable portion of the region between Norway, Bear Island, and Spitzbergen on one side, and the Faröe Islands, Iceland, and Greenland on the other. This region has a bottom temperature of from 0° to 1·6° Cent. (32° to 34·9° Fahr.), and the depth ranges from 300 to 2000 fathoms. The sea-bed, especially of the deeper portions of the area, consists of a soft, light-coloured, sticky mud, of nearly uniform composition; that is to say, composed in very large proportion of one species of porcellanous Foraminifera, *Biloculina rin-*

---

[1] "Indberetninger til Departementet for det Indre fra Professor, Dr. G. O. Sars om de af ham i Aarene, 1864--1878, austillede Undersogelser angaaende Saltwandsfiskerierne." Christiania, 1879.

gens. Professor Sars has been kind enough to send me a characteristic sample of this "*Biloculina*-mud," with the following particulars as to locality:

"Station 52—Lat. 65° 47·5′ N., Long. 3° 7′ W.; depth 1862 fathoms; temperature at the bottom 1·2° Cent." (about 34° Fahr.).

The fine impalpable silt had been partly removed before I received it, I therefore completed the cleaning by washing it thoroughly on a sieve of 120 meshes per linear inch, through which no particles larger than 0·005 of an inch in diameter could pass. The loss was about 6 per cent. of the entire weight, and, of the impalpable material thus separated, about one half was calcareous, the particles evidently consisting of the *débris* of foraminiferous shells, and the other half fine silicious sand. I have no information as to the proportion of impalpable mud in the dredged material before the preliminary washing, but as it is said to be sufficient to incorporate the whole into a sticky paste, which on being dried forms a hard, light-coloured, calcareous mass, it must be considerable. The composition of the material as I received it from Professor Sars was as follows—the proportions stated are by weight:

Biloculina ringens (one half being entire shells) . .	50	p. c.
Haplophragmium subglobosum	20	,,
Globigerinæ (the minute arctic form)	4	,,
Sand and small fragments of rock with a few Foraminifera other than the above-named	20	,,
Impalpable débris	6	,,
	100	

Assuming that the calcareous part of the impalpable mud consists of the disintegrated shells of the same species in similar proportions, the total amount of the deposit derived from surface organisms would not in this case exceed 4 per cent. even were *Globigerinæ* at all times pelagic.

The specimens of *Biloculina* are very uniform; they are of the stout, inflated, typical form, with a small admixture of the depressed carinate variety, *B. depressa*, d'Orb. Hardly less remarkable is the existence of so large a proportion of one of the comparatively small, nautiloid, arenaceous species, *Haplophragmium subglobosum;* and here again the specimens show scarcely any variation in minor characters. The *Globigerinæ* are all of the minute, subglobular, thick-shelled, arctic type, which may be fitly named *Gl. Dutertrei,* var. *borealis.* Altogether sixteen species of Foraminifera were noted; but beyond those already alluded to they were unimportant and represented by few individuals.

A sufficient number of apparently clean specimens of *Biloculina* were selected for chemical analysis, but the result gave a proportion of silica which suggested that notwithstanding the careful washing to which they had been subjected, the chamber cavities had retained a certain amount of sand. The experiment, however, was sufficient to prove that the tests contained no earthy carbonates except carbonate of lime, and no phosphates.

The analysis of *Haplophragmium subglobosum* was more satisfactory, and as it is interesting to compare the chemical composition of the test of one of the non-labyrinthic *Lituolæ* with that of a labyrinthic type such as *Cyclammina cancellata,* of which the analysis was given in a previous paper (' Quart. Journ. Mic. Sci.,' vol. xix, N.S., p. 25), I append the details. As often heretofore I am indebted to my friend, Mr. J. T. Dunn, B.Sc., for practical help in the chemical portion of the subject.

Haplophragmium subglobosum.

Silica	76·10
Peroxide of iron with some alumina	16·30
Carbonate of lime	7·30
	99·70

The alumina was not separately determined, but as it only exists in small proportion, the importance of peroxide of iron as a constituent is evident; and that the percentage is even larger than in *Cyclammina* is a noteworthy fact. No phosphoric acid was present nor was there any trace of magnesia.

In a recent letter, referring chiefly to the *Biloculina* deposit, Professor G. O. Sars states that in the portion of the Arctic Ocean lying east of the cold area already alluded to, namely, east of Finmark, Bear Island, and Spitzbergen, an entirely different bottom-fauna prevails. In this eastern area the characteristic rhizopod is the large, stellate, arenaceous type *Rhabdammina,* which exists in such abundance as to render the term "*Rhabdammina*-ooze" not inappropriate for the dredged mud.

I can scarcely conclude these preliminary papers without expressing the obligation I am under to some of my old fellow-labourers in the same field of research. But for their encouragement and ever ready help, the tedious details of mechanical work which have occupied so much of my time during the last four years or more, would have been wearisome in the extreme. I hope in the proper place to make due acknowledgment of many acts of courtesy that I cannot enumerate here, and I will now do no more than mention the names of Rev. A. M. Norman, Professor T. Rupert Jones, Dr. Carpenter, and Professor W. K. Parker, as amongst those to whom I am primarily indebted for assistance and advice.

Postscript.

Since the foregoing paper has been in print, I have received through the kindness of Herr Gustav Steinmann, a copy of his recently published memoir, " Die Foraminiferengattung Nummoloculina, n.g." Without entering into any discussion of the views therein expressed, I may just state that the *Nummoloculina contraria* of Herr Steinmann is in part, at least, the *Hauerina borealis* of the present paper (p. 46). The difficulty of distinguishing *Hauerina borealis* and *Biloculina contraria* has been already alluded to, and if the views put forward by Herr Steinmann be correct, is now satisfactorily disposed of. At the same it must be remembered, on the one hand, that between *Biloculina sphæra,* d'Orb. and *B. contraria,* d'Orb. every gradation of form is to be found in northern dredgings; and on the other, that the alar prolongation of the chamber-walls is a character shared by other species of *Hauerina.*

On the HEAD CAVITIES *and* ASSOCIATED NERVES *of* ELASMOBRANCHS. By A. MILNES MARSHALL, D.Sc., M.A., Fellow of St. John's College, Cambridge. Professor of Zoology in Owens College. (With Plates V and VI).[1]

THE discovery by Mr. Balfour[1] of the extension forwards to the head of that splitting of the mesoblast which in the trunk gives rise to the body cavity, and of the subsequent division of the cavity so formed into the series of segments which he has termed head-cavities, has given us a new and very important clue to that favourite problem of morphologists, the segmentation of the vertebrate head.

I have been led to pay special attention to the development of these head-cavities in Elasmobranchs, in order to test the accuracy of conclusions as to the morphology of certain of the cranial nerves, notably the third pair, to which I had been led by a study of their development in the chick.[2]

In the present paper I propose to treat of—(1) the development of the head-cavities, (2) the relations of the cranial nerves to these cavities, (3) certain stages in the development of those nerves which are most intimately connected with the cavities, and (4) certain stages in the development of the eye muscles.

For my material, which consists almost entirely of embryos of *Scyllium canicula,* I am indebted partly to Mr. Balfour and partly to the Managers of the Southport Aquarium. With few exceptions, the embryos were hardened in a $\frac{1}{4}$ per cent. solution of chromic acid, to which a few drops of a 1 per cent. solution of osmic acid were added. In this solution they were left for twenty-four hours, and then transferred to alcohol of 30 per cent., which was gradually increased in strength until absolute. In embryos prepared in the above manner, the brittleness due to the use of osmic acid alone is completely avoided; all the epiblastic tissues are stained a deep brown or black colour, and the nerves in particular stand out with remarkable sharpness and distinctness from the surrounding and but slightly stained mesoblast.

Much of any success I may have obtained is due to this mode of preparation, which appears to be peculiarly applicable to nerve investigation. For a knowledge of the method I am again indebted to Mr. Balfour.

The Development of the Head-Cavities.—As Balfour has

---

[1] 'Elasmobranch Fishes,' p. 206, *seq.*

[2] *Vide* this Journal, January, 1878, p. 23, *seq.*

already pointed out,[1] the separation of the layers of the mesoblast so as to give rise to a cœlomic cavity, occurs much earlier in the head than in the body. In the head this separation first occurs in the earlier part of stage D,[2] while in the body in does not commence till stages G or H, though the two layers of the mesoblast, somatic and splanchnic, are distinctly formed by stage D.

My observations on the earlier stages of development of the head cavities accord so completely with the account given by Mr. Balfour that I shall deal with the subject rather summarily, referring the reader for a fuller account to Mr. Balfour's work already cited.

Plate V, fig. 1, represents a transverse section through the head of a *Scyllium* embryo at the end of stage C; the mesoblast (*mes.*) is distinctly divided into two layers, which are just commencing to separate from one another. Balfour figures a similar section[3] at a slightly earlier age, when the mesoblast is one solid mass with no distinction into layers.

By stage D this separation of somatic and splanchnic layers, which is just commencing in fig. 1, has gone so far as to give rise to a distinct cavity in the head region on either side, while at a considerably later period (stages G or H) the separation extends further back, so as to form in the trunk the peritoneal or body cavity.

The whole of the cavity so formed may be termed cœlom; that part of it which is contained in the trunk is the body cavity, while the anterior part, situated in the head, is named by Balfour head cavity. This latter, as Balfour has pointed out,[4] "can only be looked on in the light of a direct continuation of the body or peritoneal cavity into the head."

Plate V, fig. 2, shows the condition of the head cavities at stage H, as seen in a transverse section through the hind brain. The cavities (*h.c.*) are of considerable size; their ventral ends lie against the sides of the alimentary canal (*al.*), with which they are in close contact, while dorsally they extend some distance up the sides of the brain. The section also passes through the roots (VII) of the seventh or facial nerves, whose distal ends are seen to be in very close contact with the dorsal walls of the head cavities. The walls of the cavities are formed of a single layer of short columnar or almost cubical cells.

The sections from the same embryo in front of the one figured

---

[1] Op. cit., p. 86.

[2] Throughout the present paper I have employed, in order to distinguish the different stages of development, the nomenclature proposed by Mr. Balfour in his work cited above.

[3] Op. cit., plate ix, fig. 2.

[4] Op. cit., p. 86.

show that the cavities at this stage extend forwards beyond the anterior end of the alimentary canal and end rather abruptly immediately behind the outgrowths of the fore brain that give origin to the optic vesicles. I have not found either at this or any other stage any trace of a head cavity in front of the optic vesicles. The cavities of the two sides are at stage H quite distinct from one another, though their walls are very close together anteriorly.

During stage G a pair of lateral diverticula arise from the alimentary canal, and form the rudiments of the first pair of visceral clefts—the spiracular or hyomandibular clefts. These diverticula, as they increase in size, first press the two layers of mesoblast together, so as to obliterate the head cavities opposite their points of impact, and then gradually displace the mesoblast, the hypoblast of the diverticula ultimately coming into contact with the external epiblast. After a short pause perforation of the epiblast is effected, and the visceral cleft is completed. In this way the head cavity on either side becomes divided into a part in front of the hyomandibular cleft, and a part behind this cleft.

Balfour's account of the succeeding stages, which my own observations simply confirm, is as follows:[1]—"During stage I this front section of the head cavity (the part in front of the hyomandibular cleft) grows forward and becomes divided, without the intervention of a visceral cleft, into an anterior and a posterior division. The anterior lies close to the eye and in front of the commencing mouth involution. . . . . The posterior part lies completely within the mandibular arch. . . . . As the rudiments of the successive visceral clefts are formed the posterior part of the head cavity (behind the hyomandibular cleft) becomes divided into successive sections, there being one section for each arch. Thus, the whole head cavity becomes, on each side, divided into—(1) a premandibular section; (2) a mandibular section; (3) a hyoid section; (4) sections in the branchial arches."

The obliteration of the head cavity by the rudimentary visceral clefts is well shown in Plate V, fig. 3, which represents a somewhat oblique section, passing through the hind brain (*h. b.*), auditory pits (*aud.*), and third visceral (second branchial) clefts (*al'*) of an embryo of stage I; *h. c.* is the portion of the head cavity left between the second and third visceral clefts, *i. e.* in the first branchial arch; *al'* is the diverticulum of the alimentary canal which forms the rudiment of the third visceral cleft, opposite which the two layers of mesoblast are seen to be brought into contact so as to obliterate the head cavity; and *b. c.* is the

---

[1] Op. cit., p. 206.

cœlomic cavity left between the two layers of the mesoblast further back.

In the anterior part of the head the dorsal ends of the head cavities are situated above the tops of the visceral clefts (*vide* fig. 2); consequently, after these clefts are formed, the several head cavities, while they are separate from one another below, still communicate together dorsally. At the commencement of stage I the dorsal ends of the three anterior head cavities still communicate together, but between stages I and K they become separated. This point, which is not noticed by Balfour, appears to be one of some importance, inasmuch as we have in this division of the dorsal part of the head cavity a segmentation of the mesoblast of the head, which is not directly caused by the visceral clefts, although it takes place along the same lines, or, more strictly, along dorsal prolongations of these lines.

The dorsal portions of the first three head cavities lying above the level of the tops of the visceral clefts (*vide* Plate V, fig. 9, 1, 2, 3) become, at a still later stage, cut off from the ventral portions lying in the visceral arches. The subsequent changes undergone by these dorsal and ventral portions differ materially from one another, as will be noticed later on. In the trunk we also find a division of the cœlomic cavity on either side into a dorsal or vertebral portion, which forms a series of cavities occupying the centres of the protovertebræ, and a ventral or parietal portion forming the peritoneal cavity.

It becomes now an interesting question, which, owing to insufficient material, I am unable as yet to answer definitely, whether this division of the head cœlom into dorsal and ventral portions is not strictly comparable to the division of the body cœlom into vertebral and parietal portions. I have only observed these dorsal portions in the first three head cavities—the premandibular, mandibular, and hyoidean cavities.

"Between stages I and K the anterior (premandibular) cavities of the two sides are prolonged ventralwards, and meet below the base of the fore brain. The connection between the two cavities appears to last for a considerable time, and still persists at the close of stage L."[1] This median communication between the two premandibular cavities is shown at stage L in Plate V, fig. 13, 1. Fig. 14 represents a longitudinal and vertical section very near to the middle line, and shows the premandibular cavity (1), still of considerable size. Fig. 13 represents the median section from the same embryo; at 1 is seen the median communication between the two premandibular cavities; this is small, but has a perfectly distinct and obvious lumen. This median portion of the first head cavity has interesting relations to the

---

[1] Balfour, op. cit., p. 207.

notochord and the pituitary body. As seen in the figure, the notochord (*n.*) as it runs forward beneath the hind brain tapers considerably. On reaching the level of the head cavity it becomes still more constricted; it also leaves the floor of the hind brain, becomes curiously twisted on itself, and, after running forward a short distance in the mesoblast, bends sharply downwards and backwards, its terminal portion being very closely applied to the dorsal wall of the head cavity. The figure also shows that this median portion of the head cavity is in very close relation both to the outgrowth from the fore brain to form the infundibulum (*inf.*), and to the pituitary involution from the mouth (*pit.*)

At stage M these relations remain unchanged, the sole difference being that the two walls of the head cavity have come in contact so as to obliterate the lumen. By stage O the notochord has lost its connection with the head cavity and now runs straight forwards to its termination, while the walls of the head cavity are reduced to a very thin cellular plate, lying in close contact with the infundibulum and pituitary involution. I have not followed the fate of this median cavity any further, and have not determined whether it forms any part of the adult pituitary body, though, from its position, this would appear not improbable.

In fig. 23 this median portion of the first head cavity (1) is shown in horizontal section at a stage intermediate between L and M very shortly before its obliteration.

The premandibular cavity itself at stages K and L "forms a space of considerable size, with epithelial walls of somewhat short columnar cells."[1] Its position and relations are well shown in fig. 4, and in the series of figs. 8 to 14. It lies, as shown in figs. 4 and 8, very close to the posterior surface of the eye (*o. v.*). During stage M it becomes still more closely applied to the eye; it becomes partially doubled up on itself so as to form a hollow cup, which closely invests the eye on its posterior and inner surfaces, as shown in figs. 18 and 20 (1), and figs. 34 and 35. From the walls of this cup, as will be shown later on, certain of the eye muscles are developed.

This premandibular cavity presents at certain stages a marked constriction at its ventral part, tending to partially divide the cavity into two; this is shown at stage L in fig. 9; and still more clearly at a stage intermediate between L and M in fig. 21. Whether this indicates an aborted division of this cavity into two parts, each equivalent to one of the posterior cavities, or merely a division into dorsal and ventral portions such as occurs in the hinder cavities, I have been unable to determine.

The premandibular cavity persists very much longer than any

---

[1] Balfour, op. cit., p. 206.

of the hinder cavities. Its more deeply situated portion is shown in figs. 26 and 27, at a stage between M and N; and figs. 36 and 37 show that at stage O it not only persists but is considerably larger than at the earlier stages.

The second or mandibular cavity presents during stages K and L, as shown in fig. 5 (2), and in figs. 7 to 12, a dorsal dilated portion, and (fig. 5) a laterally compressed ventral prolongation extending down the whole length of the mandibular arch. During these two stages the dorsal dilated end lies very close to the surface immediately beneath the epiblast; it lies at a more superficial level than the cavities in front of and behind it, so that in a series of longitudinal and vertical sections the most superficial sections cut this cavity alone (fig. 7, 2); the subsequent ones cut all three cavities (figs. 8 to 10); while still deeper ones cut the first and third only (fig. 12); and deeper still only the median portion of the first (fig. 13).

By the commencement of stage M the mandibular cavity is still but little altered (*vide* figs. 21, 22, and 23, 2); it is relatively smaller, but still presents a dorsal dilatation and a flattened prolongation extending down the mandibular arch. By the middle or end of stage M the dorsal portion has atrophied and disappeared; the prolongation into the mandibular arch persists longer; its walls become converted into muscles,[1] but the distal portion of the cavity still persists at stage O (*vide* fig. 34).

The third or hyoidean cavity is very similar to the second; it is from the first rather smaller, and situated, as already noticed, at a rather deeper level. Its position and relations are shown at stages K and L in figs. 4, 5, and 8 to 12 (3). It is also shown between L and M in figs. 24 and 25 (3). Like the mandibular cavity it presents a dorsal dilated part and a ventral laterally compressed portion extending down the hyoidean arch. The dorsal portion, like the corresponding part of the mandibular cavity, disappears during stage M; the ventral part persists longer, and its terminal portion is shown at the extremity of the hyoidean arch at stage M in figs. 15 and 16. Its walls like those of the mandibular cavity become ultimately converted into muscles.

Relations of Cranial Nerves to Head Cavities.—Certain of the cranial nerves have, as has already been shown by Balfour, very definite and very important relations to the head cavities, and especially to the dilated dorsal ends of the three anterior cavities. A full account will be given later on under the heads of the several nerves; here I propose merely to note the general relations.

---

[1] Balfour, op. cit., p. 208.

The main trunk of the seventh or facial nerve lies immediately behind and in very close contact with the third or hyoidean cavity; this is shown in figs. 5, 8, and 9 (VII). Similarly the main trunk of the fifth or trigeminal nerve lies wedged in between the second and third, the mandibular and hyoidean cavities, as is clearly shown in figs 5, 9, and 10 (V).

The relations of these two nerves—the seventh and the fifth—to the hyoidean and mandibular cavities are very definite, and are very early acquired; they are fully established before the close of stage I, and have been already fully described by Balfour.[1] They establish the fact that if the head cavities are to be taken as indicating head segments, about which there can be but little doubt, then these nerves must also be spoken of as segmental nerves.

I now turn to a point of very considerable interest, which has not, I believe, been noticed hitherto. Wedged in between the first and second, premandibular and mandibular cavities, and occupying a position precisely analogous to that held by the fifth and seventh nerves one or two segments further back respectively, is a ganglion, shown in figs. 4, 10, and 11 (*c. g.*). From this ganglion a nerve can be traced forwards, figs. 13 and 14 (III), to the base of the mid brain. This nerve is conclusively shown by its origin from the base of the mid brain, by its course, and by its distribution, at a slightly later stage, to certain of the eye muscles, to be the third or oculomotor nerve; while the ganglionic expansion on it, between the first and second head cavities, I believe I shall be able to prove, in a later section of this paper, to be the ciliary ganglion.

I am not aware that the third nerve has been described by previous observers in Elasmobranch embryos, but if the figures I here give of it be compared with the figures and descriptions I have previously given of the development of the same nerve in the chick,[2] it will be seen that the resemblance between the two forms is of a very striking and conclusive character.

If the relations of the fifth and seventh nerves to the second and third head cavities demonstrate the segmental value of these nerves, then the relation which I have just pointed out the third nerve bears to the first and second head cavities must also be held to demonstrate the segmental value of this nerve.

I have previously attempted, in my paper on the nerves of the chick, referred to above, to prove that the third nerve is a segmental nerve strictly equivalent to the fifth, the seventh, or any other of the segmental cranial nerves. The discovery that the third nerve has, in Elasmobranchs, from a very early period

---

[1] Op. cit., p. 197, *seq.*

[2] This Journal, January, 1878, p. 23, *seq*, and Plates II and III.

of development, the characteristic relation to the head cavities that is possessed by the fifth and seventh nerves, affords, perhaps, the strongest possible confirmation of the segmental value of the third nerve. Fig. 4 shows that this characteristic relation is acquired by the third nerve at any rate as early as stage K.

I propose now to consider certain phases in the development of these three nerves, the seventh, fifth, and third pairs. My observations on the earlier stages are very fragmentary, and, therefore, I shall omit for the present all account of their development previous to the latter part of stage I.

The Development of the Third (Oculomotor) Nerve.—I have not observed this nerve in specimens earlier than the commencement of stage K; but by this time it is already a nerve of considerable length, with its more important branches fully developed, so that it must certainly be present at a considerably earlier period. In the chick I have elsewhere shown[1] that the third nerve is one of the first nerves, if not the very first nerve, in the body to appear; and my observations on Elasmobranchs, though inconclusive on this point, yet show no reason why the same should not be the case in them.

In embryos of stage K the third nerve is a conspicuous and easily recognisable object; it arises from the base of the mid brain, not far from the mid ventral line, the roots of the two nerves being only a small distance apart. Its root, which is expanded somewhat, and has a triangular shape, contains numerous nerve-cells. From this ganglionic root (shown at stage L in fig. 14 (III) the nerve runs as a long slender stem almost horizontally backwards, then turns slightly outwards to reach the interval between the dorsal ends of the first and second head cavities, where it expands into a small ganglion (*c. g.*, fig. 4). From this ganglion two main branches arise; of these, one (III *b*, fig. 4) continues the course of the main stem of the nerve, and runs down between the first and second head cavities; the second branch (III *a*, fig. 4) runs directly forwards from the ganglion, passing along the top of the first head cavity, then along the inner side of the eye, and finally terminates at the extreme anterior end of the head, just dorsad of the olfactory pit (*olf.*).

The ganglion (*c. g.*) receives also a short but very interesting branch (V. *d.*, fig. 4) coming direct from the large ganglion at the root of the fifth nerve. This communicating branch between the third and fifth nerves is apparently that which is described and figured by Balfour as the rudiment of the ophthalmic branch

---

[1] Loc. cit., p. 27.

of the fifth.[1] I propose to consider it more fully in a later section of this paper.

The condition of the third nerve and its branches at stage L is shown in the figs. 10 to 12 and 14, representing longitudinal and vertical sections at various depths of the same embryo. In fig. 10, the most superficial of these sections, the ganglion (*c. g.*), is shown wedged in between the first and second head cavities; the figure showing, in addition, the roots of the two branches (III *a* and III *b*), already described, and also the posterior half of the communicating branch (V. *d.*) between the fifth and third nerves. In fig. 11, taken at a deeper level, the anterior end of this connecting branch is shown. Fig. 14, at a still deeper level, shows the end of the third nerve just before reaching the ganglion; while, finally, fig. 14, which is the deepest section of the series, not far from the midline, shows the greater part of the length of the nerve, with the triangular ganglion at its root of origin from the mid brain.

Towards the end of stage L, besides this ganglionic root of origin shown in fig. 14, two or three small additional roots are developed; these are very slender, are situated in front of the main ganglionic root, and differ markedly from this root by having no ganglion cells. Towards the end of stage L, then, the third nerve arises by one large ganglionic root and two or three small, slender, non-ganglionic roots placed in front of the main and original root. In stages M, N, and O, these small anteriorly situated roots become much more evident and also increase in number; they are shown at stage O in fig. 40 (III). The morphological significance of these additional roots is discussed later on.

The condition of the nerve between stages L and M is shown in the series of horizontal sections represented in figs. 22 to 24. The section shown in fig. 22 is a curiously lucky one, inasmuch as it includes the whole length of the third nerves (III) on both sides from their origins from the mid brain to the ganglia (*c. g.*) between the first and second head cavities. The roots of the two nerves are seen to arise very close together from the ventral surface of the mid brain; each nerve runs for a short distance almost directly backwards, then turns somewhat outwards, and runs to the interval between the first and second head cavities (1 and 2), where it expands into the ganglionic swelling (*c. g.*). Fig. 23, taken at a rather more dorsal level, shows the root of the third nerve on the right-hand side and the ganglia (*c. g.*) on both sides, while fig. 24, at a still more dorsal level, shows the communicating branch (V. *d.*) between the ganglia of the third and fifth (V.) nerve.

---

[1] Op. cit., pp. 197, 198, and plate xiv, figs. 9 *b* and 16 *b*.

The branches of the third nerve beyond the ganglion (*c. g.*) are shown at stage M in the series of longitudinal and vertical sections drawn in figs. 15 to 20; of which series fig. 15 is the most superficial, and figs. 19 and 20 (which belong to opposite sides of the body) the deepest. In fig. 20, the branches already described are well seen. The ganglion (*c. g.*) receives the communicating branch (V. *d.*) from the Gasserian ganglion on the fifth, while from it proceed the branches III *a.* and III *b.* already described at an earlier stage. Of these the anterior (III *a*) runs directly forward through the walls of the first head cavity (1), then along or rather through the substance of the inner wall of the eye, in front of which it continues its course straight forward, as shown in figs. 19, 18, 17, and 16 (III *a*), until it ends, as seen in fig. 15 (III *a.*), at the extreme anterior end of the head, just dorsad of the olfactory pit (*olf.*).

The second branch (III *b*) from the ganglion (*c. g.*) is practically the direct continuation of the main stem of the nerve; it runs down, as seen in figs. 19 and 20 (III *b*), in very close contact with the posterior wall of the first head cavity (the second cavity having already disappeared). This posterior wall, as will be fully described later on, is by this time converted in part into certain of the eye muscles; and this branch of the third nerve, which lies in close contact with these muscles (*vide* fig. 19) and supplies them, ends in the most ventrally situated of these muscles (*o. i.*, figs. 19 and 20).

At stage O the third nerve has still the same appearance, which is indeed almost identical with that of the adult, as is shown in the series of figures 33 to 40. Fig. 40, the deepest section of the series, shows the large ganglionic root and the smaller anterior non-ganglionic roots very clearly. From its root the nerve can be traced running backwards and outwards in figs. 38, 37, and 36, until it reaches, in fig. 36, the posterior wall of the first head cavity. Fig. 35 shows the branches (III *a*) and (III *b*), also the communicating branch from the fifth nerve; and in figs. 34 and 33, the terminal branch (III *b*) is clearly seen ending in the muscle (*o. i.*).

On comparing the condition of the third nerve here described in embryos from stage K upwards with that occurring in the adult, there can, I think, be no doubt whatever that the ganglion (*c. g.*) which lies wedged in between the first and second head cavities is the ciliary ganglion. Professor Schwalbe has recently pointed out, in a very important memoir on the morphology of the ciliary ganglion, that it is really a ganglion belonging to the main stem of the third nerve.[1] He has shown that in Elasmo-

---

[1] Schwalbe, "Ueber die morphologische Bedeutung des Ganglion Ciliare," 'Sitzungsberichte der Jenaischen Gesellschaft für Medicin und

branchs, Amphibia, and some other forms, as the Crocodile, the ciliary ganglion is in the *adult* situated in the trunk of the third nerve, and has brought forward very strong arguments for regarding this as the primitive position of this ganglion.

In *Scyllium,* as is evident from comparing Schwalbe's figures and descriptions of the adult[1] with the figures and descriptions here given of embryos, there is practically no change in the adult from the embryonic condition; in the adult, as in the embryo, the ganglion is situated in the trunk of the third nerve; it is also situated in the adult in the very same position occupied by the ganglion (*c. g.*) in the embryo, *i. e.* opposite the point where the communicating branch from the fifth joins the third, and where the third divides into the two branches (III *a* and III *b*).

Very strong evidence in support of the view advocated by Professor Schwalbe is afforded by the development of the ciliary ganglion in the chick. In the adult fowl the ciliary ganglion is not situated, as it is in the dogfish, on the trunk of the third nerve, but at the base of a short ciliary nerve arising from the third nerve. In the embryo chick, at the end of the fourth day of incubation, I have already figured[2] a ganglionic swelling on the third nerve, exactly corresponding in position, relations, and appearance with the ganglion (*c. g.*) of the *Scyllium* embryo. Professor Schwalbe, in referring to my paper, has suggested[3] that this ganglion, the existence of which has been somewhat gratuitously called in question by Professor Kölliker,[4] is the rudiment of the ciliary ganglion. I fully accept this suggestion of Professor Schwalbe's; I had, indeed, arrived at the same conclusion previous to receiving his paper, and since then I have directed my attention specially to the point, and have satisfied myself that this ganglion, which in the embryo chick is situated in the trunk of the third nerve (in the same position held by the ciliary ganglion in both the embryo and the adult *Scyllium*), becomes the ciliary ganglion of the adult, which is no longer situated in the trunk of the third but on one of its branches.

I think that this fact, that the ciliary ganglion of the chick embryo occupies the position that it retains throughout life in the Elasmobranchs and Amphibia, supplies the embryological

Naturwissenschaft,' 15 November, 1878, and "Das Ganglion Oculomotorii," 'Jenaische Zeitschrift für Naturwissenschaft,' Bd. xiii. It is to the latter paper, which is much the more complete, that I shall refer in future.

[1] Loc. cit., Taf. xiii, fig. 10.

[2] Loc. cit., plate ii, fig. 22.

[3] Loc. cit., p. 60.

[4] 'Entwicklungsgeschichte des Menschen u. der Höheren Thiere,' 1879, p. 622.

proof necessary for the complete establishment of the view in favour of which Professor Schwalbe has brought forward such a mass of anatomical evidence, viz. that the ciliary ganglion is primitively a ganglion belonging to the stem of the third nerve. For a full account of the various modifications presented by the ciliary nerves in adult Vertebrates I would refer the reader to Professor Schwalbe's very interesting memoir cited above.

On one point, however, I cannot completely agree with Professor Schwalbe, *i. e.* when he says that the ciliary ganglion is the homologue of a spinal ganglion.[1] That it corresponds in part there can be, I think, no doubt; but it seems to me preferable to regard the ganglion at the root of the third, together with the ciliary ganglion, and any intermediate ganglia that may be found (for which *vide* Schwalbe's paper) as collectively equivalent to the Gasserian ganglion, or to one of the spinal ganglia. I am disposed to view the third nerve as having been abnormally pulled out and lengthened by the rapid growth of the part of the brain with which it is connected, and to regard the whole trunk, from the root of origin to the point of division at the ciliary ganglion, as corresponding to the part of the fifth bearing the Gasserian ganglion, and to compare any ganglia that may occur on this part of the nerve to detached portions of the Gasserian ganglion, isolated by the lengthening process which the third nerve has undergone.

In naming and determining the other branches of the third nerve, those I have marked III *a* and III *b*, I have been much assisted by the very careful description given by Schwalbe of the nerve in the adult *Scyllium*.

Schwalbe[2] describes and figures the third nerve in the adult as giving off branches to the *rectus superior* and *rectus internus*, then receiving at the ciliary ganglion a branch from the fifth nerve, and then dividing behind the posterior border of the *rectus superior* into two branches, of which the first passes beneath the *rectus inferior*, supplying it with branches, and then runs forward beneath the optic nerve to the *obliquus inferior*, in which it ends. The second branch runs forward on the inner side of the eye, piercing the sclerotic; it passes beneath the *rectus superior* and *obliquus superior*, but over the optic nerve; it leaves the orbit in front by a canal above the origin of the *obliquus inferior*, and then runs forward to the anterior part of the head.

This description and the accompanying figures show that in *Scyllium* the third nerve has acquired by stage K all the principal branches of the adult, and that these branches have also acquired

[1] Loc. cit., p. 68.

[2] Loc. cit., pp. 15, 16.

their characteristic course and relations. The communicating branch from the fifth to the ciliary ganglion is undoubtedly the branch I have marked V *d.* The first of the two branches into which the nerve divides beyond the ganglion is the one marked in my figure III *b,* while the second of these branches, the one which pierces the sclerotic, passes through the orbit, lying above the optic nerve, and finally runs to the front of the head, is, beyond all doubt, the nerve I have marked III *a.*

The nerve III *a,* which runs through the orbit beneath the *rectus superior* and *obliquus superior,* and above the optic nerve, is the nerve which, in many Vertebrates, receives the name *ramus ophthalmicus profundus* of the fifth nerve. In most cases it has the appearance of a branch of the fifth nerve; this appearance I believe to be due to the communicating branch from the fifth (V *d.*) becoming directly continuous with the anterior branch of the third (III *a*) and to the nerve thus formed losing its connection with the ciliary ganglion. Should this conjecture prove to be correct it will probably be found that in the early stages of development the nerve III *a* is connected with the ciliary ganglion, and that the connection is only lost comparatively late.

Summary.—The third nerve, at stage K, arises from the mid brain by a single ganglionic root; it runs back to the interval between the first and second head cavities, where it expands into a ganglion—the ciliary ganglion. This ganglion receives a short communicating branch from the fifth nerve; beyond it the nerve divides into two branches, of which one continues the course of the main stem, and ends in the *obliquus inferior,* while the other runs forward through the orbit, and is the nerve usually described as the *ramus ophthalmicus profundus* of the fifth. My observations leave no doubt in my mind that in *Scyllium* this nerve is really a branch of the third. Finally, in the later stages, a number of slender non-ganglionic roots of origin appear in front of the original ganglionic root.

The Development of the Fifth (Trigeminal) Nerve.—At the commencement of stage K the fifth nerve arises by a single root on either side from the lower part of the side of the hind brain. Immediately beyond its root of origin it expands to form a large ganglionic swelling—the future Gasserian ganglion—the lower part of which is wedged in between the dorsal ends of the second and third head cavities. From this ganglion three branches arise:—*a.* From the upper and anterior angle of the ganglion, close to its root of origin, a nerve arises which runs forwards and upwards and will be spoken of as the ophthalmic branch of the fifth. *b.* From the lower and anterior part of the ganglion a nerve (v *d.*, fig. 4) runs forward along the top of the second head cavity to the ciliary ganglion of the third nerve (*c. g.*), this being the communicating branch between the fifth and third nerves already mentioned. *c.* From the most ventrally situated part of the ganglion a nerve runs down in the interspace between the second and third cavities, and is then continued down in the mandibular arch; this may be called the mandibular nerve.

Balfour describes only two branches of the fifth nerve at this stage,[1] of which the posterior is the mandibular branch of the above description, whilst the anterior, which he describes and figures[2] as lying in close contact with the upper wall of the second cavity, and which he names the ophthalmic branch of the fifth, is clearly the second of the three branches I have described above. The first branch, to which, for reasons to be fully stated hereafter, I prefer to give the name ophthalmic branch of the fifth, is not distinguished by Balfour from the second.

By stage L certain changes have occurred. In the first place two or three new roots of origin are now present, which were not recognisable at the earlier date. These roots, which are well shown in fig. 6 (v *f*), and also in fig. 11 (v *f*), are situated in front of the original roots of origin; they are very slender, are apparently variable in number, and differ materially from the original root by being totally devoid of ganglion cells. Another point of difference is that, opposite the ganglionic root of origin, the brain presents a distinct external prominence or bulging, well seen in figs. 6 and 11, while no such prominence occurs opposite these new roots. These additional roots increase in number during the later stages. By stage O one of them, usually the most anterior, has become considerably larger than the others (*vide* fig. 36, v *f*), though it still stands in marked contrast with the original root by having no ganglion cells, and by not presenting the external bulging of the brain at its point of origin. These roots will be again referred to later on. I have been unable to determine with certainty whether they arise as outgrowths from the brain or from the ganglion.

By stage L an anterior branch—the maxillary nerve—is given off from the mandibular nerve. This branch is also described and figured by Balfour.

Fig. 5 shows the roots of the ophthalmic and the communicating branches (v *a.* and v *d.*), and fig. 6 shows the ophthalmic branch (v *a.*), as well as the two kinds of roots of origin at stage L. Figs. 9 to 11 also show these branches in an embryo of the same age.

[1] Op. cit., p. 197.

[2] Op. cit., plate xiv, figs. 9 *b* and 16 *b*.

The several branches of the fifth nerve are shown at stage M by the series of figs. 15 to 20, of which, as already noticed, fig. 15 is the most superficial, and fig. 20 the deepest. In figs. 16 and 17 the maxillary (V *b.*) and mandibular (V *c.*) branches are shown very clearly; these two nerves lie close to the surface, though, as we shall see immediately, the maxillary is separated from the surface by the palatine branch of the seventh. In fig. 19 the communicating branch (V *d.*) between the fifth and third nerves is shown lying at a deeper level than the maxillary and mandibular nerves; it is a short nerve connecting the Gasserian and ciliary ganglia directly together. The same figure shows also two portions of the ophthalmic branch (V *a.*) of the fifth, which runs forward close to the dorsal surface of the head and immediately beneath the superficial epiblast. In fig. 20, taken from the same embryo as fig. 19, but from the opposite side, the root of the ophthalmic nerve (V *a.*) and the communicating branch (V *d.*) are well seen; the latter is seen to give off a fine branch (V *e.*), which runs upward to the muscle marked *o. s.*

Fig. 24 shows the roots of the ophthalmic and communicating branches in horizontal section; the former (V *a.*) is seen to be very close to the surface, while the latter (V *d.*) is situated more deeply.

Figs. 26 to 30 represent a series of transverse sections through the head of an embryo between stages M and N; they serve to illustrate the above description from a different point of view. The sections are all slightly oblique, so that in each section the left-hand half is in a plane a little posterior to the right-hand half. Fig. 26 passes through one of the anterior non-ganglionic roots of the fifth (V *f.*), it also shows on the left side the root of the ophthalmic nerve (V *a.*), and the whole length of the communicating branch (V *d.*) running to the ciliary ganglion (*c. g.*). On the right hand side the ophthalmic nerve (V *a.*) is seen in section.

Fig. 27 passes on the left side through the main or ganglionic root of the fifth; on the right side it corresponds to the left side of fig. 26. Fig. 28 shows on the left side the maxillary nerve (V *b.*) in nearly the whole of its length.

Finally, in figs. 32 to 35 the condition and relations of the ophthalmic branch at stage O are shown; it is seen to be a slender nerve running forward along the dorsal surface to the fore part of the head, and giving off branches (fig. 32) on its course.

Summary.—At stage K the fifth nerve arises from the hind brain by a single large ganglionic root, it expands into a large ganglionic swelling, from which three nerves arise: (a), the ophthalmic branch, which runs along the dorsal surface to the anterior part of the head; (b) the communicating branch running direct to the ciliary ganglion; (c) the mandibular branch, which runs down between the second and third head cavities, and then along the mandibular arch. By stage L two or more slender non-ganglionic roots appear in front of the original ganglionic root, and the maxillary nerve is given off as a branch from the mandibular.

The Development of the Seventh (Facial) Nerve.—The facial and auditory nerves at stage K arise by a single root from the hind brain, a short distance behind the fifth nerve. This root has the same ganglionic character as the original root of the fifth, and has a similar bulging outwards of the hind brain opposite its point of origin. The nerve divides almost immediately into an anterior part—the facial, and a posterior—the auditory nerve. The latter runs almost directly backwards, and becomes applied to the anterior wall of the auditory vesicle (figs. 7, 8, and 9 VIII *aud.*). The facial nerve gives off three main branches: (a), from its upper and anterior part a very large stout nerve arises, which runs forward along the dorsal surface of the head to its anterior end, lying immediately above the ophthalmic branch of the fifth, and immediately beneath the superficial epiblast; this I shall speak of as the ophthalmic branch of the seventh; (b) the second branch arises from the anterior part of the facial nerve just below the root of the ophthalmic branch; this, which is also a stout nerve, runs downwards and forwards, lying parallel to and immediately superficial to the maxillary branch of the fifth; it may be spoken of as the palatine nerve; (c) the main stem of the seventh is continued downwards as a stout nerve which runs along the posterior or hyoidean border of the spiracular or hyomandibular cleft. From this hyoidean branch an anterior or mandibular branch is given off at a slightly later stage, which runs over the top of the spiracular cleft, and then down in its anterior or mandibular wall.

These branches of the seventh nerve are well shown at stage M in figs. 15 to 18. The large ophthalmic branch (VII *a.*) is seen at its origin in fig. 17, while figs. 16, 18, 19, show other portions of its course; its relation to the ophthalmic branch of the fifth (V *a.*) is seen in fig. 19. The root of this ophthalmic branch of the seventh is also well shown at a slightly earlier stage (between L and M) in fig. 25 (VII *a.*), which shows how very closely it lies to the surface. It is seen in transverse section in figs. 26, 27, and 28 (VII *a.*), and finally, is shown along the whole of its course at stage O in figs. 32 to 35, these figures showing its relation to the ophthalmic branch of the fifth (V *a.*) particularly clearly.

The palatine nerve is shown at stage M in figs. 15, 16, and 17 (VII *b*); it is a stout straight nerve, lying immediately superficial to the maxillary branch of the fifth and very close to this latter; these relations are still better seen in the transverse sections shown in figs. 28 and 29; in both these figures the two nerves (VII *b.*) and (V *b.*) are seen running down side by side, and so close together as almost to touch at places.

The hyoidean branch (VII *c.*) is shown in figs. 15, 16, and 18; and finally, the mandibular branch (VII *d.*) is well seen in figs. 32, 33, and 34.

The seventh nerve is, in these stages, a very large nerve, very much larger than the fifth; two of its branches, the ophthalmic and palatine, accompany branches of the fifth, the ophthalmic and maxillary, very closely indeed, the branches of the seventh lying in both cases more superficially.

Finally, the seventh nerve never acquires additional non-ganglionic roots of origin, such as have been described as occurring in both the third and fifth nerves; this is a distinction of some importance and one to which I shall refer again further on.

Comparison of the Third, Fifth, and Seventh Nerves.—In the section on the relations of these nerves to the head cavities it has been shown that the third has the same right to be considered a segmental nerve that the fifth and seventh nerves have; and that it must, therefore, be regarded as a nerve of equal morphological importance with the latter. It is, therefore, a point of considerable importance to determine how far the several branches of these three nerves can be compared with one another.

All three nerves arise at first by single ganglionic roots; in the case of the third and fifth additional non-ganglionic roots are subsequently acquired, but in the case of the seventh no such roots are acquired. I hope to show shortly that this apparent distinction is capable of full and satisfactory explanation.

Of the three nerves the seventh is very much the largest, the third very much the smallest, the fifth, which is intermediate in position, being also intermediate in size; this statement applying not only to the main trunk, but to the individual branches as well.

In comparing the fifth and seventh nerves, there can be but little doubt that the mandibular branch of the fifth corresponds to the hyoidean branch of the seventh; and when we bear in mind that this branch in each case is the apparent direct ventral continuation of the main trunk, and in the early stages in close relation with the posterior wall of a head cavity, it will, I think, follow that the branch (III *b.*) of the third, which supplies the *rectus inferior* and *obliquus inferior*, which is the apparent direct ventral continuation of the main trunk of the third, and which is in very intimate relation with the posterior wall of the first head cavity, is the corresponding branch of the third nerve, and is the strict homologue of the branches of the fifth and seventh nerves mentioned above.

It would also appear probable, from the times of their appearance and their general relations, that the maxillary branch of the fifth and the mandibular branch of the seventh are equivalent nerves.

Mr. Balfour, who was the first to describe the remarkable ophthalmic branch of the seventh,[1] has already shown that it is strictly comparable to the ophthalmic branch of the fifth. Schwalbe[2] describes and figures the *ramus ophthalmicus superficialis* in the adult *Scyllium* as arising by two roots, a posterior *radix dorsalis* and an anterior *radix ventralis* or *profunda ;* these enter the orbit by two separate foramina, run forward through the orbit as the *portio major* and *portio minor* of the *ramus ophthalmicus superficalis*, lying dorsad of all the eye muscles, and finally, on leaving the orbit anteriorly, end in branches to the anterior part of the head. As the two nerves (VII *a.* and V *a.*) in the embryo at any stage from L upwards exactly correspond to this description, there is, I think, no reason to doubt that these branches, which I have called the ophthalmic branches of the seventh and fifth, become respectively the *portio major* and *portio minor* of the *ramus ophthalmicus superficialis* of the adult. Schwalbe's figures of the adult show that these branches acquire their final arrangement at a very early period, in fact, from their very first appearance; his figure of these ophthalmic branches in the adult represents, with almost perfect accuracy, their arrangement in embryos of stage L.

Balfour leaves the fate of the ophthalmic branch of the seventh undecided,[3] though he expresses himself as "inclined to adopt" the view of which I have attempted to demonstrate the correctness.

There appears to be no branch of the fifth corresponding to the palatine branch of the seventh; this latter is a very singular nerve, lying, as it does, so extremely close to the maxillary branch of the fifth. I thought at one time that it might

---

[1] Op. cit., p. 199.

[2] Loc. cit., p. 14.

[3] Op. cit., p. 200.

correspond to the buccal branch of the maxillary nerve, but its independent origin from the main stem of the seventh seems to disprove this.

The very remarkable communicating branch between the Gasserian and ciliary ganglia still remains for consideration; concerning it we know—(a) that it is developed very early, (b) that it is a direct connection between the main ganglia of the third and fifth nerves. I have no direct observations on its development, but am inclined to think it may be the remains of the commissure which (at any rate in the Chick,[1] and probably, also, in *Scyllium*) connects together, at an early stage, the rudiments of the third and fifth nerves. This suggestion renders it necessary for me to abandon the explanation I have previously given[2] of the ophthalmic branches of the seventh and fifth nerves as being possibly persistent rudiments of this commissure, as it is obvious that the ophthalmic branch (V *a.*) and the communicating branch (V *d.*) could not both be derived from this commissure.

Concerning the anterior branch (III *a.*) of the third nerve I feel in great doubt. It seems possible that it may be an ophthalmic branch of the third nerve, equivalent to the ophthalmic branches of the fifth or seventh, and its course and distribution certainly favour such a view. On the other hand, until the development of the fourth nerve has been satisfactorily determined, I think any attempt to determine the homologies of this branch of the third would be premature.

The Development of the Sixth (Abducens) Nerve.—Plate VI, fig. 38, represents a longitudinal vertical section through the head of a *Scyllium* embryo at stage O, the section being taken a short distance to one side of the median plane; it shows the three main divisions of the brain, the pineal gland (*pin.*) and infundibulum, also the lateral expansion (*pit.*) of the pituitary involution from the mouth. A portion of the third nerve (III) is, at a short distance from its origin, included in the section, which also shows the investing mass (*i. v.*) lying at the side of the notochord. Springing from the base of the third brain by a large number of slender roots and then running forward and losing itself in the investing mass is a nerve (VI). In the next section this nerve can be seen to come into contact with the end of a muscle (*r. e.*, fig. 37), which muscle can be traced through the more superficial sections in figs. 36, 35, 34, 33, 32, and ultimately, in sections not figured here, can be traced into contact with the eyeball, and can be recognised definitely as the

---

[1] *Vide* self, loc. cit.

[2] Loc. cit., p. 30.

rectus externus v. posterior. This nerve is identical in structure and relations with the nerve I have previously identified and described in the Chick as the sixth or abducens nerve.[1] If fig. 38 be compared with the figure I have elsewhere given of the sixth nerve in the Chick,[2] their identity will be recognised at once.

In fig. 39 an enlarged view is given of the nerve from the same section as fig. 38, in order to show its structure more clearly. The nerve itself is slender; it arises by a number of roots, eight or nine in number, arranged antero-posteriorly, so that its origin covers a considerable length of the base of the brain. Both the nerve itself and its roots consist of fusiform or still more elongated cells. *Neither in the nerve nor in any of its roots are there any ganglion cells.*

I have recognised the sixth nerve having all the characters above described, except a slightly smaller number of roots, in embryos of stage M, and also at stage L; in embryos younger than L I have failed, after most careful searching, to detect it.

In figs. 28 to 31 the sixth nerve is shown in transverse section at a stage between M and N. Fig. 28 passes through the *rectus externus* muscle on both sides, but is entirely in front of the nerve. Fig. 29, which is taken from a section a short distance further back, shows on the left side the sixth nerve cut transversely; on the right the posterior end of the rectus externus, and a small fragment of the nerve are shown; this section passes through the point at which, in fig. 38, the nerve bends down and loses itself in the investing mass.

Fig. 30 is taken still further back; it passes through the roots of origin of the sixth nerve on both sides. These roots lie entirely above the investing mass (*i. v.*), as is shown also in fig. 38. In fig. 31 the roots of the nerves (vi) are shown on a larger scale from a section belonging to the same series as figs. 28 to 30; it shows very clearly the non-ganglionic character of the roots.

Figs. 29, 30, 37, and 38, enable us to determine accurately the situation of the roots of the sixth as compared with the other nerves. Fig. 29 passes through the root of origin of the seventh nerve and through the whole length of its palatine branch (VII *h.*); it also passes through the sixth nerves just in front of their roots of origin. Fig. 30, which shows the roots of the sixth nerves, passes also through the extreme anterior ends of the auditory vesicles (*aud.*), through the main trunks of the seventh nerves, and through the auditory nerves (VIII) before they have reached the auditory vesicles.

---

[1] Loc. cit., p. 32, *seq.*

[2] Loc. cit., plate iii, fig. 25.

A comparison of these figures with figs. 37 and 38 shows that the sixth nerve arises on either side a short distance from the mid ventral line of the hind brain by a number of roots; the most anterior of these roots is situated about the level, or immediately behind the level, of the roots of the seventh nerve, and the most posterior of the roots is in front of the middle of the auditory vesicle. These roots differ totally from the root of the seventh nerve—(a) in being multiple instead of single, (b) in being devoid of ganglion cells. In these features, as well as in their point of origin from the brain, as shown in figs. 29 and 30, they bear a very close resemblance indeed to the anterior roots of a spinal nerve. Indeed, these two figures seem to me to leave no doubt that *we must regard the sixth nerve as having the same relation to the seventh that the anterior root of a spinal nerve has to its posterior root.*

In my paper on the cranial nerves of the chick I have already expressed the same opinion, and arrived at the same conclusion, as to the morphological value of the sixth nerve.

The question of the existence or non-existence of anterior roots to the cranial nerves, interesting enough before, acquires additional importance from the arguments based by Mr. Balfour on the supposed non-existence of such roots. In Amphioxus all the nerves possess single roots, which roots correspond, as Balfour has conclusively shown, to the dorsal or posterior roots of other Vertebrates. Assuming that this is the primitive condition, of which strong evidence is afforded by the actual development of the spinal nerves, Balfour, failing to find anterior roots to the cranial nerves, argues that the "retention of the primitive condition in the brain implies that this became diffentiated from the remainder of the nervous system at a very early period before the acquirement of anterior nerve roots, and that these eventually became developed only in the case of spinal nerves, and not in the case of the already highly modified cranial nerves."[1]

It will be seen that this argument amounts to little more than an explanation of the supposed absence of anterior roots to the cranial nerves, which explanation becomes, of course, unnecessary if even a single undoubted anterior root is discovered in a cranial nerve. There is no *à priori* improbability that such anterior roots should exist; on the contrary, it is, to my mind, in the highest degree improbable that the cranial nerves should in such a point have preserved a more primitive arrangement than the far less complex spinal nerves. Therefore, when we find a nerve in embryos of stage L having the characteristic position, structure, and appearance of an anterior spinal root, and bearing the same relations to the seventh nerve that an anterior spinal root

---

[1] Op. cit., p. 193.

does to a posterior root, I see no reason to doubt that this nerve (the sixth) is to be considered as the anterior root of the seventh.

The sole point of any importance in which the sixth differs from a typical anterior root is that it does not join with its posterior root, the seventh. I do not think, however, that this point can be regarded as of sufficient importance to render the comparison invalid.

It becomes now a matter of much interest to determine, if possible, whether anterior roots are present in the other cranial segmental nerves. Such roots have been described by Gegenbaur in the adult *Hexanchus*,[1] by Jackson and Clarke in the adult *Echinorhinus*,[2] by Balfour[3] (who, however, regards them as belonging really to the spinal and not the cranial nerves) in both adult and embryo examples of *Scyllium*, by myself in the embryo chick about the end of the fourth day,[4] and by others in different adult Vertebrates.

Are there any traces of such roots in the third and fifth nerves? In answer to this question I would suggest that the small anterior non-ganglionic roots, which I have described as appearing in both the third and fifth nerves during stage L, are really morphologically equivalent to anterior spinal roots. These roots closely agree with the roots of the sixth nerve in appearance; they are slender, non-ganglionic, multiple, arranged in an antero-posterior series, and appear later than the original ganglionic roots. The exact time of their appearance I have not determined with accuracy, but it is certainly noteworthy that the earliest period at which I have noticed these additional roots of the third and fifth nerves is also that at which I have first detected the sixth nerve, viz. stage L.

In the fact that their anterior roots are situated further forward than their posterior roots, the third and fifth nerves resemble the spinal nerves,[5] but differ from the seventh, whose anterior root (the sixth) we have seen to be situated a little behind the root of the seventh. This point is probably connected with the complete independence retained by the sixth nerve, and alluded to above.

The fact that the anterior and posterior roots of the third nerve arise at the same vertical level, and not one below the other, is, I think, fully explained by the great change in position which the posterior roots undergo, these posterior roots

---

[1] 'Jenaische Zeitschrift,' vol. vi.

[2] 'Journal of Anatomy and Physiology,' vol. x.

[3] Op. cit., p. 205.

[4] Loc. cit., p. 38.

[5] Balfour, op. cit., p. 160, and plate xvi, fig. 1.

arising from the brain in the adult, as shown in fig. 22, in the position held by normal anterior roots (cf. fig. 30). The same explanation may possibly apply to the fifth also.

The Development of the Eye Muscles.—I have made certain observations on the development of the eye muscles, which, though as yet imperfect, I desire to record here, because they seem to throw some light on the morphology of this very interesting and perplexing group of muscles.

In a previous section I have described the way in which the first head cavity becomes partially doubled up on itself so as to form a cup, closely embracing the inner and posterior surfaces of the eyeball, and have further mentioned that the outer wall of this cup becomes converted in part into certain of the eye muscles. I propose now to examine these changes more in detail.

Previous observers, with one very important exception, have maintained an almost complete silence concerning the development of these muscles. Balfour, in his great memoir on Elasmobranch Fishes, to which I have referred so often, states concerning the first head cavity, "I have not yet succeeded in tracing the subsequent fate of its walls, *but think it probable that they develope into the muscles of the eye*."[1] These three lines which have furnished the clue I have attempted to follow up, contain, I believe, all that has been hitherto published concerning the embryology of these muscles.

Plate V, figs. 19 and 20, represent, as already described, longitudinal and vertical sections through an embryo at stage M. The first head cavity (1) is doubled up in the manner described above, and its outer wall is converted in part into muscles. Figs. 32 to 40 show the same parts in a more advanced state of development at stage O. In figs. 34 and 35 the muscle fibres derived from this outer wall of the first head cavity are clearly divided into three sets, *upper*, *middle*, and *lower*. Of these the *middle* set, figs. 34 and 35 (*r. i.*) run horizontally forwards in close connection with the inner surface of the eyeball; with this muscle the anterior branch of the third nerve (III *a.*) is seen to be in very close relation. This muscle, which appears to be developed partly from the outer and partly from the inner wall of the first head cavity, has exactly the position and relations of the *rectus internus* of the adult. The *upper* portion (*r. s.*, fig. 34) is quite distinct from the middle portion first described; it is situated entirely above the *ramus ophthalmicus profundus* (III *a.*) it runs upwards and outwards, and terminates in the upper wall of the eyeball (fig. 32, *r. s.*) This muscle, from its course and relations, can be none other than the *rectus superior*. The

---

[1] Loc. cit., p. 208.

third or *lower* portion of the muscular mass derived from the first head cavity is situated below the *rectus internus,* and has the main trunk (III *b.*) of the third nerve running along its posterior border (*vide* fig. 33, *r. inf.*) ; from its position and relations I believe it to be the *rectus inferior.*

Immediately below the lowest portion of the first head cavity is a very conspicuous muscle (*o. i.,* figs. 19 and 20, and 32 to 35). This muscle runs forward (figs. 19, 32, 34) at a lower level than the optic nerve, but does not actually pass below it, stopping just as it reaches it. The upper and posterior end of this muscle is in close contact with the posterior wall of the first head cavity, *i. e.* with the *rectus inferior* muscle (figs. 19 and 33) ; it also receives the terminal branch of the third nerve as shown in figs. 19 and 33 (III *b.*). From the position and relations of the muscle, and especially from the fact of the third, nerve ending in it, I have no doubt that it is the *obliquus inferior* muscle; it differs from the adult mainly in its origin not being in front of the optic nerve.

Concerning the relation of this muscle to the first head cavity, my observations leave me in doubt; its dorsal end is undoubtedly from its first appearance part of the outer wall of the first head cavity; but its ventral end is situated considerably further forward than the end of the head cavity.

My observations thus enables me to state with considerable confidence that of the four eye muscles supplied by the third nerve the *rectus superior, rectus internus,* and *rectus inferior* are developed out of the walls of the first head cavity; and that the *obliquus inferior* is, very possibly, also developed from the same source.

Concerning the *obliquus superior,* my observations are much less definite. In embryos from stage M upwards, I have observed a muscle situated above the eyeball, below the ophthalmic branch of the fifth, and in front of the *rectus superior.* This muscle (*o. s.,* figs. 19 and 20, and 32 to 35) has the position and relations of the *obliquus superior,* and I believe it to be this muscle. Though it lies not far from the top of the first head cavity (figs. 19 and 20), especially in the earlier stages, I have not observed that it has any genetic relation to that cavity. The only nerve I have succeeded in tracing to this muscle is a small branch (V *e.*) shown in fig. 20, as arising from the communicating branch (V *d.*) between the fifth and third nerves, and running upwards to the under surface of the muscle in which it ends. As a communicating branch between this branch of the fifth nerve and the fourth nerve (the special nerve of the *obliquus superior*) is described by Schwalbe as existing in the adult *Scyllium,* this may be taken as evidence in favour of the muscle (*o. s.*) being as I have named it, the *obliquus superior*.

The last of the muscles of the eye, *rectus externus*, is the easiest of all to recognise and determine. It can be clearly recognised in stage M (*vide* 18 to 20, *r. e.*) as arising behind the main stem of the fifth nerve (between the fifth and seventh nerves in fact), and running forwards and outwards to the eyeball, passing to the inner side of the mandibular branch of the fifth nerve (figs. 19 and 18) and ending by being inserted into the outer and posterior wall of the eyeball. The course of the muscle and of the nerve supplying it (the sixth) is well shown at stage O in figures 32 to 38. Fig. 38 shows, as already noticed, the origin and trunk of the sixth nerve; fig. 37 shows at *r. e.* the nerve terminating in the posterior part or origin of the muscle; the muscle itself can be traced running forwards and outwards in figures 36 and 35 (*r. e.*). In fig. 34, it is seen crossing the mandibular branch of the fifth, lying to its inner side, while figs. 33 and 32 show it running forwards in front of the fifth nerve towards the eyeball.

The *rectus externus* is also shown in transverse section in figs. 28 and 29 as already described, the former figure showing its close relation to the fifth nerve.

The most important fact in connection with this muscle, and a fact proved in the most conclusive manner by the figures referred to above, is that *it has nothing whatever to do with the first head cavity*. Though it ultimately reaches the eyeball, yet, as shown in figures 18 and 32, it lies superficially to this cavity and never comes in contact with it at all. This is a point whose importance can hardly be overrated, as it furnishes us with an explanation of the fact that the *rectus externus* is supplied, not by the third nerve, but by a totally distinct nerve—the sixth. Of the four muscles supplied by the third, three are certainly, and the fourth possibly, developed from the walls of the first head cavity. The *rectus externus* has nothing to do with this cavity; it is in fact a muscle belonging to a segment further back, and consequently has a distinct and separate nerve supply.

We have seen above that the dorsal ends of the second and third cavities disappear by stage M; it will be seen from the figures that the *rectus externus*, which appears at stage M, occupies exactly the position previously held by these cavities. I have not yet succeeded in determining whether the muscle is directly developed out of the wall of one or other or both of these cavities, but when we consider that the walls of the other cavities become muscles, that this muscle appear immediately after the disappearance of the cavities, that it occupies the position previously held by the cavities, and that, finally, it is, for a time at least, the only muscle in this region, it becomes very probable that the *rectus externus* is really developed from the wall of these cavities. Whether both cavities take part in it is uncertain, but it is evident that, if this be the mode of its development, the third cavity must at any rate form part of it, inasmuch as from its earliest appearance part of the muscle (figs. 19 and 36) is situated behind the fifth nerve, and therefore behind the second cavity. In this fact we probably get the explanation of the fact that the nerve supply of the *rectus externus* is by means of the anterior root of the seventh nerve.

The morphology of the eye muscles and their nerves has long been recognised as a curious and interesting problem. I venture to hope that the account given above of their development in a very typical Vertebrate will be accepted as giving us at any rate the clue to the solution of the problem —a clue I hope to follow up more closely in a further paper.

CONTRIBUTIONS *to the* MINUTE ANATOMY *of the* NASAL MUCOUS MEMBRANE. By E. KLEIN, M.D., F.R.S., Lecturer on Histology and Embryology, in the Medical School of St. Bartholomew's Hospital. (With Plate VII.)

IN the present paper I propose to describe the structure of the organ of Jacobson, and of the mucous membrane lining the anterior portion of the nasal cavity in the adult guinea-pig, and in future papers, soon to follow, to extend these observations to the same organs in the rabbit, mouse, dog, ape, and man.

I.—*The Organ of Jacobson.*[1]

The organ of Jacobson of mammals, as is well known, is situated bilaterally in the lower or osseous part of the septum narium, or more correctly speaking, in the crista nasalis of the superior maxillary bones. Its general morphology and development has been studied by Gratiolet,[2] Born,[3] and Fleischer.[4] Its minute structure is, however, little known. A rudiment of the organ of Jacobson exists also in man, as Dursy, and especially Kölliker,[5] very clearly proves. Kölliker found it in the fœtus, in the child, and also in the adult; and in this last instance he identified it as the duct described by T. Fr. Meckel and by Sömmering in the lower part of the nasal septum. But about its histology Kölliker does not give any detailed account, owing to the want of suitable material.

I have investigated the structure of the organ of Jacobson of the adult guinea-pig, in specimens suitably prepared for microscopic examination, and I am thus enabled to give a detailed account of the parts composing it.

The preparations were obtained by placing the nasal organ, together with the upper maxillaries, after having previously removed the osseous covering in $\frac{1}{4}$ p. c. solution of osmic acid for 24 hours; after this, in absolute alcohol for 24—48 hours; a very minute quantity of hydrochloric acid is then added, and thus the bone softened. But I have obtained very good specimens by placing the fresh organ first in absolute alcohol, then in osmic acid, and then into the macerating fluid; or, the fresh organ is placed in Müller's fluid for two or three weeks, and then in a mixture of chromic acid $\frac{1}{6}$ p. c., and hydrochloric acid a few drops (about $\frac{1}{20}$ volume being always used).

---

[1] This part of the present paper is published in the 'St. Bartholomew's Hospital Reports' for 1880.

[2] 'Recherches sur l'organe de Jacobson,' Paris, 1845.

[3] 'Sitzung. d. naturw. Section, August, 1877, Schlesisch Ges. f. Vaterl. Cultur.'

[4] 'Sitzungsb. d. Phys. Med. Société̈t,' Erlangen, 1877.

[5] 'Gratulationschrift an Fr. v. Rinecker,' Leipzig, 1877.

In this animal the shape of the organ of Jacobson resembles that of other mammals (see Jacobson and Gratiolet) and of man, as described by Kölliker, being a tubular structure flattened from side to side, and leading in front into the ductus Stenonianus, which opens into the oral cavity; but its diameter is much larger than that of the latter. It terminates behind in a cæcal extremity. It is surrounded, not by the bone of the septum, but by hyaline cartilage. This latter is, however, altogether independent of the cartilage forming the upper part of the nasal septum. The cartilage surrounding the organ of Jacobson, or Jacobson's cartilage, forms a more or less complete capsule around that organ. But there are a great many places where the cartilaginous capsule is incomplete, and then the wall of the organ of Jacobson is in immediate contact with the bone, or rather its internal periosteum. This incompleteness affects more generally the lateral and lower than the median and upper parts. In some places the cartilage is reduced to a few (one or two) small thin plates for the lower and median, and two larger plates for the upper part of the organ. The cartilage always projects, for each organ, upwards as a plough-shaped plate, showing an outer convex and an upper concave surface. The connective tissue, *i. e.* the perichondrium, covering this latter surface is at the same time the tissue by which the convex lower border of the cartilaginous part of the nasal septum is fixed here. Between the two there are in some places clumps of fat-cells to be met with.

The measurements of the thickness of the cartilage of Jacobson, where it is quite complete, as in the preparation from which fig. 1, Plate VII, is taken, are these: just at the middle of the organ of Jacobson, on either side, it is about 0·072 mm., at about its lower part 0·18 mm., at the upper or plough-shaped part the diameter at its broadest part from side to side is about 0·56 mm.; the diameter of the plough-shaped project tion from the organ of Jacob soon the upper pointed extremity of the former is about 0·72 mm. The greatest transverse diameter of the lower enlargement of the cartilaginous nasal septum of fig. 1 is about 0·67 mm.

The description of the cartilage of Jacobson in the guinea-pig here given differs from that given by Gratiolet of the mammals' organ in general. His description is quite applicable to the rabbit, as I shall show in a subsequent paper, for in this animal, "l'organe de Jacobson est entouré de cornet cartilagineux."[1] In the rabbit there exists a continuous broad slit along the upper part of the wall of the organ of Jacobson, and through this slit the wall, and especially its glands, form a continuity with the mucous

---

[1] Loc. cit., p. 21.

membrane covering the cartilaginous nasal septum, but this does not hold good in the guinea-pig.

Gratiolet speaks[1] of the tissue in that slit as of "une sorte de mesentere." In the guinea-pig, however, the above-mentioned plough-shaped projection of the upper part of the cartilage of Jacobson includes one or two spacious longitudinal channels or clefts for the branches of the nerves and blood-vessels supplying the organ of Jacobson.

Figs. 1 and 2 of Plate VII give an accurate representation of the relation of the cartilage of Jacobson to the organ of Jacobson, and to the cartilaginous nasal septum. Both figures were made with the camera, and their respective preparations were obtained from the same organ of Jacobson at different places.

In fig. 1 the capsule is complete; in fig. 2 it is incomplete in some places; while in others the cartilage is reduced in thickness to a very considerable degree.

Balogh,[2] in his description and illustrations of the cartilage of Jacobson in the sheep, introduces a perfectly unnecessary and complicated terminology of the different parts of the cartilage. As far as I can understand his elaborate description (pp. 451 and 452), the cartilage does not differ much from that described by Gratiolet of other mammals. Balogh does not know of Gratiolet's work, otherwise he might have been able to follow this latter's simple description.

As mentioned above, the organ of Jacobson is flattened, and, therefore, its walls are generally considered as the lateral and median wall, the latter being the one nearest to the median line of the septum. For a better understanding we shall speak, in addition, of an upper and lower sulcus, meaning the parts where the lateral and median walls are in contact.

In the guinea-pig the outline of the transverse section is not simply oval, but is kidney-shaped, the lateral wall being pressed inwards, *i.e.* against the lumen or cavity of the organ.

The size of the organ is about the same on both sides.

Gratiolet mentions,[3] in the upper and outer part of the organ, a conspicuous projection, "un bourrelet saillant, que je ne saurais mieux comparer qu'à l'organe décrit dans l'intestin du lombric sous le nom d'intestinum in intestino."

Balogh[4] finds in the organs of the sheep a similar projection of the wall from the upper outer part, "Drusenwulst." But in the guinea-pig there is no such projection from the upper part of the wall, the lateral wall as a

---

[1] Loc. cit., p. 19.

[2] 'Sitzungsber. d. Kais. Akadem. d. Wiss.,' Vienna, volume 42, p. 449, "Das Jacobson'sche Organ des Schafes."

[3] Loc. cit., p. 20.

[4] Loc. cit., p. 457.

whole forming a slight convexity towards the lumen of the organ. Compare Figs. 1 and 2.

The following are the measurements of the different diameters, taken at a point where the cartilaginous capsule is more or less incomplete at the outer and lower part of the organ (see fig. 2).

The short transverse diameter of the organ, exclusive of the cartilage or bone, is about	0·72 mm.
The long transverse diameter of the organ, *i.e.* across the upper and lower sulcus is about . . .	1·4 ,,
The thickness of the lateral wall	0·28 to 0·38 mm.
,, ,, median wall.	0·14 mm.
The short transverse diameter of the cavity . . .	0·3 ,,
The long transverse diameter, from the upper to the lower sulcus	0·77 ,,

The thickness of the wall at the lower sulcus is about the same as that of the lateral wall, while that of the wall at the upper sulcus is slightly larger than that of the median wall.

As regards the structure :

1. The lateral wall. This consists of (*a*) the epithelium lining the cavity, (*b*) the subepithelial fibrous layer, (*c*) the layer of the cavernous tissue, (*d*) the layer of the glands. Outside these is the thin layer of fibrous tissue connecting the organ with the cartilage, or, where this is wanted, with the bone, and acting as the perichondrium or inner periosteum respectively.

a. The *epithelium* is in all respects similar to that lining the mucous membrane of the nasal cavity, being composed of a superficial layer of columnar or conical cells, between the extremities of which extend spindle-shaped or inverted conical cells, *i.e.* cells whose basis is directed towards the depth. Each of these cells possesses an oval nucleus. The superficial conical cells show on their free surface a bundle of fine cilia. The whole thickness of the epithelium, inclusive of the cilia, is 0·064 ; the length of the cilia is 0·0054 mm. Loewe[1] failed to see the cilia of the epithelium of the lateral wall of the organ of Jacobson in the rabbit, but I presume this is entirely owing to the mode of preparing the specimens. Some of the superficial epithelial cells present themselves as *goblet cells.*

Leydig mentions[2] that "ciliated epithelium" forms the boundary of the narrow lumen of the organ.

Balogh[3] does not distinguish, in the sheep's organ, the epithelium covering the lateral wall from that of the median wall, but speaks of it as a whole, and remarks that it is ciliated epithelium.

---

[1] 'Beitr. zur Anatom. d. Nase und Mundhöble,' Berlin, 1878.

[2] 'Lehrbruch d. Histologie,' p. 218.

[3] Loc. cit., p. 458.

b. A very delicate basement membrane separates the epithelium from the next or the *subepithelial fibrous layer*. This layer is chiefly composed of bundles of fibrous tissue, and in it are capillary vessels, and here and there a thin bundle of unstriped muscle cells. The thickness of this layer varies in different places; in about the middle of the lateral wall it is about 0·032 mm.

c. The next outer layer is the layer of the cavernous tissue; this layer is thickest in about the middle of the lateral wall; it is altogether wanted near the upper and lower sulcus. The thickest diameter is about 0·12 mm. But at the places in which the cartilage capsule is incomplete, the thickness of this layer is much greater, being 0·22 mm. The length of this layer varies between 0·43 and 0·61 mm.

The matrix of this layer is fibrous tissue, containing a plexus of bundles of unstriped muscle cells. The essential parts are large venous vessels connected into a plexus, the vessels running chiefly parallel to the long axis of the organ, hence in a transverse section most of them appear cut transversely. The transverse diameter of the vessels varies between 0·046 and 0·092 mm. Where the cartilage capsule is wanting, some of the vessels are as large as 0·16 mm. in diameter. These vessels take up the venous capillaries of the subepithelial fibrous layer as well as some of those of the next outer glandular layer. The efferent veins of the cavernous layer are smaller than the vessels of this layer, a character essential of a cavernous tissue.

d. The layer of glands (Jacobson's membrane adenoide, Gratiolet's membrane glanduleuse) is the next outer layer. It consists of a wide-meshed framework of connective tissue, the meshes containing the gland alveoli. A few nerve trunks are met with amongst these latter in some places: from the cavernous layer extend small bundles of unstriped muscle tissue amongst the alveoli in some places; a similar arrangement has been pointed out by me (see October number 1880 of this Journal), also for the glands of other parts of the nasal organ. This layer is thickest at the lower sulcus, where its diameter amounts to 0·22—0·28 mm.; here it occupies at the same time the whole thickness of the wall, there being here no cavernous layer. In about the middle of the lateral wall the thickness diameter of the gland layer is about 0·08 mm. It decreases towards the upper sulcus, where it becomes reduced to a single layer of alveoli.

In those places where the cartilage capsule is incomplete the glandular layer is on the whole much better developed than where this is not the case.

The gland alveoli are not confined to the lateral wall and to the membrane at the lower sulcus, but extend at this latter place also a short distance into the median wall.

There is a remarkable difference in the distribution of the glands between the organ in the guinea-pig and the rabbit, in this latter the glands being chiefly accumulated in the upper and outer position, just as it is described by Gratiolet and Balogh.

The alveoli are branched and more or less convoluted tubes of exactly the same nature as other serous glands, *e.g.* the parotid. The transverse diameter of the alveoli varies between 0·024 and 0·036 mm. The alveoli are limited by a membrana propria; they possess a very minute lumen and are lined with a single layer of polyhedral or short columnar epithelial cells, each with a spherical nucleus, situated in the outer part of the cell. The cell substance is a dense reticulum, and therefore appears as a uniformly granular protoplasm. The outline of the alveoli is not smooth, but shows numerous minute, rounded prominences, owing to some of the epithelial cells possessing a convex outer surface. And it is just in these cells that the projecting convex outer part of the cells, containing at the same time the small round nucleus, appears more uniform and better stained than the inner part, and hence the appearance is produced very similar to that presented by the cells of the alveoli of the pancreas.

The ducts are short and they take up directly the alveoli; they are lined with a single layer of columnar epithelial cells; in some instances the outer portion of the cell substance appears longitudinally striated, just like those in the salivary ducts of Pflüger. The ducts open with a narrow mouth into the *lower* sulcus, where the columnar ciliated cells of the lateral wall pass into the mouth of the former. And these mouths form indeed the boundary between the epithelium of the lateral and that of the median wall. I have seen specimens (see fig. 5) where a duct opening in the lower sulcus with a narrow mouth—about 0·012 mm. in diameter—became much distended behind this, and extended in this state into the wall of the organ at the lower sulcus to a depth of about 0·22 mm., the diameter of its lumen being here 0·1; and throughout this whole length it was lined with columnar ciliated epithelium, the length of the epithelial cells being 0·02 mm., the length of their cilia about 0·006 mm. In this same specimen from which fig. 5 is taken Jacobson's cartilage was wanting almost everywhere except at the upper sulcus.

2. The median wall. As such will be considered that part of the circumference of the organ which is not strictly limited to the median line of the nasal septum, but which is covered with a thick epithelium; this, owing to its peculiar nature, is the ‘ sensory epithelium.”

The median wall comprises much the greater half of the wall of the organ, extending almost over two thirds of the whole circumference. Its thickness is about 0·14 mm.; at the lower sulcus it decreases slightly. The most conspicuous feature in this is the sensory epithelium; its thickness is 0·1 mm. What is not epithelium, *i.e.* outside this latter, is fibrous tissue intimately connected with the perichondrium, or internal periosteum respectively. Numerous small nerve-branches are contained in the subepithelial layer, and here they may be followed as oblique or longitudinal bundles, ultimately ascending into the sensory epithelium. These bundles are derived from large branches, which are contained as groups, and in company with blood-vessels, in the channels of the plough-shaped upper part of the cartilage above mentioned. Most of the nerve-bundles are derived from the olfactory nerve, and, like this, are composed of non-medullated fibres; but there are a few small bundles of the nervous naso-palatinus of Scarpa. Gratiolet[1] has very exhaustively treated of the origin and distribution of these nerves.

As has been mentioned above, the gland alveoli at the lower sulcus extend a short distance into the median wall.

The *sensory epithelium* extends over the whole of the median wall proper, and the greater part of the adjacent sulcus superior and inferior. Its structure is this: most superficially it, viz. the epithelium as a whole, presents a faint vertical striation, the striæ being due to thinner or thicker granular-looking columnar bodies. These, on careful examination with high powers, prove to be either the thicker processes of the deeper cells reaching up to the surface, or conical, thin epithelial cells, whose basis forms part of the general surface. The conical cells are the "epithelial cells," and they are smaller and thinner, and their nucleus less distinct, than the epithelial cells of the olfactory epithelium of the nasal cavity. Each of these epithelial cells appears to be possessed of a narrow, oval, transparent nucleus. Below the layer of epithelial cells are several layers of spherical, comparatively large nuclei, well outlined, and containing a delicate reticulum. Each of these nuclei belongs to a spindle-shaped, granular-looking cell, of which one process, the outer one, is broad, but thinner than an epithelial cell, and extends as one of the above striæ between the "epithelial cells," up to the free surface, while the inner is very delicate, and directed towards the depth. These spindle-shaped cells will be spoken of as the "sensory cells;" the amount of all substance around the nucleus is always appreciably larger than in the "olfactory cells" of the olfactory region, with which they are evidently analogous.

---

[1] Loc. cit., p. 23 *et passim.*

The sensory cells vary much in size, some being nearly twice as bulky as others; the latter possess also a larger nucleus than the former. They are generally arranged in a number of layers, five to twelve and more, either uniformly occupying the lower half or two thirds of the whole epithelium, or they form groups of four, five, and more. There exists a great difference in different parts as regards the extent to which the sensory cells reach towards the surface, for there are many places in which only a narrow strip of 0·027 (the whole thickness of the epithelium being 0·1 mm.), *i.e.* only a fourth of the whole epithelium is free of them, while in other places they occupy a layer of 0·054 mm., or about one half of the whole epithelium. But it must not be therefore supposed that in the latter places the epithelial cells are much longer than in the former; this is not by any means the case; in the places where the sensory cells reach up near to the surface, the "epithelial cells," if not wholly absent, are reduced in numbers to a very great extent, the outer processes of the sensory cells almost entirely occupying their places. Towards the sulcus, superior and inferior, the epithelium as a whole decreases in thickness; at the sulcus the epithelial cells become very few, and the sensory cells almost entirely form the layer. There exists a most sharp boundary between the sensory epithelium and the ciliated columnar epithelium of the lateral wall, as mentioned above. The sensory epithelium at the extremities of the median wall is either rounded off, or it terminates with a pointed margin. On the free surface of the sensory epithelium is a delicate cuticle, similar to the cuticle of v. Brunn, in the olfactory region. Both the processes of the sensory cells and the basis of the epithelial cells appear to project over the cuticle, the former as a very minute rod-like, and the latter as a minute knob-like, homogeneous process.

Amongst the lower layers of the sensory epithelium are seen either small bundles of nerve fibres, or isolated fine fibres, both extending in an oblique direction. The termination of the nerves has not been ascertained.

On the whole, then, this sensory epithelium corresponds to the olfactory epithelium, except that, in the latter, there exists a deep layer of inverted conical "epithelial cells," *i.e.* cells, whose bases are fixed on the subepithelial basement membrane. In the sensory epithelium of the organ of Jacobson this deep layer of inverted conical epithelial cells is apparently wanting, the sensory cells reaching as far down as the subepithelial fibrous coat. Besides, as mentioned above, the "epithelial cells" of the sensory epithelium in the organ of Jacobson are much more indistinct and smaller than those of the olfactory epithelium, while the bodies of the sensory cells appear larger than those of the olfactory cells.

Balogh[1] describes the epithelium of the organ of Jacobson of the sheep, without, however, making a distinction between the sensory epithelium lining the median wall and the ciliated epithelium lining the lateral wall. According to this observer the epithelium consists of two kinds of cells—(*a*) large ciliated columnar cells, and (*b*) smaller olfactory rods (Riechstäbchen). These latter are rod-like, hyaline, and contain in their lower or deeper part a swelling produced by the nucleus. They extend to the free surface, and possess here two triangular pointed rods (Riechhärchen).

Balogh says that he has convinced himself of the connection of the epithelial cells with the processes of the connective-tissue corpuscles of the mucosa, while the "olfactory rods" are connected, through spindle-shaped cells, with the olfactory nerves. But, considering the methods with which he worked (maceration in acetic acid mixture) it is not too much to say that these assertions must be received with great reserve.

I cannot say precisely what the function of the organ of Jacobson is, since I have not made any experiments on this point. Kölliker[2] thinks that "if we do not assume that the tubes of Jacobson are simply secretory organs, their vascular thickness, their numerous glands, and the numerous branches of the nervus naso-palatinus, seem to point to it; but against this speaks their supply with very numerous olfactory nerve-fibres. There remains nothing else but to assume *that they* (Jacobson's tubes) *secrete juices and substances which act on their specific nerves, and thus enable the organism, directly, as it were, to obtain knowledge of the chemical constitution of its own juices.*" Whatever may be the precise meaning of this view of Kölliker's, it seems strange to have recourse to such a theory. Why should it not serve for the perception of specific smells, seeing that it really belongs to the olfactory nerve termination? Its communication with the oral cavity does not appear to me to be adverse to such an assumption.

In man the organ of Jacobson is only of a rudimentary nature (Kölliker) as compared with that of mammals, and do not these latter possess a power of perception of smell, about the degree and nature of which human beings can hardly have a true conception?

---

[1] Loc. cit., p. 465.

[2] Loc. cit., p. 11.

II.—*The Accessory Organ of Jacobson.*

At 8, in fig. 1 of Plate VI, a minute tube is shown in transverse section, which extends alongside the groove which, at the bottom of the nasal cavity, is contained between the septum and the alveolar process of the superior maxilla. This tube is shielded, as is shown in the figure, partly by the lower extremity of the osseous lamella that forms the support of the lower conchanarium. It, *i.e.* the tube, is met with as far as the tube of Jacobson extends, and like this is bilateral. I will call it the "*accessory organ of Jacobson;*" it is also flattened from side to side, and it includes a lumen varying in diameter, in hardened specimens, and in different places. In some places the lumen is a narrow slit, in others it is of relatively large transverse diameter. The wall of the tube consists of an epithelium and of a loose connective tissue, which above and below is thickest, and includes here a plexus of large vessels, arteries, and veins, extending chiefly in a longitudinal direction, *i. e.* parallel with the tube.

The following are the measurements of the accessory organ, the epithelium only being here considered as the wall of the tube:—At a place exactly corresponding to that in fig. 1, the tube being much compressed from side to side, I find the breadth, *i.e.* the transverse diameter of the tube from side to side—lumen and epithelium of both sides—amounts to 0·144 mm., while the height of the tube, *i.e.* the transverse diameter across the upper and lower wall, is 0·63 mm.

At another place, further backward, where the tube appears of larger size, and with a much larger lumen, the breadth of the tube (lumen and epithelium of both sides) is 0·518 mm., while the height is 1·06 mm., the transverse diameter of the lumen being 0·38 mm.

The epithelium lining the tube is stratified columnar epithelium, whose thickness is somewhat greater in the median wall, *i. e.* the wall nearer the nasal septum, than in the lateral wall. In the upper and lower sulcus it is slightly thicker than at the sides. At a place identical to that represented in fig. 1 the thickness of the epithelium of the median wall is 0·079 mm., that of the lateral wall 0·058 mm. At a place further in front, *i. e.* where the tube appears larger, the thickness of the epithelium of the median wall is 0·05 mm., of that of the lateral wall 0·04 mm.

The epithelium lining the lumen is the most conspicuous, and at the same time the thickest part of the wall. On its inner surface, *i. e.* the one facing the lumen, there exists a well marked cuticle, similar to that mentioned in the organ of Jacobson. The epithelium consists of a superficial layer of conical epithelial cells, about 0·032 mm. long, each with an oval nucleus; their free basis, which is on the surface, appears covered in some places with fine cilia, similar to those described above of the lateral wall of the organ of Jacobson. Both in the lateral and median wall I have seen in some places indications of fine cilia still connected with the surface of the epithelial cells, while in others there were no cilia in connection with the cell bases, but there were minute rods and cilia-like bodies near the surface of the cells held together by a mucous coagulum. From this it appears probable that the cilia have become detached from the surface of the cells. In some places a great many of the epithelial cells are converted into goblet cells, with or without mucus in their interior.

Away from the surface the cells are drawn out into a single or branched fine process. Between these are packed-in spindle-shaped cells, each with a spherical or slightly oval nucleus. In some places, especially in the median wall, these spindle-shaped cells are very conspicuous, there being a considerable amount of protoplasm around the nucleus, but in other places they appear very minute. Underneath these cells and forming the inner or lower boundary of the epithelium is a stratum composed of one or two, or in some places of the median wall, even three, layers of small spherical or oval nuclei, closely placed side by side; to each nucleus belongs a very narrow zone of protoplasm, hence the nuclei form in this stratum the most prominent part. They are more deeply stained than the nuclei of the other cells. Their cell protoplasm is of a polyhedral or conical or angular shape, but is always inconsiderable.

In some places of the upper and lower sulcus the spindle-shaped cells are much more numerous than the other cells, and hence the appearance is produced not unlike that of the sensory epithelium of the organ of Jacobson.

Branched lacunæ are seen extending from the subepithelia membrane into the epithelium itself, where fine canaliculi pass from the lacunæ between the individual epithelial cells, *i. e.* into the interstitial cement substance. These lacunæ and their canaliculi are best seen in oblique or horizontal sections through the epithelium of the tube, and they correspond to the lymph-canalicular system known of the epithelium of other membranes.

One of the most striking appearances is the presence, in about the middle of the epithelium, of spherical or oval cavities; each of them appears limited, and consequently separated, from the epithelial cells by a very delicate membrane, and hence may be spoken of as an intraepithelial vesicle, the smallest of them being about 0·014, the largest about 0·03 in diameter. They occur in a single layer only, both in the lateral and median walls; but towards the upper and lower sulcus their number is much diminished. In those parts where the tube is larger; *e.g.* in the front part, the number of these vesicles is greater than where the tube is smaller. Where they are most numerous they are situated so closely that they are separated from one another by epithelial masses, whose breadth is not much greater than that of themselves, *i. e.* the vesicles. The dome of these vesicles, *i. e.* the surface nearest the lumen of the tube, is covered with the columnar superficial epithelial cells only; they are here less of a conical but more of a cylindrical shape, and shorter than the cells of other places. Above the middle of the dome the epithelial cells are reduced to about 0·016 mm. in length, *i. e.* half the length of the conical cells of other places.

Each of these vesicles is connected with the internal lumen of the tube by several minute canals extending between the epithelial cells forming the dome up to the free surface. With the subepithelial membrane the vesicle is connected by a vertical or slightly oblique channel. This latter is much larger than the ones leading into the inner cavity, and may be appropriately spoken off as at the neck. Its breadth is measurable; it amounts to 0·005 mm., while its length is about 0·02 mm. The deep stratum of small epithelial cells, described above, is partly invaginated by the passage through it, of the neck of the vesicle, the small cells not reaching further than the point of connection of the neck with the vesicle. The neck, together with the vesicle, resembles a flask-shaped organ; the former contains, and is, in fact, filled with a cord-like continuation of the subepithelial membrane, and it consists of a tissue,. in which occasionally a capillary blood-vessel, a few spindle-shaped looking cells, or a nerve-fibre can be recognised. This tissue is continued into the vesicle, but fills only a small part of the cavity of this latter. In some rare instances I have seen two vesicles connected by a horizontal broad channel. The vesicles and their neck could be compared with the papillæ of other membranes, but this comparison would not be quite correct, inasmuch as in an ordinary papilla, the epithelium, as a whole, is inflected over this latter, whereas in our present instance the flask-shaped vesicle penetrates, as it were, into the layer of the epithelium.

The case of the penetration of capillary blood-vessels and pigmented cells into the epithelium of that portion of the ligamentum spirale, known as the stria vascularis in the cochlea, could be perhaps more appropriately adduced, and is in a certain sense similar to our own case. A penetration of capillary blood-vessels into the epithelium has been described by Professor Lankester[1] in the integument of the medicinal leech.

Outside the epithelium is a fibrous coat, whose thickness in the lateral and median wall is about the same, 0·05—0·06 mm. It contains minute nerve-bundles, and capillaries are found only immediately underneath the epithelium. In the lateral wall there are several arterial vessels running longitudinally; where the organ is in contact with the bone (see fig. 1), the fibrous coat is in intimate connection with the periosteum. In the lower part of the median wall, *i.e.* the one in contact with the mucous membrane of the descending inner surface of the concha (see fig. 1), the mucous membrane is more or less distinctly infiltrated with lymph-cells similar to that of the concha. At the upper and lower sulcus the tissue is very loose, and contains, as mentioned previously, large blood-vessels running in a longitudinal direction; the diameter of the largest vessels here is about 0·13 mm. Numerous elastic fibrils, extending longitudinally and connected into a network and flattened connective-tissue cells, each with an oval nucleus as well as a few minute nerve branches, are also to be met with here.

III.

The mucous membrane covering the cartilaginous septum and lining the furrow between the septum and the alveolar process of the superior maxilla in the region of the organ of Jacobson has the following structure:—Both surfaces of the cartilaginous septum are lined with an epithelium of exactly the same nature as that described above as lining the lateral wall of the organ of Jacobson, viz. stratified columnar epithelium, of which the most superficial cells are conical and possessed of cilia. Goblet cells occur also here.

The thickness of the whole epithelium is about 0·056—0·07 mm., the length of the cilia 0·006 mm.

The mucous membrane immediately underneath the epithelium is fibrous connective tissue, infiltrated in many places with lymph-corpuscles. A network of venous vessels in the mucous membrane forms a very conspicuous feature.

The mucous membrane of the septum is thickened at three definite places, owing to the presence of glands; these places are: *a,* where the septum joins the dorsum of the nose; *b,* in about the middle height of the septum; and *c,* at the point where the thick rounded lower margin of the cartilaginous septum is fixed on the cartilage of the organ of Jacobson. In Plate VII, fig. 1, these places are easily recognised. At the last place the thickening is greater than at the second, and at this

---

[1] This Journal, No. 79, New Series, p. 303.

greater than at the first. The thicknesses of the mucous membrane of the three places in the preparation from which fig. 1 is taken are 0·25, 0·29, 0·36 mm.

The glands are serous glands; there are no other glands here. Their structure is identical with that described above of the lateral wall of the organ of Jacobson. The alveoli are tubes, wavy and convoluted, some more branched than others; their transverse diameter is about 0·02 to 0·036 mm.; their lumen is very minute, and they are lined with a single layer of columnar or polyhedral, granular-looking cells, the nucleus of which is situated in the outer part of the cell.

The ducts are identical with the intralobular ducts in the salivary glands, *i. e.* Pflüger's salivary tubes; their epithelium is a single layer of beautiful columnar cells, whose outer portion is conspicuously longitudinally striated; their nucleus is spherical, and contained in about the middle of the cell.

The lumen varies between 0·0135 and 0·019, the whole diameter of the duct, including the entire epithelial wall, being between 0·04 and 0·05 mm.

The cartilage of the septum is hyaline, but the cartilage cells show this peculiarity, that in many places they contain minute fat-globules. In some places they are quite filled with them, and in specimens that have been prepared with osmic acid the cartilage cells appear then filled with black spherules. When dissolving away the fat-globules the honeycombed reticulated cell substance becomes very evident, and is identical in appearance with that presented by the epithelial cells of the alveoli of the sebaceous and Meibomian glands.

The groove between the septum and the alveolar process of the superior maxilla is lined with a thin mucous membrane, the free surface of which is covered with the same ciliated columnar (stratified) epithelium as the septum.

Small groups of alveoli of serous glands are found in very few places. The tissue of the mucous membrane is infiltrated with lymph-corpuscles; in the outer wall of the groove, *i. e.* on the alveolar process of the superior maxilla, these infiltrations amount occasionally to a distinct lymph-follicle (see fig. 1, 13). The adenoid tissue of the lymph-follicle penetrates into the epithelium of the surface, in the same way as is seen in the summits of the lymph-follicles of the Peyer's glands and on the tonsils. I measured one such follicle, oval in shape; its long diameter is about 0·28 mm., its short diameter 0·16 mm. The follicle lies close to the epithelium; this latter is here much infiltrated with adenoid tissue, but, on the whole, thinner by about one fourth than the ciliated epithelium of the neighbourhood.

IV

The mucous membrane covering the concha or turbinal bone differs in structure from the one hitherto described in the following points :—As is shown in fig. 1 beginning from *a, i.e.* immediately after passing the region of the accessory of Jacobson's organ, the mucous membrane as a whole becomes thicker, owing to their being contained in it a continuous layer of serous glands. Its thickness diameter amounts here to about 0·25 mm. But the epithelium becomes thinner (see at *a,* in fig. 1), being now composed of a superficial layer of short columnar cells without cilia, and a single deep layer of small polyhedral or conical cells. From *a* to *b,* in fig. 1, the epithelium retains the character just described ; its thickness is between about 0·016 and 0·024 as against 0·056, the thickness of the stratified columnar ciliated epithelium downwards from *a*. In the preparation from which fig. 1 was taken the epithelium altered its character at *b ;* from here over *c* to *d,* the epithelium is stratified pavement epithelium, consisting of a superficial stratum corneum like that of the epidermis, and a deep stratum Malpighii, consisting of two or three layers of polyhedral cells. Indications of minute papillæ are met with here. The whole thickness of this stratified pavement epithelium is between 0·016 and 0·024; the superficial stratum corneum is about 0·008 mm. thick. Where the stratified columnar epithelium joins the stratified pavement epithelium it is seen that, just as in other similar regions, the superficial cells of the former become suddenly shorter and are continued on the pavement epithelium as the superficial layer of scales.

But not in all places does the stratified pavement epithelium commence at *b ;* in some places the columnar non-ciliated epithelium may be followed up to *c,* being, however, in one or two places interrupted by short islands of stratified pavement epithelium ; beyond *c, i. e.* up to *d,* the epithelium is always stratified pavement epithelium.

Near the epithelium the tissue of the mucous membrane contains a plexus of large veins, chiefly running longitudinally, and the membrane is in many places infiltrated with lymph-corpuscles. The thickness of the mucous membrane at about *b* and *c* varies between 0·14 and 0·18 mm.; between *c* and *d* its thickness is 0·14 to 0·3 mm. From *a* to *d* the mucous membrane includes a continuous layer of serous glands ; they are here short tubes, generally branched and more or less convoluted. Their duct is also very wavy, and it passes in a very oblique or almost horizontal direction towards the surface, where it opens with a wide mouth, into which the surface epithelium is continued for a short distance. Where there is on the surface stratified pavement epithelium, the stratum corneum above mentioned is also continued for a short distance into the duct, as I have mentioned in my paper in the October number of this Journal, 1880.

The ducts possess a relatively large lumen, and are lined with a single layer of columnar cells, whose outer part is very conspicuously striated, and whose nucleus is spherical, and situated in about the middle of the cell; they resemble, therefore, completely the salivary tubes of Pflüger. The diameter of the ducts varies between 0·048 and 0·06 mm., the lumen being between 0·012 and 0·024 mm. The structure and size of the alveoli are in all respects identical to those described above of the aveoli of the serous glands of the other parts.

In some places the layer of these serous glands is much thicker than in others; in such cases there are bundles of unstriped muscle cells to be traced between the alveoli, forming in some places a plexus, and acting then as the matrix of the gland alveoli. The large veins, mentioned above, appear in this case also embedded in a tissue containing unstriped muscular tissue, and hence the appearances are produced not unlike those of a cavernous tissue (see my paper in this Journal, October, 1880). I have given an illustration of these relations in the 'Atlas of Histology,' plate xlvi, fig. 20.

……

Histological Notes. By E. Klein, M.D., F.R.S., Lecturer on Histology and Embryology in the Medical School of St. Bartholomew's Hospital.

Dissecting the salivary glands of the guinea-pig I noticed various points in their arrangement and structure which I do not think have been observed by others, and therefore deserve to be described.

Removing the skin of the facial and cervical regions the salivary glands present themselves in the following arrangement:

1. The parotid occupies the position as in the rabbit, dog, cat, and other mammals; it is very flat, its lobules loosely connected and scattered over a considerable area. Its colour and structure is the same as in other mammals. The lower part of the parotid, *i.e.* in the region of the angle of the inferior maxilla, but not specially marked off from the rest of the gland, is much thicker, and extends as a compact body, of about 15—20 mm. in length and 10 mm. in breadth, in a transverse direction.

Fixed to the posterior margin of this oblong thickened portion, but surrounded by its own connective-tissue capsule, is a small, oval, and somewhat flattened, whitish-looking body. Its length is about 8 mm. its breadth 5 mm. and its thickness between 2 and 3 mm. Its structure is identical with that of the submaxillary of the dog, that is, it is a mucous gland. The intralobular ducts are, like the salivary tubes of Pflüger, lined with columnar epithelial cells, whose outer portion is conspicuously fibrillar. The alveoli are branched and convoluted tubes, their relatively small lumen is lined with a single layer of columnar mucous cells of the ordinary description. There is, however, this distinction between the gland under consideration and the submaxillary of the dog, that there are no crescents in the alveoli of the former. I have carefully searched for them but failed to find them.

In a gland in which the ducts appear filled with a granular secretion the mucous cells of the alveoli show more or less distinctly two zones, an outer and an inner zone, the latter more transparent than the former. In a former paper (this Journal, April, 1879) I have called attention to a similar differentiation in the mucous cells lining the alveoli of the submaxillary of the dog.

The efferent duct lies on the side next the parotid, whose large ducts it joins.

Claude Bernard saw occasionally small mucous glands connected with the duct of the parotid of the dog. Heidenhain ('Hermann's Handbuch d. Physiologie,' V Band, p. 25) says that in the parotid gland (of the dog) he met with alveoli lined with mucous cells, but, he adds, this occurrence is not frequent.

2. Passing from the parotid along the inner side of the lower jaw we meet with a large gland, of a pale rosy colour, compact, oval, or rather pear-shaped, and holding the position of the submaxillary gland of other mammals. Its length is 15—20 mm., its breadth in the part nearest the parotid is much greater than that of the other end, in the former being about 10 mm., in the latter about 5 mm., its thickness is about 5 mm. Now, the structure of this gland coincides neither with that of the parotid of this or other animals, nor with that of the submaxillary gland of other mammals, for it is identical with that of the pancreas.

Boll ('Archiv f. Mikr. Anat.,' Band v), describes the submaxillary gland of the guinea-pig as a mixed gland, *i. e.*, its alveoli are in some places lined with mucous cells, in others with protoplasmic cells like those of the parotid. Lavdowsky ('Archiv f. Mikr. Anat.,' Band xiii, p. 286) denies this, but neither here nor on the following page (287), on which he gives a tabular classification of the various salivary glands of man and mammals, does he say of what nature the submaxillary of the guinea-pig is.

The alveoli are branched and convoluted tubes showing great inequality in size; their very minute lumen is lined with a single layer of colmmar or pyramidal cells, in each of which, just as in the pancreas, can be distinguished an outer and an inner zone; the former contains the spherical nucleus, and stains well in hæmatoxylin and carmine, while the latter looks granular and transparent, and does not stain. The former, *i. e.* the outer part, contains rod-like structures, which in some cells are very much coarser than in others. These rods are arranged longitudinally, and are distinct only near the membrana propria. I do not find, however, the centroacinar cells of Langerhans. The structure of the intralobular ducts is the same as in the sub maxillary gland.

It will be of great interest to inquire whether the secretion of this gland is similar to that of the pancreas.

Close to the outer capsule of the gland is found occasionally a minute oval lymph-follicle, ensheathed in its own connective-tissue capsule; its long diameter is about 0·6 mm., its breadth 0·18 mm. The surface of the lymph-follicle is not smooth, being notched-in at two or three places.

Attached to the posterior, or rather inner margin of the submaxillary gland, but contained within its own connective-tissue capsule, is an oval whitish gland, in size, aspect, and structure perfectly identical with the gland mentioned above in connection with the parotid; and it is this gland which has been noticed by Bermann ('Dissertat. Würzburg,' 1878) as the tubular (mucous) gland in connection with the submaxillary gland of the guinea-pig, rabbit, and other mammals.

I propose to call these two glands, viz. connected with the parotid and the submaxillary, as the *admaxillary* glands, and to distinguish the former, *i.e.* the one connected with the parotid, as the upper or superior, the latter, *i.e.* the one connected with the submaxillary gland, as the lower or inferior admaxillary gland. As a rule, I find the inferior gland in the position mentioned above;[1] in one instance (that of an animal three to four weeks old), it was found on one side more or less buried between the lobes of the submaxillary, and on the other side it could not be detected on the outer surface at all.

Measuring the diameter of the alveoli in sections through the resting glands of the same animal, hardened in spirit and stained in carmine, I find the transverse diameter of the alveoli of the parotid about 0·027 mm. and less; that of the alveoli of the admaxillary glands about 0·054 and more; and of the submaxillary gland about 0·04 mm.

Measuring the cells lining the alveoli of the same three different glands just mentioned, I find the following to be the mean sizes:

a. In the parotid, the cylindrical cells are 0·010 mm. in length, 0·007 in breadth; the pyramidal cells 0·010 in length, 0·009 in breadth next the membrana propria, 0·0036 next the lumen.

b. In the admaxillary glands, the cylindrical cells are 0·0198 mm. in length, 0·01 in breadth; the pyramidal cells 0·018 in length, 0·0136 in breadth next the membrana propria, 0·0036—0·0054 next the lumen.

c. In the submaxillary gland, the cylindrical cells are 0·0162 mm. in length, 0·009 in breadth; the pyramidal cells 0·0144 in length, 0·007 in breadth next the membrana propria, 0·003 next the lumen.

3. Attached to the front part of the submaxillary gland, and partly covering it, and often extending close to the parotid, is an oval whitish body; this is the thymus gland. As is well known, the guinea-pig does not possess a thoracic thymus; but the gland that I mentioned just now as situated bilaterally close to the submaxillary gland is found *in the young as well as in the adult*

---

[1] The position of this gland is not, however, constant, for in one instance I found it on one side in front, on the other at the inner margin of the submaxillary.

animal. In both instances its structure is the same. In the adult its length is about 11 mm., its breadth about 7 mm., and its thickness between 2 and 3 mm. The size of the gland does not vary much in the young and adult animal, and, owing to its position and lobulated nature, cannot be easily distinguished on the naked-eye inspection from the salivary glands. Its structure is identical with that of the thymus of other animals, and like this consists of the connective tissue forming the capsule and the prolongations of this into the interior as septa between the lymph-follicles. These latter are oblong, conical, cylindrical, or irregular in shape; they are of very various sizes, and their surface is either smooth: single follicles, or they are lobed, owing to more or less deep furrows, variable in number (two, three, and more up to ten), passing into the interior of the follicle: compound follicles. Where these furrows extend deep into the interior, the appearance is produced of a follicle being possessed of a number of secondary follicles, of very various sizes and shapes, and all projecting from the main body of the follicle. This appearance can be also interpreted by saying that a number of follicles have become more or less fused together.

As regards the size of the follicles, it is difficult to make exact measurements, owing their very irregular shape. The following measurements are taken from a vertical section through the gland:

One of the very large compound follices: length, 3·15 mm.; breadth, 1·01.

Another large follicle: length, 2·92; breadth 1·23 mm.

A very small follicle, the length, 1·01—1·23; breadth, 0·68 mm.

In a horizontal section, the length of a large follicle 2·7, the breadth 1·23 mm.; of a small one, the length 0·92, breadth 0·53 mm.

As regards the structure, the follicles completely coincide with those of the thymus. As in these, the outer part of the follicle *i.e.* the cortex of Watney, both in the single follicles as well as in the secondary projections of the compound follicles, stains deeper than the central part, *i.e.* the medulla of Watney, which is non-transparent, and includes fewer small lymph-corpuscles than the former; the medulla contains, like that of the follicles of the thymus in other animals, larger cells, with one, two, or more large clear nuclei, such as have been known previously, and have been more minutely investigated by Afanassiew ('Archiv f. Mikr. Anatom.' Band xiv), and Watney ('Proceedings of the Royal Society,' No. 187, 1878). Also the concentric bodies of Hassall are found in the follicles of the thymus in the guinea-pig. Their structure is the same as of those described by Afanassiew ('Archiv f. Mikr. Anat.,' Band xiv, 3rd part). Seeing that these concentric bodies occur in all the glands of adult animals that I have examined, and seeing that the structure of the follicles remains the same, *i.e.* their tissue does not undergo the fatty and connective-tissue degeneration that always occurs in the follicles during the involution of the thymus gland in other animals, it follows that Afanassiew's view of the degeneration of the follicles being caused, or rather initiated, by the concentric bodies cannot be sustained.

On the INTRA-CELLULAR DIGESTION *and* ENDODERM *of* LIMNOCODIUM. By E. RAY LANKESTER, M.A., F. R.S. With Plates VIII, IX, X.

No observation made within the last three years appears to me to have greater importance and general significance for the progress of Biology than the discovery of the inception of solid food particles by endoderm cells in the Planarians and Nematophorous Cœlentera, by Elias Metschnikoff.

The actual history of this discovery appears to date from the observations of Lieberkühn on Spongilla ('Müller's Archiv,' 1857). The first observer to suggest the existence of intra-cellular digestion in an organism other than one of the Protozoa or of the Porifera, was Allman, who, in his memoir on Myriothela ('Philosoph. Transact.,' vol. 165, 1875, p. 552), describes a thin layer of protoplasm as occurring on the free surface of the endoderm, and observes that "its occurrence, with its pseudopodial extensions, on the gastric surface of the animal, is full of interest, and suggests a close analogy between the absorptive action of the gastric surface and amœboid reception of nutriment."

Next we have a note by Metschnikoff in the 'Zoolog. Anzeiger,' 1878, p. 387, in which the inception of solid food particles by the cells lining the alimentary canal of certain Planarians is described, and in the 'Zeitsch. wiss. Zoologie,' 1879, p. 371, the same author describes similar observations on Sponges.

Led by these observations of Metschnikoff, Jeffery Parker came to the conclusion that a similar mode of digestion obtains in Hydra. In his paper on the histology of *Hydra fusca,* published in the 'Proceedings of the Royal Society,' 1880, and in this Journal, April, 1880, Parker carefully describes the amœboid character of the endoderm cells of Hydra as seen in sections, the extent and activity of their movements during life having been previously insufficiently recognised. Dark-coloured irregular granules of various sizes are found within these cells, and were noted by Kleinenberg to vary in number with the state of nutrition of the animal. Parker is convinced that these bodies are food particles, taken into the protoplasm of the cells, from the partially disintegrated bodies of the *Entomostraca* in the digestive cavity. The clearest case of ingestion of solid particles observed by Parker was when a diatom was seen to be completely embedded in the protoplasm of a cell. Parker very judiciously observes that it is quite possible that a preliminary disintegration of the animals taken in is performed by juices secreted by the endoderm cells; but the final digestion seems to take place in the actual protoplasm of the cells, into which the food particles are taken in the solid form. He does not suggest how the digested material is distributed to the other cells of the Hydra.

Lastly, we have a brief *résume* from Metschnikoff in the 'Zoolog. Anzeiger,' No. 56, May, 1880, of a series of observations on the subject of intra-cellular digestion, carried on by him in the spring at the Zoological Station at Naples.

Metschnikoff made use of carmine powder, which he observed to penetrate the endoderm cells in many Hydroid polyps and Hydromedusæ (Plumularia, Tubularia, Eucope, Oceania, Tiara, Praya, Forskalia, Hippopodius, Pelagia, Beroe among Ctenophora and Sagartia and Aiptasia among Anthozoa.) In the Trachymedusæ *Liriope, Carmarina, Cunina,* Metschnikoff failed to establish the occurrence of intra-cellular digestion. It will be observed that the method employed by Metschnikoff is not altogether a conclusive one. The majority of forms studied by him, like the Hydra studied by Parker, are opaque, and, consequently, it was not possible to watch the process of ingestion during life. In *Praya,* however, Metschnikoff studied a transparent form, and was able to observe the throwing out of pseudopodia by the endoderm cells, and their fusion into a plasmodium. Even here, however, it seems that there is still room for doubt as to whether the pseudopodia are really active in digestion, for Metschnikoff only speaks of their penetration by carmine particles. It is exceedingly probable that when his observations appear at greater length, we shall find that they include the fact of inception of natural food materials, such as Algæ, disintegrated Entomostraca, &c. The mere penetration of minute particles like those of powdered carmine into amœboid cells would not in itself indicate a natural process of intra-cellular digestion. Such a penetration of carmine particles into the amœboid corpuscles of vertebrate blood is well known, and does not in that case lead to the inference of normally occurring intra-cellular digestion.

On this account I think some importance attaches to the observations which I made last summer on the intra-cellular digestion of Limnocodium, the fresh-water Medusa discovered in the lily-house of the Botanical Gardens, Regent's Park, London. I was able, in this animal, on account of its exceeding transparency, to study the endoderm cells during life, and to establish the fact of the inception of *natural* food materials by those cells.

I have since made a careful study of the endoderm of various regions of this Medusa's body in specimens preserved in osmic acid.

The series of questions which arise in connection with this phenomenon of intra-cellular digestion are so numerous and important that it is quite certain that the most complete study of the endoderm of the various regions of the digestive tract is necessary before the phenomenon can be rightly appreciated. The following considerations, amongst others, are those which naturally present themselves to an observer as indications directing his inquiries.

PRELIMINARY CONSIDERATIONS.

1. Supposing it to be established that some of the endoderm cells in Hydroid and Anthozoan polyps are capable of ingesting solid food particles, the question arises whether this is an occasional and accidental phenomenon, or whether it is a normal and definitely fixed function of such endoderm cells.

2. The question also occurs as to whether all the endoderm cells have this property, or whether it is limited to certain groups of these cells, whilst a distinct kind of activity (possibly similar to that of the gastric cells of other animals) is assigned to other cells in the same animal.

3. Further, it is of fundamental importance to ascertain what becomes of food particles ingested by amœboid endoderm cells. Are these particles *digested* by these cells as food particles are by an Amœba? or are they again ejected unchanged.

4. Supposing the food particles to be digested—that is to say, dissolved and converted into diffusible peptones or analogous substances—what becomes of such peptones? Are they simply retained by the endoderm cell for its own nutrition? or are they passed by that cell away from its surface to subjacent cells, which thus are nourished by a process of diffusion? or, again, are the products of digestion *returned* by the endoderm cell to the alimentary tract, and carried thence by its ramifications, as a nutrient fluid, into various regions of the body?

5. Are there any special kinds of food particles which are ingested in the solid form by certain endoderm cells, whilst other food materials are dissolved and distributed by diffusion, in the same way as are albuminoids and carbo-hydrates, by the alimentary organs of Vertebrata. Is there any ground for supposing that the ingestion of fats in a particulate form by Vertebrata is a survival of the intracellular digestion now established as occurring in Cœlentera and Planarians?

When we take into consideration the structure of Hydra it seems possible that the sole nutrition of the ectoderm cells is, by means of the products of digestion, elaborated by endoderm cells, such products passing through from the endoderm cells to ectoderm cells by osmosis. And we have definite observations of Metschnikoff (in the case of Ctenophora) upon the passage of carmine particles, away from the endoderm cells which took them up, into mesoderm cells lying beneath them, which favour the notion of such a passage. It may, however, be noted that the carmine particles do not appear in this case to have been digested—that is, chemically changed and dissolved—and hence the passage of the particles in question is a phenomenon similar, in essential respects, to the passage of fat particles unchanged through cells on the surface of the intestinal villi of Vertebrates to subjacent cells and cell spaces.

On the other hand, when we try to bring the structure of the Medusæ, with their elaborate gastro-vascular canal system, into relation with the facts of intra-cellular digestion, we find it impossible to admit that the nutrition of the organism can be carried on by the mere osmotic passage of nutrient matters from those cells which are active as intra-cellular digesters to subjacent cells. Metschnikoff has observed that in many Cœlentera the intra-cellularly digestive cells are limited in number and position, and this fact I can fully establish by my observations on Limnocodium. Hence the regions in which subjacent cells can be nourished by superjacent intra-cellularly digestive cells is exceedingly limited. The products of the digestive activity of the intra-cellularly digesting endoderm cells are in all probability, in the Medusæ, returned to the alimentary canal, and carried on by the agency of the gastro-vascular canals into the remoter parts of the organism.

Bearing in mind these considerations we may proceed to an examination of the endoderm of the gastric and gastro-vascular cavities of Limnocodium.

INTRA-CELLULAR DIGESTION IN THE PROXIMAL REGION OF THE GASTRIC TUBE OBSERVED DURING LIFE.

The manubrium of Limnocodium is a somewhat quadrangular tube, which depends during life below the margin of the umbrella. Its cavity, the stomach, presents a considerable difference in the structure of its lining cells, the gastric endoderm, in different regions. Where the four angles of the stomach-tube are inserted into the umbrella they are slightly produced, and give rise to the four radiating canals. The enlarged angles of the stomach are lined by peculiar cells, in which I observed an intra-cellular digestion to be proceeding during the observation of living specimens. In Plate VIII, figs. 1 and 2, two drawings of this intra-cellularly digestive endoderm, taken from living specimens, are reproduced. The cells are seen to form a widely-open meshwork, large spaces occurring between neighbouring cells, the cells being connected by ridges, which traverse the spaces. The cells themselves appear to be naked, and their protoplasm is irregularly aggregated so as to form masses with pseudopodia-like processes, and also clothing the ridges connecting cell with cell. Spherical nuclei (*a*), with spherical nucleoli, are placed at intervals in the protoplasm, and have a uniform appearance and size, which is characteristic of the endodermal nuclei throughout the gastric and gastro-vascular area. They measure about $\frac{1}{5500}$th inch in diameter, whilst the spaces in the meshwork are on an average about $\frac{1}{2000}$th by $\frac{1}{1000}$th of an inch in the smallest and largest diameter. In the neighbourhood of the nuclei are numerous dense-looking masses of an ill-defined shape, which give to the cell-substance a certain opacity (*b*). In fig. 2 there are also seen vacuole-like spaces or clearer portions of the cell-substance, containing very dark minute granules.

In both figures there are seen embedded in the protoplasmic net-work green unicellular organisms.

In fig. 1 a large Euglena-like form (*x*) is embedded in a plasmodium formed by the confluence of cell-substance from some four or five cells. In the upper part of the figure two Protococci are seen embedded in pseudopodia-like processes of the cell network. The one to the left (*y*) is in a state of disintegration, that to the right (*z*) has not yet been altered appreciably.

In fig. 2 the letter *x* points to an ingested organism, which has been almost entirely broken up and its colouring matter lost; *y* marks a Protococcus reduced to the condition of a few coloured granules, whilst *z* is placed near a recently ingested Protococcus.

I did not observe the movement of the pseudopodia-like lobes of this protoplasmic network during life, nor the actual process of the entry of a solid food particle into its substance.

I may mention in this connection that the proximal region of the stomach in many specimens of Limnocodium was infested by a remarkable little free smimming, yet tubicolous Rotifer, which carried its tube about with it as it swam.

This parasite appeared to escape altogether the embraces of the amœboid endoderm cells, as well as to be unaffected by the digestive secretions, if any such were present.

Structure of the Gastric Endoderm of various Regions, as seen on Treatment with Reagents.

The true structure of the endoderm of the gastric tube becomes evident when specimens which have been treated with osmic acid are stained with picro-carmine and examined under the highest powers of the microscope by means of teazing and sections. The meshwork of amœboid cells in which intra-cellular digestion takes place is seen to be confined to the four proximal angles of the gastric tube.

Endoderm of radial canals.—The endoderm suddenly changes its character at the commencement of the radial canals (see Plate IX, fig. 8 *w*), and in these continuations of the gastric chamber, instead of a network, we find closely-set nuclei, the cell areas not distinctly marked off from one another and the protoplasm free from granulations. These cells as seen in the living condition are ciliated.

The nuclei are precisely similar in form and size to those of the gastric tube, and take up the carmine staining in a way which is characteristic of the endoderm nuclei in general (see Plate).

Endoderm of the ring-canal.—I have in my former paper on Limnocodium (this Journal, July, 1880) described and figured (Plate XXX, fig. 6) the modification of the endoderm cells on the abumbral wall of the marginal ring-canal. The cells of the adumbral wall are like those of the ring-canals. The cells of the abumbral wall are modified by the deposit of block-like masses of a dense substance within them, which usually obscure the nuclei. These cells also have a remarkably angular and irregular form. They form the representative in Limnocodium of the cartilaginous marginal ring of Trachymedusæ, and are drawn out into lobes which are continuous with the roots of the tentacles. The endoderm of the gonads (genital pouches) has a similar structure to that of the abumbral wall of the ring-canal.

Endoderm of the gonads.—A portion of this part of the endoderm is drawn in Plate IX, fig. 9. It quite closely resembles that of the abumbral wall of the ring-canal. The block-like deposits within the cells and the dark colour which the whole layer had assumed under the influence of osmic acid were sufficient to obscure the nuclei, which accordingly are not seen in the drawing.

Endoderm of the middle third of the gastric tube.—This is represented in Plate X, figs. 1 and 2. Over a comparatively small area the cells present a uniform hexagonal pavement when viewed from their free surface (Plate X, fig. 2). The nuclei have the same size and character as in the other endoderm cells, but the cell substance is small in quantity and of a homogeneous appearance. Here and there in this and in other parts of the gastric tube, nematocysts are scattered in considerable numbers. They sometimes are embedded in the endoderm (*g g*) so as to present a spherical appearance, and the first explanation of their appearance here which suggests itself, is that they have been developed in endoderm cells. But the fact that they are scattered very irregularly and occur in all regions of the gastric tube sporadically is against this view. Further the absence of any cells of the endoderm in which stages of the development of such nematocysts can be made out is also against the view that they are developed here. Lastly, the facts that they are precisely similar in appearance and size to the nematocysts of the tentacles, and that actual bits of ectoderm cells containing three or four nematocysts side by side may be observed occasionally in the gastric tube, are in favour of the view that the nematocysts occurring in the gastric endoderm have been swallowed by the Medusa with its prey, and have become embedded in the soft endoderm fortuitously.

This explanation has been offered by Mr. Marcus Hartogg (see this Journal, 1880) of the similar occurrence of nematocysts in the endoderm cells of Hydra; and for the present case, as well as that of Hydra, it seems to me to be satisfactory, though it must be remembered that there is no great improbability connected with the development of nematocysts by endoderm cells unless the mesenterial filaments of the Anthozoa can be shown to have an ectodermal origin.

Above and below the limited region of homogeneous hexagonal cells the endoderm of the middle third of the gastric tube exhibits two distinct concomitant modifications (Plate X, fig. 1).

1. Some of the cells are enlarged and highly granular (*b*), in fact have become secretion cells or unicellular glands.

2. The cells are no longer continuous, but here and there the cell-pavement is deficient, actual gaps of greater or less size (*f*) making their appearance between neighbouring cells.

Endoderm of the oral third of the gastric tube.—The endoderm of the oral region presents a condition which may be considered as a development of that last described. In Plate IX, fig. 3, a piece is represented. All the cells are here either fully developed as secretion cells (*b*), large clear bodies about the $\frac{1}{1500}$th inch in diameter, or are on their way to this condition (*h*). The nuclei have the characteristic form and size (*a*). The intercellular spaces (*f*) are very small and few, whilst surrounding the enlarged secretion cells and enclosing the yet young secretion cells is a sort of laminated matrix (*d*). This matrix is to be regarded as an intercellular substance of a horny or gelatiginous character. It forms a complete framework to the whole series of cells, enveloping each of the more fully-grown secretion cells in a distinct capsule, which is broken through on the free surface of the endoderm by circular apertures (Plate IX, fig. 4) corresponding each to a ripe secretion cell.

The nuclei of the ripe secretion cells are less defined than those of the younger cells, and I am inclined to think that they undergo atrophy, and that the whole secretion cell, when its chemical metamorphosis is complete, is passed into the gastric cavity. I am also led to believe that this takes place *periodically* by the following observation.

Whilst in some specimens of Limnocodium studied by me the oral gastric endoderm presented uniformly the appearance represented in Plate IX, fig. 3, yet in another batch of specimens it had uniformly a very different appearance, which is drawn in Plate IX, fig. 6. In this case all the sites which in the former example were occupied by large-sized secretion cells are empty (*f*). The framework (*d*) remains, and projecting into the empty spaces, as though destined in their turn to occupy them, are small secretion cells (*b*).

I can only interpret these appearances on the supposition that the large cells are shed when ripe, and that the next generation grow out into the spaces left, whilst a third generation is developed from the scattered cells, with at present little protoplasm, and merely indicated by the nuclei (*a a*). And, further, it seems that the ripening and shedding of the secretion cells must take place in the whole of the oral gastric endoderm simultaneously.

It is possible that a periodicity of this kind may be inherent in the growth and development of these cells. It is also exceedingly likely that the simultaneous clearing off of all the ripe secretion cells is due to some special act of the Medusa. It is likely that the act of feeding, of seizing prey, such as Entomostraca (on which the Medusa was frequently seen to feed), would be the determining cause of the clearing out of the secretion cells.

This hypothesis is borne out by some further facts, to be related below.

Whether it be accepted or not, it is clear that we have a copious secretion produced by the oral-gastric endoderm, and it is in the highest degree probable that this secretion has the action of a ferment or of a solvent upon the larger food masses taken into its gastric tube by Limnocodium.

A modification of the endoderm, not unlike this of the oral-gastric region of Limnocodium, is described by Claus in *Charybdæa marsupialis*, that most interesting of all Medusæ. In his admirable memoir on Charybdæa ('Arbeiten des Zoolog. Instituts zu Wien,' 1878) Claus gives, in his plate iv, figs. 36 and 37, drawings of endoderm from the oral portion of the gastric tube, closely resembling that figured by me in

Plate IX, fig. 3. Claus distinguishes two kinds of gland cells, corresponding to what I believe to be young and old stages of one kind of gland cell. A difference exists in the fact that in Charybdæa ciliated cells are interspersed among the gland cells, whilst such do not appear to be present in the same region in Limnocodium.

Endoderm of the proximal third of the gastric tube.—As we pass upwards towards the umbrella, along the walls of the gastric tube, the endoderm cells gradually open out, leaving intercellular spaces, and where the tube expands slightly in the horizontal plane the characters exhibited in Plate IX, figs. 1 and 2, are assumed. This is the region which has already been described above in the living condition, and in which intra-cellular digestion takes place.

A comparison of figs. 1 and 2, Plate IX, with figs. 3 and 6 of the same plate, shows that we have in this region the same elements of form to deal with as in the oral region, but somewhat differently characterised. There are large inter-cellular spaces (*f*), which are marked off by a somewhat fibrillated or laminated framework (*d*); spherical nuclei, which take the carmine staining, are scattered irregularly, and have surrounding them a protoplasmic cell substance, which is very deficient in some parts, and is aggregated in other parts; it appears to be continuous throughout, and is not marked off into separate cells corresponding to the individual nuclei. Two nuclei are often closely approximated, indicating recent division, but I have not met with any in process of division.

Corresponding to the secretion cells of the oral-gastric endoderm are circular or oblong groups of oval bodies of a refringent substance (*b*), which appear to correspond to the groups of large granules seen in the living specimens. As now seen (after the action of reagents), these groups appear to be formed by oval droplets of a homogeneous transparent substance, which stain of a pale-pink colour with picro-carmine, and are strongly emarginated by the difference of refractive index between their substance and that of the material in which they are deposited. Whilst representing, in position and size, the secretion cells of the oral-gastric endoderm, these bodies have a different structure from those cells, and the substance which stains pink is unlike anything present in that region.

Large vacuole-like spaces also occur (*e e*), in which a few dark granules and irregular particles may be observed, whilst the substance filling the vacuole is transparent, and stains pink with picro-carmine. It also appears to have been *precipitatea* as a homogeneous or excessively finely granular solid by the action of the reagents.

The substance filling the vacuoles (*e*) is apparently identical with the substance filling the numerous oval spaces of the bodies (*b b*). At the same time there can be little doubt, from the comparison of the prepared specimens with the living, that the vacuoles are *food vacuoles,* viz. spaces into which solid food materials have been taken and digested. Accordingly the material which they contain is an albuminous substance resulting from the digestion of those food particles.

From these considerations it seems not improbable that the pink substance of the bodies (*b*) is also an albuminous substance resulting from digestive activity.

I submit as suggestions for further examination, when the histology and physiology of the endodorm is attempted in other Medusæ, that these bodies (*b*) are either points at which numerous small food particles have been incepted and digested by the protoplasm, or, what is more probable, that they are portions of the protoplasm of this remarkable meshwork which are especially active in "working up" the products of intra-cellular digestion, and that they periodically *discharge the albuminous product of digestion and elaboration* into the gastric chamber, whence it passes into the radial canals and marginal canal to nourish the outstanding parts of the organism.

That albuminous substances in a digested state must pass into these canals, either in this way or as the result of the digestion of a portion of the food by juices secreted into the gastric cavity, appears obvious when the limited number and area of the intra-cellularly digestive cells is considered.

Projecting into the spaces (*f*) of the meshwork are pseudopodia-like processes (*c* in figs. 1 and 2, Plate IX); these are not only given off from the larger masses of cell-substances, but appear to spread along the fibro-laminar trabeculæ (*d*) of the meshwork, and whilst clothing the trabeculæ, and often projecting from them into the inter-cellular spaces, also keep the protoplasm of neighbouring masses in continuity.

Just as in the oral-gastric endoderm, two very different conditions of nourishment and activity were observed, so here in the endoderm of the proximal end of the gastric tube—which I will call the ingestive endoderm—there were two very different conditions which came under my observation. *The two conditions of the ingestive endoderm were definitely related to the two conditions of the oral endoderm.* When the oral endoderm presented the condition of abundant large secretion cells filling up the inter-cellular spaces (Plate IX, fig. 3), then the ingestive endoderm had the appearance just described (Plate IX, figs. 1 and 2). It was active in throwing out pseudopodia into the large inter-cellular spaces, and was feeding upon the small particles (such as Protococci and Euglenæ) which chance threw in its way. In fact, whilst the oral endoderm was full and unshed, the ingestive endoderm at the other end of the gastric tube was half-starved, with great inter-cellular spaces and eager pseusopodial processes, making the best of bad times, and taking up materials previously unprepared.

In those specimens, however, in which the oral endoderm had shed its secretion cells, and in which I have supposed that an act of swallowing some large prey had recently taken place—in these the ingestive endoderm of the proximal end was totally changed in appearance. It was gorged with finely granular matter; its inter-cellular spaces had almost entirely disappeared in consequence of the swelling out of the protoplasm, now remarkable for its granular structure.

The appearance is represented in Plate XI, fig. 5. The masses of oval metamorphic products (*b b*) are still present, but the spaces are reduced to a few small chinks (*f*). The trabeculæ of the framework are no longer visible, owing to the swelling of the protoplasm and its granular opaque character; they are concealed by the contiguous edges of the enlarged masses of protoplasm.

I conceive this change to be due to the absorption by the ingestive cells of a very abundant supply of albuminous matters obtained by the digestion in the cavity of the gastric tube of a Daphnia, Cyclops, or some such form. The raw products of gastric digestion—partly dissolved partly in the form of fine particles—would, it may be assumed, be taken up by the amœboid ingestive cells, just as are the rarer living food particles in times of dearth when so copious a feast as that afforded by a Daphnia is not forthcoming.

As to the return of the ingestive endoderm to its meshwork state, with pseudopodia ready for the inception of large food-bodies, I have no observations to offer, and I will not speculate further upon the possible activity of the ingestive endoderm in elaborating the food matters taken in by it.

It is a matter for regret that the fresh-water Medusa died down in the lily-house tank a few weeks after its discovery, so that I have not been able to follow up experimentally some of the suggestions which the study of the endoderm has afforded me. It would be an easy matter with Limnocodium and, indeed, with other small Medusæ, to determine experimentally the condition of the endoderm cells of different regions of the gastric tube *before*, *during*, and *after* the introduction into that tube of an Entomostracous Crustacean.

The observations and interpretations which I have put forward in the preceding pages cannot be regarded as more than an early contribution to the subject of intra-cellular digestion and the comparative physiology of digestion in general, which I do not doubt is about to be investigated with new vigour and interest, in consequence of Metschnikoff's researches.

SUMMARY.

1. The cells of the endoderm of the gastric tube and gastro-vascular canals differ very considerably in form and in the chemical metamorphosis of its substance in different regions.

2. The nuclei are alike in all as to size and form, excepting in the cells of the abumbral wall of the marginal canal and the similar cells of the endoderm of the genital pouches.

3. These latter are angular, close-set cells, with dense block-like deposits in their protoplasm concealing the nucleus.

4. The cells of the radial canals are close set and ciliate with sparse, hyaline protoplasm.

5. The endoderm of the gastric tube is divisible into three regions: *a*, the oral, *b*, the mid-gastric and *c*, the ingestive or proximal.

6. Only the cells of the proximal region exhibit intra-cellular digestion.

7. The cells of the oral region produce a secretion by their development as secretion cells (goblet cells of Claus).

8. The cells of the mid-region are inactive.

9. The cells of the proximal region appear, under certain circumstances, as an open meshwork giving off amœboid processes, by means of which they take in solid food particles.

10. Under the same circumstances the secretion-cells of the oral region are richly developed and in place.

11. Under other circumstances the cells of the oral region appear to have been, to a large extent, shed, leaving inter-cellular spaces.

12. When this is the case, the secretion-cells of the proximal are swollen and granular, and the inter-cellular spaces of the meshwork obliterated.

13. It is inferred that the latter circumstances are the result of the taking into the gastric tube of relatively large prey; whilst the former condition is one of comparative fasting, in which such small food bodies as may be ingested by the endoderm of the proximal region are proportionately valuable to the organism.

ADDENDUM ON THE ENDODERM OF THE TENTACLES.

In Plate X, fig. 3, a surface view is given of one of the smallest sized tentacles, for the purpose of showing the mode in which the thread-cells are clustered in groups upon its surface.

These groups appear to have a spiral arrangement, more or less definitely expressed. In fig. 6 two thread-cells are represented with ejected filament, showing the series of six small barbs at its base. In fig. 4 an optical median longitudinal section of the tentacle is drawn, in order to show definitely the character and arrangement of the endoderm cells. The specimen from which the drawing was taken had been treated with osmic acid and picro-carmine. An actual transverse section of a similar tentacle is shown in fig. 5. The endoderm cells consist of a dense, highly-refringent substance, which is somewhat wrinkled by the action of the reagent. The nuclei are a little smaller than those of the gastric endoderm. In some cases a small amount of granular cell substance may be seen radiating from the nucleus, but the whole cell body otherwise has been metamorphosed into a homogeneous cartilaginoid substance. There is no continuous lumen, although the cells are disposed in a single series around the axis of the tentacle, and leave, on shrinking, a small space where their adaxial surfaces should come into contact. This potential lumen appears not to be continuous, even in the specimens treated by reagents, and in living specimens it has no existence.

A structureless lamella (Stutz-lamella) (*c*) adheres closely to the endoderm cells. Subjacent to the ectoderm cells are the very fine transversely-striped muscular processes (*d*), which are developed on their inner faces, not only here but in the case of the subumbrellar ectoderm and of the ectoderm of the adumbrellar surface of the velum.

On the MICROMETRIC NUMERATION *of the* BLOOD-CORPUSCLES *and the* ESTIMATION *of their* HÆMOGLOBIN.

BY MRS. ERNEST HART.

THE micrometric numeration of the blood-corpuscles and the estimation of hæmoglobin are operations which, though of comparatively recent introduction, have rapidly passed out of the sphere of laboratory experiment into practical use as exact methods of physiological and clinical investigation. Those who have worked at this subject cannot, however, have proceeded far without discovering that the methods and instruments hitherto in use are inconveniently imperfect and vitiated by numerous sources of error. Some recent improvements by M. Malassez, assistant in the Laboratory of Histology in the Collége de France, appear to me to have done much to remove these disadvantages.

Before proceeding, however, to describe the new Corpuscle-Counter which M. Malassez has just introduced, it may be well to say a few words on the methods and instruments usually employed for the numeration of the corpuscles. The three which have been hitherto in general use are those known as the instruments of Malassez, Hayem, and Gowers. In the method first invented by Malassez (the *Compte-Globules Capillaire*) 100 parts of a 5 per cent. solution of sulphate of soda are mixed in a special instrument called the *Melangeur Potain* (Fig. 1) with one part of blood. This solution is then drawn into an extremely fine capillary tube. The calibre of this tube is known ; hence the volume of the fluid which the tube contains in a given length, say in 500, 400, or 300 micro-millimeters is also known.[1] This volume is some fraction of a cubic millimeter. It follows that the volume multiplied by the denominator of that fraction will equal a cubic millimeter. The multiplier is written on a glass plate, on which the capillary tube is mounted. Before using the instrument the eye-piece of the microscope must be exchanged for an eye-piece containing a micrometer divided into a number of square millimeters. Then by means of a stage micrometer, the microscope must be graduated, so that ten of the square millimeters of the eye-piece correspond exactly to the arbitrary length (500μ, 400μ, or 300μ) fixed upon. A mark being then put on the tube of the microscope, this magnifying power—the lens being always the same—can be easily found again. The process and calculation are then

---

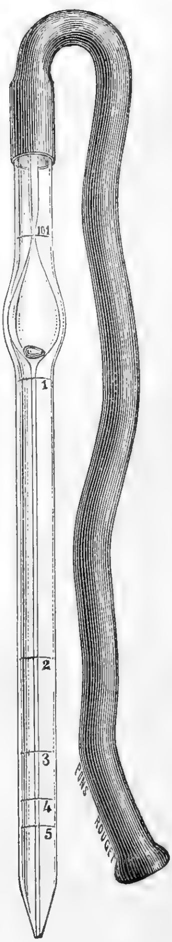
[1] This unit, the thousandth of a millimeter, is expressed by the Greek μ.

very simple. The solution of blood, after being well shaken in the Mixer, is drawn up into the tube by capillary attraction, and the number of corpuscles contained in the given length of the tube is counted. This is rendered extremely easy by the aid of the small squares of the ocular micrometer. The number obtained is multiplied by the denominator written, as already mentioned, on the glass plate, and the product multiplied by 100 or 200, according to the strength of the solution used, gives the number of the corpuscles per cubic millimeter. Thus, supposing that, in a length of 500μ, the volume $=\frac{1}{150}$ of a cubic millimeter, and that a 1 per cent. solution occupying this space contains 300 corpuscles, then

$$300 \times 150 \times 100 = 4{,}500{,}000$$

the number of corpuscles per cubic millimeter of blood. The calculation, however, is not so simple as this in practice, since the multiplier, of course, will seldom be a round number.

FIG. 1.



M. Ranvier, in his ‘Traité Technique d’Histologie,’ says that this method, considering the short time it takes, gives, of all those hitherto known the best results. It is certainly very accurate, but it has two great disadvantages. In the first place, owing to the necessity of undertaking the somewhat difficult task of graduating the microscope, the same *Compte-Globules* must always be used with the same microscope and the same lens; hence its use clinically is obviously very much curtailed. Secondly, the extreme delicacy of the instrument is a serious drawback. Though a person with ordinary manipulative skill may learn to use this instrument, it requires more than ordinary care to keep the minute capillary tube absolutely clean and this is positively necessary, since a minute particle of dirt, or a few dried corpuscles left in the tube, will vitiate the accuracy of the results. My own experience is that, though I am able to make correct observations with this instrument in the pure air of Paris, I am unable in London, where nothing is clean, not even distilled water, to keep the tube quite free from dirt.

Hayem's method differs altogether from that of Malassez. The unit is arrived at by means of a cell $\frac{1}{5}$ mm. deep, of which an area of $\frac{1}{20}$ of a square mm. is marked off. This gives us, therefore, $\frac{1}{5} \times \frac{1}{20} = \frac{1}{100}$ of a mm^3. The area of $\frac{1}{20}$ square mm. (which is, of course, $\frac{1}{20}$ of 1,000,000 square μ) is obtained by using an ocular micrometer, on which is drawn an oblong, 5 mm. long by 4 mm. wide, and divided into 20 squares. By means of a stage micrometer the microscope is graduated so that the 5 mm. exactly correspond to an objective length of 250μ. The mixture of blood and the preserving fluid is made at a strength of 8 per 1000. The method of mixing is that invented by Vierordt. A pipette, holding 8 cubic mm., is used to measure the blood; another, of a calibre of 992 cubic mm., to measure the preserving solution. The two fluids are mixed in an open glass vessel by means of a glass rod. The same rod is also used to deposit the drop on the slide. The cover-glass is kept *in situ* by the capillary attraction existing between two moist glass surfaces, a drop of water or saliva being placed at the edge of the cover-glass, and allowed to run under it. The chief objections to this instrument are the uncertain depth of the cell, the clumsy method of mixing, the possible elevation of the cover-glass by allowing too much water to run under, it and also the same objection made to Malassez's capillary *Compte-Globules* just considered, namely, that, owing to the necessity of graduating the microscope, it is of limited use as a clinical instrument.

In Gowers' Hæmacytometer,[1] which is a modification of Hayem's, a very decided improvement is made. In the depth of the cell and in the old-fashioned mode of mixing, it is identical with that of Hayem; the solution of blood used being, however, at 5 per 1000 instead of at 8 per 1000. The improvement consists in measuring the area and drawing the squares in which the corpuscles are to be counted upon the floor of the cell itself. Squares, with sides $\frac{1}{10}$ of a mm. long, are drawn on the floor of the cell. The area of each, therefore, is $\frac{1}{100}$ of a sq. mm. The cell having a depth of $\frac{1}{5}$ mm., and any 10 squares an area of $\frac{1}{10}$ of a sq. mm., the cubic contents of any ten squares taken within the cell will be—

---

[1] "On the Numeration of the Blood-corpuscles," by Dr. Gowers, 'Lancet,' Dec., 1877.

$$\tfrac{1}{10} \times \tfrac{1}{5} = \tfrac{1}{50} \text{ mm}^3.$$

The number of corpuscles observed in ten squares, therefore, multiplied by fifty will give the number in a cubic millimeter of the solution; and that multiplied by $\frac{1000}{5}$ will give the result for a cubic millimeter of blood.

For example, if the number of red corpuscles counted on ten squares is 500, the calculation is simply

$$500 \times 50 \times \frac{1000}{5} = 500 \times 10{,}000 = 5{,}000{,}000 \text{ per mm.}^3,$$

or, in other words, there is nothing to be done except to add 0000 to the number found by counting. No graduation of the microscope is required, so that the instrument can be used anywhere and with any lens. This renders it more convenient as a clinical instrument, and it is therefore that which is in general use in the English hospitals. It gives approximately accurate clinical results. I must, however, point out that it is liable to four serious sources of error, which destroy the value of observations made with it from an absolute and scientific point of view. These sources of error are—the uncertain depth of the cell; the inequality of the surface of the cover-glass; the method of placing the cover-glass on the drop; and the means used to make the mixture and to place the drop in the cell. Since a paper by two American physicians was published,[1] showing how careful observations may be vitiated by the variation in the depth of the cell in different instruments, the error in the depth has been written on the slide. In the Hæmacytometer which I habitually use the cell has a depth of 190μ, instead of 200μ. This error necessitates a troublesome correction in each calculation. The correction is made by multiplying the number of corpuscles obtained by 20 and dividing by 19; for let *a* equal the number of corpuscles in a mm.^{2} multiplied by the actual depth of the cell,

$$190\mu : \text{a} :: 200\mu : x.$$

This method of correction which is that recommended is, however, irksome when a great number of observations have to be made. I wish now to suggest that it may be altogether avoided by directing the instrument maker to graduate the pipette or mixer, whichever may be used, not, as at present, on the assumption that the depth of the cell accurately measures 200μ, and therefore that a solution of 5

---

[1] "Blood-Cell Counting: a Series of Observations with the Hématimètre of M.M. Hayem and Nachet, and the Hæmacytometer of Dr. Gowers." By Drs. Henry and Naucrede.—'Boston Med. and Surg. Journ.,' April, 1879.

per 1000 should be used to ensure correct results, but so to graduate it as to make a solution of such a strength that, having previously ascertained the actual depth of the cell an area of $\frac{1}{10}$ mm.^{2} multiplied by this depth shall give $\frac{1}{50}$ mm.^{3} In this way the necessity for arithmetical correction of each observation is avoided, the special adjustment of the pipette affording a correction which applies to all observations made with the instrument. Thus, taking my own Hæmacytometer as an example, if, instead of using a 5 per 1000 solution, a 5 per 950 solution were used, *i. e.* 5 parts of blood to 945 of the diluting fluid, the result would be absolutely the same as if the depth of the cell were correct, or as if the error were corrected by calculation. Thus supposing 500 corpuscles to be contained in ten of the squares,

$$500 \times 10 \times \frac{1000}{190} \times \frac{950}{5} = 5{,}000{,}000.$$

This device will work equally well whatever the error in the cell may be, if the following rule be adhered to:—Multiply the actual number of μ in the depth of the cell by 5 and take the product as the number of parts of the solution of blood and diluting fluid to be used, the number of the parts of blood remaining constant at five—

$$190\mu \times 5 = 950$$

or, still more generally, the number of parts of blood being fixed, and the actual depth of the cell in μ being known, the product of these two numbers, minus the number of the parts of blood, will give the necessary number of parts of diluting fluid required.

With the pipette or mixer graduated according to these rules, it will only be necessary to add 0000 to the number of corpuscles counted in ten squares.

I commend this suggestion to the notice of all who are using Gowers' instrument, as its adoption will greatly facilitate the attainment of correct results.

Secondly, as to the error caused by the inequality of the surface of the cover-glass. Any ordinary cover-glass is used to flatten the drop to an uniform height. Now, as every histologist knows, cover-glasses are rarely of an uniform flatness; they are generally either slightly convex or concave, hence the layer of fluid is likely to be thicker in some places than in others, and consequently a count made in one part of the cell may give very different results from one made in another. To remedy this defect in my instrument, I have had ground a perfectly flat cover-glass.

Thirdly, the mode of placing the cover-glass on the cell is faulty; whether it is dropped on horizontally or laid on gently at an inclined plane, the uniform diffusion of the corpuscles through the fluid is disturbed.

Fourthly, in the method of mixing and placing the drop on the cell, errors are caused by the white corpuscles adhering to the sides of the vessels used for mixing, and by evaporation from the little open cup in which the solution is kept. Further, in placing the drop on the slide, unless the manœuvre is very quickly executed, the red corpuscles gravitate to the bottom of the drop, and are thus deposited and form a thicker collection in the centre of the drop than at the periphery. The white corpuscles also, by adhering to the glass rod, introduce a source of error in estimating the right proportion between white and red corpuscles.

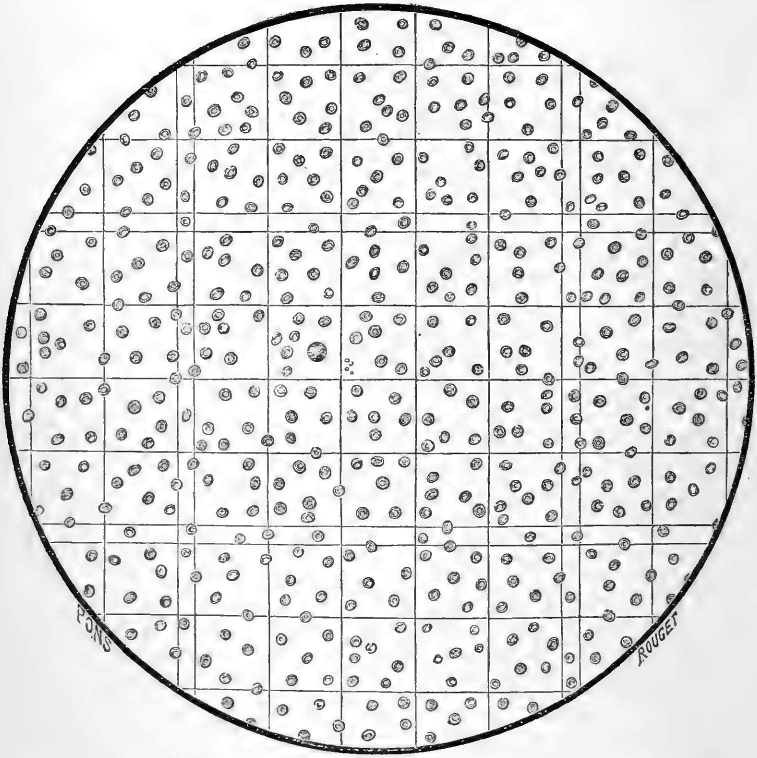
It is, I think, to be regretted that, in introducing this really useful clinical instrument, Dr. Gowers should have adopted the old, clumsy, and discarded method of making the solution, instead of using Potain's Mixer, the use and value of which were already known. By this mixer a solution of blood at 100, 200, 300, 400, or 500, as desired, is made in a *closed* vessel, evaporation thus being prevented; the drop is deposited on the slide whilst the corpuscles are in rapid motion and before they have had time to gravitate to the bottom of the drop. For the last eighteen months I have, when using Gowers' Hæmacytometer, substituted Potain's Mixer in the place of the apparatus provided, and with the result of obtaining much more uniform counts in different parts of the cell, whereas previously the want of uniformity was often very marked.

By the means I have indicated, namely, by correcting the error in the depth of the cell, by substituting a perfectly flat cover-glass for one that may or may not be flat, and by using Potain's Mixer for making the solution, a useful and nearly accurate clinical instrument can be made of Gowers' Hæmacytometer. As it is at present arranged, the results obtained by it are often misleading, unless the mean of a great number of counts be taken. Single observations are likely to lead to the most fallacious conclusions, and are not at all trustworthy, whether for scientific or clinical data.

In Malassez's new *Compte-Globules* he has adopted the great improvement introduced by Gowers, of drawing the squares on the surface of the slide. He has moreover succeeded, by many ingenious contrivances in carefully avoiding all the sources of error in Hayem's and Gowers' instruments above enumerated, to several of which I had occasion to call his attention. This new MICROMETRIC GRA-

DUATED CORPUSCLE-COUNTER with WET CHAMBER (*Compte-Globules à chamhre humide graduée micrometrique*[1]) consists of a thick nickel slide, in the centre of which is a circular groove enclosing a glass cylinder about a centimeter in diameter. Outside this groove are three pointed metal screws, equidistant from each other. The elevation of these points above the surface of the metal slide is exactly $\frac{1}{5}$ mm. In the centre of the glass surface, limited by the groove, are drawn the squares, in which the corpuscles are counted. These have a side of $\frac{1}{20}$ mm., and they are arranged in groups of 20, each group having a length of $\frac{5}{20} = \frac{1}{4}$ mm., and a width of $\frac{4}{20} = \frac{1}{5}$ mm., and an area, therefore, of $\frac{1}{5} \times \frac{1}{4} = \frac{1}{20}$ square mm. Each group of 20 squares is separated from adjoining groups by a double line (Fig. 2). The peripheral

FIG. 2.

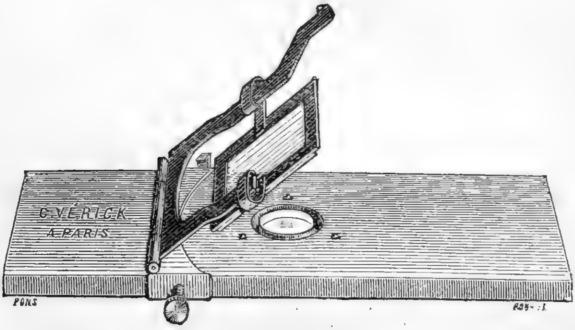


---

[1] "Sur les Perfectionnements les plus récents apportés aux Méthodes et aux Appareils de Numération des Globules Sanguins, et sur un nouveau Compte-Globules," par L. Malassez, 'Arch. de Phy.'

parts of the ruled space are simply divided into rectangles, $\frac{1}{4}$ mm. long and $\frac{1}{5}$ mm. wide. The cover-glass, which is ground accurately flat, is attached, by moistening the edges slightly with saliva, to a frame fixed to the sides of the slide. By an ingenious and delicate rack movement of this frame the cover glass is lowered without delay, and *in a horizontal position* down upon the drop. The slide carrying the frame is represented in Fig. 3.

FIG. 3.



To make a numeration, the solution is made in Potain's Mixer at the strength of 1 per 100, 200, 300, 400, or 500, as desired; and, whilst being rapidly agitated, a drop is placed in the centre of the ruled space, and the cover-glass, having been previously attached to the frame, is lowered and clipped, so as to rest firmly on the points of the screws. To prevent evaporation, if desired to keep the preparation any length of time, a drop of water should be placed at the edge of the cover-glass, and allowed to run under and fill the vacant space between its edge and the groove. The *red* corpuscles that are lying within a group of 20 squares are then counted. These 20 squares, it will be remembered, have an area of $\frac{1}{20}$ mm.^{2}, and the depth of the fluid being $\frac{1}{5}$ mm., the quantity of the solution under review will be $\frac{1}{20} \times \frac{1}{5} = \frac{1}{100}$ of a mm.^{3} The number of corpuscles seen, therefore, has to be multiplied by 100, and then again by the number representing the strength of the solution, and the product will be as before the number of corpuscles in a cubic millimeter of blood.

Thus, for example, let the solution be 1 per 200, and let 250 corpuscles be found on an area of $\frac{1}{20}$ mm.^{2}; then—

$$250 \times 100 \times 200 = 5,000,000.$$

Thus, to the number of corpuscles counted, if the solution be 1 per cent., it is only necessary to add 0000, but if the strength of the solution be less it is necessary to multiply the number of corpuscles by the figure representing the dilution before adding 0000. To correctly estimate the number of *white* corpuscles per cubic millimeter a much larger area must be taken, and for this purpose the rectangles of $\frac{1}{20}$ square mm. have been drawn on the slide. The number of white corpuscles found in ten of these large rectangles must be counted. If in a 1 per cent. solution the number of white corpuscles in ten of these large rectangles is found to be thirty, then we know, as above shown, that the volume of the solution counted is—

$$10 \times \frac{1}{20} \times \frac{1}{5} \text{ or } 10 \times \frac{1}{100} = \frac{1}{10} \text{ mm.}^3$$

The number counted, therefore, multiplied by 10 and then by 100, will give at once the number of white corpuscles in a cubic mm. of blood; or, in other words, it is only necessary, for a one per cent. solution, to count and add 000. For example:

$$30 \times 10 \times 100 = 30{,}000.$$

This method of estimating the number of white corpucles will be felt by every worker at this subject to be a great gain, for on this point none of the previous instruments gave any but the roughest approximate results, likely to give rise to the most delusive conclusions. To sum up, the advantages of this new *Compte-Globules* over that first introduced by M. Malassez are that it can be used clinically with any microscope, that no particular skill is required to use it, and only ordinary care to keep it clean and in order. Over other clinical Corpuscle-Counters it possesses the merits—of making the layer of fluid accurately $\frac{1}{5}$ mm. in depth, so that there are no corrections to make; of having the squares ruled to the smallest size yet found possible, so that the numeration is exceedingly easy and not fatiguing to the eyesight; of making an exact computation of the number of white corpuscles per cubic mm.; and, lastly, by means of the rack movement of the carrier of the cover-glass, and by the use of the Melangeur Potain, of preserving the homogeneity of the drop when placed on the slide and flattened to the depth of $\frac{1}{5}$ mm.

The counting of blood-corpuscles is now so common and frequent an operation in clinical medicine, and its value in assisting diagnosis and treatment is so well recognised, that I feel sure that insistence on the minute details and scrupulous care necessary to ensure correct and reliable results will not be thought trivial.

Corpuscle counting is, however, only one stage in the optical investigation into the state of the blood. To arrive at an opinion on which diagnosis and treatment should be based, it is necessary to estimate the amount of hæmoglobin as well. In an elaborate paper[1] of Malassez (of which I published an abstract in the 'London Medical Record' of 1879), all the various methods employed for estimating hæmoglobin are described at length. In nearly all of these an arbitrary standard of colour is taken as normal, and the blood to be examined is compared with it. In Malassez's Hæmochromometer there is no arbitrary standard; each degree of the coloured standard solution to which the blood is compared corresponds to a blood containing a certain estimated amount of hæmoglobin per cubic mm., and having the power of absorbing a certain known amount of oxygen.

These figures have all been ascertained by a prolonged series of experiments; here therefore, there is no guessing that the amount of hæmoglobin may be above or below the normal, for we are able to ascertain the actual amount of hæmoglobin in a cubic mm. of blood, and also the respiratory power of the same unit. But M. Malassez points out that it is not only necessary to ascertain the amount of hæmoglobin per cubic mm., but that we should learn in what state of division it exists, namely, what is the amount contained in each corpuscle. Welcker considers that there is always a constant relation physiologically between the richness of the blood in corpuscles and in hæmoglobin; Hayem and Johann Duncan have, however, discovered that, pathologically, particularly in anæmia and chlorosis, the relations are disturbed, the number of corpuscles often resting normal, the hæmoglobin being less than normal. The way of arriving at the amount of hæmoglobin per corpuscle is, by M. Malassez's method, extremely simple. The number of corpuscles in a cubic mm. of blood is first counted, and by the hæmochromometer the amount of hæmoglobin per cubic mm. is estimated. The latter figure divided by the former gives the amount of hæmoglobin per corpuscle. Thus, a blood containing 5,000,000 corpuscles per cubic mm., and 0·125 mlgr. of hæmoglobin per mm.^3 gives $\frac{0{\cdot}125}{5{,}000{,}000} = {\cdot}000{,}000{,}025$ mlgr., *i. e.* $\frac{25}{1{,}000{,}000}$ of a $\frac{1}{1{,}000{,}000}$ of a gramme, or, as it is commonly written,

---

[1] "Sur les diverses Méthodes de Dosage de L'Hémoglobine et sur un nouveau Colorimètre," par L. Malassez, 'Arch. de Phy.,' 1877.

25 $\mu\mu$ gr. The result in terms of $\mu\mu$ gr., however, may be found in a moment by simply dividing 125 by 5 = 25, and disregarding all ciphers.

In an extremely interesting research,[1] M. Malassez found that, pathologically, the estimation of the hæmoglobin per corpuscle gave very significant indications. In a case, which he quotes, of chlorosis which improved under treatment, the actual number of corpuscles per cubic mm. diminished, the amonnt of hæmoglobin per corpuscle, almost doubling, however, in the same time; mere corpuscle counting here would have given an erroneous inference. In a series of experiments on fowls kept first at liberty in the open air, and then in unhealthy conditions in a courtyard, it was found that though the corpuscles did not notably diminish in number, the hæmoglobin per corpuscle fell from 48 $\mu\mu$ gr. to 33 $\mu\mu$ gr. On examining a great number of animals he found that the lower in the scale one descends the larger the amount of hæmoglobin per corpuscle, so that it might be too hastily assumed that the blood of the lower animals was richer in hæmoglobin than that of the higher. At one end of the scale stands man with a mean normal of 30 $\mu\mu$ gr., and at the other the Proteus with 1066·6 $\mu\mu$ gr. But the corpuscle of the Proteus is 127 times the volume of that of the human subject. The true ratio between them can only be ascertained by knowing the amount of hæmoglobin contained in an unit of corpuscular substance. The unit taken is μ^3. To obtain this, the volume of the corpuscle must be known. Welcker, by an elaborate process, ascertained the mean volume of the corpuscles of a few animals as standards of comparison. These measurements being accepted as accurate, the amount of hæmoglobin per corpuscle is divided by the mean volume of the corpuscles, and the product is the amount of hæmoglobin per μ^3 of corpuscular substance.

From the following table it will be seen that though the quantity of hæmoglobin per corpuscle may increase from the higher to the lower animals, the true ratio of comparison is the unit of corpuscular richness in hæmoglobin, and that this, on the contrary, rises in passing from the lower to the higher animals:

Welcker's ingenious method of ascertaining the value of

---

[1] "Sur la Richesse en Hémoglobine des Globules rouges du Sang," par L. Malassez, 'Arch. de Phy.'

	Quantity of hæmoglobin per corpuscle.	Volume of the corpuscle.	Quantity of hæmoglobin per μ cube of corpuscular substance.
	$\mu\mu$ gr.	μ cube.	$\mu\mu$ gr.
Man	30	72	0·4166
Pigeon	52	125	0·4160
Lizard	70	201	0·3483
Russian frog	216	629	0·3432
Proteus	1066	9200	0·1159

the volume of the corpuscles in μ^3 is, however, quite out of the question in clinical work, and as no simpler method has at present been devised, we must it appears to me, be at present content to ascertain the mean area of the corpuscles in μ^2, and to take as our unit of corpuscular substance μ^2 multiplied by the unknown thickness of the corpuscle, on the assumption that this is uniform throughout, and always the same. Actually we know this not to be the case, as normal corpuscles are biconcave and not flat, and in pathological conditions they vary in form, and possibly in thickness. However, let the constant representing the supposed thickness (or more accurately the factor by which we should multiply the diameter to obtain the volume) be called τ. Our arbitrary unit of corpuscular substance will, therefore, be $\tau\mu^2$. The results in the way of comparison will only be liable to error in so far as the corpuscles vary in thickness. As, however, this variation is immeasurable by our present instruments, it may be taken that the unit $\tau\mu^2$ will give for all practical purposes a sufficient approximation to the truth.

To obtain the mean area of the corpuscles in any given specimen of human blood, the mean diameter of the corpuscles must first be ascertained. A simple method of obtaining this is to graduate the microscope so that an image thrown by the camera lucida at a certain fixed distance magnifies exactly 1000 diameters. The corpuscles having been rapidly fixed and dried by exposing them to the action of heat, or better still, to the vapour of osmic acid, their image is thrown by the camera lucida on to white paper, care being taken to correct the errors of refraction.[1] The outlines of the corpuscles are then traced in pencil, and their diameters

---

[1] 'Note sur la Mesure des Grossissements Microscopiques,' par L Malassez. 'Correction des Déformations produites par les Chambres Claires de Milne-Edwards et de Nachet,' par L. Malassez.

measured by a millimeter rule. The resulting numbers give the diameters of the corpuscles in micro-millimeters. Of course the mean of a great number of measurements must be taken. I generally take the mean of fifty measurements. The area in μ^2 is obtained by the well known formula of πr^2.

To take an example:

$$\text{Let } 7{\cdot}60\mu = \text{the diameter of a normal corpuscle;}$$

$$\text{and } 3{\cdot}8\mu = \text{the radius: then}$$

$$\pi r^2 = \pi.\ 14{\cdot}44\mu^2 = 45{\cdot}36\mu^2.$$

45. $36\mu^2$ is therefore the area of a normal human corpuscle. To obtain the unit of corpuscular richness, *i. e.* the amount of hæmoglobin in a volume of $\tau\mu^2$, the amount of hæmoglobin per corpuscle must be divided by the area.

In the following table, which I have prepared, these calculations, and others to which reference has been made above, have been worked out. The number of corpuscles in a cubic millimeter is taken as invariable in the examples of normal and anæmic blood (this is not infrequently the case); all the other figures vary, however, from the normal in different degrees, the unit of corpuscular richness being finally the only figure that gives the exact ratio between the normal and pathological states.

	No. of corpuscles per mm.^{3} of blood.	Hæmoglobin per mm.^{3} of blood.	Hæmoglobin per corpuscle.	No. of corpuscles per mil. gr. of hæmoglobin.	Diameter of corpuscle.	Area of corpuscle.	Hæmoglobin per unit $\tau\mu_2$ of corpuscular substance.
		m. gr.	μμ gr.		μ.	μ.^{2}	μμ gr.
The corpuscles in a normal state of health .	4,500,000	0·134	29·77	33,580,000	7·60	45·36	0·66
Slight anæmia, with diminution of the size of the corpuscle .	4,500,000	0·082	18·66	54,878,048	6·50	33·18	0·56
Marked anæmia, with increase of the size of the corpuscle . .	4,500,000	0·062	13·77	72,580,645	8·20	52·81	0·26

Pathologically these exact or minute analyses are most interesting, though their outcome clinically and therapeutically is yet obscure. But we may hope that a minute study of the state of the blood in the cachexia of cancer and syphilis, in pernicious and simple anæmia, in chlorosis and leucocythæmia and in other wasting diseases, may lead to an exact knowledge of the pathological changes, revealing the causes at work and that this knowledge may form a rational basis of treatment.

PRELIMINARY ACCOUNT *of the* DEVELOPMENT *of the* LAMPREYS.

By W. B. SCOTT, M.A., Assistant in Palæontology, Princeton, New Jersey.

FOR nearly a year (1879-80) I was occupied in the laboratory of Professor Gegenbaur in working out the development of the Lampreys, the results of which investigations will appear shortly in a more extended form. It seemed, however, desirable to give a preliminary account of the results which have been obtained, in order, as far as possible, to direct the attention of morphologists to the general character of the development of this important and little understood group of Vertebrates. Professor Gegenbaur very kindly placed at my disposal the splendid material which was so laboriously and skilfully collected and prepared by the late Dr. Calberla. This consists of an immense series of preserved embryos and larvæ, and of many thousand prepared sections; and, in addition to these, I have further obtained a fine series of larvæ, for which I am indebted to the friendly offices of Professor Wiedersheim.

Since the publication of my little article in the 'Zoologischer Anzeiger,' 1880, No. 63, I have obtained an article by Professors Kupffer and Benecke,[1] which has made it necessary to alter slightly, though not essentially, the statement concerning the maturation of the ovum contained in my paper. According to Calberla[2] the conversion of the germinal vesicle into the female pro-nucleus takes place simultaneously with the metamorphosis of the larva into the sexual animal. I was led to doubt this statement for many reasons, and believed that the germinal vesicle persisted unchanged until nearly the time of laying. This result is fully confirmed and extended by those of Kupffer and Benecke. According to these observers there are two polar bodies formed, one before and one after impregnation. The one which I mentioned as being present in the ovum already segmented into four corresponds to the latter of these bodies. The first of them I did not find, as no fresh material of the proper stage was accessible to me. According to Kupffer and Benecke this first polar body is a nucleus-like body surrounded by a membrane, and embedded in granular protoplasm; it is nearly always eccentric in position. The authors just mentioned consider it highly probable that this body is a derivation of the germinal

---

[1] C. Kupffer and B. Benecke, 'Der Vorgang der Befruchtung am Ei der Neunaugin,' Königsberg, 1878.

[2] E. Calberla, 'Der Befruchtungsvorgang beim Ei von Petromyzon Planeri. Zeitschr. für wissensch. Zoologie,' B. xxx.

vesicle. In short, the maturation of the egg, it seems, offers no such great peculiarities as Calberla supposed.

Segmentation.—According to my results this process takes place as Max Schultze[1] has described it, *i. e.* as in the case of the frog, or rather that of the newt, and not, as Calberla supposed, in such a way that epiblast and hypoblast are distinguished by the first division. At the end of segmentation the ovum is very similar to that of the triton, or sturgeon, of a corresponding stage. There are two kinds of blastomeres, the larger form the lower half of the egg, the smaller ones the upper half. The quantity of food-yolk is less than in the eggs just mentioned; the segmentation cavity is extraordinarily large, and lies almost entirely in the upper half of the egg; its cover is made up of several layers of cells, of which only the exterior will develop into epiblast. The presence of cells in the roof of the segmentation cavity, which will eventually belong to hypoblast, occurs otherwise only in such eggs as have a very large quantity of food-yolk, *e. g.* that of *Accipenser*.[2] In general, epiblast and hypoblast are roughly distinct at the end of segmentation, but the strict differentiation, as well as the foundation of the mesoblast, is produced by the well-known process of invagination. This is preceded by the thinning out of the roof of the segmentation cavity, which now consists, for the greater part of its extent, of a single layer of cells. The invagination is precisely similar to that of the newt.[3] By this means are formed in the median dorsal line two layers of cells, the epi- and hypo-blast, whilst on the sides we find these two and a third, the mesoblast. In the head and anterior part of the body the germinal layers are formed only in this way, while in the greater part of the body the ventral part of the mesoblast, and by far the greater portion of the hypoblast, arise by differentiation of the yolk-cells.

This description of the formation of the germinal layers is very different to that which Calberla[4] gives. The discussion of his views would, however, lead us too far, and so it must be deferred.

I can completely confirm Calberla's results as to the formation of the notochord. It is formed from the invaginated hypoblast alone, but when it becomes detached from this layer it grows considerably further forward in the head than the hypoblast of the alimentary canal extends.

---

[1] M. Schultze, 'Die Entwicklung von Petromyzon Planeri,' Haarlem, 1856.

[2] Salensky, 'Development of the Sturgeon' (Russian), Part 1, Kasan, 1879.

[3] Scott and Osborn, "On Some Points in the Early Development of the Newt," this Journal, 1879.

[4] Calberla, "Zur Entwicklung des Medullarohres und der Chorda dorsalis, etc.," 'Morph. Jahrbuch,' Bd. iii.

The alimentary cavity (Urdarmhöhle) is formed by invagination. In the head region this cavity becomes the lumen of the permanent alimentary canal, but in the body there arises a new and much larger lumen. The blastopore is enclosed by the medullary folds, and a neuro-enteric canal is thus formed. As Professor Benecke[1] has already discovered, the anus is a new formation.

The *visceral clefts* arise as diverticula of the hypoblast of the throat towards the external skin, which is resorbed at these points. At a much later period a shallow sinking of the epiblast is formed into which all the gill-slits open. It is plain, therefore, that the epiblast has no share in the formation of the gills, which arise as vascular processes of the walls of these diverticula. *Eight* pairs of diverticula arise, of which the first pair very soon disappear, and so far as I have been able to discover, never pierce the skin at all; and I could not find traces of them in larvæ more than a day or two old. The arch of the first pair bears no gills, but its presence is very important for the proper understanding of the skeletal and other parts of the head, as well as for the settling of the disputed question of the systematic position of the Cyclostomata. Professor Huxley[2] has described a hyomandibular cleft in quite old larvæ, but I have not been able to verify his observations on this point.

The *mid-gut* is at first completely filled up with yolk-cells, which do not begin to be absorbed until the larva has reached a length of about 6 mm. In the front end more cells are absorbed, in the hind parts very few disappear, and the epithelium of the alimentary canal is at first remarkably high, but the cells gradually become very much flattened. A deep fold in the wall of the mid-gut makes its appearance in larvæ of about 7 mm., in which fold there is a special aggregation of mesoblast cells. This fold is the valve, and is very similar to the valve of Chimæra as well as to the first rudiment of the spiral valve in the Elasmobranchs.

The *hind-gut* is distinguished from the mid-gut by the absence of the valve, and further by the circumstance that it loses the yolk-cells, which fill it, at a very early period, while the embryo is still unhatched, in adaptation to the function of the excretory organs, which already develop an opening into the end-gut and through the anus outwards.

In general it may be said that the alimentary canal suffers a gradual degeneration in the course of development. The canal is relatively largest and most important in larvæ of 7—10 mm.,

---

[1] Benecke, quoted by Kupffer, 'Zoolog. Anzeiger,' No. 59.

[2] Huxley, 'Proc. Roy. Soc.,' No. 157, p. 129.

while it has become extremely small in the sexual animal, and is almost obliterated by the enormously swollen genital glands.

The *mouth* is one of the most peculiar organs of the entire organism, and in its development, I believe, the key to many of the peculiarities of the Cyclostomata is to be found. It arises as a simple sinking in of the epiblast, which becomes gradually deeper until it finally touches the hypoblast at the blind anterior end of the alimentary canal. The next step is the perforation of the two membranes, which seems to take place in the usual way; but I have not been able to follow all the steps in the process. The great peculiarities of the mouth-parts which we have mentioned, lie in the lips, &c., which surround the cavity, and will be more properly treated of in connection with the mesoblast.

The *epidermis* is at first single-layered, and does not divide itself into two layers until after the hatching of the larva.

The *central nervous system* has, so far as the earlier stages are concerned, been carefully investigated by Calberla ('Morph. Jahrb.,' Bd. iii), and I can confirm his results in all points, except that the division into two layers of the epiblastic cells concerned in the formation of the cerebro-spinal axis does not seem to be so clear as he made it out. A shallow groove appears in the dorsal surface of the embryo, and the epiblast cells in the neighbourhood of this groove begin to divide themselves rapidly into two layers, and by their multiplication form a strong solid keel which projects inwards towards the hypoblast. The keel is soon detached from the general epiblast, becomes oval in section, and upon the fourteenth day after hatching develops a lumen by the separation of some of the cells. Such a peculiar formation of the medullary cord is to be found only in the Teleosteans. Now, it has been suggested by other investigators that the Teleostean egg has undergone a reduction in bulk through the partial loss of the food material. If we assume such a reduction in bulk in the egg of *Petromyzon*, we shall be able to explain not only the surprising correspondence of two such widely separated groups, but also the presence of hypoblast cells in the roof of the segmentation cavity in the egg of *Petromyzon*, which is supplied with such a small amount of food material.

The *brain* arises at first as a club-shaped swelling of the anterior end of the nervous axis, and is very small and simple. Soon, however, the rudiment becomes divided by shallow constrictions into three divisions, of which the posterior is by far the longest, the anterior the shortest. The walls of the brain are everywhere uniform; thickenings and thinnings of separate parts do not occur till a much later period. The rudiment of the cerebral hemispheres is a simple unpaired bud, which later becomes divided into lateral halves. At first only a lateral development of the extraordinarily small rudiment takes place, so that the epiphysis lies between the hemispheres and almost at the anterior edge of the brain. In the later stages, and especially after the metamorphosis, the cerebrum grows longer and the epiphysis comes to lie behind it. The differentiation of the olfactory lobes takes place comparatively late in larval life. The epiphysis and infundibulum are diverticula of the roof and floor respectively of the posterior part of the fore brain. The pituitary body is developed as a solid cord of cells which are invaginated from the epiblast, together with a single median invagination for the olfactory pit. Only the posterior part of this invagination is concerned in the formation of the pituitary body. These cells soon lose all connection with the olfactory apparatus, and become divided by connective-tissue bundles into solid follicles; in the sexual animal the gland lies above the naso-palatal passage.

In the mid-brain the lateral thickenings and thinnings of the dorsal median line are especially to be noticed. This division thus receives a bilobed roof. The brain is at first straight and shows no tendency to flex itself. The cranial flexure never attains a very great degree, about a right angle, and is partially corrected by an actual extension in the reverse direction. There is also an additional *apparent* correction which is caused by the great development of the upper lip.

The rudiments of all the higher *organs of sense* appear during embryonic life before the epiblast has divided itself into two layers. The eye develops in essentials just as in the Gnathostoma and needs no especial explanation. The optic vesicle is, however, remarkable for its length, and for the fact that only a part of the anterior wall of the vesicle becomes the retina. The lens arises as a local invagination of the single-layered epiblast. The auditory vesicle develops as an invagination of thickened epiblast cells, which gradually deepens, becomes spherical, and detaches itself from the skin. Its later development presents some details of interest; which, however, must be reserved for the full paper.

The olfactory organs are, for *Petromyzon,* of especial interest. I cannot confirm Calberla's[1] result as to the paired origin of the olfactory pit, on the contrary, according to my observations (and here I am in accord with Dr. Götte, as he informs me by letter) this pit is single from the very first. The first stage is a shallow sinking in of the epiblast at the anterior end of the head

---

[1] Calberla, 'Amtl. Bericht der 50 Versamml. d. deutschen Naturforscher, &c.,' Munich, 1877, p. 188.

immediately above the mouth; then the epiblast cells which border the pit above are thickened and form a layer of epithelium which is perfectly continuous, and takes up the whole of the anterior part of the head. On account of the cranial flexure this epithelium looks directly downwards. The pit becomes gradually deepened, but the olfactory epithelium remains still on the surface of the head; soon, however, it has a deeper position, and shows only a small triangular opening on the exterior. Late in larval life the epithelium of the olfactory organ develops the well-known folds which show a definite paired arrangement. The palato-nasal passage does not attain any considerable length until after the metamorphosis; and its rudiment is from the first single. The paired olfactory nerves show, however, that that organ was at one time paired, and that a later fusion of the two pits took place. If the two pits of the Sturgeon (O. Salensky, l. c., Taf. ix, fig. 84), which lie at the anterior part of the head and not laterally as in the Elasmobranchs, were brought nearer the middle line, we would have almost exactly the condition of *Petromyzon*. But in the latter type the paired stage has been over-lept in the course of development.

The *mesoblast*, in the earlier stages, has in general very much the relations that are observed in the Elasmobranchs, or still more like those of *Triton;* but it is worthy of notice that in *Petromyzon* the first pair of protovertebræ follows closely upon the auditory vesicles instead of leaving a considerable interval free between them as in the Elasmobranchs. The protovertebræ develop muscle-plates which gradually grow forwards and overlap each other; the anterior ones grow over the head as far as the olfactory capsule, and by processes of division develop the muscles of the head. But since these myotomes belong morphologically to the trunk they cannot be regarded as guides to the segmentation of the head.

In the head the mesoderm undergoes another segmentation, giving rise to one segment between every two gill-slits and two in front of the first gill-slit, just as in the Elasmobranchs and Urodeles. These segments surround a central cavity and correspond exactly to what Balfour has called "head-cavities." These segments develop the gill muscles, and I believe the first pair give rise to the muscles of the eye. In the trunk the development of the mesoblast, its splitting formation of the pleuro-peritoneal cavity, &c., shows no essential deviations from the conditions found in the Elasmobranchs and Urodeles. The internal muscles of the sucking-disc appear to develop themselves directly from indifferent mesoblast cells. The formation of the sucking-disc is very striking, and its peculiarities appear very early. At first the upper lip appears as a low rounded ridge, between the mouth and olfactory pit, which seen in longitudinal vertical section, has the shape of a right-angled triangle, the hypotenuse of which i bounded by the olfactory pit. This stage is found in embryo of about the eighteenth day. In very small larvæ the lower edge of the ridge (*i. e.* the edge next the mouth) has begun to grow very rapidly, and is curved downwards and backwards. The mouth has still a completely ventral situation; the correction of the cranial flexure brings it further and further forwards, and at the end of this the upper lip, which is now still more lengthened, turns about an angle of nearly 180°, so that the edge of the lip, which formerly pointed directly backwards, now points directly forwards.

This gives us the characteristic terminal mouth of the Cyclostomata, the formation of which brings the olfactory pit to the upper side of the head. According to Max Schultze's results, the mouth is fitted for its sucking function in almost the very smallest larvæ.

This peculiar mouth would, therefore, appear to be one of the first deviations from the normal character which developed itself in this group, and the change which the mouth undergoes is followed by, and I think the connection is a causal one, many other alterations, *e.g.* the situation and fusion of the olfactory organs. The change of the mouth into a sucking apparatus makes necessary an alteration of the mechanism of breathing. The water of respiration can no longer stream through the mouth to the gills and out of the gill-slits, but must stream in and out of the gill-slits; and this makes necessary a change in the musclès and skeleton of the gill apparatus. These changes cannot be discussed here, we can only indicate the general importance of the formation of the mouth. In addition to the modifications already mentioned, there must be remembered the *new* formations, the supporting cartilages for the sucking-disc, none of which are present until after the metamorphosis of the larva into the sexual animal, as well as changes in the course of the cranial nerves, which are easy to follow in the course of development. In short, *I find in the change of form of the mouth a key to the solution of the problem of the head and its organs in the Cyclostomata.*

Urino-genital system.—My observations upon the excretory system are somewhat more complete than those of W. Müller ('Jen. Zeitschr.,' B. ix), but they confirm all his results. The "segmental duct" (I use this term of Balfour's to translate the "Kopfnierengang") is formed as a solid cord in the lateral part of the mesoblast, which is not taken up in the formation of the protovertebræ. This solid cord appears on about the fourteenth day of embryonic life; it soon shows a lumen, and anteriorly opens into the pleuro-peritoneal cavity. At the anterior end of this duct are formed a series of ciliated tubes opening by wide funnel mouths into the body-cavity, and on the other side by narrow tubes into the duct. A glomerulus is then formed on each side of the mesentery, just as in the Amphibia, and the whole organ thus developed is the head kidney (Müller's Vorniere). Although I cannot prove it with absolute certainty, it is yet in the highest degree probable that the ciliated tubules are a development of the duct itself. The duct empties into the now empty hind gut even in the embryos. It is to be especially noted that the head kidneys form for quite a long period the only excretory apparatus of the larva, the first rudiments of the Wolffian bodies not appearing until the larvæ have reached a length of 9 mm. These rudiments are metameric involutions of the peritoneal epithelium, at first solid, which soon become hollow and open into the body cavity and the segmental duct; they differ from those of the Elasmobranchs in being lateral to the ducts. As Müller has shown, the head kidneys gradually become atrophied and disappear.

The segmental ducts at first open separately into the hind gut near the anus; shortly before the metamorphosis they come close together and form a common canal. The anal opening becomes longer, and finally a part of the hind gut becomes constricted off and forms the sinus *urino-genitalis,* and receives a separate external opening. The wall of the sinus is perforated at two points to form the abdominal pores.

My investigations upon the genital organs are yet far from complete. As far as they have yet gone, they agree with the results of Müller.

Princeton, *November* 29*th*, 1880.

On some APPEARANCES *of the* RED BLOOD-CORPUSCLES *of* MAN *and other* VERTEBRATA. By G. F. DOWDESWELL, B.A. (Cantab), F.C.S., F.L.S., &c.

SOME time since, in examining the action of septic matter, I observed that when blood of man or the dog was treated on the warm stage of the microscope with an aqueous extract of putrid muscle, the red corpuscles shortly exhibited a curious phenomenon, throwing out from their surfaces numerous processes, which, in some cases formed a rosary of minute beads, in others fine undivided filaments, generally terminating in one or more droplets, and assuming a bifurcated or racemose appearance; they were of very variable size and form, from mere diminutive globules or protuberances on its surface up to five or six times in length the diameter of the corpuscle. These processes were evidently contractile, sometimes, from a considerable length, retracting suddenly into a globule, or being withdrawn entirely into the substance of the parent corpuscle; the detached particles, too, would coalesce into one larger globule. After a time, varying, according to circumstances, from a few minutes up to half an hour or so, they all became detached, forming a number of small spherical bodies of various size, undistinguishable from Micrococci; or short slender filaments, identically similar in appearance to Bacteria or Vibrios, and in incessant molecular movement swarmed over the field of view. About the same time, usually, the hull or stroma of the red corpuscles became colourless and difficult to distinguish, for all the reagents with which I obtained these processes dissolve out the hæmoglobin. In the finer filaments and minute globules any colouration is difficult to distinguish clearly, but when these coalesce into a larger body the colour becomes apparent, and it is most evident in the large processes which are formed in the blood of the frog, under the action of a 5-per cent. solution of ammonium chromate in the cold. When the colouring matter of the red corpuscles is dissolved out, and they disappear, the processes are also lost to sight; but upon treatment of the preparation with magenta or other staining fluid, both the hull of the red corpuscles and the processes too, become stained, and again apparent. On first observing this phenomenon I was in doubt whether it was to be regarded as a physiological and vital process, or merely as a physical one. Shortly afterwards Francis Darwin's paper on the "Protoplasmic Filaments of the Teasel"[1] appeared, and it seemed to me that there must be an intimate connection between

---

[1] This Journal, N. S., No. lxvii, July, 1877, pp. 245—272.

the two phenomena, so essentially similar in character. I then instituted a series of experiments with solutions of different salts and various reagents, under different conditions, with a view to ascertain the laws which regulate these appearances. I subsequently found that the same processes in the red corpuscles of the blood of man had been described and figured some years previously by Dr. William Addison, F.R.S.[1] The results I obtained myself agreed in the main with those described by him. It is sufficient here to mention that I found they occurred most readily, in the blood of mammals, when treated with a mixture of one part of pale sherry wine and one part of a 10-per cent. solution of sod. sulph., at a temperature of about 98° Fahr., or somewhat lower. The sp. gr. of this mixture is about 1·008, and its reaction acid; on neutralisation it fails to produce any processes from the red corpuscles. In an aqueous solution of sugar 2·5 per cent. sod. sulph. 10 per cent., alch. 15 per cent., and ac. acet. 1 per cent., they are also readily formed at about the same temperature. By treatment with some sherry wines alone they are produced, though not very readily. A slight acidity of the reagent usually favours their production, as does the addition of 10 to 15 per cent. alcohol; though neither of these is absolutely essential, and the variety of reagents and mixtures which produce them is endless. According to the sp. gr. of the solution in which they are produced, the temperature, and other circumstances, their form and duration is modified. The temperature most favorable for their production, in the larger number of cases, is somewhat below 98° Fahr., and that in all the mammalia that I have examined alike; above that temperature they are quickly dissolved, and much below it, with most reagents, they are formed slowly and imperfectly, if at all. A solution of urea, as first stated by Kölliker, and others after him, will produce these appearances in the blood both of frogs and of mammals. This is most readily effected by drying a drop of the solution upon a slide, putting the blood upon this, and covering it. Salt solution, 0·6 per cent., as described in a recent paper, produces these appearances well in defibrinated frog's blood on the warm stage. By this treatment, too, the nucleus of the red corpuscle is sometimes very clearly shown, and the reticular fibres which it contains, with the limiting membrane which encloses it, in places penetrated by the fibres, as recently described and figured by Dr. Klein[2] in this Journal, and by Fleming,[3] are very apparent.

---

[1] This Journal, N. S., vol. i, 1861, pp. 81—89, and 'Proc. R. Soc.,' vol. x, 1859, pp. 186—189.

[2] Vol. xviii, N. S., 1878, pp. 314—339, and vol. xix, N. S., pp. 125—175, and ib. pp. 404—420.

[3] 'Archiv für Mikros. Anat.,' Bd. xvi, 1879, s. 302—436, Bd. xviii, 1880, pp. 151—259.

A solution of picric acid shows the structure of the nucleus well, and with this permanent preparations may be made. As far as I am aware, the intimate structure of the nucleus of the red blood-corpuscle has not been described, excepting by Dr. Schmidt, in the blood of Amphiuma[1] and some other animals. He describes and figures the nucleus as granular, and invests the corpuscle itself with a membrane, which, whatever it may be in the case of Amphiuma, appears certainly not to be so in other animals; its existence seems to be clearly incompatible with the production of the above-described processes, as much as with the well-known experiment of Dr. Beale, of breaking up by pressure with the point of a needle, on the covering-glass, a red corpuscle into several small droplets. In frog's blood, too, its absence seems clearly demonstrated by treating it in the cold with a 5 per cent. solution of ammon. chromate, when the corpuscles are at first little altered in appearance, excepting that the nucleus becomes pale and distinct. After some minutes protuberances appear on different parts of the periphery of the corpuscle; some of these are then extended, and form long processes, two or three times the diameter of the corpuscle, of very appreciable thickness, and distinctly coloured; the size of some of these amounts to a material portion of the corpuscle, the membrane of which, if it existed, must be ruptured by their protrusion, and would be clearly apparent under an amplification of 1000 diameters or upwards, but nothing of the kind can be seen. The processes formed in this case are frequently retracted again completely, even the largest of them, and the appearances are most interesting and instructive; after a very short time the processes disappear, are retracted or detached; the corpuscles then become circular and colourless. Under the influence of this reagent the corpuscles seem to become more plastic than normally, in the same manner as when subjected to heat.

As above-mentioned, I found that these appearances were first recorded by Dr. William Addison, in 1861 (loc. cit.). He describes the action of acids and alkalis, of various salts, and other reagents upon the blood. He obtained the processes in question most readily by treating the blood upon the slide with sherry wine, either by itself or with the addition of different salts, and found that neither quinine, morphia, nor strychnine, added to the preparation, nor even the vegetable alkaloids in large proportions, prevented their appearance, but that a very small proportion of bichloride of mercury did so effectually. Dr. Addison gives a plate with the different forms of the processes admirably figured, and his paper forms a very complete account of them.

[1] 'Journ. R. Mic. Soc.,' vol. i, 1878, pp. 57 and 97.

In 1863 Klebs,[1] observing the blood of dead animals warmed to bodily temperature, observed points projecting from the surface of the red corpuscles, the larger of which often divided into two parts, the corpuscles themselves becoming distorted. The description is very meagre, and the appearances may be little more than the prickly, or as it has been termed the hedgehog form of the corpuscles.

In the same year Rindfleisch[2] published some experimental observations on the blood. He found that in extravasated blood of the frog the red corpuscles became round, and a portion of their contents, as he describes it, protruded, forming filamentous processes, or a rosary of red-coloured droplets, on the surface of the corpuscles; these he considers are protruded through pores or other openings in the cell-wall, the droplets of which they consist being held together by a viscid substance. He further states that these appearances may also be produced by a concentrated solution of urea.

The first mention of the effect of urea on the red blood-corpuscles which I have seen is by Dr. T. L. Huenefeld, in a work published in 1840,[3] in which he describes the action of a great number of reagents, and states that a solution of pure urea does not seem to have much effect on the red blood-corpuscles of man or the pig, beyond that it dissolves out the colouring matter very quickly, leaving only portions of the hull and the nucleus visible.

In 1864, Dr. Beale[4] describes and figures the changes of form in the red blood-corpuscles of man from heat, the processes and appearances presented, though more varied than those described above, are obviously of the same character. The paper was written in support of the author's theory of formed and living matter. In the same year Preyer[5] describes the appearances in extravasated blood of the frog on the warm stage. Long processes are formed, and globules which become detached and sometimes reunite with the parent corpuscle. He remarks that the action of urea will produce similar appearances, which only differ slightly in colour, and makes the observation, that in the blood of frogs at breeding time, nuclei evidently dividing are found; these he figures. The processes above described he also finds in the blood of frogs on the warm stage without any reagent.

---

[1] 'Centralblatt. f. ci. Medicinisch Wissen.,' Bd. i, 1863, s. 851.

[2] 'Experimentalen Studien über des Blutes,' Leipsig, 1863.

[3] 'Der Chemismus in der thierischen Organisation,' Leipsig, 1840.

[4] This Journal, N. S., vol. xii, p. 32, 1865.

[5] "Ueber Amœboide Blut-Korperchen," Virch. Archiv,' Bd. xxx, s. 433, 1865.

The same appearances caused by heat are next described by Max Schultz.[1] He found that on the warm stage of the microscope the changes of form commenced first at 52° C. in the blood of man and various mammals, on reaching which temperature the corpuscles immediately change and break up into many parts of various sizes, and are dispersed, dancing through the serum in lively motion, or throw out filaments of various lengths and form, which too become detached and move about in the surrounding medium, like Vibrios. Here also a plate is given, the representations in which agree exactly with the appearances above described as caused by the action of reagents, both on the warm stage and in the cold. No one can doubt the phenomena being exactly the same.

In 1871, Professor E. Ray Lankester, in an article upon the structure of the red blood-corpuscles,[2] describing the effect of various reagents upon them, records the pseudopodial-like processes which occur in the blood of the frog on treatment by ammonia gas, and the fluidity which it seems to occasion in the human red corpuscles, resulting in the production of long threads or processes from the corpuscles, and the separation of minute particles from them. Drawings are given of these, which likewise agree exactly with the appearances before described.

Quite recently two papers describing these appearances in the red blood-corpuscles have appeared, the one is by Dr. Rudolph Arndt,[3] who first endeavours to show that the nucleus which occurs in the red blood-corpuscles of Fish, Amphibia, and some other Vertebrata, is an artificial production caused by the action of reagents or pathological changes, which has no existence normally, though when formed it is an independent contractile body, which shows amœboid movements (!); and that consequently there is no integral difference between the ovoid red blood-corpuscles of the Amphibia, &c., and the round corpuscles of man and other mammalia, which do not usually show any nucleus, though he considers that by the action of reagents or certain changed conditions, they too show nuclei of the same nature as those of the ovoid red corpuscles, mere aggregations of their protoplasmic constituents, as shown by Bœtticher,[4] who treated them with alcohol and acetic acid, or with a solution of bichloride of mercury in alcohol; the appearances so induced, however, if carefully regarded, can never be mistaken for identical with the nuclei of the ovoid red corpuscles; the latter, as already

---

[1] 'Arch. f. Mikros. Anat.,' Bd. i, s. 25, 1865.

[2] This Journal, N. S., vol. ii, pp. 361—387.

[3] Virchow, 'Archiv f. Path. Anat.,' Bd. lxxviii, H. 1, s. 7, 1879.

[4] 'Arch. f. Mikros. Anat.,' Bd. xiv, s. 73—94, 1877.

mentioned, in some cases showing very distinctly, an elaborate structure, a network of internuclear fibres, some of them perforating the limiting membrane, which is very distinct, and its appearance quite inconsistent with its being merely a pathological change as asserted by the author; moreover, the appearances caused by treatment after Bœtticher's methods have been clearly and fully explained, and accounted for in a paper in a subsequent number of the same journal.[1] In some cases, too, the nuclei may be seen clearly dividing, though as my own observations were made chiefly upon summer and autumn frogs these instances were few. Preyer, however,[2] describes and figures this division of the nucleus in the blood of frogs during the breeding season. After discussing the question of the contractility of the red corpuscles, and whether they possess a cell wall, in the proper sense of the term, the author describes the appearances which are the immediate subject of this paper. Following Preyer, he first examined extravasated frogs' blood, and found numerous processes, short points, and long straight filaments, in short, exactly the appearances above described; and mentions their production similarly by the action of urea and of heat. Also that similar processes are thrown out in cases of fever, especially typhus; and that with a bodily temperature of 39-40° C., they occur at the temperature of the chamber; and in recurrent fever, as first described by Haidenreich in 1871, long filamentous processes of great length, extending over the whole field of the microscope, appear. These have been regarded as independent organisms, a form of Bacteria, and named *Spirochætæ Obermeieri*, or *Recurrentis*, as which they are described by Cohn.[3] These the author has observed sometimes to coalesce again with the parent corpuscle, and disappear. On this account he regards them as portions of the protoplasm of the corpuscle, which have become detached and endowed with independent vitality and spontaneous movement, but are not mere parasites. This view appears to be well founded, and supported by the circumstance, previously recorded, of their intermittent appearance in the blood, and that when present in vast numbers under the microscope, they again

---

[1] If the red corpuscles of human blood be treated with a five per cent sol. amm. chromate, the superficial appearance of a nucleus is produced, at least as distinct as that which Bœtticher's methods create: the discs become "cup-shaped," or more accurately, the shape of a soft felt hat, with the margin of the brim turned over, or under, all round; and as they float about and turn over, it becomes evident that the colourless or pale appearance of a nucleus is occasioned by the central portion of the layer of the corpuscle, when lying flat and looked down upon, being seen single and consequently pale.

[2] Loc. cit., *supra*.

[3] 'Beit. z. Biol. d. Pflanzen,' Bd. i, H. 3, 1875, s. 196.

shortly all disappear; and still further, by the fact stated by Cohn (loc. cit.), that they are dissolved by potash, which is contrary to the properties of all known Bacteria, the resistance of which to alkalis and acids is regarded as their chief and most reliable characteristic. That they are portions of the protoplasmic substances of the corpuscle appears to be evident; as such they would possess contractility as long as they retained the vital properties of protoplasm, though in such attenuated filaments, the movements which they exhibit may be merely physical, the result of the currents which are never absent from a preparation of blood under the microscope, unless it be sealed, or until coagulation occur.

The next and latest description of these processes is in an excellent paper by Gaule,[1] which describes their appearances in defibrinated frogs' blood, treated on the warm stage at a temperature of 30° to 32° C. with a solution sod. chor. 0·4 to 0·8 per cent.; the method here adoped for defibrinating the blood was by shaking it up sharply in a mixing glass with salt solution and a little mercury, which subsides and carries the fibrin with it; a drop of the supernatant fluid is then placed on the warm stage, treated with more salt solution, and sealed. The author describes the formation of these processes from the large majority of the corpuscles; a staff-shaped body first appears in which some bright granules or striæ are visible, this elongates, becomes detached, pointed at both ends, and commencing a spiral movement, wriggles about over the field of view; coming in contact with other blood-corpuscles it adheres to them, dragging them after it, strongly resembling in appearance minute worms, but, as the author observes, in outward appearance only, and not in their real nature. In length these bodies equal about half the diameter of a red blood-corpuscle, but their character varies with circumstances, the vigour of the frog, the strength of the solution, &c. The author concludes that these are processes evolved from the constituent protoplasm, the stroma of the corpuscle. I can confirm the author's results in all respects. I have not found it necessary to defibrinate the blood to obtain these appearances, though to do so, simply whipping it is sufficient; nor is it necessary to seal the preparation, though this, by preventing evaporation, may somewhat prolong their duration.

Similar appearances have been described in other protoplasmic bodies, not only in the white blood-corpuscles, but in Amœbæ (Protozoa) too,[2] under the action of dilute salt solution; in some

---

[1] 'Archiv f. Anat. u. Physiol.,' 1880, Th. 1 and 2, s. 41—57. "Ueber Würmchen," etc., von J. Gaule.

[2] Dr. Vincent Czernay, "Beobacht. über Amœben.," 'Arch. f. Mikros. Anat.,' Bd. v, 1869, s. 158—166.

instances filamentous, or nodular processes, long pseudopodia, rosaries, or detached particles, were formed as in the case of the red blood-corpuscles; these appearances occurred during vitality, and on treatment with plain water the organisms regained their normal appearance and movement.

Thus it will be seen that these appearances have been observed and described by many during the last twenty years; that they are one and all of essentially the same character, there can be no doubt; it appears to me—that is, that they are essentially processes of the constituent protoplasm of the red corpuscles, a phenomenon of its contractility. Many who have observed and described the appearances seem to have been unaware that they had previously been described by others, as I was myself when first I noticed them; for this reason I have thought it would be useful to collate and record the observations already made upon this subject. The appearances are remarkable and highly interesting, as affording evidence of the constitution of the red corpuscles; no one, I think, after watching their evolution as above described, more especially under the action of ammonium chromate, can have any doubt as to their true nature.

NOTES AND MEMORANDA.

Medusæ and Hydroid Polyps living in Fresh Water.—With reference to the interesting note on this subject in the October issue of this Journal I should like to make a few remarks. It is there said, "Curiously enough, Mr. Romanes has found that marine Medusæ are not so injuriously affected by brine as the Limnocodium is by sea water. The fact, however, is less astonishing when we remember that the percentage of saline matter in solution in sea water is many hundred times what it is in average pond water, whilst the strongest brine has not a percentage of saline matter many times in excess of that of sea water" (p. 483).

Now, this is certainly one way of looking at the matter, but I doubt whether it is the fairest way. The percentage of salt held in solution by "average pond water" is really so small that it probably exerts no physiological influence of any kind on a Medusa, and, therefore, for purposes of physiological reasoning, ought not to be considered as a unit for comparison with higher percentages which do exert a physiological influence. It ought rather to be considered as a vanishing quantity or zero, so far, at least, as the Medusæ are concerned. Therefore, it seems to me that a fairer unit to take is the one which I had in my mind, although I did not explicitly state it, while writing my article to 'Nature' of June 24th, to which you refer. This unit is the percentage of salt which Limnocodium can tolerate for an indefinite time without manifesting a change in any of its physiological processes. What we want is a physiological, not a chemical, test of the percentage of salt that we are to consider as our unit, and this, it seems to me, can only be rendered by estimating the percentage of salt that first begins to exercise any perceptible influence upon the animal. This amount I found to be about $\frac{1}{5}$ per cent. Taking, therefore, ordinary sea water as having 3 per cent. of salt, and a saturated solution 36 per cent., we have as our proportions $\frac{1}{5} : 3 :: 3 : 36$; or $1 : 15 :: 15 : 180$; or $1 : 15 :: 1 : 12$. This shows that, if we take the above as our unit, the estimated change of conditions which a freshwater Medusa undergoes on being transferred to the sea water is pretty nearly the same as that which a sea-water

Medusa undergoes when transferred to brine of saturated strength.

If, then, we desire to draw any comparison at all, and if there is anything "astonishing" in the fact that a quickly fatal issue follows in the one case, while no harm results in the other, I should, nevertheless, still prefer adhering to the unit fixed by physiological conditions rather than to that supplied by chemical analysis.

In the same note of the 'Quarterly Journal of Microscopical Science' there appears a very interesting statement by Mr. Moseley, and another by Professor Agassiz, regarding the occurrence of marine Medusæ in "quite brackish," or even "almost fresh," water. One remark made by Professor Agassiz in this connection appears to demand some notice from me. He says: "So far as my experience goes, it is not conclusive of so fatal an action of fresh water on Medusæ as Romanes would lead us to believe in," &c., proceeding to relate his own observations on sundry species of Medusæ which live in the estuary of the Charles River. Now, as Professor Agassiz must have failed to refer to the observations which he thus appears to stigmatise as inaccurate, I will ask you to be kind enough, for his information, to quote them *in extenso*. For this purpose I give below an extract from the 'Philosophical Transactions,' in which they occur, and from which it will be seen that I have made no experiments or statements with reference to the effects of *brackish* water, either in estuaries or elsewhere. My experiments consisted merely in *suddenly* transferring Medusæ from sea water to *perfectly fresh* water. It certainly does surprise me to learn that *Sarsia*, *Tiaropsis*, and *Aurelia* are able to thrive in water that "tastes but little of salt;" but the fact in no way touches any of my published results. I can only conclude from it that a *gradual* transition from salt to comparatively fresh water not giving rise to such rapid osmosis is not so injurious to Medusæ as I should have expected from the morbid effects of sudden transition. The whole subject is thus shown well worthy of further experimental inquiry; but, so far, the following are the only experiments that I have conducted with reference to it.—GEORGE J. ROMANES.

Extract from 'Phil. Trans.,' vol. 167, p. 744.

As fresh water exerts a very deadly influence on the Medusæ, this seems the most appropriate place for describing its action. Such a description has already been given by Professor L. Agassiz, but it is erroneous. He writes: "Taking up in a spoonful of sea water a fresh *Sarsia* in full activity, when swimming most energetically, and emptying it into a tumbler full of fresh water of the same temperature, the little animal will at once drop like a ball to the bottom of the glass and remain for ever motionless—killed instantencously by the mere difference or the density of the two media."[1] As regards the appearance presented by *Sarsia* when subjected to "this little experiment," the account just quoted is partly correct; but Professor Agassiz must have been over-hasty in concluding that, because the animals seemed to be thus "killed instantaneously," such was really the case. Nothing, indeed, could be more natural than this conclusion; for not only is the contrast between the active swimming motions of the *Sarsia* in the sea water and their sudden cessation of all motion in the fresh water very suggestive of instantaneous death, but, a short time after immersion in the latter, their contractile tissues, as Professor Agassiz observed, became opalescent and whitish. Nevertheless, if he had taken the precaution of again transferring the *Sarsia* to sea water, he would have found that the previous exposure to fresh water had not had the effects which he ascribes to it. After a variable time his specimens would have resumed their swimming-motions; and although these might have had their vigour somewhat impaired, the animals would have continued to live for an indefinite time—in fact quite as long as other specimens which have never been removed from the sea water. Even after five minutes' immersion in fresh water, *Sarsia* will revive feebly on being again restored to sea water, although it may be two or three hours before they do so; they may then, however, live as long as other specimens. In many cases *Sarsia* will revive even after ten minutes' exposure; but the time required for recovery is then very long, and the subsequent pulsations are of an exceedingly feeble character. I never knew a specimen survive an exposure of fifteen minutes.[2] In not a few cases, after immersion in fresh water, the animal continues to pulsate feebly for some little time; and, in all cases, irritability of the contractile tissues persists for a little while after spontaneity has ceased. The opalescence above referred to principally affects the polypite, tentacles, and margin of the nectocalyx. While in fresh water the polypite and tentacles of *Sarsia* are strongly retracted.

Thinking it a curious circumstance that the mere absence of the few mineral substances that occur in sea water should exert

---

[1] 'Mem. American Acad. Arts and Sciences,' 1850, p. 229.

[2] The covered-eye Medusæ survive a longer immersion than the naked-eyed—*Aurelia aurita*, for instance, requiring from a quarter to half an hour's exposure before being placed beyond recovery. Moreover the cessation of spontaneity on the first immersion is not so sudden as it is in the case of the naked-eyed Medusæ—the pulsations continuing for about five minutes, during which time they become weaker and weaker in so gradual a manner that it is hard to tell exactly when they first cease.

so profound and deadly an influence on the nervo-muscular tissues of the Medusæ, I was led to try some further experiments to ascertain whether it is, as Agassiz affirms, to the mere difference in density between the fresh and the sea water, or to the absence of the particular mineral substances in question, that the deleterious influence of fresh water is to be ascribed. Although my experiments led to no very instructive conclusion, they are, I think, worth stating.

I first tried dissolving chloride of sodium in fresh water till the latter was of the same density as sea water. *Sarsiæ* dropped into such a solution continued to live for a great number of hours; but they were conspicuously enfeebled, keeping for the most part at the bottom of the vessel, and having the vigour of their swimming-motions greatly impaired. The tentacles and polypite were strongly retracted, as in the case of exposure to fresh water, and the tissues also became slightly opalescent. Thinking that perhaps a fairer test would be only to add as much chloride of sodium to the fresh water as occurs in sea water, I did so; but the result was much the same. On now adding sulphate of magnesium, however, to the amount normally present in sea water, the *Sarsiæ* became more active. I next tried the effects of chloride of sodium dissolved in fresh water to the point of saturation, or nearly so. The *Sarsiæ*, of course, floated to the surface, and they immediately began to show symptoms of torpidity. The latter became rapidly more and more pronounced, till spontaneity was quite suspended. The animals, however, were not dead, nor did they die for many hours—their irritability continuing unimpaired, although their spontaneity had so completely ceased. The tentacles and polypite were exceedingly relaxed, which is an interesting fact, as being the converse of that which occurs in water containing too small a proportion of salt. Lastly, to give the density hypothesis a still more complete trial, I dissolved various neutral salts and other substances, such as sugar, &c., in fresh water till it was of the density of sea-water; but in all cases, on immersing *Sarsiæ* in such solutions, death was as rapid as that which followed their immersion in fresh water."

Terminology of Reproductive Organs and Classification of Thallophytes.—Our readers are requested to substitute the following table for the one given on pp. 419, 420 of the last number of the "Journal."

We take this opportunity of thanking many correspondents for their kind and encouraging criticisms; and of making the following corrections and emendations.

The most recent investigations of the *Chytridiaceæ* point to their systematic position among the Zygomycetes next to the Mucorinis rather than among the Oomycetes. A corresponding correction should be made on p. 409, lines 8—5 from bottom.

Berthold's discovery of the conjugation of zoospores (zoozygospheres) in *Dasycladus,* following the similar observation of De Bary and Strasburger in *Acetabularia,* points to the probable location of the whole family of *Dasycladeæ* among the Zygophyceæ, near to Botrydieæ, rather than among the Oophyceæ.

On p. 415, l. 21 from bottom, "Desmidieæ" should be Diatomaceæ.

On p. 418, l. 21, the Uredineæ and Ustilagineæ should be named, in addition to the Basidiomycetes, as Carpomycetes, in which the sexual organs (at least the female ones) are at present unknown.

ALFRED W. BENNETT.
GEORGE MURRAY.

Reproductive Organs of Thallophytes.

	Female.	*Non-sexual.*
PROTOPHYTA.		Chlamydospore. Sporangium. Sporangiospore.
MYXOMYCETES.		Zoosporangium. Zoospore
MUCORINI.	Zygogonium. Zygosphere. Zygosperm.	Chlamydospore. Sporangium. Sporangiospore.
PERONOSPOREÆ.	Oogonium. Oosphere. Oosperm.	Conidiospore. Zoosporangium. Zoospore.
SAPROLEGNIEÆ.		Zoosporangium. Zoospore.
UREDINEÆ.		Teleutospore. Sporidium. Accidiospore. Uredospore.
USTILAGINEÆ.		Teleutospore. Sporidium.
BASIDIOMYCETES.		Basidium. Basidiospore.
ASCOMYCETES, including LICHENES.	Trichogonium. Carpogonium. Carposphere. Carposperm.	Conidiospore. Stylospore. Ascus. Ascospore. Polyspore. Merispore.

	Female.	*Non-sexual.*
ZYGOPHYCEÆ.	Zygogonium.	Zoosporangium.
	Zygosphere.	Zoospore.
	Zoozygosphere.	Megazoospore.
	Zygosperm.	Auxospore.
	Hypnosperm.	Hypnosporangium.
		Hypnospore.
		Parthenospore.
OOPHYCEÆ.	Oogonium.	Zoosporangium.
	Oosphere.	Zoospore.
	Oosperm.	Parthenospore.
	Conceptacle.	Androspore.
	Hypnosperm.	Hypnospore.
CARPOPHYCEÆ.	Trichogonium.	Zoosporangium.
	Carpogonium.	Zoospore.
	Carposphere.	Tetraspore.
	Carposperm.	Octospore.
	Cystocarp.	Carpospore.

MEMOIRS.

The MINUTE ANATOMY *of the* BRACHIATE ECHINODERMS.

By P. HERBERT CARPENTER, M.A., Assistant-Master at Eton College. With Plates XI and XII.

IN the following pages I propose to give some account of the work that has been done during the last few years upon the minute anatomy of the Starfishes, Ophiurids, and Crinoids. I do not intend to touch upon the question of the skeleton at all, as I have already discussed its morphology in the pages of this Journal.[1] At present I aim only at giving an intelligible account of what appear to be well-established discoveries in the anatomy and physiology of the nervous, vascular, and generative systems of these three groups.

By far the most important of the recent researches in this subject are those of Ludwig.[2] Others may have devoted more attention to particular groups, but no one has worked so extensively at increasing our general store of facts in Echinoderm morphology as he has; while, at the same time, his observations are by far the most accurate and trustworthy of any that have been recorded. Although there are one or two theoretical points with respect to which our opinions are entirely different, I have no hesitation in saying that I have the utmost confidence in his facts. There are, of course, many anatomical peculiarities that were more or less perfectly elucidated by his predecessors, but on the whole, no one has done so much as he has towards correlating, systematising, and verifying or correcting the more or less conflicting observations of other investigators.

---

[1] "The Oral and Apical Systems of the Echinoderms," 'Quart. Journ. Micr. Sci.,' vols. xviii and xix. "Some disputed points in Echinoderm Morphology," 'Quart. Journ. Micr. Sci.,' vol. xx.

[2] "Morphologische Studien an Echinodermen," Leipzig, 1877—79. These were originally published as separate papers in the 'Zeitschrift für Wissenschaftliche Zoologie,' Bände xxviii, seqq.

Were it not for his work on the 'Anatomy of the Starfishes,' the observations of three other authors, which were published in 1876, would only have increased the confusion that already existed in this subject. This has, in fact, been the case with respect to the Urchins and Holothurians, though in a less degree, and it is now very desirable that the minute anatomy of both these groups should be reinvestigated by the light of our present knowledge of the other Echinoderms. It is more than probable that the results of such investigation would necessitate the giving up of many of our present ideas concerning the nervous and vascular systems of these two orders.

It will perhaps be best if we commence our studies with the Starfishes, which are probably the best known among the Echinoderms. Having acquired some understanding of their typical structure, we can proceed to consider that of the Ophiurids, and finally that of the Crinoids, which presents many singular deviations from the ordinary Echinoderm type. I shall not attempt to do much more than describe the actual anatomical facts, leaving almost entirely out of consideration any identification of particular parts with the structures mentioned by the older anatomists. This (if required) must be sought for in the original memoirs themselves, a list of which is to be found at the conclusion of this paper.

Figure 3 on Plate XI is a diagrammatic transverse section, representing the structure of a Starfish arm. On the left side the section is supposed to pass through a vertebral ossicle, while on the right it passes between two successive ossicles. The pyloric cæcum (*p.c.*) is omitted from the left side for the sake of clearness, while on the right the genital gland (*ov.*) is not represented. The integument lining the ambulacral groove between the rows of tube feet differs considerably from that covering the rest of the exterior of the body. It is usually more or less raised into a median ridge, and consists of two principal layers, an outer cellular and an inner fibrillar one. The latter (figs. 3 and 5, *n.*) is thickest in the middle line, but thins away laterally, while the former (*a.e.*) is merely a modified portion of the general external epithelium of the arm (E), with which it is continuous at the sides of the groove. This ambulacral epithelium consists of columnar cells, each bearing cilia, which seem to pass through delicate pores in the superficial cuticular layer. The cells are closely packed, and their nuclei are situated at different heights, so as to produce the appearance of there being several different layers of cells

(17, 21).[1] The cell bodies are supported upon long rods, the bases of which are often forked, and rest upon the connective-tissue membrane that separates the ambulacral epithelium from the vascular apparatus above it (fig. 5, *ct.*). Between these vertical rods lie the longitudinal fibres of the subepithelial layer (*n.*), to which a nervous character is generally assigned (21, 33). Intercalated among the fibrils are small masses of nucleated protoplasm, which are continuous with their substance, and sometimes mark the points at which they divide. Ludwig regards these small cells as nerve-cells, but they escaped the notice of Lange, who was consequently led to assign a nervous character to a cellular layer on the upper (*i. e.* dorsal) side of the above-mentioned connective-tissue membrane (fig. 6, *ep.*).

The observations of Ludwig and Teuscher, however, prove that Lange's masses of nerve-cells are merely local thickenings of the epithelium lining the radial perihæmal canals. They are not constant in all Asterids, and when present are not continuous through the ray. A similar thickening in the peristome of *Asteracanthion rubens* is represented in fig. 6, *ep.*

Above and within the nerve band is the blood-vascular apparatus of the ambulacrum, which is very complicated in its arrangement. Between the basement membrane supporting the ambulacral epithelium (fig. 5, *ct*) and the band of connective tissue that bears the lower transverse muscles of the arm, immediately under the water-vessel, is a relatively large space, which extends through the whole length of the ambulacrum. It is divided into two lateral portions by a perforated vertical septum, also longitudinal (fig. 5, *v.s.*), which supports the radial blood-vessel (fig. 3, *b.*). The latter, which is often somewhat plexiform in character, was more or less perfectly known to Hoffmann, Greeff, Lange, and Teuscher, but its true relations were first elucidated by Ludwig. The space already mentioned (fig. 3, *r.p.*) between the water-vessel above and the ambulacral epithelium below, which is traversed by the perforated longitudinal septum, was named by Ludwig the "perihæmal canal." It had been previously called the nerve-vessel or nerve-canal, and was supposed to form an integral part of the blood-vascular system.[2] Now,

---

[1] The numbers in brackets refer to a list of recent memoirs on Echinoderm anatomy, which is printed at the end of this paper.

[2] In his "Anatomy of the Invertebrata" Prof. Huxley speaks of the perihæmal canal as the ambulacral neural canal, and expresses great doubt as to whether it really belongs to a special system of blood-vessels. The later observations of Ludwig render the old view no longer tenable.

however, it is regarded by Ludwig merely as a derivative of the body-cavity. At the intervals between the successive vertebræ its upper third is crossed by a series of transverse septa (fig. 5, *t. s.*). The longitudinal septum has a slight horizontal expansion at the level of the radial blood-vessel, which increases in size at the origin of each transverse septum, but never reaches the side of the perihæmal canal. Hence the latter is nowhere completely divided into three or four sets of chambers, as was formerly supposed. It gives off lateral extensions, which embrace the bases of the tube feet, and unite on their outer sides to form longitudinal canals (21). These, which were formerly regarded as lateral auxiliaries of the radial blood-vessels, are in connection with an extensive lacunar system in the body wall (fig. 3, *lac.*), which can be injected from the radial perihæmal canal, and appears, like the latter, to be a derivative of the body-cavity, or perhaps, of the embryonic blastocœl.

In the peristomial area of the disc the radial, nervous, and vascular trunks unite into their respective oral rings (Pl. XI, figs. 5, 6, 8). The nerve-ring (*n. r.*) in the lip contains circular fibres packed among the rod-like bases of the epithelial cells. The basement membrane which supports them also forms the wall of the perihæmal ring-canal,[1] into which there project the cellular masses described by Lange as nervous (fig. 6, *ep.*). The ring-canal itself is divided into two parts, an inner and an outer one, by an annular continuation of the longitudinal vertical septum in the radial canal (figs. 5, 6, 8, *s.*). This is situated rather obliquely, and supports the oral blood-vascular plexus (*o. b.*). The inner canal (*i. p.*) is the oral blood-vascular ring of Tiedemann, while the outer one (*o. p.*) is the orange-coloured vessel described by him. The latter is connected by interradial canalicular extensions with a widely-spread canal system, situated between the two layers which form the body wall, just as in the arms. This system is also connected, as will be seen later on, with the perihæmal canals surrounding the genital vessels (12, 21). Both the inner and the outer perihæmal ring-canals have received various names from Greeff, Hoffmann, Teuscher, and Lange, who all regarded them, together with their radial extensions, as integral parts of the blood-vascular system. The real oral blood-vascular ring was seen by Tiedemann, Greeff, and Teuscher, but its true relations were only imperfectly known to them. Greeff conjectured what Ludwig has since

---

[1] This is the "circular neural canal" mentioned in Huxley's 'Invertebrata.'

demonstrated, viz. the connection of this ring with a large plexiform bundle of vessels (figs. 2, 7, 8, *c.p.*), which ascends by the side of the stone-canal (*s. c.*) and joins an aboral blood-vascular ring (fig. 2, *a. b.*). This organ was discovered by Spix, and described by Tiedemann as a heart or heart-like canal, by Hoffmann as a glandular body, by Greeff as a gill-like organ, and then again as a heart by Teuscher and Ludwig, though the latter author has since abandoned the use of this term for the Echinoderms generally, and now speaks only of the central plexus. The organ in question is enclosed, together with the stone-canal, in a large tubular space, which is supported by one of the interradial falciform bands (figs. 7 and 8, *a.p.*). A person standing in the dorso-ventral axis of the Starfish with his feet in the oral ring, and facing the stone-canal, would see the central plexus to the right of it. It consists (21, 33) of a close network of vessels, partly dividing and partly anastomosing, the walls of which contain connective-tissue fibres, and perhaps muscle fibres also. The lumina of the vessels of the central plexus, and likewise of those in the oral and aboral rings connected with it, are filled up by large, brownish, cellular bodies of a peculiar character, which are also described as occurring in the cœlom and in the water-vascular system. Tiedemann described the central plexus as responding to stimulation by feeble contractions, and Hoffmann observed it contracting rhythmically, the same peculiarity characterising the two smaller plexiform bundles (fig. 2, *p. b.*) which proceed to the stomach from the aboral ring at the point where the central plexus joins it.

The central plexus, like the rest of the blood-vascular system, is surrounded by a perihæmal canal, which is the tubular space mentioned above as enclosing the central plexus together with the stone-canal (figs. 7, 8, *a. p.*). It may be termed the axial perihæmal canal. Its central end has been shown by Teuscher and Ludwig to arise from the inner perihæmal ring-canal (figs. 5, 6, 8, *i.p.*). This is only separated by a perforated septum (*s*) from the outer perihæmal ring (*o. p.*) which unites the perihæmal canals of all the rays.

The so-called "heart" was described by Tiedemann as terminating dorsally in an aboral vascular ring, from which proceed (1) ten genital vessels, (2) ten vessels to the pyloric cæca, (3) two gastric vessels, the plexiform bundles mentioned above. These results were confirmed by Greeff and Hoffmann, except as regards the supposed vessels of group (2). These had been previously shown by the late Professor

Sharpey to be merely the spaces between the two folds of mesentery in which each pyloric cæcum is slung (fig. 3, *im.*). They are only partially separated portions of the general body cavity with which they are connected in the disc.

Greeff, Hoffmann, and Teuscher were able to inject the tubular space enclosing the "heart" and stone-canal from this aboral ring described by Tiedemann; and Greeff described the lumen of the ring as partially filled up by a hollow fold, which exhibits the same structural characters as the "heart" and the gastric vascular bundles. As in the case of the oral ring, the hollow fold discovered by Greeff in Tiedemann's anal ring has been shown by Ludwig to constitute the true aboral ring or annular plexus. It is more or less filled up with the brown cellular elements already mentioned as occurring in the central plexus, the dorsal end of which joins it (fig. 2, *a. b.*). The space around it is no part of the true blood-vascular system, as supposed by Tiedemann and Greeff, but the perihæmal canal corresponding to the aboral blood-vascular ring. This of course explains the injection of the tubular space from Tiedemann's ring, as it is merely the perihæmal canal of the central plexus which joins the true aboral ring, but does not terminate in it; for it passes upwards and attaches itself to the under surface of the disc just outside the madreporite (fig. 2, *x*).

Just in the same manner the structures hitherto described as the two gastric vessels are merely the perihæmal canals corresponding to the real vessels which they enclose (fig. 2, *p. b.*). The ultimate ramifications of the latter are not known, but they contain the same brown cells as the rest of the blood-vascular system.

Up to the time of Ludwig, the true vessels of the generative organs had never been properly observed; the structures described under that name by Tiedemann and others being merely their perihæmal canals. They are ten in number, arising from the aboral ring (fig. 2, *g. v.*), and each expanding into a sinus around one of the spreading genital glands (fig. 3, *g.*). The perihæmal canals enclosing them, which start from Tiedemann's ring, are directly connected with the lacunar system in the body wall. Hoffmann was led to suppose that in the Starfishes devoid of the interbrachial genital openings described by Müller and Troschel, the perihæmal canals are in immediate connection with the internal cavities of the glands, the blood having direct access to the follicles. He imagined these canals to serve as ducts, the ova passing along them into Tiedemann's ring, thence into the tubular space and out to the exterior through the pores in the madreporite. Greeff recognised that the so-called genital vessels (*i. e.* perihæmal canals) surrounded the glands; but he supposed the glands to open into them, and that the sexual products passed out by the interbrachial pores, which would thus place the blood-vascular system in direct communication with the external water.

Ludwig's later observations have shown that definite genital openings are present in all Starfishes, varying in number from ten upwards. They sometimes extend out on to the arms, so that the presence of individual openings for the numerous separate glands in the arms of *Brisinga* is no longer such a striking peculiarity as it was formerly supposed. Each genital pore (fig. *3, g. p.*) leads into a distinct efferent canal, which pierces the perihæmal canal and its contained vessel, and enters the cavity of the gland, to which it serves as a duct. In most cases these pores are on the dorsal surface of the arm, but in *Asterina gibbosa* they are ventral (25).

It will be seen from what has been said above, that the pores of the madreporic plate were supposed by Hoffmann to lead into the tubular space around the stone-canal as well as into the stone-canal itself. He likewise believed the marginal pores of the madreporite to lead directly into the cœlom. This connection of the water-vascular and the blood-vascular systems through the pores of the madreporite with the exterior and with one another was also believed in by Greeff and Teuscher. Their views rested principally upon the results of injection *not* checked by the section-method, which are necessarily liable to much error. Ludwig, however, after making sections in three planes through the madreporic plate, satisfied himself of the truth of the older views of Sharpey, L. Agassiz, Müller, and Tiedemann, viz. that the pores of the madreporite lead simply and soley into the stone-canal. The interior of the plate is traversed by pore canals, which correspond in position with the radiating furrows on its upper surface, and communicate with them by short vertical tubules. These are lined by a pavement epithelium, which becomes columnar and ciliated at their openings on the surface, and likewise in the actual stone-canal itself, into the top of which the radial pore canals open (fig. 7, *p.*). At the aboral edge of the attachment of the stone-canal to the madreporite is a lateral diverticulum of the former, into which some of the collecting tubes of the madreporite open (12, 21). It is occasionally double or even triple, but its wall is never calcified like that of the stone-canal, and it is lined by pavement epithelium. On the other hand, the epithelial cells lining the stone-canal are high and ciliated, and its walls more or less plicated, in some species very much so; the plications being supported by calcareous rings of various forms. Towards its ventral end all the plication ceases and it joins the water-vascular ring as a simple tube. So far as we know with certainty, the water-vascular and blood-vascular systems are entirely distinct, though the injections of Hoffmann and Greeff have led them to believe in a communication between the two systems in the region of the disc. At any rate the old view, which was based chiefly on the results of injections, is no longer tenable, viz. that the two are connected by the ten "brown bodies of Tiedemann," small eminences resting on the water-vascular ring (fig. 6, T). Ludwig finds these structures to be lateral diverticula of the water-vascular ring, which are lined by an epithelium of cuboid cells, and contain the brown cellular bodies that have been described as present in the blood-vascular system. But there is no connection between these diverticula and either the perihæmal ring-canal (fig. 6, *i. p.*, *o. p.*) or the true oral blood-vascular ring (*o. b.*).

The origin of the lateral trunks from the radial water-vessels is protected by a valvular arrangement discovered by Jourdain, the effect of which is to prevent a reflux into the radial vessels after contraction of the ambulacral vesicles for the purpose of expanding their corresponding tube feet. Lange and Ludwig have found this structure to be universally distributed in the Asterids, and Ludwig describes it as also present in Ophiurids, and in some Urchins (16, 17, 21, 27).

Although Sars' observations had led him to believe that *Brisinga* is entirely devoid of a special blood-vascular system distinct from the cœlom, Ludwig has shown that in this as in other structural characters it is a true Asterid, having a dorsal and ventral ring and a central plexus connecting them. This organ was known to Sars, together with the perihæmal space around it, and also the oral perihæmal ring with its radial prolongations, but he did not regard the latter as in any proper sense entitled to be called blood-vessels, while he altogether denied the existence of any aboral blood-vascular system.

2. *Ophiuroidea.*

The minute anatomy of the Ophiurids has been investigated by Lange, Teuscher, and Simroth, and most recently by Ludwig, who has shown that they correspond closely with the Asterids in the essential details of their organisation.

Müller's discovery of the madreporic opening on one of the mouth shields of *Ophioglypha lacertosa,* which has been generally overlooked, has been confirmed by Ludwig and extended to other Ophiurids. There is usually only one pore which leads into a bent canal with lateral diverticula, and lined by ciliated epithelium. Some *Ophiuræ* and many Euryalids have several pores on one mouth shield, while in *Trichaster elegans* there is one pore in each interradius (22).

Above the madreporite lie the stone-canal and central plexus, which are together enclosed in a perihæmal space that, with its contents, was described as the stone-canal by Müller and Teuscher. The real stone-canal (water-tube), which was first recognised as such by Simroth, is lined as in the Asterids by a ciliated columnar epithelium, and it opens just above the madreporite into an ampulla-like cavity lined by pavement epithelium. Ludwig believes the pore canal of the madreporite to open into this space as its fellow does in the Asterids, but he has never been able to prove it, though he has demonstrated the connection of the upper end of the water-tube with the water-vascular ring (Pl. XI, fig. 4, *w.r.*). This bears either no Polian vesicles at all, or four, one for each of the remaining interradii (P.), or numerous blind tubular diverticula of various shapes, which are merely modified Polian vesicles (27, 29, 32). In most cases the ring gives off bifurcating trunks which supply the large buccal feet (*b.f'.*).

The blood-vascular system of the Ophiurids, like that of the Asterids, consists of dorsal and ventral rings united by a central plexus (Pl. XI, fig. 1). The former sends off branches to the genital glands (*g. v.*), while from the latter there arise the radial blood-vessels (figs. 1 and 4, *b.*). These last were discovered and correctly described by Lange, but Teuscher at first supposed them to be parts of the nervous system. They lie immediately above the nerve band (fig. 4, *n.*), and send off branches to the tube feet which are accompanied by nerves. Between the blood-vessels and the water-vessels of the arms are the perihæmal[1] canals of the former (fig. 4, *r. p.*), which are connected laterally with the contracted remnants of the extension of the body-cavity into the arm that remain between the body wall and the vertebræ (27, 32).

In the disc the radial perihæmal canal communicates as in the Asterids with the outer of the two perihæmal ring-

---

[1] Neural canals, Huxley.

canals, which has been hitherto described as the blood-vascular ring (fig. 4, *o. p.*). The real oral ring, however, from which the radial vessels originate (*o. b.*), is in close connection with the nerve ring (*n. r.*) and joins the upper end of the central plexus, which ascends from the mouth shield alongside the water-tube. As already mentioned, these two are enclosed within a perihæmal space which is connected with the inner perihæmal ring-canal, just as is the case with the axial perihæmal canal of the Asterids. Its other end opens into the perihæmal canal of the aboral ring (fig. 4, *p. h.*), the disposition of which is very singular. Although naturally belonging to the dorsal portion of the disc, only a part of it is to be found there, viz. those sections of the ring which lie beneath the radial shields (fig. 1, $a.\ b_3$.; fig. 4, *a. b.*). Resting on the mouth shields in the five interradial spaces are five other sections of the ring ($a.\ b_1$.), with one of which the central plexus is connected. The five radial and dorsal sections are connected with the five interradial and ventral ones by ten descending limbs, which pass downwards at the sides of the rays (fig. 1, $a.\ b_2$). Just before leaving their dorsal position these give off the ten principal genital vessels (fig. 1, *g. v.*), branches from which surround the glandular cæca situated between the genital clefts and the rays. The cæca, which are situated between every two clefts, receive their blood supply by lateral branches of the ten descending limbs at the sides of the rays ($a.\ b_2$). All the separate cæca are connected by a cellular cord concealed within the vascular ring, which is regarded by Ludwig as a sterile portion of the generative apparatus comparable to the so-called *rachis* of the Crinoids.

It has been hitherto supposed that the genital glands of the Ophiurids "pour their products into the peritoneal cavity, which communicates freely with the exterior by vertically elongated apertures placed interradially on its margins."[1] Ludwig's observations, however, show that this view, which was advocated chiefly by Müller, does not altogether agree with the facts. He confirms the earlier discoveries of della Chiaje and Rathke, to the effect that the so-called genital clefts lead into a sac situated within the cœlom but not communicating with it, and receiving the ducts of the genital glands. There are ten of these "bursæ" in the disc, two in each interradius, which lie close alongside the radial skeleton (fig. I, B). Each is a thin-skinned invagination of the body wall, the adradial lower portion of which is supported by one of the so-called genital

---

[1] Huxley's 'Invertebrata,' p. 504.

plates (*Bu′.*); while the abradial margin of the cleft is formed by a continuation of the general scaly covering of the ventral perisome (*Bu″.*). The walls of the sac sometimes contain small calcareous plates. Into each bursa open the isolated genital tubes (*G.*), some of which occupy the space between it and the ray, while others are situated on its abradial side, extending nearly up to its dorsal surface. Each tube is surrounded by a blood space, which is derived either directly from the aboral ring, as in the case of the abradial tubes, or from the genital vessels (adradial tubes). The genital products are not set free into the body-cavity, as was formerly supposed, but merely into the bursæ from which they reach the exterior. It is possible that the bursæ are also respiratory organs, serving the same purpose as the external respiratory cæca of the Asterids; while in some viviparous species they serve as marsupial cavities.[1]

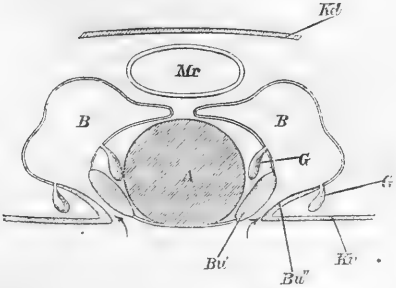


Fig. 1.—Diagrammatic vertical section across a radius of an *Ophioglypha* near the edge of the disc. *Kd.* dorsal; *Kv.* ventral body-wall; *Mr.* radial diverticulum of the stomach; *A.* portion of the arm included in the disc; *B.* bursa; *Bu′.* the adradial edge of the bursal cleft, with the bursal (genital) plate; *Bu″*, its abradial edge, with the scales of the ventral perisome; *G.* genital tubes. The arrows point to the bursal clefts. (Copied from Ludwig.)

Among recent Echinoderms there is no structure at all comparable to these bursæ of the Ophiurids, but Ludwig points out their resemblance to the hydrospires of the Blastoids, and especially those of *Orophocrinus* (*Codonites*). In this genus the hydrospires have no special spiracular openings at the apex as in *Pentremites*. There are also

---

[1] Studer has recently pointed out (‘Zool. Anzeiger,’ No. 67, p. 526) that he described these bursæ as marsupial pouches as long ago as 1876 (‘Antarkt. Echinodermen. Monatsber. d. Berlin. Akad.,’ 1876). In *Ophiomyxa vivipara* every pouch contains two or three fully-developed young starfishes, each enclosed in a thin membrane like a chorion. *Ophiacantha vivipara* has fourteen clefts and the same number of pouches, in each of which there may be three young ones.

no rows of pores at the sides of the ambulacra. But the hydrospires communicate with the exterior by ten interradial slits, which extend along the sides of the ambulacra very much as the bursal clefts do in the recent Ophiurids. The abradial walls of the bursæ also exhibit traces of the plicated structure which is so characteristic of the hydrospires of the Blastoids. This comparison is one of great interest from many points of view, but a discussion of the questions it involves would be out of place here.

Müller's views respecting the nervous system of the Ophiurids, although attacked by Lange, have been abundantly confirmed by Teuscher, Simroth, and Ludwig. All these four observers describe a band of tissue (fig. 4, *n*) which lies immediately above the under arm plates (S_1—S_6, &c.), and is composed of two layers, an outer cellular and an inner fibrillar one. Lange considers this to be merely a portion of the integument, and has described as nervous a so-called ganglionated cord lying above it, very much as he did in the case of the Asterids. The other observers have shown the untenability of this view, the "ganglion cells" being merely portions of the epithelial wall of the perihæmal canal, while the "longitudinal commissures" are portions of the membranous septum which separates the latter from the real nerve band. Each radial nerve gives off two branches to the bursæ, which leave it just on the aboral side of the origin of the nerves proceeding to the second pair of buccal feet. The radial nerves are connected in the disc with an oral ring (27, 29), which is immediately contiguous to the blood-vascular ring and to the outer oral perihæmal ring-canal (fig. 4, *n. r.*). With the latter are connected five interradial spaces, one of which lies on the adoral side of the external interradial muscle of every oral angle, and is connected with the cœlom. Separated from the outer ring-canal by a septum[1] is the inner one (fig. 4, *i.p.*), from which arises the axial perihæmal canal that encloses the central plexus and sand-canal, just as is the case with its fellow in the Asterids. The perihæmal systems of the two groups are essentially similar, except that the Ophiurids lack the lacunar system which is so abundantly developed within the integument of the Asterids.

---

[1] It is evident from fig. 4 that the inner and outer perihæmal ring-canals of the Ophiurids are much more distinctly separated than they are in the Asterids. The former is separated from the body-cavity by the septum marked *s*, which must not be confounded with the perforated septum *within* the ring-canal of the Asterids; that supports the oral blood-vascular ring (figs. 5, 6, 8, *s*).

3. *Crinoidea.*

The Crinoids occupy a very curious position among the Echinoderms. There are certain structural peculiarities in which they resemble the Starfishes much more than the Holothurians do, and yet they possess a complicated system of organs which is absolutely without parallel in the other Echinoderms.

The ambulacral epithelium lining the food grooves is essentially similar to that in the ambulacra of the Starfishes. It consists of closely packed columnar cells, each with a cuticle and numerous cilia. Their lower ends are pointed and are apparently connected with some vertical fibres, among which lie the longitudinal fibrillar bundles of the ambulacral nerve. These vertical fibres, which arise from the thin membrane separating the nerve and radial blood-vessel, are probably merely of a connective-tissue character. Sometimes in *Antedon Eschrichtii* there is a second connective-tissue lamella which separates the nerve from the epithelium above it, and supports the lower ends of the epithelium cells (Pl. XII, fig. 10, *n.*), but its presence does not appear to be constant.

Intercalated among the longitudinal fibrils are many very minute cells, just as in the ambulacral nerves of the Starfishes and Ophiurids, which have a close histological resemblance to the sub-epithelial bands of the Crinoids. Lateral branches proceed from the ambulacral nerve to the successive tentacular groups, but there are no representatives of the muscle nerves which occur in the Ophiurids.

Between the water-vessel and the ambulacral nerve the middle line of the arm (or pinnule) is occupied by the radial blood-vessel, just as is the case in the Starfishes (Pl. XII, figs. 9, 10, 11, *b.*), but it is not enclosed in any perihæmal space, and is usually of very small size, so as occasionally to escape notice altogether. On the other hand, it is sometimes relatively very large, as in *Actinometra nigra.* Immediately beneath it is the radial water-vessel (figs. 9—11, *w.*), from which lateral branches proceed alternately on opposite sides of the arm to the different tentacular groups, each branch being accompanied by corresponding ones from the blood-vessel and ambulacral nerve. The three radial trunks are continued over the disc (figs. 14, 15) to the peristome, where they unite into their respective circumoral rings.

Depending from the water-vascular ring into the body-cavity are a number of small tubules, open below and lined by ciliated columnar epithelium (fig. 14, *w. t.*). Their number varies considerably in adult *Comatulæ*, reaching thirty or more in each interradius of *Antedon rosacea;* but in *Rhizocrinus* and in the young *Antedon* there is but one in each interradius (20), while in the early stages of the Pentacrinoid larva of *Antedon* there is only one, which is always situated in the same interradius as the foregut (26). Corresponding to these water-tubes ("Steinkanäle," Ludwig), the ventral perisome, whether soft or plated, is pierced by a variable number of water-pores, which lead from the exterior directly into the body-cavity (figs. 14, 15, *w. p.*). They are the external openings of small canals lined by columnar epithelium, and expanding almost immediately into enlargements in which the epithelium is ciliated. The inner end of the canal beyond the enlargement is lined by pavement epithelium, and opens into the body-cavity. The number of these water-pores is very large, but very variable, and they are not limited to the disc, for I have found them on the lower parts of the arms (fig. 15), and even on the proximal pinnules. In both these cases they open into that section of the body-cavity which surrounds the generative apparatus, and is known as the genital canal. Ludwig has estimated their number in the adult *Antedon rosacea* at about 1500, while in the young *Antedon* and in *Rhizocrinus* there is but one in each interradius (20), and in the Pentacrinoid larva there is only one which pierces the lateral margin of the oral plate that is in the same interradius as the single water-tube (26).

By means of the water-pores, body-cavity, and water-tubes, therefore, the ambulacral system of a Crinoid is placed in communication with the external water; and Ludwig regards the water-pore and water-tube together as conjointly representing the madreporic apparatus and stone-canal of the Starfishes and Ophiurids. A multiplication of these organs may occur in both groups, which is merely carried somewhat further in the Crinoids, and the nature of the epithelial lining of these afferent channels is the same in all three groups. It remains, however, yet to be proved that the disconnected water-pore and water-tube of the *Antedon* larva are morphologically equivalent to the stone-canal and madreporic apparatus of the other Echinoderms, though it is certainly exceedingly probable that they are so. At present we do not know enough of the earlier stages of their development to be quite sure of their homology with their undoubted analogues in the other Echinoderms.

Between the dorsal skeleton of the arms and pinnules and the water-vessels on their ventral side, are three tubular prolongations of the body-cavity (figs. 9—12). The middle one, into which open the water-pores on the arms and pinnules, is the genital canal already mentioned (figs. 9, 10, 12, 15, *g. c.*). The canals above and below it, which communicate with one another at the end of each arm or pinnule, are known respectively as the ventral or subtentacular (*s. t. c.*), and the dorsal or cœliac (*c. c.*). They are sometimes connected in the pinnules by a series of lateral trunks, as seen in fig. 12. In those Crinoids which have a central mouth (*Antedon, Pentacrinus*), the subtentacular canals all arise from a large central space in the axis of the visceral mass, around which the digestive tube is coiled.[1] In *Actinometra*, however, which has an excentric mouth, the subtentacular canals of the disc gradually become indistinguishable from the general body-cavity, and there is no distinct axial cœlom. Currents proceed through the subtentacular canals to the tips of the arms and pinnules, and return to the disc again by the cœliac canals (3). These currents are due to the action of cilia, which are not uniformly distributed, but are localised in little cups on the top of each pinnule joint that supports the lower part of the cœliac canal (figs. 9, 11, 12 *ci. c.*). The genital and cœliac canals are continuous respectively with the ventral and dorsal portions of the circumvisceral division of the body-cavity, *i.e.* the space between what have been called the visceral and parietal layers of the peritoneum (3, 8). In *Actinometra* the subtentacular canals accompany the genital ones, and enter the ventral division of this space, the whole of which is laid open when the disc is removed from the calyx.

The blood-vascular system of the Crinoids differs in several important points from that of the Starfishes and Ophiurids. In the first place there is no definite aboral ring, although there is a representative of the central plexus, the so-called "heart" of the Starfish. This organ consists of an irregular lobate bundle of vessels, which rises out of the calyx into the lower portion of the visceral mass. In *Antedon* and *Pentacrinus* it ascends nearly in the vertical axis of the body, in the immediate neighbourhood of the axial cœlom, to the point at which the latter subdivides into the five subtentacular canals of the disc. Its further course is exceedingly difficult to follow. Both Ludwig and myself believe it to be

---

[1] This is very well shown in Ludwig's diagrammatic vertical section of an *Antedon* (19, pl. xix). His figure (which is reproduced in the 'Popular Science Review,' July, 1880) may be advantageously compared with the two similar ones illustrating the Anatomy of *Actinometra* which accompany this paper (Pl. XII, figs. 14 and 15).

connected with some ramified tubes depending into the cœlom from the oral blood-vascular ring (7, 19), but we have been unable to obtain conclusive evidence as to this point.

The genital glands of the Crinoids, like those of the Starfishes, are enclosed in blood spaces (figs. 9, 12, *g.*), the individual ovaries or testes at the sides of each arm being connected by a "genital cord," the so-called "rachis" (3, 5, 19, 28), with its enclosing blood-vessel (fig. 10, *g. c′.*). These genital cords are merely undeveloped portions of the genital glands, which serve to unite the isolated genital tubes, just as is the case in the Ophiurids (19, 27). In this group the genital cords of all the rays are connected by a circular one within the aboral blood-vascular ring; but in the Crinoids they, or rather the vessels enclosing them, unite into an extensive network immediately beneath the ventral surface of the disc, consisting principally of larger trunks beneath the subtentacular canals (figs. 14, 15, *g.v.*), which unite laterally with one another and with those of adjacent rays. This network is believed by Dr. Carpenter and by Ludwig to be connected with the central plexus, though neither of them has succeeded in demonstrating this fact in the adult *Antedon*. I have fortunately been able to obtain one series of sections, which furnishes the necessary proof, while Dr. Carpenter's observations of the advanced larval stages have yielded similar results (3, 7). He found the central plexus subdividing by the side of the œsophageal funnel into five diverging branches, one of which passes into each ray beneath the commencing subtentacular canal, precisely in the position of the genital rachis of the arms.

I have spoken above of some ramified tubes depending into the cœlom from the oral blood-vascular ring, with which both Ludwig and I believe the central plexus of *Antedon* to be connected. In Ludwig's otherwise excellent figure of a section through the peristome of *Antedon rosacea* (19, Taf. xvi, fig. 39) these tubes are represented as much less complex in their character than they are in some parts (at any rate) of the peristome. Both in *Ant. rosacea* and in *Ant. Eschrichtii* I find a great part of the lip to be occupied by a large spongy mass, which consists of a dense plexus of closely interlaced tubes with an epithelial lining. Some of them are connected with the oral blood-vascular ring, and others, I believe, with the vessels of the central plexus. In *Actinometra* this labial plexus is much larger behind the mouth (*i. e.* between it and the anus) than in front of it (fig. 14, *l.p.*). It is especially developed towards the left or eastern angle of the mouth,[1] where it gives off a large bundle of vessels. These pass downwards and backwards, slightly above the gullet (figs. 14, 15, *ae. b.*), to near the centre of the visceral mass, where they communicate with the lower portion of the intervisceral network of vessels that is spread out, as in *Antedon* (19), over the coils of the intestine. This œsophageal bundle is seen in longitudinal section in fig. 14 (*ae. b.*), and cut transversely in fig. 15, which represents a cross-section through the disc of *Actinometra polymorpha,* seen from its anterior face, so that the structures which are (W) in the figure are (E) in the disc, and *vice versâ.*

The labial plexus also gives origin to the genital vessels which extend outwards beneath the ambulacra of the disc (figs. 14, 15, *g. v.*); and from the smaller portion of it that lies in front of and below the mouth there passes backwards, slightly below and on the right (W) of the gullet, a more compact bundle of vessels, which enlarges slightly beneath the centre of the visceral mass, bends directly downwards, and enters the calyx (figs. 14, 15, *c. p.*). This corresponds to the central plexus of *Antedon,* and, like it (19), is connected laterally with a close network of intervisceral blood-vessels at the lower part of the visceral mass, that also receives the vessels of the œsophageal bundle, as shown in fig. 14. Vessels extend backwards from this network, and also to the right and left, so as to completely surround the complicated windings of the alimentary canal. Some of them are quite large, and frequently contain coagulum. They interpenetrate the rather close network of connective tissue that fills up all the unoccupied portions of the body-cavity (figs. 14, 15, *c.*).

Up to this point we have found the Crinoids to conform, on the whole, to the type of the Starfishes and Ophiurids. There is, it is true, no real aboral ring, unless the labial plexus with which the genital vessels are connected may be considered as generally homologous with the aboral ring that connects the genital vessels of Starfishes and Ophiurids; but no detailed comparison of the two is at all possible. The Crinoids are also unprovided with any definite perihæmal system. For even supposing that Ludwig is correct in regarding the genital canal as the perihæmal canal of the genital vessels (27), there is absolutely no trace of any perihæmal canal around the radial blood-vessel. Neither is it easy to define any special portions of the body-cavity as

---

[1] The mouth is supposed to be N, or pointing forwards away from the observer.

perihæmal canals for the oral ring and the central plexus, though Ludwig speaks generally of the circumvisceral cœlom as representing the perihæmal canals of the Asterids (21).

We have seen that the central plexus which unites the oral and aboral rings of the Starfishes does not terminate in the latter, but passes on towards the dorsal portion of the disc, attaching itself to the under surface of the perisome, just outside the madreporite. In the Crinoids, however, the case is different. Towards the lower portion of the visceral mass the vessels of the central bundle group themselves into an inner set, surrounded by a ring of five vessels, which are radially disposed. After passing down the central funnel between the first radials (figs. 14, 15 *R.*$_1$), and traversing the median opening of the rosette (*r.*), these five vessels expand into five large chambers (*ch.*), which are regularly arranged around the central vascular axis. The structure thus formed, which is known as the "chambered organ," is lodged within the cavity of the centro-dorsal piece (*cd.*) of *Comatula*, and is covered in by the rosette (*r*); but in the stalked Crinoids it is situated in some portion of the space enclosed within the basals and radials (7, 8).

This chambered organ is enclosed in a fibrillar envelope (N), processes of which extend through all the joints of the rays and arms (figs. 9, 11—15, *a.*) and also into the cirrhi, that are borne on the centro-dorsal piece of *Comatula* (*ci*). Each of these fibrillar cirrhus cords encloses a small vessel (*ci. v.*). Those of the oldest or first-formed cirrhi are derived from the vessels of the central axis of the chambered organ, while the vessels of the last-formed marginal cirrhi arise directly from the five peripheral chambers. These chambers are not completely closed below, each having a small opening in its floor close to the central axis. This opening is a relic of the larval stage of existence, when the five chambers and the vessels of the central axis were prolonged downwards into the stem. In the stalked Crinoids this condition is permanent through life. The stem of *Pentacrinus, Rhizocrinus,* or *Bathycrinus* contains a central vascular axis enclosed in a downward extension of the fibrillar envelope of the chambered organ (7, 8, 20). Like the chambered organ this axis consists of five peripheral vessels surrounding a core of smaller ones, and from these peripheral vessels are derived the central vessels within the cirrhi borne by the stem. In *Pentacrinus* these cirrhi are borne by special nodal segments which occur at more or less regular intervals all down the stem. The five large peripheral vessels expand slightly in each nodal joint, and each gives off one cirrhus vessel; hence, every nodal joint contains, as it were, a small edition of the chambered organ situated in the calyx, which was considered as a "heart" by Müller, and is apparently still so regarded by Greeff (13, 14). According to this view, therefore, there must be a "heart" at every node of the stem of *Pentacrinus*, the length of which is sometimes very considerable. In the fossil *P. briareus*, for example, with its 50—70 feet of stem, the number of these "hearts" must have been rather large.

The axial cords enclosing the cirrhus vessels of *Comatula* (figs. 14, 15, *ci. v.*) are given off from the lower or dorsal portion of the fibrillar envelope of the chambered organ. From each of its ventral interradial angles a large cord passes upwards and outwards (figs. 13, 14), and forks almost immediately. The right branch of one fork, and the left branch of its neighbour, enter two adjacent openings on the inner face of each first radial. They run side by side through its central canal and on into the third radial, where each of them forks (fig. 13). The two right branches enter the central canal of the skeleton of the right arm, while the left branches enter that of the left arm, to form their respective axial cords. Before leaving the third radial, however, these two cords are united by a transverse commissure. There are also commissures in the first radials. The two cords which each contains are united with one another and with those of adjacent radials by one continuous circular commissure (figs. 14, 15, *c. co.*), lodged like the other cords in special canals.

It is very difficult to determine whether these cords enclose vessels as the axial cords of the cirrhi do. Greeff (14) has attempted injection but without satisfactory results. The appearances sometimes presented by transverse sections seem to indicate that each cord consists of two lateral fibrillar masses enclosing a central structure, which may possibly be a vessel, though I have never been able to satisfy myself that it has a lumen. This may, however, be the result of a post-mortem contraction, while both Ludwig and myself have met with coagulum, not only in the plexiform tissue forming the organic basis of the skeleton, but also in the substance of the axial cords themselves. This rather tends to support Ludwig's idea that whether the cords contain distinct vessels or not, they do serve as the medium by which a nutritive fluid is able to enter the substance of the skeleton.

Histologically, these cords have a remarkable resemblance to the ambulacral nerves of the Crinoids and of the other Echinoderms, consisting as they do of longitudinal fibres and minute intercalated cells. This resemblance is so considerable that Baudelot (1) was only prevented from describing these axial cords as the nerves of the Crinoids (as Dr. Carpenter had previously done (2) though unknown to him) by the fact of their occupying a dorsal and not a ventral position. Experiment shows that all the movements of the arms are dependent upon the integrity of their axial cords, and upon the connection of these cords with the central fibrillar envelope of the chambered organ, but that they are entirely unaffected by section of the ambulacral nerve (2, 3, 4). Removal of the chambered organ immediately stops the swimming movements of all the arms; they become rigidly straightened out by the action of their dorsal elastic ligaments, which the muscles are powerless to antagonise. But irritation of the chambered organ by a needle passed down the central funnel of the calyx of an eviscerated specimen causes all the arms to be suddenly and simultaneously closed over the calyx.

On the other hand, this last experiment shows that the movements of the skeleton are entirely independent of the oral nervous ring. This is contained in the visceral mass which is very readily removed from the calyx, but the swimming movements are altogether unaffected by this operation. They are performed as perfectly by the eviscerated calyx with its attached arms as by the entire and uninjured animal.

Further, in many *Actinometræ,* more or fewer, sometimes more than half the arms are unprovided with an ambulacral nerve (6, 8, 9). Some of the ambulacral grooves radiating outwards from the peristome become slowly obliterated by the gradual, or occasionally sudden, approximation and union of their sides. Their ciliated epithelium with the subjacent nerve and blood-vessel become completely lost, and at the same time the water-vessel becomes very much reduced in size, and ceases to be connected with any lateral tentacular apparatus (Pl. XII, fig. 12, *w.*). Nevertheless, these ungrooved and nerveless arms take the same part in the swimming movements as those in which the ambulacra are entirely normal. They are usually those which are borne on the radii behind the mouth; but in some *Actinometræ,* with two hundred or more arms, there are ungrooved arms upon each radius (9).

The ambulacral nervous system, therefore, has no relation whatever to these movements. This is not to be wondered at, as, unlike its homologue in the Ophiurids, it has no connection whatever with the muscles. It may possibly have some influence on the creeping movements which the isolated visceral mass has been observed to perform, but the movements of the organism as a whole are independent of it.

These movements are effected by the contractions of pairs of muscular bundles (Pl. XII, figs. 14, 15, *m.*) which connect the successive joints of the arms, and sometimes also of the pinnules. Some tropical *Comatulæ* have from 50 to 200 arms, each consisting of from 150 to 200 joints, and bearing a corresponding number of pinnules, the joints of which are also united by pairs of muscular bundles. Nevertheless, all this immense muscular system, the action of which is so perfectly co-ordinated when the animal swims, is generally supposed to be uncontrolled by any nervous apparatus. Fifteen years ago, however, long before the discovery of the ambulacral nerves, Dr. Carpenter suggested that the fibrillar envelope of the chambered organ and the axial cords of the rays and arms connected with it, constituted a nervous system governing the movements of the animal (2, 3, 4). He was led to this view by the discovery that the axial cords give off pairs of branches which ramify upon the ends of the muscular bundles (fig. 14, *a. m.*). But in spite of the strong anatomical and physiological evidence in its favour, his suggestion has been by no means generally accepted. This is probably due to the morphological difficulties which it involves. Ludwig discusses it (19, 20) but declines to accept it, as he finds a difficulty in admitting that the Crinoids have an antiambulacral nervous system, of which we know as yet no homologue in the other Echinoderms. Nevertheless, he does not hesitate to regard the chambered organ and its connections as parts of a blood-vascular system, to which there is nothing comparable in the other Echinoderms. In the two leading German text-books[1] the nervous system of the Crinoids is described as essentially similar to that of the Asterids, and not a word is said as to the physiological importance of the axial cords, though in any other case the experimental evidence above mentioned would be at once admitted as a proof of their nervous nature. Both Claus and Gegenbaur, however, ignore it entirely.

The branches of the axial cords which Dr. Carpenter traced on the ends of the muscular bundles are merely portions of a large system of branches connected with these

---

[1] *e.g.* Claus's 'Grundzüge der Zoologie' and Gegenbaur's 'Grundriss der Vergleichenden Anatomie.'

cords (5, 6). In the centre of each joint of the ray, arm, or pinnule, the cord enlarges slightly and gives off four branches, or occasionally more (Pl. XII, figs. 11, 12, *a'*). One pair runs towards the dorsal surface of the arm and breaks up into successive subdivisions, which can be traced no farther. The other pair extends towards the ventral side, branching freely on its way. Some of its subdivisions spread out upon the ends of the muscular bundles, as described by Dr. Carpenter. This is shown in one of the first radials of the section represented in fig. 14. In the arms and pinnules other branches proceed further towards the ventral side, and may be traced into the small marginal leaflets bordering the ambulacral groove. In figures 11 and 12 on Pl. XII, I have embodied the results of the study of series of sections through grooved and ungrooved pinnules of *Actinometra polymorpha*. In this species the perisome contains a number of more or less regularly arranged spaces in the connective tissue, and the branches of the axial cords run in the partitions between them. They have no regular mode of subdivision, no two pinnules being exactly alike; while they are not symmetrical on the two sides of the same pinnule. Mindful of Hubrecht's discoveries respecting the nervous system of the Nemertines, I have endeavoured to determine whether the smaller branches unite outside these spaces so as to form a continuous layer, but I have utterly failed in following them to any distance, and am quite unable to say how they end. Fresher specimens, which have not been eight years in spirit, might possibly yield more favorable results, but none of those which I have been able to examine are at all suited for very minute histological work.

The branches of the axial cords mentioned above seem to have escaped the notice of the German observers, who either make no mention of them at all or intimate that they have failed to find them. They are, it is true, better developed in some of the large tropical *Comatulæ* than in the European species; but it is not very difficult to obtain a section of an arm or pinnule of *Antedon rosacea* or *A. celtica*, in which one of the ventral branches may be followed all the way from the axial cord to the end of one of the marginal leaflets, where it becomes lost. The existence of these widely-spreading branches requires a more satisfactory explanation of the character of the axial cords than that given by Ludwig (19, 20). He regards them as permanently uncalcified remnants of the connective-tissue basis of the skeleton, and supposes them to effect the transmission of nutritive fluid from the chambered organ into the arms and pinnules. I quite believe that this is part of their function, but if it be the whole, why do they give off such regular branches, the terminations of which are altogether outside the skeleton? Ludwig does not attempt to explain this difficulty.

It is true that no connection has been actually traced between the fibrils of the branches of these axial cords and the individual muscular fibres. But how rarely has this connection been seen in any Invertebrate. No one has yet demonstrated a direct connection between the muscle-fibres of the Ophiurids and the nerves proceeding to them; while there is no more evidence for the nervous character of the ambulacral nerves of the Asterids than for that of the axial cords of the Crinoids. In fact, in one respect, there is less, for the ambulacral nerves of the Starfishes, like those of the Crinoids, give off no muscle branches whatever. But it must be remembered that the movements of a Starfish are far more sluggish than those of a Crinoid, and the number of muscles called into play to effect the movements very considerably less; while the influence of the axial cords upon the muscular contractions of the Crinoids is undoubted.

The chief argument against the nervous nature of these cords is the morphological difficulty inseparable from this view of them. Another objection to it is the presence of the fibrillar sheath around the vessels of the cirrhi, and in the stalked Crinoids around the central vascular axis of the stem (19, 20), in which there are no muscular bundles. Dr. Carpenter's position, however, despite this assault upon it, seems to me a stronger one than that held by those who do not question the experimental evidence detailed above, but decline to accept the conclusions which, in any other case, would naturally be deduced from it.

The morphological difficulty accompanying Dr. Carpenter's view is another question altogether. It is not so very much greater than that involved by the existence of the chambered organ and its connections at the dorsal end of the central plexus of the blood-vascular system. The representative of this organ in the other Echinoderms ends quite simply without even the most imperfect indication of any such structures in connection with it. Both structurally and as regards its dual function the whole of this apparatus is without a parallel in the other Echinoderms, and there is no place for it in the "archetype" of the group as at present conceived, which is simply that of the Urchins and Starfishes.

Recent work on the Crinoids has shown that in some respects, *e.g.* the possession of water-vascular, blood-vascular, and ambulacral nervous systems, they agree with the other Echinoderms in a manner undreamt of ten years ago, when their position in the group was a very uncertain one. But other equally well established results are ignored or denied, because they do not accord with preconceived notions of what Crinoid structure ought to be in order to agree with the type which has been deduced from the study of Urchins and Starfishes without the Crinoids ever being considered at all. Leuckart's separation of the stalked Echinoderms from the other members of the subkingdom under the name *Pelmatozoa,* might, with advantage, be more generally adopted than it is.

Literature.

1.—*Baudelot, E.*, "Études générales sur le système nerveux. Contribution à l'histoire du système nerveux des Échinodermes," 'Arch. de Zool. expérim. et générale,' tome i, pp. 177—216.

2.—*Carpenter, W. B.*, "Researches on the Structure, Physiology, and Development of *Antedon rosaceus,*" part i, 'Phil. Trans.,' vol. 15 6 pp. 671—756, plates xxxi—xliii.

3.—*Carpenter, W. B.*, "On the Structure, Physiology, and Development of *Antedon rosaceus,*" 'Proc. Roy. Soc.,' No. 166, 1876, pp. 211—, 231, plates 8 and 9.

4.—*Carpenter, W. B.*, "Supplemental Note to the above Paper," 'Proc. Roy. Soc.,' No. 169, 1876.

5.—*Carpenter, P. Herbert*, "Remarks on the Anatomy of the Arms of the Crinoids," part i, 'Journ. of Anat. and Physiol.,' vol. x, April, 1876, pp. 571—585.

6.—*Carpenter, P. Herbert*, the same, part ii, 'Journ. of Anat. and Physiol.,' vol. xi, October, 1876, pp. 87—95.

7.—*Carpenter, P. Herbert*, "On some Points in the Anatomy of *Pentacrinus* and *Rhizocrinus,*" 'Journ. of Anat. and Physiol.,' vol xii, October, 1877, pp. 35—53.

8.—*Carpenter, P. Herbert*, "On the Genus *Actinometra,* Müll., with a Morphological Account of a New Species, *Act. polymorpha,* from the Philippine Islands," 'Trans. Linn. Soc.,' Sec. Series, 'Zoology,' vol. ii, pp. 1—122, plates i—viii.

9.—*Carpenter, P. Herbert*, "Preliminary Report upon the *Comatulæ* of the 'Challenger' Expedition," 'Proc. Roy. Soc.,' No. 194, 1879, pp. 383—395.

10.—*Greeff, R.*, "Ueber den Bau der Echinodermen," 1. Mittheilung. 'Sitzungsb. d. Gesellsch. z. Beförd. der gesam. Naturwiss. zu Marburg,' No. 8, 1871.

11.—*Greeff, R.*, the same, 2. Mittheilung. 'Marburg Sitzungsberichte," No. 6, 1872, pp. 93—101.

12.—*Greeff, R.*, the same, 3. Mittheilung. 'Marburg Sitzungsberichte,' No. 11, 1872, pp. 155—169.

13.—*Greeff, R.*, the same, 4. Mittheilung. "Ueber den Bau der Crinoideen,' 'Marburg Sitzungsberichte.' No. 1, 1876, pp. 16—29.

14.—*Greeff, R.*, the same, 5. Mittheilung. "Ueber das Herz der Crinoideen," 'Marburg Sitzungsberichte,' No. 5, 1876, pp. 88—95.

15.—*Hoffman, C. K.*, "Zur Anatomie der Asteriden," 'Niederländisches Archiv. für Zoologie,' Band ii, 1873, pp. 1—32, Taf. i, ii.

16.—*Jourdain, S.*, “Recherches sur l’appareil circulatoire de l’Étoile de mer commune (Asteracanthion rubens),” ‘Comptes rendus,’ tome lxv, 1867, pp. 1002—1004.

17.—*Lange, W.*, “Beitrag zur Anatomie und Histiologie der Asterien und Ophiuren,” ‘Morphol. Jahrb.,’ Band ii, 1876, pp. 241—286, Taf. xv—xviii.

18.—*Lange, W.*, “Bemerkungen zum Beitrag,” &c., ‘Morphol. Jahrb.,’ Band iii, 1877, pp. 449—452.

19.—*Ludwig, Hubert*, “Beiträge zur Anatomie der Crinoideen,” ‘Zeitschr. f. wiss. Zool.,’ Band xxviii, pp. 255—355, Taf. xii—xix.

20.—*Ludwig, Hubert*, “Zur Anatomie des *Rhizocrinus Lofotensis*, M. Sars.,” ‘Zeitschr. f. wiss. Zool.,’ Band xxix, pp. 47—76, Taf. v, vi.

21.—*Ludwig, Hubert*, “Beiträge zur Anatomie der Asteriden,” ‘Zeitschr. f. wiss. Zool.,’ Band xxx, pp. 99—162, Taf. v—viii.

22.—*Ludwig, Hubert*, “Trichaster elegans,” ‘Zeitschr. f. wiss.Zool.,’ Band xxxi, pp. 59—67, Taf. v.

23.—*Ludwig, Hubert*, “Zur Kenntniss der Gattung Brisinga,” ‘Zeitschr. f. wiss. Zool.,’ Band xxxi, pp. 216—234, Taf. xv.

24.—*Ludwig, Hubert*, “Beiträge zur Anatomie der Ophiuren,” ‘Zeitschr. f. wiss. Zool.,’ Band xxxi, pp. 346—394, Taf. xxiv—xxvii.

25.—*Ludwig, Hubert*, “Ueber die Genitalorgane der Asterina gibbosa,” ‘Zeitschr. f. wiss. Zool.,’ Band xxxi, pp. 395—400, Taf. xxviii.

26.—*Ludwig, Hubert*, “Ueber den primären Steinkanal der Crinoideen nebst vergleichend anatomischen Bemerkungen über die Echinodermen überhaupt,” ‘Zeitschr. f. wiss. Zool.,’ Band xxxiv, pp. 310—323, Taf. xii and xiii.

27.—*Ludwig, Hubert*, “Neue Beiträge zur Anatomie der Ophiuren,” ‘Zeitschr. f. wiss. Zool.,’ Band xxxi, pp. 333—365, Taf. xiv—xvi.

28.—*Semper, C.*, “Kurze anatomische Bemerkungen über Comatula,” ‘Arbeiten aus dem Zoolog-zootom. Institut zu Würzburg,’ Band i, 1874, pp. 259—263, translated, with an *addendum*, by Dr. Carpenter, in the ‘Ann. and Mag. Nat. Hist.,’ September, 1875.

29.—*Simroth, H.*, “Anatomie und Schizogonie der *Ophiactis virens*, Sars,” Theil i, ‘Zeitschr. f. wiss. Zool.,’ Band xxvii, pp. 417—485, and pp. 555—560, Taf. xxxi—xxxv.

30.—*Simroth, H.*, the same, Theil ii, ‘Zeitschr. f. wiss. Zool.,’ Band xxviii, pp. 419—526, Taf. xxii—xxv.

31.—*Teuscher, R.*, “Beiträge zur Anatomie der Echinodermen, i, *Comatula Mediterranea*,” ‘Jenaische Zeitschr. f. Naturwissensch,’ Band x, pp. 243—262, Taf. vii.

32.—*Teuscher, R.*, “Beiträge, &c., ii, Ophiuridæ,” ‘Jenaische Zeitschr.,’ Band x, pp. 263—280, Taf. viii.

33.—*Teuscher, R.*, “Beiträge, &c., iii, Asteridæ,” ‘Jenaische Zeitschr.,’ Band x, pp. 493—514, Taf. xviii and xix.

On YOUNG STAGES *of* LIMNOCODIUM *and* GERYONIA. By E. RAY LANKESTER, M.A., F.R.S., Jodrell Professor of Zoology in University College, London. With Plate XIII.

METSCHNIKOFF, in a paper in the 'Zeitsch. wiss. Zoologie,' 1874, p. 17, describes and figures the development of Geryonia from the egg up to the formation of tentacles and an umbrella cavity. It is, however, obvious from his figures and description that certain steps in the development were not followed by him with precision—and some of his observations are supplemented, whilst others are contradicted, by those of Fol ('Jen. Zeitsch.,' vol. vii). It appears to be certain, from the observations of both Metschnikoff and Fol, that in Geryonia the endoderm forms by delamination[1]—the embryo being, when it has so formed, a nearly spherical "diblastula" without orifice of any description (Metschnikoff's plate ii, fig. 7). The outer layer now grows away from the inner, which remains in the form of a lenticular sac, closely applied to one pole of the ectodermal sphere. From this point forwards the accounts of Haeckel ('Jenaische Zeitschrift,' vol. ii), of Metschnikoff, and of Fol, are diverse. A mouth is formed by a breaking through into the endodermal sac, and a sub-umbrellar cavity is formed, and also a velum and tentacles; but the accounts given of the mode of origin of these parts is not conclusive in any one of the authors' memoirs above cited. Haeckel observed Geryonia embryos, in which he definitely states that the sub-umbrellar cavity existed in the form of a closed sac. Metschnikoff thinks this an erroneous observation, and due to a mistaking of the endoderm sac for the sub-umbrellar cavity. According to Metschnikoff, the ectoderm and endoderm are ruptured at the central point where the enclosed endoderm sac is resting on the inner wall of the larger ectodermal sac, and the ectoderm is *invaginated* to form the sub-umbrellar cavity. At the same time a ring grows up at some distance from the mouth thus formed, which becomes the margin of the umbrella, and gives off, on its adoral side, the velum; on its aboral side, the first tentacles.

Fol's account of the formation of the sub-umbrellar cavity substantially differs from that of Metschnikoff in that he does not derive the sub-umbrellar cavity from an invagination,

---

[1] It is not possible to reject this well-established fact, as the brothers Hertwig do, apparently without hesitation ('Cœlomtheorie,' 1881).

but states that its walls form as an upgrowth around the oral area, after the mouth has been formed by a rupture of the ectoderm and endoderm at the centre of that area.

The observations which I have made upon young stages of the freshwater Trachomedusa, *Limnocodium Sowerbii*, lead me to think it probable that, after all, Haeckel's *observations* are correct, and that the sub-umbrellar cavity is formed as a closed space between two layers of the ectoderm. It would not by any means be necessary to accept the interpretation of appearances given by Haeckel to the effect, viz. that the endodermal sac is the sub-umbrellar cavity, but we have to suppose a step in development intermediate between his fig. 28 and 29 (of Taf. iv, 'Jenaische Zeitschrift,' vol. ii). In his fig. 28 the internal sac which is drawn *is* the archenteron or endodermal sac. In figs. 29 and 30 an ectodermal sac (formed, I would suggest, by a hollowing out of the ectoderm, probably without any opening to the exterior) has taken the place of the primitive endodermal sac, which has become flattened and otherwise modified to form the stomach and gastrovascular canal system (see Fol's fig. 17, plate xxv, 'Jen. Zeits.,' vol. vii). The probability of the correctness of Haeckel's observations and inference as to the *first appearance of the sub-umbrellar cavity as a closed sac*, is not only supported by my observations on Limnocodium, in which this certainly is its condition at one period of its development, but when we examine carefully the accounts given by both Metschnikoff and Fol, we find that it is precisely at the critical period which would enable them to deal decisively with Haeckel's observations that their series of embryonic Geryoniæ in both cases is deficient. Neither Metschnikoff nor Fol have seen stages corresponding to Haeckel's figs. 29 and 30, *with only four tentacles.* They both give series in which there is a sudden break at what is the critical period for this matter; they pass at once from the condition in which no tentacles are present to that in which six are already manifest. (Fol's figure 18 of an embryo 82 hours old, with no tentacles, is succeeded by figure 19 of an embryo, 156 hours old, with six tentacles; and Metschnikoff's figure 10, which represents an embryo about 50 hours old, having no tentacles, is followed by figure 11, representing, as he states, an embryo 190 hours old, and possessing six tentacles.)

It seems to me obvious from these facts that one of the most important stages in the development of Geryonia is still almost entirely unknown, and, accordingly, the stage in the development of Limnocodium which I am about to describe, corresponding as it does in a measure to the missing stage in the Geryonia series, must not be judged by reference to a scheme of Trachomedusan development supposed to be already ascertained—for such a scheme does not really exist upon any proper basis. Rather, it appears the Limnocodium embryo may throw light on the imperfectly-known Geryonia development, and give credibility to the important observations of Haeckel, which have been too lightly dismissed by Fol and Metschnikoff.

I was only able to observe Limnocodium embryos of three different ages, and those apparently very close to one another. I have already figured two of those embryos (this Journal, 1880), and reproduce the woodcuts on the present occasion. The third embryo is figured in Plate XIII.

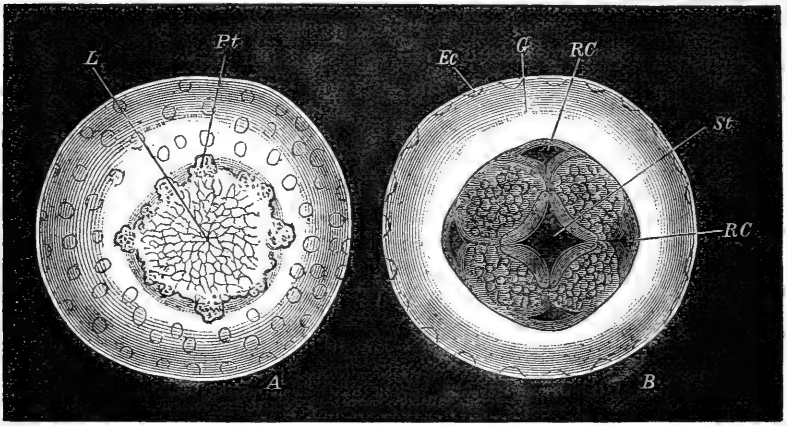


FIG. 1.—Embryo of *Limnocodium Sowerbii*, $\frac{1}{30}$th of an inch in diameter. *A*. Surface view of oral pole. *B*. Optical section of same specimen in a plane at right angles to the oro-apical axis. *Pt*. Per-radial tentacle. *L*. Præumbral lid. *RC*. Radial canal. *St*. Stomach. *Ec*. Ectoderm. *G*. Jelly of the disc.

The youngest stage observed by me is shown in the woodcut fig. 1. At the tentacular pole is seen (A, L) a circular plate of small cells absolutely imperforate and closing in the "sub-umbral cavity," or "sub-umbrellar cavity"—as is shown by the optical section of the next stage, which is but a little more advanced (fig. 2 B). This plate I call "the præumbral lid." It is surrounded by the rudiments of eight tentacles—four of which (the per-radial tentacles) are somewhat larger than the other four. In neither of these specimens are there any indications of the marginal bodies (tentaculocysts) nor of a velum. Within the sub-umbral cavity—which is capacious—we find the manubrium provided at its extremity with a mouth, which thus opens into the closed space of the sub-umbrella. The cavity of the manubrium (stomach) can be readily traced by optical sections and followed to the four radial canals which run along the adumbral wall of the umbrella—that which it is proper to call "umbrella" being the lateral wall of the sub-umbral cavity.

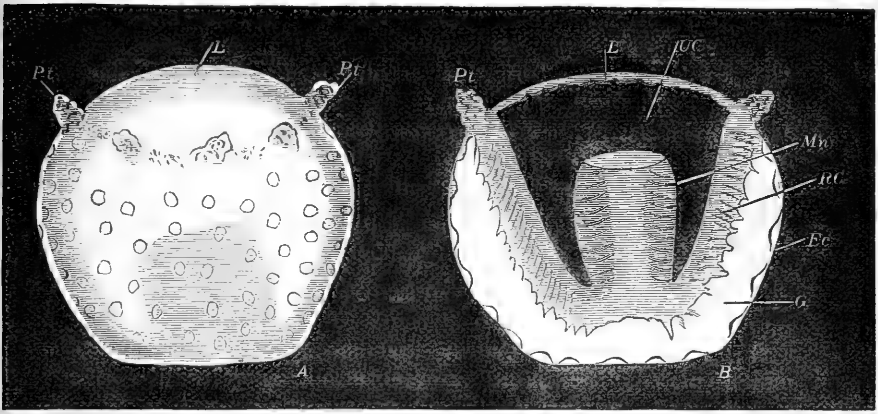


Fig. 2.—Embryo of *Limnocodium Sowerbii*, a very little more advanced, *A.* Surface lateral view. *B.* Optical section of the same specimen in a plane including the oro-apical axis. *Pt.* Per-radial tentacles. *L.* Præumbral lid. *UC.* Sub-umbrella cavity. *Mn.* Manubrium. *RC.* Radial canal. *Ec.* Ectoderm. *G.* Jelly-like substance of the disc.

There is no indication at this stage of the sub-umbral cavity having been formed by an invagination, the orifice of which has closed up; and although it, or its representative, *may* thus form by invagination and become closed up in the gonophores and modified Medusæ of some Hydromedusæ, I see no reason to doubt that it has formed in Limnocodium, as it does in Hydractinia, namely, by a splitting in the thickened ectoderm (Ed. Van Beneden). The comparison of the development of the directly developing Medusæ with that of the variously modified Medusæ of hydroid colonies will, I cannot doubt, furnish an explanation of the phenomena which are observed in both series.

If we now pass to the later stage which is represented in Pl. XIII, we find that although the young animal is but very little larger in size, and has still only eight tentacles, yet certain changes of importance have occurred.

In the first place, as seen in Pl. XIII, fig. 1, the præumbral lid is now perforate. A minute opening has appeared at its centre (*b*).

The præumbral lid does not, however, as might be expected, proceed to develop into the velum.[1] On the contrary, the velum is *already* present as a distinct, highly muscular fold, rising from the inner border of the ring which carries the eight tentacles (Pl. XIII, fig. 1 *a*). Its movements are very active and constant, consisting in an alternation of undulations of contraction and expansion—the latter movement causing it to completely close in and hide the præumbral lid, whilst its free margins come into contact centrally, as seen in Pl. XIII, fig. 4 and fig. 5. In Pl. XIII, fig. 3, the tentacular pole of the young Limnocodium is shown with the velum completely expanded and its free margin brought into such close contact at the centre of the tentacular area that its existence is not at first suspected. Suddenly, whilst this condition is under observation, the velum is seen to roll back centrifugally and to expose the præumbral lid, as shown in Pl. XIII, fig. 1.

It is necessary to state that there is no confusion here of the true oral surface of the manubrium with the præumbral lid. By causing the specimen to roll over, or by deeper focussing, the manubrium, with its mouth, can be brought into view lying at some distance below the perforated præumbral lid within the sub-umbral cavity—just as it was seen in the earlier stage (woodcut, fig. 2 B).

Besides the velum and the perforation in the præumbral lid, the tentaculocysts (marginal bodies) have now commenced to develop. Two are present—one a little more advanced in development than the other—and are seen in Pl. XIII, figs. 1 and 2, *ot.*, and in fig. 6. An endodermal axis and an ectodermal cortex are present, but the secondary investing capsule, or velar canal, is not yet indicated.

Besides the movements of the velum there are very active and sudden movements of the whole umbrella, and also slower movements of contraction and expansion, which give to the embryo the irregular outline depicted in the plate. At the stage now under description, striated muscular fibres can be detected in the wall of the umbrella (Pl. XIII, fig. 2 *e e*). The radial canals are large and give evidence of ciliation of their lining cells.

---

[1] The distinction between præumbral lid and velum—which develops later than the lid itself and from its periphery—is borne out by Metschnikoff's observations on Geryonia.

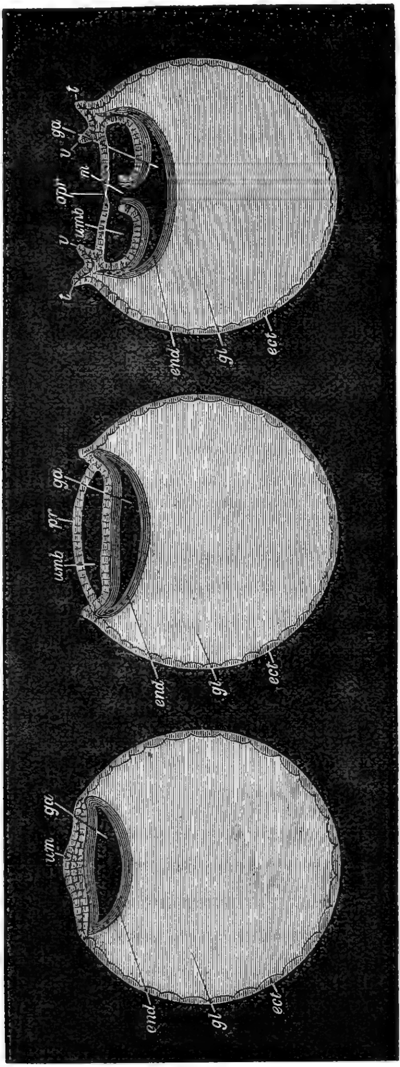
Scheme of development of Geryonia and Limnocodium.—Such being the facts with regard to a limited period of the developmental history of Limnocodium, they can, I think, best be brought into harmony with the observations of Haeckel, Metschnikoff, and Fol on Geryonia by introducing between the earlier and later stages described by the two latter observers a hypothetical stage, as exhibited in the woodcuts, figs. 3, 4, 5. Fig. 3 is simply a schematic representation of the stage (three days and a half) drawn by Fol in his plate xxv, fig. 18, and shows the thickened ectoderm, which he calls "the oral plate," but which I shall call "the umbrella plate." Fig. 5 is a schematic section of an eight or ten days' embryo based upon Metschnikoff's fig. 14 (pl. ii, 'Zeitsch. wiss. Zool.,' vol. xxiv). Metschnikoff believes that the sub-umbral cavity is formed by an invagination, which at the same time gives rise to the oral aperture. His drawings, which are by no means decisive, appear to admit of the interpretation of a formation of this cavity by the splitting of the umbrella plate: especially I would refer to his fig. 11, where the enteric sac and the sub-umbral space are seen as two lenticular bodies closely applied to one another. In any case I have no doubt that Metschnikoff's figures are far more nearly representative of the process which goes on in the formation of the sub-umbral space in Geryonia than are Fol's; and the account of the process which he has based upon his observations, though, as I think, erroneous (owing to the absence of observations on embryos between the 50th and the 190th hour), are yet by no means so wide of the truth as those of Fol, who has completely failed to give even an approximately correct account of the matter.

We may now interpose between the stages represented by figs. 3 and 5 a hypothetical representation of the stage not observed by Metschnikoff or Fol, basing our suggestion upon the fact observed with regard to the young Limnocodium, namely—that the sub-umbral cavity is large and well developed at a stage when it has no opening to the exterior, but is completely closed in by an imperforate præumbral lid. Such a stage is represented in the woodcut, fig. 4. There is little room for doubting that the series 3, 4, 5 is true for Limnocodium, and that 3 and 5 are true for Geryonia.

It will not be a difficult task to decide by the special examination of Geryonia embryos whether the aperture in the præumbral lid seen in stage 5 forms *after* the sub-umbral cavity and the oral perforation of the manubrium are complete, as it does in Limnocodium, or whether, as

Metschnikoff supposed (but did not demonstrate), the aperture in the præumbral lid is an orifice of invagination, by which the sub-umbral cavity and the perforation of the manubrium have been brought about. In the latter case an important but readily intelligible difference between Limnocodium and Geryonia would be established.

Fig. 3. Fig. 4. Fig. 5.



Diagrams representing three stages in the development of one of the Trachomedusæ.

end. Endoderm. *ect.* Ectoderm. *gl.* Gelatinous substance of the disc. *um.* Umbrella-plate. *umb.* Subumbral cavity. *ga.* Gastric cavity. *pr.* Præumbral lid. *opr.* Commencing rupture of the præumbral lid. *m.* Mouth. *t.* Tentacle. *v.* Velum.

Relation to the morphology of Ctenophora and of Medusoid Gonophors.—I have already alluded to the reciprocal illustration afforded by the development of the sub-umbral cavity in such gonophors as those of Hydractinia, and in medusæ developing directly from the egg.

The exceedingly important and (as I think) convincing reference of the Ctenophora to the Hydromedusa type, made by Haeckel ('Sitzungsber. der Jenaichen Gesells.,' 1879, p. 70), is in no small degree rendered acceptable by the existence of directly-developing Medusæ in which the sub-umbral space has the form of a closed chamber, or of a sac with but a narrow opening to the exterior. This sub-umbral sac appears to correspond with the so-called stomach of the Ctenophora, and its opening with the so-called mouth of those forms. In both cases it is lined by ectoderm, and develops quite independently of the endodermal cells, which give rise to the stomach and canals of the Hydromedusæ on the one hand, and to the infundibulum and canals of the Ctenophora on the other hand.

Balfour ('Embryology,' vol. i, p. 145) compares the ectodermally-originating "stomach" of Ctenophora to a stomodæum,[1] and more especially to the stomodæum of Acraspedote Medusæ (Scyphomedusæ) and Anthozoa.

But if this assimilation be well founded, the stomodæum of Scyphomedusæ and of Anthozoa will be morphological equivalents of the sub-umbral space (sub-umbrellar cavity) of Hydromedusæ; and the question presents itself as to whether the stomodæum of the higher groups—with its wide dimensions and contractile walls—may not be similarly identified. Against such a view there appears to be a strong argument in the very general presence in higher animals of a "proctodæum"—an anal ectodermal invagination, parallel in its origin to that connected with the mouth. It may, however, be worth while to examine the facts of morphology in reference to this suggestion.

---

[1] The name applied by me and adopted by Balfour for the ectodermal invagination which gives origin to the mouth and first portion of the alimentary tract in all Metazoa except some Cœlentera. See this Journal, October, 1876.

The ORIGIN *and* SIGNIFICANCE *of the* METAMORPHOSIS *of* ACTINOTROCHA. By EDMUND B. WILSON, Fellow in Biology, Johns Hopkins University. With Plates XIV, XV.

ABOUT thirty-five years ago Johannes Müller described a very curious free-swimming pelagic animal which he captured at the surface of the sea at Heligoland, in the North Sea, giving to it the name *Actinotrocha,* in allusion to its beautiful circlet of ciliated swimming arms. The striking appearance, and very peculiar structure of this creature subsequently attracted the attention of many observers. Wagener described its anatomy, supposing it to be an adult animal Krohn subsequently discovered, however, that *Actinotrocha* passes by a sudden metamorphosis into a Gephyrean worm; and some years later Schneider made out the true nature of this very remarkable process. The life-history was at length completed by Kowalevsky, who, by raising the eggs, proved that the adult worm is the singular Gephyrean *Phoronis,* which had long been of especial interest as forming a supposed transition from the Polyzoa to the Annelides. The matter was then carefully revised by Metschnikoff, who studied another species found in the Mediterranean Sea, and published in 1871 a valuable paper setting forth the results of his observations.

No attempts were made in these papers to offer any explanation of the origin and significance of the singular change undergone by the larva in its passage to the adult form. I was thus led, during the summer of 1879, while enjoying the facilities afforded at the Chesapeake Zoological Laboratory, to undertake a renewed study of the subject with two species of *Actinotrocha* commonly occurring in Chesapeake Bay. As a result of this study, an hypothesis has suggested itself that seems to me to afford a reasonable explanation of the steps by which the adult structure and strange metamorphosis of *Phoronis* may have been acquired. Moreover, a study of this particular case places in a very clear light some of the phenomena of metamorphosis in general, and is conducive to an understanding of the causes which have led to certain remarkable methods of development in a number of animal groups. In many cases of metamorphosis the phenomena of growth are so complex that it is extremely difficult to form any definite conception of the exact way in which they have been brought about.

In the development of *Actinotrocha*, however, we find a metamorphosis involving profound and remarkable changes of structure, and yet produced by very simple processes of growth. The problem to be solved is much simplified and comparatively definite—reduced as it were to its lowest terms. While the contrast between the structure and habits of the larva and those of the adult is so striking as to show very clearly the general causes which have brought about the metamorphosis, it is possible, I believe, to get some idea of the exact way in which these causes may have operated in producing the peculiar transformation which the creature undergoes.

In order to render these considerations intelligible, it will be necessary to give some description of the metamorphosis. The first part of this paper is therefore devoted to such a description, based upon my own observations and those of Metschnikoff. Although the account of the Russian embryologist is very complete and satisfactory, my own observations are not without some value as supplying many hitherto unobserved details of the process, and from having been made upon two distinct species, neither of which can be satisfactorily identified with that studied by Metschnikoff. Certain discrepancies will be noted further on. I succeeded, moreover, in keeping the young *Phoronis* alive during several weeks—until they had, in fact, assumed the characteristic appearance of the full-grown animal. For the sake of completeness, a few of Metschnikoff's figures of the earlier stages have been introduced (figs. 1, 4.).

PART I.

It may be convenient to preface the following description, with a short summary of the more important points. The larva has a somewhat elongated body with a series of long ciliated arms nearly encircling the body behind the mouth, and a narrow belt of long cilia just anterior to the anus, which is situated at the posterior end of the body. The mouth is on the ventral side near the anterior end, and is overarched by a very large hood-like expansion of the body wall, the præoral lobe. The hood is richly ciliated and serves to produce currents of water flowing towards the mouth, which bring with them particles of food.

The only internal organ at first is the digestive canal, consisting of a distinct œsophagus, a capacious stomach, and a longer or shorter intestine. In time, however, the ventral wall of the body thickens at a point in the middle line just behind the circlet of the arms. This thickening is apparently produced by an infolding of the body wall; at any rate, it soon becomes hollow and pouch-like, and communicates with the exterior by means of a small opening, while fibrous or muscular connections between it and the stomach walls may be observed. The pouch grows rapidly, and at length comes to occupy a large part of the perivisceral cavity, doubling back and forth, and becoming transversely folded on its inner wall.

When the pouch has attained its complete development the larva becomes sluggish, sinks to the bottom, and the body usually contracts forcibly, so that it becomes shortened and rounded, and the walls become tense from the internal pressure thus produced. Suddenly, at what Metschnikoff appropriately terms the "critical stage" of the metamorphosis, the pouch turns itself outward through its external opening, unrolling like the finger of a glove or the eye tentacle of a snail. As it unrolls, the middle part of the digestive canal is drawn out into its cavity, thus forming a long U-shaped loop. At the same time the larval body shrinks together, and is doubled up toward the dorsal side, so that the mouth and anus are brought close together. The hood is withdrawn into the œsophagus, leaving only a small remnant, which overhangs the mouth and persists as an epistome. The greater part of each swimming arm undergoes a kind of granular disintegration and drops off, leaving only a small thickened basal portion, which becomes directed forwards, and subsequently grows into the corresponding tentacle of the adult *Phoronis*. The larval body fuses completely with the everted pouch, which now constitutes the greater part of the body of the worm. The subsequent external changes consist merely in the elongation of the tentacles and a great increase in their number, and the elongation of the body. The worm secretes for itself a membranous tube, in which it dwells, protruding only the extremity with its crown of tentacles. The resemblance of the latter to the lophophore of a hippocrepian Polyzoön is wonderfully close, though this resemblance is undoubtedly adaptive and secondary. A clear idea of the metamorphosis may be gained from the diagrammatic figs. 15 to 19.

Figure 1 represents a lateral view of the first stage figured by Metschnikoff. At *h.* is the hood; *m.* is the mouth leading by a short œsophagus to the stomach (*st.*). The latter communicates by a short intestine with the anus (*a*). Rudiments of two swimming arms are seen at *a′*, *a.″* Fig. 2 gives a ventral view of the same; rudiments of the second pair of arms are seen at a'', a''. Fig. 3 is a corresponding view of a somewhat later stage with the first four arms now plainly indicated. The body is everywhere richly ciliated and the cilia are distinctly longer along the margin of the hood and at the tips of the arms. Fig. 4 represents a side view of a considerably later stage, with five pairs of well-developed, though still short, arms. A number of large black pigment spots have appeared. Neither the præ-anal belt of cilia, nor the ventral pouch, have yet been developed. These four figures are copied from Metschnikoff.

The following figures represent different stages in the development of the two Chesapeake species, which for convenience sake may be designated *A* and *B*. The two species have well-marked specific differences, but their metamorphoses are almost identical, and agree in the main with that of the form studied by Metschnikoff. *A* is characterised by a very short intestine and a correspondingly stout body, and shows a considerable resemblance to the form studied by Metschnikoff (figs. 5, 7, 11). *B* has a long slender intestine, and the posterior part of the body is correspondingly elongated. At the upper lateral portions of the stomach are two rounded glandular lobes, one on either side of the œsophagus. The hood, in the later stages at any rate, has a prominent conical ciliated elevation on the median line in front. This form is apparently identical with *Actinotrocha branchiata*, the species originally discovered by Johannes Müller.

Figure 5 represents form *A* shortly after the appearance of the pouch which is seen in optical section at *p* on the ventral side. The arms are sixteen in number; they diminish regularly in length toward the dorsal side, thus indicating the order in which they have appeared. On the extreme dorsal side the series is interrupted by a considerable interval. The arms are nearly solid, but contain narrow central channels, which are diverticula from the perivisceral cavity. There are a number of pigment spots irregularly scattered over the body. The præ-anal belt of cilia (*a. r.*) has appeared, the dorsal vessel is seen at *v.*, and an accumulation of pseud-hæmal corpuscles has appeared at *cor.* on the wall of the stomach. By flattening out the creature under a compressor the opening into the pouch becomes apparent (fig. 7).

Figure 6 represents the corresponding stage of the other species as seen from the opposite side, showing the pouch (*p.*) lying in the perivisceral cavity between the stomach and the body wall. It shows the ventral pseud-hæmal vessel with rudiments of the contractile cæca (*c.*), which are so striking a peculiarity of the adult circulatory apparatus.

Figure 8 represents a considerably later stage, in which is shown a great advance in the development of the pouch. Bending at first toward one side of the body it folds upon itself, and returns to the opposite side, then turns sharply downward along the intestine, and finally bends upward again near its extremity. Its inner walls are transversely folded. The arms have increased to the number of twenty, and are thickened on the lower side just at the base, to form the first rudiments of the permanent tentacles. There are four masses of pseud-hæmal corpuscles, two lying at each side of the stomach.

Figure 9 gives a dorsal view of the same stage, to show the arrangement of the arms on the dorsal side. The folds of the pouch have here a somewhat different arrangement, and cannot be clearly followed. The rudiments of the tentacles are shown at *p. a.*, and the broad contractile dorsal vessel at *v.*

Figure 10 represents a full-grown larva of *B* immediately before its metamorphosis. The folds of the pouch are voluminous, and occupy a large part of the perivisceral cavity; they have nearly the same disposition as in fig. 8. The tentacular rudiments are very distinct, and have become partly independent of the larval arms. Both dorsal and ventral pseud-hæmal vessels are well developed and, like the cæcal appendages, are actively contractile. The masses of corpuscles (*cor.*) are large and very conspicuous from their red colour. Form *A* agrees essentially with *B* at this stage, though the body is much stouter, and the rudiments of the tentacles are much smaller and more closely united to the larval arms.

The two species differ slightly as to the manner in which the metamorphosis is effected. In the case of *A* the larva sinks to the bottom, contracts very strongly, and the evagination of the pouch occupies but a few minutes. The process occupies in the case of *B* a much longer time, and the body is only slightly or, at first, not at all contracted.

Figure 11 represents the latter species at the *critical* stage, with the pouch about half unrolled. The inner extremity of the latter still extends to the back of the stomach. At *b*, *b*, are seen some of the fibrous connections between the intestinal walls and those of the pouch.

Figure 12 represents the creature with the pouch (the whole of which is not shown) wholly unrolled. The hood has been withdrawn into the œsophagus, the larval arms have dropped off, and the short tentacles are directed straight forwards, and form a circlet surrounding the mouth. The posterior portion of the larval body (*p.*), with the anus at its extremity (*a.*), is still distinct, and is bent at right angles to the long axis of the pouch, which must now be called the body proper. In time it becomes bent still further upwards, and, at the same time, is gradually withdrawn into and fuses with the remainder of the body. Ultimately it quite disappears, and the anus remains as an opening on the side of the body, immediately behind the circlet of tentacles.

Figure 13 represents *A* about twenty-four hours after the metamorphosis, when the larval body has completely fused with that of the adult. The body is soft and extensible, and, though it has numerous transverse folds when contracted, there is no indication of metamerism. The aboral extremity is extremely changeable in shape; two forms commonly assumed are shown. The surface is everywhere finely granular, and becomes distinctly tuberculose towards the aboral extremity.

New tentacles are henceforward constantly budded forth at the dorsal side of the lophophore, at about the rate of a new pair every day, until their number becomes a hundred or more, and they increase gradually in length.

Figure 14 represents the same individual as that shown in fig. 13 twenty-two days after metamorphosis, and practically in the adult condition. The tentacles are richly ciliated, and have exactly the same appearance and perform the same movements as in the Polyzoa. Each has, however, a cæcal vessel from the pseud-hæmal circumoral ring, and performs a distinctly respiratory function. The pseud-hæmal fluid is very remarkable from the presence of numerous large, oval, nucleated corpuscles, which look not very unlike the red corpuscles of frogs' blood, though of less regular shape. They are also red in colour, and render all the branches of the pseud-hæmal system very conspicuous, so that their arrangement may be very easily made out. In neither of the Chesapeake species is there a vessel running along the intestine towards the anus, although such a vessel is figured by Metschnikoff. Nor can I agree with this observer that the corpuscles of the pseud-hæmal fluid are developed free in the perivisceral cavity to be drawn into the pseud-hæmal vessels at the time of metamorphosis. There cannot be the least doubt that in both our species these corpuscles are developed in solid masses adhering to the stomach walls, near the base of the tentacles, and I believe them to a rise within the cavity of a sinus, which becomes the circumoral ring of the adult. They never float freely in the perivisceral cavity, and cannot for a moment be confounded with the true blood-corpuscles of this cavity. During the metamorphosis these masses suddenly break up, and the corpuscles are almost immediately carried along within the vessels by the peristaltic contractions of the latter. During the later larval stages they are sometimes elongated towards each other, and connected by a narrow band containing a few corpuscles (see fig. 10) ; and repeated observations of the metamorphosis has convinced me that, for our species at least, Metschnikoff's account is incorrect. Owing to the failure of attempts to make satisfactory sections of the larvæ I have been unable absolutely to demonstrate this point, which is of considerable importance from its bearings on the relation between the pseud-hæmal system and the body cavity.

PART II.

Before advancing speculations as to the origin and significance of this most remarkable course of development, it is necessary to dwell for a moment on the systematic relations of *Phoronis,* and on certain structural features of the group to which it belongs. It is pretty well agreed that *Phoronis* is a Gephyrean, although a greatly modified and specialised representative of this peculiar group. Although the peculiarities of its development are so great as to lead so high an authority as Mr. Balfour to question the correctness of this identification, I believe, nevertheless, that these doubts are not well-founded. In all essential anatomical characters, so far as they are known, *Phoronis* agrees closely with such forms as *Sipunculus* or *Phascolosoma.* Its most striking characters, such, for example, as the close proximity of the mouth and anus and the high development of the oral tentacles, are simply exaggerations of characters possessed by the last-named genera. Almost all of the adult characters are readily explicable as the result of extreme adaptation to a strictly tubicolous life. In regard to the development, I shall endeavour to show that its peculiarities are almost certainly due to secondary adaptations, correlated with the highly specialised structure of the adult. Many facts show that it is not worth while to attach much importance to so inconstant and variable a character as the arrangement of ciliated belts in pelagic larvæ. But if this point were of importance, their arrangement in *Actinotrocha* would not present any great difficulties in the way of regarding the creature as a true Gephyrean larva. If the circlet of swimming arms were reduced to a simple postoral belt and the præoral lobe reduced in size, the larva would agree fairly well with the larva of *Phascolosoma* or *Bonellia*. If, in support of this view, it is necessary to consider the longer cilia along the margin of the præoral lobe as representing a præoral belt comparable to that of the *Echiurus* larva, I see no valid objection to so considering them.

Accepting, then, the view that *Phoronis* is a true Gephyrean, we may note that among these animals two series of forms may be distinguished, which differ from each other in the course of the alimentary canal and the position of the anus. This division of the group is not necessarily a "natural" one; for our present purpose this point is quite immaterial. In one series, represented by such forms as *Bonellia, Thalassema, Echiurus,* the mouth and anus are at opposite extremities of the body, and the alimentary canal, aside from secondary flexures, pursues a straight course from one to the other. In the other series, including *Phascolosoma, Sipunculus, Phoronis,* and others, the anus is near the mouth on the apparently dorsal side of the body; and the alimentary canal is bent upon itself to form a long U-shaped loop, which is usually complicated by secondary flexures, as in the first series. The *Actinotrochan* larva of *Phoronis,* aside from its specially acquired swimming apparatus, corresponds pretty closely with such a form as *Thalassema,* representing the first series. And the larva may itself fairly be taken as a representative of this series. The presence of swimming arms is no obstacle to this view, for these are, clearly, provisions to increase the surface for the attachment of locomotor cilia, and can have no ancestral significance.[1]

Through its metamorphosis the larva becomes an extreme representative of the second series, the anus undergoing, apparently, a transfer from one extremity of the body to a point very near the opposite extremity, beside the mouth, and the intestine acquiring a corresponding flexure.

From these and other more general considerations, it is evident that the displacement of the anus and corresponding flexure among the Gephyrea in general are derivative characters which, in all probability, were originally acquired to

---

[1] Professor Lankester's comparison of these arms to the "branchiotroch" of Echinoderm larvæ and other forms can be valid only if taken in the most general sense, as was no doubt intended, and not as indicative of any special relationship to those forms.

adapt the animals more perfectly to a tubicolous life. We have seen how this shifting of the anus is effected in the individual development or ontogeny of the animal; and it is plain that in considering how this mothod of development has been acquired, the first question to answer is as to how this shifting was effected in past time during the phylogeny, as the ancestors of the animal passed from a free-swimming, or at least wandering, mode of life to a strictly sedentary tubicolous one.

The answers to this question are possible. The first is that the anus gradually moved dorsalwards, and then forwards to its present position behind the mouth. The second, which is the view here maintained, is that the worm upon assuming the tubicolous mode of life developed a habit of bending the body into a U-shape, bringing the posterior part of the body alongside the anterior part in order to discharge the excreta at the mouth of the tube, or for some similar reason. External signs of this flexure becoming in time obliterated by coalescence of the two limbs of the U, the body once more assumed a straight linear form, but with the anus and mouth now near the same extremity.

Obviously, there are many difficulties in the way of the supposition that the anus gradually moved forwards along the dorsal side. This process seems impossible if we assume the worm to have meanwhile retained its tubicolous habit; for only a very great displacement could be of any advantage, and it is impossible to suppose that such a displacement could occur as a natural variation of structure. To assume an intermediate mode of life during which the creature should live, for instance, in mud or sand, would not obviate this difficulty, but would do away with the need for any displacement at all. The existence of forms like *Phascolosoma,* which live in sand or mud, and yet have a greatly displaced anus, does not meet the objection; for there is some reason to believe that such forms may be considered descendants of strictly tubicolous forms which for some reason—perhaps increase of size—have abandoned their former mode of life.

On the other hand, the flexure hypothesis has considerable evidence in its favour. Among the tubicolous Annelides there are forms—*e. g. Sabellaria*—which habitually flex the body, as I suppose the ancestors of *Phoronis* to have done. Such a form is shown in fig. 20, which represents a large *Sabellaria* from the North Carolina coast. It is very noteworthy that the direction of the flexure is constant and toward the dorsal aspect of the body; so that if in time external signs of the flexure should disappear, the anus would have exactly the same position it occupies in *Phoronis*, and the intestine would have the same disposition. Admitting that such a flexure took place, it seems very probable, *a priori*, that the two parts of the body thus brought into proximity would tend in time to coalesce, and thus to destroy the appearance of flexure. In certain analogous cases such a flexure and fusion plainly seems to have occurred. A clear and convincing illustration is afforded by certain Holothurians; for here a record of the change has been preserved in the rows of ambulacral suckers. These retain their flexed course, and thus give us external evidences of the history of the animal, which are wanting in *Phoronis*. Such forms as *Pentacta* or *Synapta* are perfectly straight, exhibit a striking though not absolutely perfect radial symmetry, and the radii of the body are of equal length. In other forms, like *Cucumaria*, which, living in sand, habitually bend the body sharply in the middle to bring both extremities to the surface, the body has lost something of its radial symmetry. The side of the body towards which the flexure is made is perceptibly shorter than the other, and the body cannot be fully straightened. In other words, the flexure is partially obliterated by coalescence of the body walls, it has become organic. Here, again, the direction of the flexure is constant, being towards the bivium. A further step is seen in forms like *Psolus* or *Lophothuria*, where the radial symmetry has become profoundly modified, and the mouth and cloaca are perhaps twice as far apart on the side of the trivium as on the opposite side. Whether the next stage, which would be in shape something like an Ascidian, exists among the Holothurians I do not know. But the extreme of the series is found in *Rhopalodina*, where the mouth and cloacal opening are in immediate proximity, and the body is considerably elongated in a direction *transverse* to the original long axis. That this structure was really brought about by the complete flexure of a primitively straight and radially symmetrical form is shown by the persistence of this flexure, almost unaltered in the ambulacral vessels, at first pointed out by Hermann Ludwig.

Another illustration is afforded by the Chætopod larva *Mitraria*. In this well-known form the digestive tube is completely doubled upon itself, so that the mouth and anus are in immediate proximity. We must believe that here, too, the entire body has undergone an actual or virtual flexure corresponding to that of the intestine; for in a later stage a deep infolding of the body wall grows in between the mouth and anus, so that the previously concealed flexure becomes apparent. It may be added that the flexure subsequently straightens out and the worm assumes the ordinary form. This mode of development is the converse of what I take to have been the ancestral history of *Phoronis*.

These facts are not very full, yet they are enough to demonstrate the possibility of such a process of transformation as that which has been assumed to have taken place among the Gephyrea. The case would be stronger if there were in existence any forms intermediate between the two series of Gephyrean forms. But it must be admitted that no such forms are now known. In certain species, it is true, the mouth and anus are much more widely separated than in *Phoronis*. These forms show no external evidences of any flexure of the body; and if they were really intermediate forms their structure would furnish a strong argument against the flexure hypothesis. But it must be noted that in many of these forms the anterior or oral part of the body walls may be invaginated to form a sort of false œsophagus with the true mouth at its bottom. And it seems probable that the considerable interval between the mouth and anus exists simply in order to permit such invagination to take place without involving the latter opening. If this surmise be correct, the forms in question have really been derived from others which had the mouth and anus closely approximated, and therefore they are not intermediate forms at all, and present no difficulties in the way of the view which has been advanced.

Assuming, then, that the peculiar structure of *Phoronis* has resulted from the flexure of a primitively straight form, we may proceed to a consideration of the metamorphosis as viewed in this light. In the first place, what general causes have rendered this sudden and violent metamorphosis serviceable to the animal? The answer lies in the circumstance that the animal is subjected, during its larval existence, to conditions of life utterly unlike those by which it is surrounded when adult. At first pelagic and actively free-swimming, it subsequently assumes a purely sedentary mode of life, inhabiting a fixed tube which it never leaves, and in which movement is limited to comparatively sluggish extensions and contractions of the body. In passing from one existence to the other the animal must undergo a complete revolution in its mode of life; as a matter of course, this profound change can be possible only if accompanied by a corresponding modification of structure. In fact, the larva and adult are extremely different in appearance and in structure. Each is very perfectly adapted to its own special set of conditions; and since the two sets of conditions are very unlike, the corresponding structural adaptations are also very unlike. Now the animal must somehow exchange the one set of characters for the other; and of course the most advantageous method of exchange will be preserved. If the larva were converted into the adult by a process of continuous growth, gradually losing its larval peculiarities while acquiring the adult feature, evidently an intermediate or transition period would result, during which the animal would be imperfectly adapted to either larval or adult life, and yet would require all its energies to keep up the structural changes going on. It would either be at a great disadvantage during this period or would have to acquire still another set of compensating adaptations answering to the requirements of the transition stage. Examples of such adaptations are common, *e.g.* among many insects where the changes undergone are so profound, apparently, as to render transition stages inevitable.

For these reasons, it seems manifest that it would be advantageous for the animal to pass rapidly and directly from the larval structure to the adult, thus escaping as far as possible the disadvantages entailed by a state of transition or the expenditure of energy demanded by the acquisition of secondary adaptations. The metamorphosis of *Actinotrocha* secures to it this advantage, and it seems clear that this is why the process of sudden transformation has been acquired.

Passing on now to consider *how* the process may have been acquired, the bearing of the speculations concerning the way in which the anus originally left its primitive terminal position becomes apparent. If my theory of this process is correct, it is evident that in the adult worm the dorsal region is nearly obliterated, being represented only by the short interval between the mouth and the anus; while the ventral region includes nearly the whole length of what is apparently the dorsal surface as well as the whole "ventral" surface. In the larva, however, the dorsal and ventral region are of nearly equal extent; so that in assuming the adult condition the ventral region must undergo extensive increase through some process of growth. From what has been said above, it is evident that advantage would be derived if this growth could take place in such a way as not to impair the adaptation of the larva to its conditions of life. A simple ventral outgrowth of the body wall, carrying with it a loop of the intestine, would destroy the balance of the larval body, render the præanal belt of cilia useless, and increase the size of the body, so that the swimming apparatus would be insufficient, and the pelagic life would have to be abandoned for a period of transition. On the other hand, if the ventral wall, as it grew, should become so folded on itself as to preserve the outlines of the body unaltered, an extensive growth would be possible, without the need for any transition period. Abundant room for such an infolding is afforded by the spacious perivisceral cavity of *Actinotrocha*, and the pouch may be regarded as having been derived in this way. Strictly parallel cases among other animals are not numerous. Perhaps an imperfect illustration may be drawn from the foldings in the embryonic wings of insects which render them capable of being stowed away in a very small space in the pupa, and yet permit their rapid expansion at the time when the imago state is assumed.

A much more striking instance is afforded by the metamorphosis of certain Polyzoa. We owe to Barrois (see 'Annales des Sciences Naturelles,' August, 1880) the very interesting observations that the so-called "internal sac," which has been considered as the rudiment of some portion of the alimentary canal, becomes evaginated at the time of metamorphosis and then forms a large peduncle (*plague quadrangulaire*), by means of which the larva first attaches itself. The similarity of this process to the metamorphosis of *Actinotrocha* is obvious; and it seems equally clear that it has arisen for the same reason. The larval Polyzoon thus makes ready for the act of attachment without detriment to its own adaptation to free-swimming life.

The infolding in the larvæ of the ancestors of *Phoronis* must have been at first slight and more or less indefinite, becoming more extensive and definite as the dorsal region of the adult diminished in consequence of the gradual obliteration of the flexure. From such a primitive infolding the pouch of *Actinotrocha* could by natural selection be readily derived. The external opening would tend to become restricted for the exclusion of parasites or foreign bodies, and the gradual deepening of the infolding would at length reduce it to the form of a long pouch. The fibrous or muscular connection between the pouch and the walls of the alimentary canal are perhaps homologous with some of the similar fibres connecting the stomach wall and body wall in such Gephyrea as *Thalassema* or *Bonellia*, which are in turn comparable to the septa of the Annelides.

A little consideration shows that the metamorphosis itself is in reality a sudden and extreme dorsal flexure of the body, which may be considered as the ontogenetic repetition of a habit of adult ancestral forms. It differs from the latter, however, in the immediate disappearance of external signs of the flexure, while in the phylogeny this disappearance probably took place very gradually. Fig. 13 shows the larval body bent at right angles towards the dorsal side.

A few further remarks may be added in regard to the systematic position of *Phoronis*. Its very striking resemblance to a simple hippocrepian Polyzoon is entirely secondary, and is a very curious illustration of the acquisition of almost exactly similar structures to meet similar conditions by organisms having no near genetic relation. As already stated, the Polyzoon-like character is a result of strictly tubicolous life; and the latter, again, may depend in part upon the absence of structures, like the complex setæ of tubicolous Annelides, adapted to effect the ready protrusion or withdrawal of the body. Schneider's comparison of *Actinotrocha* to the Polyzoon larva *Cyphonautes* is purely superficial, and affords no proof whatever of relationship between the Polyzoa and Gephyrea. Further, his notion of the relation of *Phoronis* to its larva, which he compares to that existing between the polyzoon polypide and the cystid from which it develops, is manifestly based on a misunderstanding of the real nature of the metamorphosis, for the adult cannot be said, in any sense, to arise by a process of gemmation upon the larva.

The significance of the life-history of *Phoronis* becomes apparent when we pass to a consideration of some of the remarkable metamorphoses undergone by other animals. As already pointed out, these metamorphoses are in most cases of so complex a character that it is impossible to follow out in imagination their past history as circumstantially as we are able to follow the history of *Phoronis*. Nevertheless, we can trace the same principle in many of these cases, and in a few instances a somewhat similar process. For example, in the well-known and very remarkable case of *Pilidium* the animal is subjected, during its development, to two entirely different environments. During the earlier part of its life it is, like *Actinotrocha*, an active free-swimming animal. At a later period it leads an utterly different life, burrowing in mud or sand, living upon different food with new enemies, and subjected to many other new influences. Correlated with these great differences of environment we find so great a difference of structure between the larva and the adult that no one, without knowing the development, could suspect their identity. They are of wholly unlike shape, have acquired independent and dissimilar sensory and locomotor organs, protective characters, and possibly even distinct nervous systems. Notwithstanding these very great differences, the peculiar development of the Nemertine within the larval body obviates entirely the necessity for intermediate stages with their attendant disadvantages, and permits what may fairly be called an instantaneous leap from one condition of adaptation to another. As in the case of *Actinotrocha*, a complete preparation for the adult condition is gradually made, by the formation of a large part of the adult structure from portions of the larval body wall, which are infolded into the perivisceral cavity, and there go on developing without altering the external features of the larva. There are four such invaginations in *Pilidium*, and they undergo much more profound and extensive structural changes than does the single invagination of *Actinotrocha*. Yet it seems to me that they may have had a not very unlike origin. It is tolerably clear that the same general cause has determined their development, and I think the two processes of growth may be classed together.

We find something similar, again, among the Echinoderms, which are especially interesting as exhibiting a series of larval forms undergoing various degrees of metamorphosis. Here, too, in some cases, when the metamorphosis is very decided, the first rudiment of the adult arises from an infolding of the larval body wall, though more commonly no such invagination occurs. But in either case the adult develops within the body of the larva in such a way as to preserve almost unaltered those external features of the latter, by means of which it comes into direct relations with the surrounding medium. The tendency is towards the avoidance or abbreviation of transition stages by maintaining the larval adaptation unimpaired as long as possible. In this case transition stages are not entirely avoided, for it seems impossible to quite bridge over in this way the very great structural differences between the larva and the adult.

It is a significant fact that the development of the Holothurians is simpler and more direct than that of the Starfish, Sea-urchins, or Ophiurans. This seems to depend on the fact that the adults of the former group are soft-bodied, with flexible walls, and not very different in shape from the larvæ. Thus, a direct transformation of the larvæ into the adults is possible without the intervention of a very prolonged or distinct intermediate stage, while the rigid and regular coverings of the other groups involves a greater metamorphosis of the larva, which accordingly seems to require more gradual preparation. This can be made only during a period of transition or by means of some special mode of internal development. And the latter method, being more advantageous, has been acquired and preserved. To trace out exactly how this process has been acquired seems impracticable at present, owing to the want of sufficiently minute and extended knowledge of the exact conditions of the larval and adult life. But it seems quite possible that a complete explanation of these interesting phenomena will in time become possible on this principle of the abbreviation of transition stages.

The significance of insect metamorphoses has been so well elucidated, that I will add only a few words on this subject. In this group the structural differences between larva and adult are, in many cases, of such a nature that a transition stage cannot be avoided; and we find this stage provided, in many cases, with the most elaborate adaptive structures to compensate for the disadvantage thus necessitated. It is a very interesting fact, as Mr. Balfour well remarks, that the intermediate (pupa) stage as thus protected "has become secondarily adapted to play a part in the economy of the species quite different from that to which it owes its origin," *i. e.* the protection of the creature during the inclement season of the year, as when the insect passes the winter in the pupa state.

Although a transition stage occurs, it is ordinarily assumed, so far as external features are concerned, by a sudden change and not by gradual modification of the larva; and the change from pupa to imago is equally abrupt. Sir John Lubbock's explanation of the suddenness of these changes is that it results from the hardness of the external skin, which renders gradual modification impossible. This is clear enough in the change from pupa to imago, but it seems insufficient to explain the sudden change from the soft-skinned larva to the pupa. This is rather to be explained as a result of the advantage of maintaining complete adaptation to larval life up to as late a period as possible. It is very interesting to note that in some cases of decided metamorphosis the abruptness of change is facilitated by the development of portions of the adult organism from invaginations of the larval skin —the imaginal discs—so that an internal preparation is made by a process which has analogies with the development of *Actinotrocha, Pilidium,* and *Echinoderms,* and may have had a somewhat similar origin. The infoldings undergo so complex changes and become so highly specialised in the insects (as in *Pilidium*) that it is not easy from a consideration of their development in this group alone to understand how they at first arose. But the simplicity of the process in *Actinotrocha* renders it possible to suppose that the imaginal discs of insects may be the result of the gradual complication of simple infoldings of the body wall, first acquired, as in Actinotrocha, to preserve the larval external form.

The general conclusions to which a study of these phenomena of metamorphosis leads may be thus stated. Abrupt metamorphosis has resulted from the advantage derived from the abbreviation or avoidance of periods of imperfect adaptation. It is effected through acceleration of the development of certain portions of the larval organism with reference to a future adult state, while the remaining portions are specialised to meet the conditions of larval life. The organism thus becomes specialised simultaneously in two divergent directions, and in such a way that the two courses of development do not interfere with each other. The metamorphosis consists in a sudden assumption of functional activity by the adult set of structures and the simultaneous cessation or transformation of activity in the larval set. This may be effected either by rapidly absorbing or bodily casting off the larval structures or by a more or less complete transformation of them into adult structures; while a greater or less number of larval structures may persist nearly unaltered as the corresponding structures of the adult.

I gladly avail myself of this opportunity to make my acknowledgments to Dr. Brooks for his constant aid and counsel both to myself and to my fellow workers at the Zoological Laboratory.

BALTIMORE; *December,* 1880.

A FURTHER CONTRIBUTION *to the* MINUTE ANATOMY *of the* ORGAN *of* JACOBSON *in the* GUINEA-PIG. By E. KLEIN, M.D., F.R.S., Lecturer on Histology and Embryology in the Medical School of St. Bartholomew's Hospital. (With Plates XVI, XVII.)

IN my last paper on this subject (this Journal, January, 1881), I have given a detailed description of the relations and minute structure of the Organ of Jacobson in the Guinea-pig. This description, although referring to the greater part of the Jacobson's tubes, did not embrace the relations and structure of the anterior and posterior extremity of these organs. Extended observations have placed me in the position to give now a detailed account also of these parts in the adult guinea-pig.

Jacobson, Gratiolet, Balogh, Fleischer and others, describe the organ of Jacobson of mammals as opening into the ductus Stenonianus, and in my first paper on this subject (this Journal, January, 1881) I had also accepted this view for the guinea-pig; but on more careful examination, with a view to definitely ascertain whether this be the case, I made the unexpected observation that the tubes (organs) of Jacobson do not open into the Stenonian ducts but stand in a free and open communication with the anterior part of the nasal cavity, or more correctly speaking, with the lower nasal furrow, that is, the furrow along the bottom of the nasal septum. As is well known the organs of Jacobson develop as diverticula of the front part of the primary nasal pits. Later on, in the mammal, they are placed in communication with the Stenonian canals, while their communication with the nasal cavity is lost. But in the adult guinea-pig there is no such relation, since the communication with the nasal cavity persists, and this opening of the tubes of Jacobson into the latter will be spoken of below as the mouth of the organ of Jacobson. That this persistence of an open communication of the Jacobson's organs with the nasal cavity is of great importance I need hardly say, and I would only remind the reader that it is exactly the assumed non-existence of such a communication with the nasal cavity, which made it very difficult to ascribe to these organs in the mammal a satisfactory function in conformity with their rich supply with olfactory nerve branches and with the presence of their sensory epithelium. This communication being now found in the guinea-pig[1] there is of course no difficulty whatever in ascribing to the organ of Jacobson in this animal a function similar to that of the olfactory mucous membrane, but probably of a more specific nature, as I have already hinted at in my last communication. Whether in other mammals this communication with the nasal cavity persists, will be a subject to which I soon hope to devote myself.

Examining a series of vertical transverse sections through the front part of the nasal organ of the guinea-pig, the organ having been previously suitably prepared (see my paper in this Journal for January, 1881), we meet with appearances of the several parts, very accurately shown in the figures 1 to 6 of Plates XVI and XVII.

Fig. 1 is a transverse section through the most anterior part of the nasal organ. The relation of the cartilaginous septum to the lower turbinated bone, or lower concha nasalis, and further, of this to the alveolar process of the superior maxilla, are very well illustrated, the drawings being all made with the camera lucida, the relative proportions and sizes are all very accurately preserved. It will be seen in fig. 1 that the cartilaginous septum nasale forms an unbroken continuity with the cartilage representing here, *i. e.* in the front part of the nasal organ, the support of the lower concha. This continuity is brought about, on each side, by a curved, more or less trough-shaped cartilage plate extending on the inner surface of the alveolar process of the superior maxilla to the angular piece of cartilage that forms the support of the lower concha. But this continuation is more or less broken as we pass a little way backwards (see fig. 2). Here we find the above-named curved cartilage plate more or less separated from the lower margin of the septum as well as from the cartilage piece of the concha.

In my former communication I have used the term "concha" of the German anatomists for the "turbinated bone" of English text books. I consider the former term preferable, because it gives to the organ a name independent of its structure. In our case, *i. e.* in the front part of the nasal organ where there is no bone contained in it yet, it would be incorrect to speak of a turbinated 'bone.'

In fig. 3, which is taken a little behind fig. 2, this separation is complete, and, in addition, we find also the carti-

---

[1] I am in a position to assert also for the rabbit such a free communication of the organ of Jacobson with the nasal cavity.

lage of the concha reduced considerably in extent, being now for the greater part replaced by bone, with the exception of an oblong angular piece, which extends above the naso-lachrymal duct (see below).

In the sections still further behind that of which fig. 3 is a representation the individuality of the above curved cartilage plate is well seen. Its shape and relation to the septum are now different from that before mentioned, as is easily understood from a reference to this fig. 3, and it is, therefore, unnecessary to enter into any lengthy description. This curved cartilage plate is the front end of the cartilage surrounding the Jacobson's organ, *i. e.* the Jacobson's cartilage, and as such is referred to in the figures at 3. While in the parts delineated in figs. 1, 2, and 3 the concave surface of Jacobson's cartilage is directed upwards, forming, at the same time, the bottom of the lower nasal furrow (see the figures), in the parts further backwards, such as are shown in figs. 4 and 5, the cartilage becomes so changed that its concave surface is directed outwards, while its convex surface is in close contact with the lower margin of the cartilage of the septum, but separated from this latter by the respective perichondrium and a small amount of loose connective tissue with fat cells (see the figures).

As is also well shown in the drawings, the two extremities of Jacobson's cartilage, especially the upper one, become very bulky as we pass further backwards; (see fig. 6). Still more backwards the Jacobson's cartilage assumes the shape of a more or less complete capsule around the organ of Jacobson on each side, such as I have described and figured in my previous memoir, and for this reason I need not further enter into this subject.

Towards the posterior extremity of Jacobson's organ the cartilage so alters that the lower part gradually altogether ceases, and only the upper plough-shaped portion (see fig. 2 in my former memoir) remains; its channels, including the large afferent branches of the vessels and nerves of the organ of Jacobson become gradually enlarged, and finally all traces of the cartilage are lost. But this total disappearance of the cartilage of Jacobson occurs long before the hind or cæcal extremity of the organ of Jacobson is reached, its place being taken by the bone of the nasal crista; see figs. 7.

Another point to be noticed in figs. 4, 6, and 7 of the present memoir is the relation of the upper maxilla, or, more correctly speaking, of the nasal crista, to the Jacobson's cartilage. Beginning with a part illustrated in fig. 4 we see at 11 the first indication of the nasal crista of the upper maxilla, in the shape of a thin lamella of bone, extending along the convex surface of Jacobson's cartilage. It soon becomes very conspicuous, covering not only the convex surface of the cartilage, but gradually embracing the greater part of the circumference of the organ of Jacobson, as shown in figs. 1 and 2 of my paper in the January number of this Journal. In the posterior portion of the organ of Jacobson, viz. where the cartilage of Jacobson is wanting, as has been just mentioned, the former, *i. e.* the organ of Jacobson, is entirely enclosed in the osseous substance of the nasal crista, as is shown in fig. 7 of the present memoir.

In figs. 1, 2, and 3, *i. e.* in the anterior portion of the nasal organ, the lower nasal furrow (4) is lined with columnar ciliated epithelium, like that of the septum; it has been minutely described in my former memoir, and need not, therefore, be referred to here any further. The very bottom of the furrow, however, is lined with stratified pavement epithelium, of which the superficial layers consist of sqamous epithelial cells, each possessing a flattened nucleus. In the preparations from which the above figures are taken these superficial layers are more or less loosely attached to the rest of the epithelium, hence appear as if desquamating. The stratified epithelium, as a whole, is stained very much deeper in these preparations than the columnar epithelium, and is therefore very conspicuous in the drawings.

Such is the state of the epithelium at the bottom of the furrow in the front part (figs. 1, 2, and 3). But, going further back, we find that this part of the furrow, viz. the one lined with stratified pavement epithelium changes its position, inasmuch as it does not now occupy the very bottom of the furrow, but gradually extends inwards and upwards towards the concave surface of the Jacobson's cartilage. An inspection of figs. 4 and 5 will make this easily understood. At 12 in these figures the changed position of the part covered with stratified pavement epithelium is well seen.

Now, this pit, viz. marked 12, is the commencement of the organ or tube of Jacobson, or, more correctly speaking, the anterior opening of this latter into the nasal furrow; we shall call it the mouth of Jacobson's organ. Its diameter is about 0·05 mm., not including the lining epithelium, whose thickness is about 0·057 mm.

A little way further back (see fig. 5) we meet already with the well-formed tube of Jacobson, and we still recognise here the stratified pavement epithelium, occupying, as must be expected from what has been said before, the upper part of the tube; the lower part is lined with columnar ciliated epithelium, like that of the nasal furrow. A little further back the epithelium lining the tube is uniform on all sides, being ciliated columnar epithelium, such as has been minutely described in my former paper as lining the lateral wall of the organ of Jacobson.

Still further back (see fig. 6) we meet with this important alteration in the epithelium, that the median wall is lined with the thick sensory epithelium minutely described in my first paper. The tube, as a whole, has not, however, got yet its characteristic shape, *i. e.* so compressed from side to side, that its lumen is kidney shaped in transverse section (see figs. 1 and 2 in my first paper); this shape is, however, soon obtained, and the organ retains it to near its posterior extremity. Before this, however, is reached the direction of the lumen, and consequently of the walls, is more or less oblique and slanting downwards and inwards. In this oblique position we find chiefly the epithelium and the cavernous layer.

As regards the structure of the wall of the tube of Jacobson in its commencement, it is similar to that of the nasal furrow, viz. the subepithelial membrane is connective tissue infiltrated with numerous lymph-corpuscles, and resembling more or less diffuse adenoid tissue.

Already at the mouth of Jacobson's tube (12 in figs. 4 and 5) we find large veins forming a plexus and representing the rudiment of the cavernous tissue described in my first paper. This plexus becomes more developed as we pass backwards, and from its first appearance, *i. e.* at the mouth of Jacobson's organ, to the posterior extremity of this (see below), does not encroach on the median wall, *i.e.* the wall nearest the septum. In the parts from which fig. 6 is taken, but not further in front, we find between the vessels of the cavernous tissue already bundles of non-striped muscular tissue.

Longitudinal sections through the organ of Jacobson prove that a considerable posterior portion is without any cartilage, and is enclosed altogether in the bone of the crista nasalis, as mentioned above.

The posterior cæcal extremity of the organ is slightly curved inwards and upwards; it is smaller in diameter than the rest of the organ; its lumen is circular in cross section. The median wall of this extremity is not lined with any sensory epithelium, this having previously suddenly come to an end; we find here everywhere only columnar epithelium, similar to that of the lateral wall of the preceding parts.

In my first paper I have shown that the layer of (serous) glands is situated outside the cavernous layer, and extends in the lateral wall from the lower sulcus to the upper sulcus, but its bulk lies about the lower sulcus, into which the ducts chiefly open (see figs. 1 and 2 of my former paper). As we pass backwards, but long before the extremity of the organ is reached, we find the area in which the glands extend considerably increased, for many glands are now found also about the upper sulcus, and their ducts open into this latter. In the part which we just before described as the posterior extremity, viz. the narrow hind part with the cylindrical lumen, and without any sensory epithelium on the median wall, we find the layer of glands forming a complete investment and occupying almost the whole periphery of the organ (see fig. 7).

Another difference between the posterior section of the organ and the rest lies in the change the cavernous tissue undergoes in that extremity. As is shown in fig. 7, its position is not the same that it was before (compare figs. 1 and 2 of my first memoir), viz. occupying the lateral wall only, for, owing to the altered position of the lumen—this latter being not vertically but obliquely placed—the cavernous tissue now occupies the lower wall. At the same time the number of vessels and the amount of non-striped muscle tissue are greatly increased, although the individual vessels are not of so large a size as in preceding portions of the organ.

The plexus of non-striped muscular tissue forming the matrix of the cavernous layer is best developed in the extremity itself, and, together with the plexus of veins of the cavernous layer and the gland layer outside, extends for some little distance behind the cæcal end. In longitudinal sections comprising the posterior portion of the organ of Jacobson, these plexuses of bundles of non-striped muscular cells, and of veins between them, is very finely seen.

The tube which I described and figured in fig. 1[1] in my first memoir was there named the "accessory organ of Jacobson." But this is the naso-lachrymal duct; this was suggested to me through the kindness of Dr. Allen Thomson, by Prof. Kölliker, of Würzburg, and on careful dissection I have convinced myself that this is really the case.

It extends anteriorly and posteriorly considerably beyond the region of the organ of Jacobson. Anteriorly the naso-

---

[1] In the text, p. 106, it is erroneously referred to as "at 8 in fig. 1," it should read "at 9 in fig. 1."

lachrymal ducts are met with, in vertical transverse sections through the nasal organ, some distance in front of Jacobson's organs, as minute tubes, in the same position and of the same nature as described in my first memoir, as will be easily understood on comparing the figures 1, 2, 3, and 4 accompanying the present paper. Also backwards for a very long distance beyond the region of the organ of Jacobson about 10—12 mm.; the naso-lachrymal ducts retain the same position, viz. between the nasal furrow and the alveolar process of the upper maxilla. As I mentioned in my first paper, this duct is slightly flattened from side to side, and the diameter enlarges considerably posteriorly; in the region of the hind extremity of the organ of Jacobson the diameters of the naso-lachrymal duct (5 in fig. 7) are these:

Horizontal transverse diameter of the whole duct .	1·01 mm.
Vertical transverse diameter ,, ,, . .	2·81 mm.
Horizontal transverse diameter of the lumen .	0·60 mm.
Vertical ,, ,, ,, . . .	1·01 mm.
Vertical (thickness) diameter of the lower wall .	0·7875 mm.
Vertical (thickness) diameter of the upper wall .	1·01 mm.

Behind the region of the organ of Jacobson the naso-lachrymal ducts increase still more in diameter, the transverse diameter of the lumen alone amounting in the broadest part of the tube to 1·125 mm.; they become at the same time cylindrical, *i.e.* less flattened from side to side. As regards the structure of the naso-lachrymal duct I have little to add to my previous description as far as that part is concerned, that belongs to the region of the organ of Jacobson. In the whole extent of the duct I find in close proximity to the epithelium lining the interior of the duct, and described minutely in my first memoir, but separated from it (epithelium) by a thin fibrous layer, *i. e.* the subepithelial fibrous layer, a plexus of large veins, most of these running a longitudinal course. The wall of these veins is exceedingly thin and appears to consist almost entirely of the endothelial membrane lining the lumen. Had I not seen the blood capillaries in connection with them, and had I not found some of them containing blood, I might easily have mistaken them for lymphatics. This plexus, which I will call the "subepithelial venous plexus," is well seen in the region of the posterior extremity of the organ of Jacobson (see figs. 9, 10); it reaches its greatest development in the region behind the organ of Jacobson. In fig. 7 it is also noticed that the rest of the wall of the naso-lachrymal duct is made up of a very loose fibrous connective tissue; the thickness of the wall is greatest in an upward and downward vertical direction. Scattered lymph-corpuscles and more or less well-defined accumulations of them, *i.e.* true lymph-follicles, are met with in the upper wall of the duct close to the epithelium (see figs. 7 and 8).

Henle[1] mentions in the lower portion of the human naso-lachrymal ducts the richness of its wall in lymphatic tissue and in a cavernous tissue, this latter being a continuation of the cavernous tissue of the lower nasal concha.

In fig. 8 I have given an accurate representation of the wall of the naso-lachrymal duct, and it will be here seen that the stratified columnar epithelium, with the vascular intraepithelial vesicles or cavities, the subepithelial fibrous layer, and the subepithelial venous plexus are well marked. I cannot ascertain whether the superficial cells bear cilia or not. In my last paper I have given several reasons why I think that they are ciliated but I have not been able to get further in this matter. Henle[2] maintains that in man the epithelium lining the duct is simple columnar ciliated epithelium as against R. Maier,[3] who describes it as stratified columnar non-ciliated epithelium.

The thickness of the epithelium is here about 0·068, the thickness of the sub-epithelial fibrous layer is 0·012—0·016 mm., and the diameter of the veins of the sub-epithelial plexus is between 0·028 and 0·04 mm.

The rest of the wall of the duct, in fig. 8, shows an artery ascending towards the internal surface and connected at its capillary end with a lymph-follicle.

The lymph-follicle, which I described and figured in fig. 1 of my first memoir, is not in reality a solitary follicle but is one of a patch of lymph-follicles extending in a longitudinal direction, and therefore seen in the figure in transverse section. Besides these follicles I find one large patch of lymph-follicles on each side of, and close to the epithelium lining the lateral wall of the lower nasal furrow a little behind the region of the organ of Jacobson. The long diameter of this patch, *i.e.* anterio-posteriorly, is about 3·4 mm., the thickness diameter, *i.e.* from side to side is about 0·45 mm. The individual follicles of the patch are spherical, closely placed side by side, and in some places their outlines are indistinct; the diameter of the largest follicles is about 0·3—0·45 mm.

Both the upper and lower wall of the duct contains a

---

[1] 'Eingeweidelehre,' ii, p. 713.

[2] L. c., p. 712.

[3] 'Ueber d. Bau d. Thränenorgane,' Freiburg, 1859, p. 31.

great many wavy and more or less convoluted arterial vessels connected with one another; in fig. 7 they are seen cut in various directions. The capillaries proceeding from these arteries are contained in the subepithelial fibrous layer above mentioned, and some of the capillaries enter the intraepithelial spaces described in my first memoir. The capillaries lead into the subepithelial venous plexus, and small veins come out from this plexus and pass into the outer portion of the wall of the naso-lachrymal duct.

In the embryo guinea-pig that I have examined hitherto, at a time when the organ of Jacobson is well developed, also the naso-lachrymal ducts are distinctly visible, bearing the same position and relation to the surrounding parts as in the adult animal.

An embryo guinea-pig, whose total length from vertex of the head to the tail was 4 centimètres, while the long diameter of the head, *i. e.* from the tip of the nose to the occiput was 2 centimètres, has been the material from which I have made vertical transverse sections through the nasal organ. In a section through the anterior parts, containing the Jacobson's organ as closed tubes, it is noticed that the cartilage of Jacobson forms almost a complete capsule round the organ of Jacobson, the lateral and median wall of the latter being easily distinguishable. Sections made of parts a little further back show the cartilage capsule of Jacobson open on the upper part, so as to allow the entrance of the nervous and vascular branches; the inner or median lamella of the capsule is at the same time much higher than the outer one, and in this state the shape of the cartilage resembles very much that of the adult rabbit, as I shall have soon an opportunity of showing.

The relations of Jacobson's cartilage to the nasal septum in the front part of the nasal organ and the opening of the anterior extremity of the organ of Jacobson into the nasal furrow, are exactly the same in this embryo as described above of the adult animal. There is, however, this interesting condition of the lower nasal furrow throughout the front part of the nasal organ, viz. that the furrow itself is not formed yet, but the stratified epithelium lining the median and lateral wall of the septum is fused into one solid membrane. But there are smaller or larger cavities visible in this membrane; by confluence of these along a definite middle plane the furrow becomes gradually established.

The shape and relation of the naso-lachrymal ducts to the cartilage of the concha and to the upper maxilla is the same as in the adult animal. The epithelium lining it is stratified epithelium composed of a deep layer of columnar cells, and inside these are a good many layers of polyhedral cells.

In the front part the lumen of the duct is not at all distinct; in the posterior portion of the duct the lumen becomes very conspicuous, and the epithelium resembles, in all respects, stratified pavement epithelium, the superficial cells being, however, not much flattened.

I measured the thickness of the epithelium lining the naso-lachrymal duct in this embryo, and I find it to be about 0·068 mm., the lumen of the duct being 0 22—0·03 mm. In a part behind the region of the organ of Jacobson the thickness of the epithelium is smaller, being 0·056, while the lumen is 0·12 mm.

For the sake of completeness I append here a few points concerning the structure of the Stenonian ducts, which, as is well known, pass closely side by side, and in the median line, from the nasal to the oral cavity.

In the adult guinea-pig the nasal extremity of each of these ducts is an oblong cleft lined with a continuation of the mucous membrane of the lower nasal furrow; the epithelium is ciliated columnar epithelium, and underneath this is a membrane very vascular and infiltrated with lymph-corpuscles. There are no secreting glands, mucous or other, visible in this part, and the mucous membrane is in immediate contact with the bone of the palate.

As the ducts approach the oral cavity, but while still in the bone of the palate, they become much smaller, but in a transverse section are still cleft-like, and lined with stratified pavement epithelium, whose superficial cells are polyhedral. Having passed the bone and approaching the oral cavity, the ducts become still smaller, they remain cleft-like, and are lined with stratified pavement epithelium, whose superficial cells are as much flattened as those of the palatine mucous membrane. The mucosa underneath the epithelium of the ducts is dense fibrous tissue, and there are indications of minute papillæ. There is now already to be seen a trace of the Stenonian cartilage in the periphery of the wall of the ducts.

Still nearer towards the oral cavity, while the ducts enlarge in diameter, and while their shape becomes more cylindrical, the above rudiment of the cartilage forms now for each duct a curved plate, semicircular in transverse section, whose concave surface embraces the outer part of the wall of the duct.

The two ducts being close side by side, it follows that the two semilunar cartilages meet at their extremities, and thus form nearly a complete capsule for the two ducts (see figures 9 and 10). There is a smaller or larger apparently isolated nodule of cartilage found between the two ducts.

Just before the ducts open into the oral cavity the lumen becomes a little smaller, cylindrical, and there are here well-developed papillæ, such as those of the palatine mucous membrane. The Stenonian cartilages have become confluent with their extremities, and present themselves now in transverse section as a beautiful heart-shaped capsule, in each of whose cavities lies one of the ducts, the apex of the heart being directed forwards, the notch backwards (see fig. 10). In connection with the apex one or more small pieces of cartilage are seen extending into the tissue separating the two ducts.

As regards its structure, the Stenonian cartilage differs widely from that of the cartilage of the nasal septum and of Jacobson's cartilage, the Stenonian cartilage being elastic cartilage, *i. e.* dense networks of elastic fibrils, forming a sort of capsule around the individual cartilage cells. These latter are remarkable for being identical in appearance with well-formed fat-cells. Of the cartilage of the septum I have mentioned, in my first paper, that in many places the cartilage-cells are filled with numbers of minute fat globules, an appearance well known of some other hyaline cartilages; but here, *i. e.* in the Stenonian cartilages, we find each cartilage cell filled with one large oil globule. Being elastic or reticular cartilage there is, then, a great similarity between the Stenonian cartilage of the guinea-pig and the cartilage of the epiglottis of some animals (rabbit, cat, dog), where the cells are likewise transformed into fat-cells.

According to Balogh[1], the Stenonian cartilage in the sheep is hyaline, and the epithelium lining the duct, speaking of the Stenonian duct only as situated in the mucous membrane of the palatum durum, is stratified and composed of flattened and more or less hexagonal cells.

Kölliker[2] found, in the adult human subject, the duct lined with ciliated columnar epithelium. Both Balogh, for the Stenonian duct of the sheep, and Kölliker, for that of man, mention mucous glands embedded in the wall of the duct and opening into its lumen.

In the guinea-pig I have searched in vain for these glands;

[1] L. c., p. 456.

[2] L. c., p. 7.

except an occasional fold-like unevenness, I cannot find anything of glands.

The measurements which I made of transverse sections through the Stenonian ducts in the guinea-pig are these:

a. Near their nasal opening:

Anterio-posterior transverse diameter	3·8 mm.
Transverse diameter from side to side	0·51 mm.
Transverse diameter of lumen	0·38 mm.
Thickness of epithelium (column ciliated)	0·0675 mm.

b. While in the bone of the palate:

Anterio-posterior transverse diameter	2·02—2·7 mm.
Transverse diameter from side to side	0·335—0·4 mm.
Transverse diameter of lumen	0·38 mm.
Thickness of epithelium (column ciliated)	0·0675 mm.

c. Having passed the bone of the palate:

Anterio-posterior transverse diameter	1·125 mm.
Transverse diameter from side to side	0·2475—0·29 mm.
Transverse diameter of lumen	0·1125 m.m.
Thickness of epithelium (stratified pavement)	0·0675—0·09 mm.

d. Further towards the oral cavity, but surrounded laterally by the semilunar Stenonian cartilage:

Anterio-posterior transverse diameter	1·01 mm.
Transverse diameter from side to side	0·61—0·63 mm.
Transverse diameter of lumen	0·495 mm.
Thickness of epithelium (stratified pavement)	0·06—0·007 mm.
Thickness of the cartilage	0·11—0·13 mm.
Shown connecting the two extremities of the semi-lunar cartilage	1·35 mm.

e. Near the oral extremity, the duct being now cylindrical and the Stenonian cartilage at the two ducts fused into a heart-shaped capsule:

Transverse diameter	0·69—0·7 mm.
Lumen	0·56 mm.
Thickness of stratified pavement epithelium	0·064 mm.
Thickness of cartilage, laterally	0·15—0·16 mm.

In all these measurements it must be understood that the transverse diameters, *i.e.* the anterio-posterior as well as that from side to side, comprise the lumen and the lining epithelium only, the fibrous coat forming the proper wall of the ducts not being included.

Histological Notes. By E. Klein, M.D., F.R.S.

I. Examining sections through the kidney, hardened in methylated alcohol, of the white mouse, I noticed that the epithelium lining the first portion of the convoluted tubule, *i.e.* the one following the Malpighian corpuscle, is *ciliated*, in some instances for a longer, in others for a shorter distance, the cilia being, of course, on the side facing the lumen of the tubule. The epithelium lining the capsule of the Malpighian corpuscle nearest to the neck of the tubule, for about a third or fourth of the circumference of the capsule, is composed of polyhedral or short columnar cells, identical in appearance with those of the convoluted tubule, the remainder of the capsule being lined with flattened squamous cells, commonly found lining the whole capsule of the Malpighian corpuscle in other mammals and man.

In some instances, also these polyhedral or columnar cells of the capsule are possessed of cilia. These cilia are visible only in relatively few convoluted tubules, and, as mentioned above, in portions that are nearest to the Malpighian corpuscles.

The cells to whom these cilia belong show, in some instances, Heidenhain's rod-like structures in the outer portion of their substance, in other instances they, viz. the rod-like structures, are not well shown, and still in others, they extend more or less distinctly through the whole cell substance.

The cells themselves are polyhedral or short columnar, varying in height, *i. e.* in the diameter from the lumen to the membrana propria of the tubule, between 0·005 and 0·007 mm.

The cilia are, in most instances, exceedingly fine, and measure in length between 0·0036 and 0·005 mm.

In some places the whole mass of cilia are more or less knitted together, and can be then made out only with high powers as fine striations; in others they appear more isolated, and are then, of course, easiest seen. But in all instances there is a marked boundary line between the cell substance and the cilia, these latter penetrating, however, into the former.

In several instances where the rod-like structures of the cell substance are distinct, I see the cilia directly continuous with them, although, as a rule, the latter are much finer than the former.

Had I found these ciliated cells in an isolated state, *i.e.* after teasing out little bits of kidney substance, I should have taken them to be identical with the cells figured by Heidenhain ('Hermann's Physiologie,' v, p. 285, fig. 67) of the kidney of the rat, but in our case the cilia are projecting from the cell substance into the lumen of the tubule, the cell substance showing, in addition, the rods of Heidenhain, and there being a definite boundary line between the cilia and the cell substance.

The following are measurements made on tubules lined with ciliated epithelium:

1.	Transverse diameter of the whole tube (lumen and wall) in cross section	0·0414	mm. by 0·0486 mm.
	Thickness of the epithelial lining without the cilia	0·0054	,,
	Length of the cilia	0·0036	,,
2.	Transverse diameter of the tube	0·0396	,,
	Thickness of the epithelium	0·0054	,,
	Length of the cilia	0·0036	,,
3.	Transverse diameter of the tube	0·0324	,,
	Thickness of the epithelium	0·0072	,,
	Length of the cilia	0·004	,,
4.	Transverse diameter of the tube	0·032	,, by 0·045 mm.
	Thickness of the epithelium	0·0054	,,
	Length of the cilia	0·0038	,,
5.	Transverse diameter of the tube	0·036	,,
	Thickness of the epithelium	0·0072	,, on one side.
	Length of cilia	0·0036	,, ,,
	Thickness of the epithelium	0·005	,, on the other side.
	Length of the cilia	0·005	,, ,,

Ciliated epithelium, as far as I can find in the literature of the kidney, has not been seen in the mammal's organ; in that of reptiles, amphibia, and fishes as is well known (Bowman, Remak, Kölliker, and others), ciliated epithelium is of a constant occurrence in the capsule of the Malpighian corpuscle, and especially in the long thin neck of the urinary tubule; the cilia are here of very great length, and their movement has been observed in the fresh state by Duncan (Sitzungsber. d. Akad. d. Wiss, Wien Abtheil. 56). and Spengel ('Arbeit. aus d. Zoolog. Instit.,' Wurzburg, 1876). I have tried to see the movement of the cilia in sections through the fresh kidney of the mouse, but have not hitherto been successful. The sections were made immediately after killing the animal and were examined in aqueous humor, but the urinary tubules of the cortex were always so contracted that of a lumen of the tubules, and consequently of the above-named short and fine cilia, or of the movement of these latter nothing could be seen.

II. Teasing out fresh muscular tissue of the heart of the mouse, isolated fibrillæ of the fibres can be obtained; they (fibrillæ) appear of a regular moniliform character, so that they resemble a chain of large micrococci; and indeed I had at first taken them for such, having by accident found them in a drop of blood drawn from the ventricle of the heart by a capillary tube that had been pierced through the wall of the ventricle. On looking, however, through the same blood preparation I met, besides the above isolated ones, two, three, and more such chains joined together *sideways,* up to whole muscular fibres; so that about their nature, viz. as primitive fibrillæ of muscular fibres, there could be no doubt. Now, the interesting fact that could be made out here was this: passing from a single isolated fibrilla to bits of muscular fibres composed of two, three, and more such fibrillæ, the transverse striation of these several bits of muscular fibres was unmistakably due to the moniliform nature of the fibrillæ, that is to say, corresponding to the varicosities of the fibrillæ were the dark transverse stripes, while corresponding to the parts between the varicosities were the light transverse stripes, or in other words, the dark stripes were made up of the varicosities, the light stripes of the intervening constricted portions of the fibrillæ. This is, I think, a strong corroboration of Dr. Haycraft's view on the cause of the cross striation in muscular fibres, as described minutely by him in this number of the journal.

On the DEVELOPMENT *of* CERTAIN MICROSCOPIC ORGANISMS OCCURRING *in the* INTESTINAL CANAL.[1] By D. D. CUNNINGHAM, M.B., Surgeon-Major, Indian Medical Service; Fellow of the Calcutta University. With Plate XVIII.

I. *The Monads and Amœbæ of the Human Digestive Canal.*

AT a time when the association of special parasites with morbid states of their host is readily interpreted as evidence in favour of current theories regarding the parasitic origin of disease, any exact information regarding the true significance of the phenomenon in particular cases may serve a useful purpose. It has, therefore, seemed desirable to endeavour to ascertain the nature of certain parasitic forms, which, in this country at all events, are specially related to cholera and certain other conditions in which the contents of the intestinal canal are of an abnormal character, and to determine the relation which they bear to those conditions. The intestinal contents in such cases, like those in health and in other forms of disease, abound in organisms of various kinds, but are specially characterised by the frequency with which they contain excessive numbers of what may be, provisionally, termed monads and Amœbæ. The excessive development of these bodies certainly bears a definite relation to the existence of abnormal conditions in the host, and the aim of the present paper is to show what the real nature of this relation is.

The account given of the life-history, and of the mutual relations of the various bodies described, is founded on a prolonged course of investigation, including a series of cultivation-experiments, carried out with the aid of various media and extending continuously over a period of more than a year, in order to determine the influence exerted on the course of development by variations in external conditions. To give a full account of all the observations would have occupied excessive space and have tended to obscure the general results of the inquiry in masses of detail. It has, therefore, seemed advisable, as far as possible, to avoid histories of individual experiments, and merely to introduce occasional illustrative cases regarding particular phenomena. All the more general statements are, however, founded on notes recorded during the course of the investigation and not on mere memory of results, and are there-

---

[1] This paper appeared as an appendix to the 'Fifteenth Annual Report of the Sanitary Commissioner with the Government of India.'

fore free from the source of fallacy, at all events, however much they may be affected by errors of interpretation.

The discovery of the occurrence of monad forms in choleraic and other excreta dates many years back. Davaine appears to have been the first to observe them. In the year 1854 he published an account of his observations regarding their presence in choleraic excreta during the epidemic of 1853, 1854.[1] He states that he encountered them in many cases and frequently in very great abundance, and he records the occurrence of what he regarded as a smaller variety of the same organism in typhoid excreta. Subsequently Lambl described and figured similar bodies present in the gelatinous mucoid discharges occurring in cases of diarrhœa in children,[2] and Ekeckrantz recorded their occurrence in cases of diarrhœa.[3] In the year 1870 Dr. Timothy Lewis published his observations on the occurrence of monads and ambœboid organisms in choleraic and other excreta,[4] and Tham recorded the occurrence of monads in chronic diarrhœa.[5] In the following year an account of my own observations on the same subject was published, in which various forms of monad and amœboid forms were described.[6] In 1875 Marchand recorded the occurrence of monads in a case of typhoid fever,[7] and, in 1878, Zunker described two forms as occurring in severe cases of intestinal disease.[8]

The question next presents itself, whether all the observations refer to one specific organism, or whether more than one species is referred to. Leuckart, in the recently published edition of his work on 'Human Parasites,' believes that the latter is the case, and, while referring the organisms described in most cases to the genus Cercomonas, distinguishes those described by Marchand, and certain of those recorded by Zunker as belonging to the genus Trichomonas. As the result of prolonged observation of the varying forms which the organisms occurring in excreta in this country present, I must confess that I am inclined to believe in the specific identity of the parasite in all the recorded cases.

---

[1] 'Compt. rend. Soc. Biolog.,' 1854.

[2] 'Prager Vierteljahrsscurift für praktische Heilkunde,' 1859, Bd. 61, S. 51.

[3] "Bidrag till känndomen om de i menniskans tarmkanal förekommande Infusorier:" 'Nordisk med. Arkiv,' Bd. I.

[4] Appendix A. 'Sixth Annual Report of the Sanitary Commissioner with the Government of India,' Calcutta, 1870.

[5] 'Tänna fall af Cercomonas, Upsala läkare fören. förhandl.,' Bd. v, p. 691.

[6] Appendix B. 'Seventh Annual Report of the Sanitary Commissioner with the Government of India,' Calcutta, 1870.

[7] 'Archiv für pathol. Anatomie,' 1875, Bd. 64, S. 294.

[8] 'Zeitschrift für practische Medicin,' 1878, No. 1, S. 1

As previously mentioned, the occurrence of amœbal organisms in choleraic and other excreta was recorded by Dr. Lewis and myself, in the years 1870, 1871.[1] The only other accurately recorded observation regarding them appears to be that of Lösch, published in 1875, in which organisms associated with a dysenteric condition of the large intestine, are assumed to have been the specific cause of the disease, and have received the name of *Amœba coli.*[2]

In so far as morphological characters are concerned, the monads observed in excretal materials in Calcutta in some cases agree with the genus Cercomonas, and seemingly represent the so-called *Cercomonas intestinalis*, Dav. In others, however, they seem rather to correspond with the genus Trichomonas, taking the characters of the latter as defined by Stein. This authority denies the presence of any true lateral cilia, and maintains that the appearance of a ciliated border is deceptive, and really due to the rapid emission of successive protrusions from the soft body substance of the organism.[3] Whether this be true of all the organisms at present united under the generic term Trichomonas I am unable to form any opinion from practical experience; but certainly the phenomena pointed out by Leuckart in reference to *Trichomonas bactrachorum* are opposed to such a belief. In reference to the organisms with which we are at present concerned, however, I can with the utmost confidence assert that Stein's description is strictly applicable. This being so, it is clear that, unless the lateral cilioid protrusions are constantly emitted, which is certainly not the case, the same organism must at different times present the characters of both genera, Cercomonas and Trichomonas. Not only does our parasite do so, but it presents a protean series of immediate forms, as well as another series connecting it with the form-genus Amœba. This latter series presents characters practically undistinguishable from those of the swarm-spores of the Myxomycetes, a group of organisms with which the parasite, as we shall find, presents other close points of agreement.

Before going further, it may be well to describe the characters of these monads, or, as they may with more propriety be termed, zoospores, a little more closely, even though I have comparatively little to add to the descriptions of them previously given by both Dr. Lewis and myself several years ago. In regard to form, it is difficult to make any definite statement in reference to organisms which in this respect exhibit no constancy,

---

[1] Op. cit.

[2] 'Archiv für pathol. Anat.,' 1875, Bd. 65, S. 196.

[3] Quoted in Leuckart's 'Die Parasiten des Menschen und die von ihnen herrührenden Krankheiten,' Zweite Auflage, 1879, S. 311.

but are continually varying in consequence of both intrinsic and extrinsic influences. The body in most cases is a mere fragment of naked protoplasm, with no differentiated covering, and with hardly, if any, indications of a differentiation of ectosarc and endosarc. Owing to this and to their minute size, it is almost impossible to determine with any certainty many points regarding it when in a state of full activity. Nearly all reagents almost immediately produce destructive changes, leading on rapidly to disintegration and disappearance, and even slight changes in the medium, such as depression of temperature or dilution with water, are sufficient to arrest activity and induce disintegration.

When in full activity they present a more or less fusiform or pear-shaped outline (Plate XVIII, fig. 18). The size varies so greatly, according to the nature of the medium and to the rate at which processes of multiplication by division occur, that it is useless to attempt any very precise statement regarding it. In three cases in which special notes of measurements were taken, the results were as follows:

8·2 μ.[1] × 7·2 μ.
7·2 μ. × 3·7 μ.
9·2 μ.

It is almost as difficult to make any definite statement as to the number of flagella which is to be regarded as normal, as it appears to vary from one to three, or even four, in some cases. In such an undifferentiated organism the flagella differ little from pseudopodia, and their formation and retraction may frequently be observed in some of the more sluggish states of the body when movement is comparatively slow. This being the case, it is clear that any constancy in their numbers is hardly to be looked for. The posterior extremity of the body sometimes ends in a point, sometimes is more or less rounded, and frequently is provided with a caudal process, or trailing filament of very varying length and thickness. This appears to be connected with the method of nutrition proper to the organism, which is identical with that described by Cienkowski as prevailing among certain of the Monadinæ.[2] That portion of the body opposite to the site of the flagella, and therefore the posterior portion in reference to motion, is the point through which nutritive materials are absorbed into the body-mass. The process may sometimes be very clearly observed where the nutritive body, as is occasionally the case, consists of an amœboid body or of a red blood-corpuscle. When a zoospore is about to be

---

[1] μ = Micromillimètre = ·001 mm.

[2] "Beiträge zur Kenntniss der Monaden," 'Archiv für mikrosk. Anatomie,' Bd. I, 1865, S. 203.

nourished at the expense of an active amœboid organism, it becomes adherent to it by its posterior extremity. The Amœba at first continues to progress freely, but soon ceases to do so, assumes a spherical and motionless condition, and is dragged passively about by the energetic flagellary movements of the zoospore. Gradually a diminution in its bulk becomes manifest, the body of the zoospore at the same time becoming distinctly plumper and more refractive, and as the process continues the whole, or almost the whole, of the Amœba disappears, and its plunderer swims off to seek a new victim. Sometimes several zoospores unite in plundering one Amœba, which is jerked irregularly about by their opposed movements. In the case where red blood-corpuscles are the source of nutriment, as is frequently the case in choleraic evacuations, the progress of the process may be followed readily by the colouring of the zoospore-body by the absorbed hæmoglobin. In some cases portions of corpuscles, too, seem to be absorbed *en masse,* though it is difficult to be quite certain on this point, due to appearances arising from surface adhesion to the transparent bodies of the zoospores.

Owing to the constant movements presented by the parasites when in full activity, it is difficult to come to any definite conclusion regarding the frequency with which they possesss a differentiated nucleus or contractile vesicle. That they do do so in some cases there can be no doubt, but in many the most careful and prolonged examinations fail to reveal the presence of either structure. That the presence or absence of a distinct contractile vesicle is not a matter of essential specific importance, is the conclusion which Hertwig and Lesser seem to arrive at as the result of their study of the Rhizopoda,[1] and the phenomena presented by the organisms at present under consideration certainly corroborate this conclusion. The presence of a contractile vesicle appears to be an inconstant character, determined, in some cases at all events, by conditions of the nutritive medium. With regard to the constancy of a nucleus as a specific character, it is necessary to speak with some reserve, as the presence of such a structure may readily escape observation in such minute organisms as the excretal zoospores. This is more especially likely to occur where a distinct nucleolus is not present and where the nucleus is merely represented by a clear vacuolar area in the body-mass. That these conditions may replace one another in one and the same organism is, as we shall see hereafter, unquestionable, bodies which at one period merely show a clearer nuclear area subsequently showing a conspicuous

---

[1] "Ueber Rhizopoden und denselben nahestehende Organismen," 'Archiv für mikr. Anat.,' Bd. X. Suppl.

nucleolus within this. In certain cases, as Dr. Lewis pointed out in 1870, the excretal zoospores do show clear areas which are probably of a nuclear nature, and in others either previous to or after treatment with reagents, and specially with Liquor Iodi, a nucleoloid particle is rendered manifest. Without feeling justified in stating it as a positive fact, I am strongly inclined to regard the presence or absence of a nucleus as connected in the present case rather with developmental than specific character. The body of the parasite varies considerably in appearance in different cases, and at different times in one and the same specimen, being sometimes almost homogeneous and in others distinctly granular.

After continuing in full activity for some time, the zoospores sometimes pass into a condition in which they exhibit very free amœboid changes of form, accompanied by frequent retraction and protrusion of flagella. Frequently connected with this stage, but sometimes also occurring as a mere interlude in the condition of maximum activity, processes of multiplication by division take place. Division is preceded by a temporary cessation of activity, the flagella being retracted and the body assuming a more or less spherical form (Plate XVIII, fig. 17 *k*). The outline soon become oval, a constriction now appears transverse to the long axis of the body and rapidly deepens, and a new flagellum appears at either pole and begins to act with energy (Plate XVIII, fig. 17, *h*, *i*). The central contraction continues to increase in depth, and ultimately the two segments remain connected merely by a narrow neck, which, due to their energetic struggles, is soon reduced to a thread (Plate XVIII, fig. 17, *j*), and finally gives way, so as to allow the two twin zoospores to part company and swim off freely in the medium.

In other cases a retardation of movement is the antecedent to the death of the zoospores, as may frequently be observed when unfavorable alterations are naturally or artificially taking place in the medium. It is in these cases that they come to present features causing them to agree with Stein's description of those in Trichomonas. The movement ceases to be one of energetic rotatory advance and assumes a jerking character. This jerking is due to the emission of lateral pseudopodial protrusions in rapid succession. Where the emission, as is frequently the case at first, is very rapid, an appearance arises as though the body possessed a lateral row of cilia. As, however, a gradual retardation sets in, the true nature of the phenomenon can be readily determined. It is now seen that distinct, slender pseudopodial processes, often of considerable length, are emitted from the side of the body, and sweeping round in a curve are again retracted. Sometimes two are visible at once, a fresh one beginning to be emitted ere the entire disappearance of its predecessor. The pseudopodia gradually diminish in size as time goes on and finally disappear, the last traces of their formation being represented by mere wave-like undulations of the body-margin. The flagella have been retracted some time previously, and the zoospore finally remains as a mere rounded or oval particle of protoplasm, which rapidly breaks down into a molecular flake and disappears.

The presence of zoospores is by no means confined to choleraic excreta. They certainly, as a rule, are present in such excreta in much larger amount and much greater activity than in other cases, but in many cases of intestinal disease of other nature they may readily be detected, and even in cases where no abnormal condition exists, they are very frequently present in small numbers. Although this is the case, they may readily escape observation, and this for several reasons. In the first place, they are frequently inactive; and secondly, even where they are not so, the nature of the medium is such as to prevent their free movement. Moreover, they are so easily and prejudicially affected by changes in the medium, that the means employed to facilitate their detection very often defeat their own end. Thus, the addition of water is in almost all cases enough almost immediately to secure the abolition of motion, and very rapidly to lead to disintegration and disappearance of the zoospores. The two media which I have found most adapted to secure the demonstration of their presence are, first, the alkaline fluid of choleraic excreta; and second, a solution of cow dung. In either case, before using the media, it is of course necessary to filter and boil them in order to exclude *débris* and organisms which they may contain. In my first observations I always employed the choleraic fluid, but latterly I have entirely abandoned this in favour of the solution of cow dung, which seems to be peculiarly favorable to the zoospores.

The presence of the zoospores in the excreta is then a phenomenon not peculiar to cholera, or indeed to any diseased condition. On the other hand, certain diseased conditions of the excreta appear certainly to be incompatible with their presence. As I previously pointed out,[1] cases of acid diarrhœa associated with the presence of growing fungal elements are characterised by the absence of any traces of the zoospores. This in itself is sufficient to show that mere fluidity of the medium is not the only condition necessary for the occurrence of these organisms. That this is the case is also proved by their entire absence in many cases of dysentery. A much more important determinant

---

[1] Appendix B, 'Seventh Annual Report of the Sanitary Commissioner with the Government of India,' Calcutta, 1871.

seems to lie in the reaction of the medium, an acid reaction repressing, and an alkaline one favouring, their presence and development. At the same time, however, certain forms of alkalinity, associated with the excessive development of other organic forms, are almost as repressive as acidity; but, as a rule, there can be no doubt that alkalinity of the medium is one of the necessary conditions for their presence in any considerable numbers, and that any excessive acidity is most destructive to them.

This alone is sufficient to account for the extent to which their presence in the excreta in health has escaped notice. In health the excreta, as a rule, present a faintly acid reaction when perfectly fresh, but the degree of acidity increases so rapidly that within a short time the medium becomes quite unadapted to the requirements of the zoospores, which consequently die and disappear. Even where the materials, when fresh, are neutral, or, as is sometimes the case, exhibit a mixed reaction with alkalinity more permanent than acidity, the rapidity of the development of an intensely acid condition is very great, so that, unless examined at once, they may show no traces of zoospores. There is also another circumstance which must be regarded as probably accounting in part for the rarity with which these organisms have been detected in Europe, namely, that a depression in the temperature of the medium, as is the case with many other organisms, exerts a most rapid and prejudicial effect on them. During the hot weather months in India this influence exerts hardly any appreciable effect, but in the cold season it comes into play more or less distinctly.

While considering the subject of the influence of the condition of the medium on the vitality of the zoospores, it may be well to examine a little more closely the phenomena attendant on the decomposition of normal alvine excreta in this country. These phenomena exhibit a wonderful uniformity, as shown by the records of very numerous observations conducted at all times of the year, and at intervals of several years' duration. When exposed to rapid drying, comparatively little change beyond increased acidity has time to take place. When, on the other hand, the materials retain their moisture, as, for example, when they are reserved in an isolated moist chamber, a definite series of phenomena manifest itself with great regularity. The first change appreciable consists, as before said, in a very rapid increase in acidity, so that the material, after the lapse of twenty-four hours, shows an intense and permanent acid reaction. This condition is associated with a change in the colour of the basis, specially when exposed to the air, a darkening and reddening being more or less distinctly manifested. At the close of forty-eight hours the material is intensely and permanently acid. If the surface be examined closely at this stage, it will almost invariably be found to be covered with numerous short, erect hyaline points, which on microscopic examination are resolved into filaments of *Oidium lactis*, beginning to break up into conidial segments, and arising from a series of elongated horizontal tubes traversing the superficial portion of the basis (Fig. 1). Twenty-four hours later the surface is universally covered with a thick shaggy grey coating consisting of dense masses of conidia.

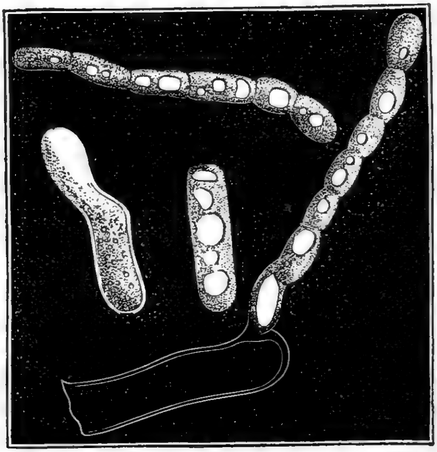


FIG. 1.—Filaments and cells of Oidium × 1000.

The following are the notes recorded in reference to these phenomena in one case, which may be taken as typical of the normal course under similar conditions. A portion of perfectly fresh normal alvine excreta was placed in a carefully cleaned capsule in a moist chamber at 12 noon. The reaction of the material was distinctly acid. Microscopically, it consisted of the usual elements. Twenty-four hours later it had acquired a reddish tinge, and the acidity was greatly increased. After another interval of twenty-four hours it was covered by a delicate whitish bloom, due to the presence of myriads of short, erect, projecting fungal filaments, which on microscopic examination were found to present the characteristic features of young conidial filaments of *Oidium lactis*. The reaction was now violently acid. The average breadth of the fungal elements was 5 μ. Many of the filaments were of considerable length, and showed no traces of division; while in others, all stages of that process were clearly manifested, and numerous free conidia represented results of its completion. After separation the conidia seem, as a rule, to become broader, and their extremities, which are at first in many cases more or less truncate, assume a rounded convex outline. In some cases the filaments appear to divide dichotomously at the extremity. The superficial layers at the basis were full of long, horizontal, sparsely-branched filaments, from which short vertical twigs arose, became aerial, and ultimately split up into conidial segments. After another interval of twenty-four hours the reaction of the basis was transiently but distinctly alkaline, and the surface was clothed with a thick, shaggy, greyish-yellow coating of curious gelatinous, acutely conical tufts, composed of dense masses of oidial conidia and filaments. Most of the conidia were very short and broad, many being nearly spherical. They were full of dense shining protoplasm, only showing at utmost one or two minute vacuolar spaces. On being sown on a suitable medium, they rapidly germinated, undergoing a great increase in size, accompanied by extensive vacuolation ere doing so. The short rounded conidia measured from 9·4 × 6·1 μ to 6·5 × 6·0 or 5·5 μ. The longer joints measured 23 × 5·5 μ, and all intermediate forms connected the two series with one another. On the following day the reaction of the basis was distinctly and permanently alkaline, and no farther development of fungi occurred in it.

That the Oidium in this and other cases owed its origin to fungal elements intrinsic to the basis, and not to extrinsic ones accidentally introduced from without, was proved by the following facts :—1*st*. *Oidium lactis* is not a form which tended otherwise to occur spontaneously in any of the localities in which the experiments were conducted. 2*nd*. Boiling the excreta previous to isolation was invariably followed by a failure in the appearance of the fungus, and this not as the result of any change causing them to become an unsuitable medium for it, as an abundant crop appeared as usual on introducing oidial elements artificially. It cannot, moreover, be assumed that in the experiments on the effect of boiling, the excreta were originally fortuitously an unsuitable medium, as check experiments were tried with unboiled portions of the same material, which constantly resulted in the occurrence of the normal development. That the phenomenon is not one dependent on casual peculiarities of a particular season was shown by its uniformity at intervals of several years' duration. Besides the experiments on a large scale in ordinary moist chambers, others were tried in which minute fragments of the material were hermetically sealed in wax cells, and the sequence of events in these cases was precisely of a similar nature. There can, I think, be little doubt that the digestive canal in man in this country normally contains the reproductive elements of *Oidium lactis,* just as that of the cow normally contains those of *Pilobolus crystallinus* and other stercoreous fungi.

The development of the Oidium is, as we have seen, coincident with a great increase in the acidity of the basis, and the question naturally suggests itself, how far the two phenomena are causally connected, and how far the increased acidity is due to a fermentive action dependent on the growth of the fungal elements. That it is partially—but only partially—dependent on this appears to be clear from the result of a series of experiments in which neither Oidium nor any other mould-fungus was developed, and in which, at the same time, a distinct but temporary increase in acidity sometimes manifested itself. The notes recorded regarding one of these cases are as follows:—A portion of fresh acid alvine excretion was boiled and set in a moist chamber. Twenty-four hours later there was a distinct increase in the degree of acidity. On the following day it exhibited a mixed reaction, being faintly and transiently acid when first applied to the test paper, and the acidity passing off and being replaced by permanent alkalinity on drying. On the next day all trace of acidity had disappeared, and a permanent and distinctly alkaline condition was present. In other cases, however, and these constituted a great majority, the reaction at the close of twenty-four hours either remained unaltered, or indicated an increase in alkalinity, and after forty-eight hours' reservation an alkaline condition was almost invariably strongly pronounced.

The increase in acidity never approached in degree that associated with the development of Oidium, and the phenomenon, where present, may, I believe, be regarded rather as an evidence of diminished manufacture of alkaline products than of any positive increase in acid-formation. The reasons for this belief are the following:—The appearance of alkalinity in the materials, whether boiled or unboiled, is associated with an enormous development of bacterial elements. During the stage of acidity normally coinciding with the development of Oidium, bacterial development seems to be suppressed or very greatly retarded, and it is only when the fungal development ceases that it comes actively into play. Prolonged boiling also causes an immediate suppression of bacterial development for the time, and at the same time permanently suppresses the oidial elements. If, then, any volatile acid or alkaline elements are originally present, a development of either acid or alkali may seem to occur, due really to alterations in the relative proportions of the products incident on the escape of volatile compounds and not on any increased formation.

As noted above, while the suppression of fungal elements by boiling is complete and permanent, an abundant development of bacteria almost invariably afterwards occurs, even in cases where the greatest precautions are taken to secure the exclusion of extrinsic elements. The following notes were recorded of the phenomena in one case of this kind :—A portion of normal excreta was boiled for half an hour, and set whilst boiling in a moist chamber. This remained closed for forty-eight hours, and the specimen was then examined. The reaction was alkaline, and the surface of the medium was found to swarm with minute active bacilli (Fig. 2). On the following day the majority of the bacilli had passed into the still condition, and formed a thick, grey, creamy layer covering the surface. The individual rods measured about 3·5 μ in length. They were either scattered singly or were associated in series of two, three, and sometimes of four. Their breadth was about 0·92 μ. Many of the still ones had already passed on into spore formation, and in doing so seemed to become somewhat shorter and thicker (Fig. 3). Subsequently the whole of them assumed this condition, and ultimately the bacillar coating was replaced by a thick gelatinous layer of the free spores. No traces of *Oidium* or of other fungi ever manifested themselves.

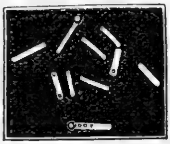


FIG. 2.—Excretal Bacilli × 1000.

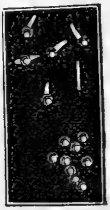


FIG. 3.—Bacilli passing into spore formation × 1000.

During the great increase of acidity occurring coincidently with the development of Oidium the zoospores rapidly become motionless, disintegrate, and disappear. The rate at which they do so is curiously rapid. Frequently within an hour a portion of the material, which at first showed numerous characteristic and active zoospores, retains no recognisable traces of their presence, and in some cases a period of ten minutes is sufficient to produce most marked changes. That this effect is not to be ascribed to change in temperature of the medium is demonstrated by the fact that when isolated portions are kept saturated with suitable fluids of an alkaline nature, such as the fluid of choleraic excreta or solution of cow-dung, the zoospores retain their activity, and even increase considerably in number, due to processes of division, for hours and even for days. This was most clearly shown by a prolonged series of experiments, in which the phenomena in such saturated portions, isolated beneath cover-glasses, were compared with those occurring in the material from which they were derived when left to undergo the normal course of changes.

A certain degree of concentration of the basis seems also, in most cases, to be essential to the continued life of the zoospores, as while those which remained in the interspaces between the solids of the basis continued in uninterrupted activity, others which found their way by their own movements, or by the action of currents, into the peripheral fluid of the preparations, as a rule, rapidly went through the series of changes previously described, and passed on into disintegration. The changes occurring in the natural basis seem to be completely fatal to the zoospores, as no reappearance of them was ever observed to occur after the medium had passed on into the alkaline condition, though, as we shall subsequently find, it is then thoroughly adapted to them when artificially introduced.

The zoospores are not the only infusorial organisms which are prejudicially affected by the initial fermentive changes occurring in the excreta, for the amœboid bodies and the bacteria are similarly affected. Leaving the effects produced on the former for future consideration, it may be well here to examine those in the case of the bacteria a little more closely. The first point to note regarding them is that the phenomena differ from those observed in the case of the zoospores. There is no evidence here of any complete destruction of the organisms. There is merely a temporary suppression of development succeeded by excessive activity of it. The phenomena are parallel to those occurring as the result of prolonged boiling of the medium. Whether, however, we are to regard the subsequent development as due to renewed activity in preformed bacterial elements which have merely passed into temporary inaction due to the state of the medium, or whether we are to suppose that these are destroyed, and are to regard those subsequently appearing as the product of spores, remains an open question. In any case, while there is no reappearance of zoospores, an excessive development of bacterial elements invariably succeeds that of *Oidium.*

While discussing questions relative to the occurrence of bacteria in the excreta, it may be noted that the results of the present series of observations are entirely opposed, in certain respects, to those at which Nägeli appears to have arrived in Europe. In his work 'Die niederen Pilze in ihre Beziehungen zu den Infections-krankheiten und der Gesundheitspflege,' he affirms that, although bacterial elements are constantly present in very large numbers within the digestive canal, they are invariably inactive, and on this view he accounts for the absence of ill effects coincident with a constant source of infection of the system at large, by what he regards as pathogenic agents. He affirms that it is inconceivable that bacteria should enter the system from the digestive canal—"*weil sie nämlich im Magen und im Darmkanal zuerst durch die freien Säuren dann durch die Salze der Galle geschwächt und bewegungsunfähig gemacht sind.*" This statement can, I believe, only be founded on general principles, and not on actual observations, unless, indeed, the latter give very different results in Europe from what they do in India. In India there can be no doubt that the lower portion of the intestinal canal very frequently, indeed, normally, contains very large numbers of active bacteria. After becoming acquainted with this very sweeping statement of Nägeli's I made an extended series of special observations on this point. The results arrived at were as follows:—A very large proportion of the alvine excretal matter, on its escape from the body, is composed of immense accumulations of bacteria. In very many cases these are in active motion, and in others begin to move at once, whenever a suitable fluid is employed to dilute the basis. Movement is not confined to cases in which the reaction of the basis is alkaline or neutral; it very frequently is present coincidently with distinct acidity. Fluidity of the materials naturally favours the movement, but the capacity for active movement is in very many cases merely concealed, and not absent when the basis is much concentrated, as is clearly shown by its immediate occurrence on dilution. Depression of temperature of the basis causes temporary cessation of activity, as demonstrated by experiments conducted when the air temperature was comparatively low, in which repeated disappearance and reappearance of movement occurred coincidently with depression of the temperature of the material below that of the body and its subsequent elevation to it, the movements referred to being not, of course, mere molecular movements, which might be ascribed to movements established in the fluid, but active darting progressive ones. The addition of water to the basis at once causes an abolition of movement.

It must, I believe, be due to the effects of depression of temperature and employment of unsuitable media for dilution that active bacteria have been asserted not to be present within the intestinal canal. In certain portions, at all events, of the intestinal canal they are almost invariably present in great numbers in an active condition, and the belief that an incapacity for movements prevents their entrance into the system, therefore, falls to the ground.

Even where present in great numbers and extreme activity, a total disappearance of movement in the bacteria coincides with the development of acid coincident with the appearance of *Oidium*. Only when the latter has matured, and coincidently with the appearance of alkalinity, do active bacteria again present themselves. When once they begin to appear, however, their development goes on with intense activity, and quickly runs through its various stages to the formation of the so-called "spores."

The amœbal organisms occurring in the excreta remain to be considered. Like the zoospores, they occur in the excreta during health, as well as in cases of cholera and other morbid conditions affecting the intestinal canal. Their presence seems to be determined by the same conditions as those regulating the presence of the zoospores; only, due to the readiness with which they assume an encysted condition, and thereby are enabled to resist the influence of detrimental conditions (Fig. 4) they may possibly be rather more constantly detected in one or other form than the other bodies are. Owing to their having assumed an encysted state, they may be recognised in media where they could not maintain activity, and even for considerable periods in such as have proved fatal to them, the strong capsule of the cyst preventing the content-protoplasm from undergoing disintegration for some time after its vitality has been destroyed. Due to this, in examining excreta for amœbal organisms, we must be prepared to recognise both still and active conditions, and in regard to bodies representing the former, it is necessary to guard carefully against mistaking them for oily particles, or *vice versâ*.

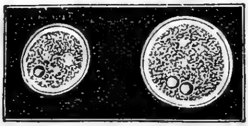


FIG. 4.—Encysted excretal Amœba × 1000.

In both the active and encysted condition they exhibit great variations in size, the variation in this respect being specially marked in active specimens, as in different media and at different times in the same one they not only seem to vary in absolute bulk, due to differences in nutrition, but they also vary in the form and extent of the pseudopodial extensions of their substance. In some cases isolated thick pseudopodia alone are emitted from the more on spherical body (Plate XVIII, fig. 19), while in others this condition is exchanged for one in which the body is spread out in all directions into an irregular, constantly changing protoplasmic flake. Between these two extremes a connecting series of intermediate forms exists, and the transition from one to the other through these can frequently be observed to take place in individual specimens. In the encysted condition, when their form is more or less spherical or elliptical, they frequently attain a diameter of 25 μ or even more, and they may range downward from this until the diameter only amounts to 8 μ.

The body-substance is sometimes almost homogeneous, at others more or less distinctly granular, due seemingly to the presence of extraneous nutritive matter. Changeable vacuoles, often of considerable size, may or may not be present; but a true contractile vesicle seems to be almost always, if not invariably, wanting so long as they are retained in the original medium.

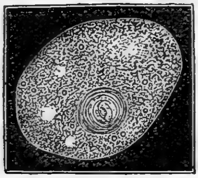


FIG. 5.—Large nucleated excretal Amœba × 1000.

As in the case of the zoospores, considerable variation exists in regard to the presence of a defined nucleus. In some cases no recognisable traces of such a structure are present, but in others a permanent clear nuclear area is visible. This may or may not include an evident nucleolus (Fig. 5). When present, the latter may attain a diameter of 7 to 9 μ. It is circular and apparently discoid, but in some cases may appear annular from the presence of a thickened margin.

The degree of movement which the Amœbæ present in different cases varies greatly. In some the movement is extremely energetic, the body forcing its way rapidly between the surrounding masses of *debris*. In others it is confined to changes of form and slow emissions and retraction of protrusions without change of place, and in still others the only signs of it present are the gradual appearance and disappearance of vacuoles, or other indications of content-change. Any direct multiplication of Amœbæ by division does not appear to occur, or if it do so, must occur with extreme infrequency, as, though carefully watched for in very many cases, it was never seen to take place.

In many normal excreta in which Amœbæ are present in considerable numbers, they are all in a state of inactivity, and more or less completely encysted. In such cases they may frequently be roused to activity by the addition of suitable media which are found in the same liquids which have been already indicated as adapted to the zoosporic bodies. Even when seemingly most distinctly encysted no trace of an envelope is left behind on the emergence of the Amœbæ, the cell-wall apparently undergoing complete resolution during the process. When they have emerged, the Amœbæ do not exhibit such extreme susceptibility to changes in external conditions as the zoospores do, for they may often be seen to make excursions in the peripheral zone of nutritive fluid in diluted preparations without showing any symptoms of immediate detriment. In some cases the still and encysted Amœbæ present in the excreta cannot be roused to activity by an addition of nutritive fluid.

Like the zoospores, the Amœbæ are very rapidly affected by the changes normally occurring in the excreta after their exit from the body. The rate at which this occurs is, perhaps, not quite so rapid as in the cases of the zoospores, but the final result, in so far as the vitality of the organism is concerned, is the same. With the increased acidity and the development of *Oidium* all activity ceases and the organisms either encyst or break up and disappear. When encystment occurs they remain for long recognisable in the medium, and may often be detected in the latter even after the acid fermentation has run its course, and has been succeeded by the alkaline one. So far as vitality is concerned, the result is the same, however, whether encystment occur or not. The acid stage is fatal to them, and they never revive with the development of the alkaline one. As in the case of the zoospores, so with the Amœbæ, no reappearance ever seems to take place in excreta which have passed through the acid fermentation, unless due to the introduction of extraneous germs, and this although the medium, when once it has become alkaline, is eminently suitable to them.

In addition to those which can be recognised as encysted or still Amœbæ, there is another class of bodies frequently present in the excreta which were for long a subject of investigation ere their true nature could be determined. These bodies are, I believe, identical with certain of the bodies long ago observed by Drs. Swayne and Brittan,[1] and subsequently described by Professor Hallier as spores in his celebrated treatise on the

---

[1] "Account of Certain Organic Cells peculiar to the Evacuations of Cholera," 'Lancet,' 1849, pp. 368—398; 'London Medical Gazette,' 1849.

fungoid origin of cholera.[1] They consist of spherical or elliptical cells of various sizes, ranging from 3·5 to 9·2 μ in diameter, and frequently characterised by the brightly refractive oily appearance of their contents (Plate XVIII, figs. 20, 21). This latter character is not by any means an invariable one, however, for in other cases they are finely clouded or molecular, and with more or less distinct vacuolation; and the passage from the one condition to the other may readily be observed to take place specially under the influence of changes in the nature of the medium. They appear to consist of a very delicate membranous sac enclosing a mass of varying content-matter. They may either occur scattered singly through the basis or may be associated in groups, and, in the latter case, may sometimes be observed to be connected with one another by a delicate gelatinous, intercellular basis, which, I take it, represents the structure described by Hallier as the sporogenic cyst (Plate XVIII, fig. 20). As a rule, their occurrence is associated with that of zoospores and Amœbæ, but in some excreta they are present apart from such bodies. The number present in the excreta during health varies very considerably. In some cases of intestinal disorder they are present in increased numbers, but never apparently are they so very abundant as *in certain* cases of cholera.

In the normal excreta in health they are very transitory, disappearing like the zoospores very rapidly with the increasing acidity of the medium, so that specimens which when quite recent showed an abundance of them may, within the course of twenty-four hours, retain no traces of their presence. Like the zoospores, too, they are very susceptible to the influence of other changes in their surroundings, rapidly disappearing when they happened to be washed out into the ring of nutritive fluid surrounding the thicker portion of a preparation. Owing to their rapid disappearance from the unmixed excreta, it is hopeless to attempt their continuous investigation without the aid of suitable nutritive media; and of these, that which I have found to act most satisfactorily is the solution of cow dung which has been already mentioned as adapted to the requirements of the zoospores and Amœbæ. Under the influence of this they may often be preserved for several days, and continued observations of various developmental changes occurring in them may thus be carried out. The results of such cultivations seem to me to have clearly shown that these enigmatic cells are reproductive bodies belonging to the Amœbæ, and forming a connecting link between these and the zoospores.

A suspicion that they really were products of reproductive processes occurring in the Amœbæ was originally aroused by

---

[1] 'Das Cholera Contagium,' Leipzig, 1867.

certain cases in which the fresh excreta contained Amœbæ, within which varying numbers of bodies, indistinguishable from them, were present (Fig. 6). As, however, the amœbæ were in some cases still active, and only differed from their compeers in not being provided with a distinct nucleus, it appeared at first very doubtful whether the phenomenon was not due rather to the ingestion of extraneous bodies than to any process of intrinsic development; although, on the other hand, it seemed strange that in isolated cases such an ingestion should have occurred simultaneously in numerous Amœbæ, while in the vast majority of cases in which the latter coexisted with the sporoid cells, no evidence of the occurrence of any such process presented itself. Further observations appeared clearly to show that whatever interpretation ought to be put on the above described phenomenon, the sporoid cells really are developed from the Amœbæ. The process of formation normally occurs coincidently with the cessation of activity in the parent, so that it is possible that in those cases in which sporoid cells were present within active Amœbæ, they may have been derived from without. As, however, the preparations in which the phenomenon was observed had been treated with nutritive fluid, it is quite possible that it was due to an abnormal resumption of activity in Amœbæ which had passed into the preliminary stages of reproductive multiplication. The phenomenon may, in fact, have possibly been parallel to those observed in the sclerotia of the Myxomycetes under the influence of favorable nutritive conditions. The plasmodia of the latter organisms, in passing into the sclerotial state, break up into a multitude of distinct spheres, each of which is capable of independent activity, and of emerging as a distinct amœboid body when separated from its neighbours and introduced into a suitable medium, but which may also melt together to reform a common plasmodium when the sclerotium, as a whole, is exposed to conditions favouring its activity. As the whole of the body-substance of the Amœbæ is not expended in the formation of the sporoid cells, a portion remaining in the form of a common gelatinous investment, and as the latter, cer-

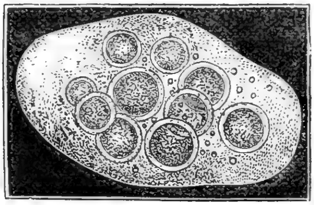


FIG. 6.—Large excretal Amœba containing sporoid bodies × 1000.

tainly in some cases, retains a certain degree of contractility and capacity of altering its form for some time after the formation of the reproductive cells has begun, I am inclined to regard the latter explanation of the phenomena in these exceptional cases as the true one.

The process of spore formation is not preceded by any true encystment; the parent body merely loses its active progressive movements and form-changes, and a very delicate surface layer becomes, in some degree, differentiated upon it. The content-substance within this next begins to show a constriction, dividing it into two lobes, and a gradual extension of the process ends in the separation of these as independent masses (Fig. 7). The entirety of the material of the parent does not, however, as before mentioned, seem to be expended thus; but a varying amount remains as a gelatinous intercellular matrix, which blends with the surface layer (Pl. XVIII, fig. 20). Under favorable circumstances, each of the daughter bodies originally formed, as described above, in its turn divides into two, and in this way groups of sporoids, containing large numbers of individual cells, may be actually observed to arise in the course of forty-eight to seventy-two hours (Fig. 8). Each of the bodies thus formed by division acquires a delicate investing layer, but the process of

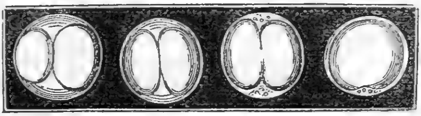


FIG. 7.—Formation of sporoids × 1000.

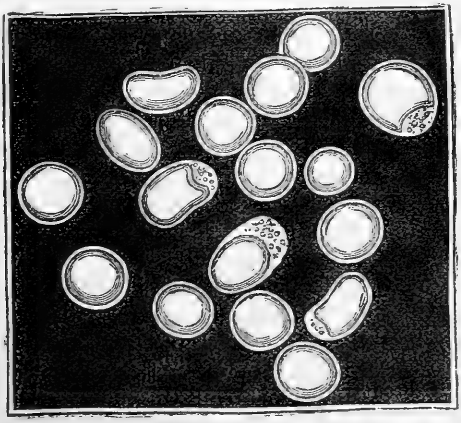


FIG. 8.—Group of sporoids developed from an Amœba × 1000.

division may undergo arrest at almost any stage, and fusion of the partially differentiated bodies may then take place. The extreme variation in the size of the sporoids in different excreta thus finds an easy explanation in the processes by which they are formed. Where the processes of formation go on undisturbed in the medium, as, for instance, where the development occurs in specimens beneath a cover glass, the sporoids remain aggregated in groups embedded in their gelatinous bases, and exactly resembling those described and figured by Hallier. Due, however, to the tenuity of the matrix, they are readily detached from one another, and scattered under the influence of slight mechanical disturbances, and hence in the excreta in their nutural condition it is rare to encounter any save isolated individuals.

The sporoid cells thus arising by processes of division within the Amœbæ, we have next to inquire into their subsequent history. In cultivations of excreta, in which their development has been followed thus far, no further vital change appears to take place within them. The medium, sooner or later, seems to become unsuited to them, and they disintegrate and disappear. It is different, however, in the case of spores which have been developed within the intestinal canal of the host, for many of these, when exposed to favorable influences, appear to give origin to zoospores. The phenomenon of the origin of the latter may frequently be observed in specimens of fresh excreta, which have been treated with nutritive fluid. Under such circumstances media, which at first contained an abundance of sporoid cells and no zoospores, may within a few hours be found to contain hardly any of the former and numbers of the latter; the proportion of zoospores present at the close of the observation being in direct relation to the numbers of sporoids originally present and the proportion in which they have subsequently disappeared. It is difficult precisely to follow the stages in the process, as it only takes place in the thicker portions of the preparation—the sporoid cells, as before said, being rapidly destroyed when washed out into the surrounding fluid—but the cell wall of the sporoid seems to become gradually softened and absorbed, and does not remain behind as distinct cyst. It has unfortunately never been possible continuously to follow out the transition of any individual Amœbæ into a mass of sporoid cells, and the resolution of the latter into zoospores. Amœbæ have been seen to give rise to sporoids in some cases, and the origin of zoospores from bodies apparently in every respect indentical with these has been observed in others, but a link is still wanting in order to render the observation quite complete. In spite of this, however, there can, I believe, be little doubt that the

Amœbæ, sporoid cells, and zoospores really constitute stages in one cycle of development, more especially when certain observations, an account of which will be met with farther on, are taken into account. Any direct origin of Amœbæ, from characteristic sporoid cells in human excreta, does not seem to take place.

Allowing, then, in the meantime, that the characteristic zoospores, Amœbæ, and sporoid cells occurring as parasites within the human digestive canal are all members of the developmental cycle of one specific organism, how can we account for the extreme frequency with which they are present? Taking the very great susceptibility of the organisms to the influence of external conditions, and the fact that the media in which they escape from the body seem, as a rule, to undergo changes certainly fatal to them, it appears at first sight very difficult to do so satisfactorily. We might, indeed, take refuge in the supposition that, after the germs have once obtained an entrance, they remain persistently within the body, giving rise to constantly recurring generations of the parasite, but such an hypothesis is hardly consistent with the fact that in the case of a given individual they may appear suddenly after considerable intervals of apparently entire absence, and after persisting for varying periods may again vanish, only to reappear as before at a later period. We should, therefore, be compelled farther to assume that periodical retentions, either of the germs or of the developed organisms, take place somewhere within the body, and alternate with uncertain periods of discharge, or that their appearance and disappearance from the excreta is determined solely by conditions in the latter allowing or preventing their persistence in the contents of the lower portion of the digestive canal.

While allowing the possibility of such explanations, I do not regard them as correct, but believe that the appearance and disappearance of the parasitic forms are due to the successive introduction of extraneous elements and the subsequent discharge of the result of their development. It is as difficult to give a definite opinion as to the precise source of these organisms as it is to state whence the oidial and bacterial elements of the intestinal contents are derived. They are, as has already been pointed out, almost constantly present in varying numbers in the intestinal canal, and are in all probability introduced with ingesta of various kinds.

II. *The Intestinal Monads and Amœbæ of Cows and Horses.*

It is now more than five years since, whilst studying the development of *Pilobolus crystallinus,* I first encountered what it appears may be regarded as the perfect fructifying or reproductive bodies of these intestinal organisms. In a specimen of recent cow dung, which had been reserved in a moist chamber for twenty-four hours, the surface was found to be studded with a multitude of minute glistening white spherules, adhering to projecting points of the basis (Pl. XVIII, fig. 1). At first sight these were regarded as basal dilatations of *Pilobolus,* in which an abnormal suppression of colouring had occurred, but on submitting them to microscopic examination this was found not to be the case. They were entirely unconnected with the mycelial tubes of the fungus which subsequently produced an abundant crop of normal fructification, and did not resemble the basal dilatations in structure, consisting of a membraneous sac crowded with spore-like bodies. These were circular, flattened, and biconcave, closely resembling red blood-corpuscles in general appearance. On being introduced into a solution of cow dung they rapidly became spherical, a contractile vesicle appeared within them and began to pulsate, and they sooner or later, as a rule, gave exit to minute amœbal bodies, which crawled off freely in the fluid, generally leaving a delicate cyst behind them in doing so. In other cases, however, in place of being resolved into Amœbæ, they appeared to give origin to flagellate zoosporic bodies. Similar phenomena were observed at various subsequent periods, and the sporangic bodies being not unnaturally regarded as representing some low Myxomycete form, a repeated but futile search was made for the presence of plasmodia corresponding with them. Subsequently the appearance of these sporangic bodies came to be recognised as a normal and almost invariable event in specimens of cow dung reserved for the study of stercoreous fungi. The essential condition ensuring their appearance seemed to be that the basis should have been secured and set for cultivation whilst still quite recent, older samples almost invariably failing to produce a crop, or only producing a very scanty one. As the result of numerous experiments, it was ascertained that the appearance of these sporangia preceded that of any other form of fungal fructification, occurring, as a rule, within twenty-four or forty-eight hours from the commencement of a cultivation of perfectly fresh material, and being succeeded by that of various fungi in the following order:—*Pilobolus crystallinus, Ascobolus* sp., various species of Gymnoasci, *Peziza* sp., *Coprinus* sp. These fungi may be regarded as the regular and almost invariable results of the cultivation of fresh cow dung in this part of India, while occasionally other forms are interpolated in the series, as, for instance, a *Syncephalis,* which sometimes attacks the *Pilobolus.* Taking the normal series of developments, the sequence of events is shown in the following table:

Sequence and Periods of Development of Fructifications appearing on Fresh Cow Dung.

White sporangia	after 24 hours.
Pilobolus crystallinus	,, 72 ,,
Ascobolus sp.	about 5 days.
Various Gymnoascal forms	,, ,,
Peziza sp.	,, 6 ,,
Coprinus sp.	,; 3 weeks.

Further investigations showed that the occurrence of these sporangia was not limited to cow dung, but also frequently occurred on horse dung too, where, as on the other medium, it preceded that of *Pilobolus*, or other forms of mucorine fungi.

Even after the study of these sporangioid bodies was specially undertaken, it was not until after many months of continuous investigation that their true nature and mode of origin were satisfactorily determined, and that their relation to organisms, seemingly identical with the parasitic zoospores and Amœbæ of the human excreta, was ascertained. In studying the developments normally occurring in reserved specimens of cow dung as compared with human excreta, one of the most conspicuous differences presented by the media is that the former has no tendency to pass through the acid fermentation so constantly affecting the latter medium. Perfectly fresh specimens are either neutral or faintly alkaline in reaction, and when kept under observation exhibit a constant progressive development of alkalinity, so as to become strongly alkaline within twenty-four or forty-eight hours—a condition which they retain for indefinite subsequent periods. Another point distinguishing vaccine from human excreta lies in the relative amount of bacterial elements originally present in them; for while, as we have already seen, a very large proportion of the mass of the latter medium is formed of these elements, the proportion of them present in a developed form in the former appears normally to be very small. Farther when the medium follows a normal course in reference to the organic developments occurring in it, there is at no period that excessive multiplication of bacterial elements so characteristic of the later stages of decomposition in human excreta, the numbers and succession of fungal organisms appearing to a great extent to exhaust the nutritive properties of the basis. The two commonest forms of bacteria occurring in cultivations of cow dung are shown in the accompanying figures (Figs. 9, 10).

The evidences of exhaustion of the basis, in so far as concerns certain of the organic developments which have occurred in any abundance of it, is unequivocal, each of them appearing in its turn and then absolutely and permanently dying out. The phenomenon is specially marked in reference to the sporangioid bodies and *Pilobolus,* which, as we have already seen, are the first forms which make their appearance. Crops of sporangia are rarely produced for more than two or, at utmost, three days, and crops of *Pilobolus* for more than six or seven, and in this case it is only for two or three that the development is abundant. We have an enormous primary production of reproductive bodies and an absolute, or almost absolute, failure of any further development due to these. Where the primary crop has been abundant, it is very questionable whether the reproductive elements then produced ever germinate in the same medium in which they were produced; for although, as before said, successive crops of fructification appear for two or three days, these apparently all belong to one generation. This certainly is the case in so far as Pilobolus is concerned; there is no evidence of successive generations of mycelia, and the successive crops of fruit are in many cases visibly the result of unequal rapidity in the development occurring in basal dilutations contemporaneously developed. The phenomenon is clearly one of exhaustion of the nutritive basis, and not due to defective germinal energy or the reproductive elements; for we have only to transfer some of them to a suitable fresh basis to secure their immediate development. While in the primary basis we find the surface covered with masses of reproductive elements totally incapable of farther development, so long as they remain there, we have only to transfer a few of them to an unexhausted

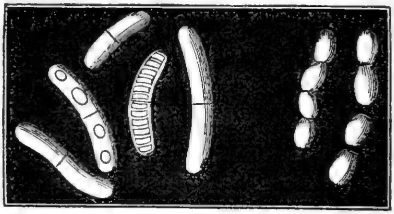


Fig. 9.—Large form of Bacterium common in cow dung × 1000.

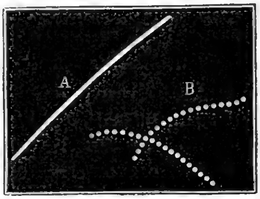


Fig. 10.—Slender Bacterium common in cow dung × 1000.

basis in order to secure an abundant production of a fresh generation. This is of course merely the parallel of what we find occurring in regard to the *Oidium* and bacteria of human excreta, where a solitary and excessive development of the organisms occurs, terminating in the production of innumerable reproductive elements incapable of germination until transferred to a fresh medium. In the case of cow dung, we certainly cannot ascribe the failure of excessive bacterial development to exhaustion by organisms of the same nature; but the variety and succession of other organisms which are developed may, perhaps, practically produce a similar result.

While fresh cow dung is relatively deficient in bacteria, it is by no means devoid of distinct organisms generally. On the contrary, we find it almost invariably containing a very large number of zoosporoid bodies (Pl. XVIII, fig. 17), and sometimes smaller numbers of other infusorial forms of various kinds. After prolonged study of the zoospores under various circumstances, I am unable to indicate any constant differences to distinguish them from those of human excreta. Like the latter, they exhibit numerous varieties in form and in character of movements, but none of these are peculiar to them. They seem also to be similarly affected by reagents artificially added to the basis, or by spontaneous changes taking place in it, and altogether there can, I believe, be no doubt of the identity of the organisms in both media. Although so constantly present, they may, like those of the human excreta, readily escape detection, unless suitable precautions are taken in preparing the specimens for examination. A dilution of the basis with pure water is frequently so rapidly fatal to them as almost entirely to conceal their presence, and even other solutions more favorable to them must be added with caution so as to avoid too abrupt a change of conditions. The best method of treating preparations, with a view to their detection, is to spread out a minute portion of the basis in a thin layer on a slide, then apply a cover glass, and (having first focussed a field containing a view of a portion of the margin of the layer) to introduce some strong solution of the same dung from which the specimen was procured, and which has been previously filtered, boiled, and allowed to cool. On doing this, the organisms may be observed emerging from the margin of the basis, and, after swimming actively in the fluid for a short time, gradually passing on into the series of changes described as occurring in those of human excreta under similar circumstances. Here, too, we find that those which, in place of emerging into the peripheral fluid, enter some of the interstitial spaces existing between the solids of the basis, retain their vitality much longer than their neighbours.

The number of zoospores which may be detected in this way is in many cases very remarkable. The size of individual specimens varies much, which is no doubt greatly dependent on the frequency with which processes of division recur in them. Very often they measure about 10 μ in length by 5 or 6 μ in breadth. The number of flagella with which they are provided also varies from one to three or four; whilst in full activity, neither nucleus nor contractile vesicle can be detected as a rule, and after treatment with Liquor Iodi they may or may not exhibit a nucleoloid particle. The latter reagent generally induces a peculiar series of phenomena. The body gradually loses its natural fusiform, or pear-shaped outline, and becomes circular and motionless. Shortly after it has ceased to move, a large vesicular protrusion is emitted at one or other point from the somewhat granular body, and general disintegration soon sets in. There is no evidence of any differentiated surface layer, and the flagella appear to be merely transitory and changeable protrusions of the protoplasm. The point opposite to the flagellar site seems to be that through which nutritive materials are absorbed, the body becoming attached to foreign particles by it, and being sometimes drawn out in consequence into a caudal process or filament of variable magnitude. All the characters which the zoospores here present are, in short, identical with those occurring in the human parasite. The processes of multiplication are also similar, consisting in transverse division preceded by diminution or temporary arrest in activity, and the phenomena attending diminished vitality and disintegrative disappearance follow the same course.

While, however, the human and vaccine parasites appear to be identical in nature, their presence in the excreta is followed by different results. In the case of the human parasites we have already seen that a rapid and complete process of destruction sets in coincident with the changes normally occurring in their medium after its exit from the body, but this does not hold good in the case of the vaccine parasite. That it does not is probably due to the absence of any fermentive change in the medium corresponding with the acid development coincident with the appearance of *Oidium*. In place of disappearing from the medium the organisms in the cow dung, after continuing to multiply by division for some time, seem to pass on to further stages of development through which they are enabled to give origin to reproductive bodies providing for the perpetuation and diffusion of the species, or where conditions are unsuited to this, to resting forms capable of renewed activity on again encountering favorable conditions. The fully developed reproductive bodies consist of the sporangia, which have been already mentioned, the resting conditions are represented by encysted zoospores or much more frequently by encysted amœboid bodies.

The following notes recorded in reference to the phenomena observed in a specimen of cow dung are generally typical of those in numerous other experiments of a similar nature. Some perfectly fresh cow dung was procured and set in a moist chamber at 10.30 a.m. The material was moist, faintly alkaline in reaction, and swarmed with large active zoospores. Five hours later a second preparation was taken from the specimen. In this an even greater number of active zoospores was present than in the first, and a certain number of still ones was also recognisable. A third preparation, procured seven hours later, showed no active zoospores but an abundance of still ones of oval and rounded form. After another interval of six hours a fourth specimen was taken and found to resemble the previous one in character save that a distinct contractile vesicle was visible in many of the cells. At this time (dawn) no signs of sporangia were visible, but a few hours subsequently they appeared in great abundance, while preparations of the basis showed an abundance of active amœboid bodies of various sizes, ranging from that of the still zoospores upwards.

Similar phenomena repeat themselves with monotonous uniformity in successive experiments. Again and again we find a basis abounding with zoospores; increase in the numbers of these bodies for some time; a cessation in their activity; the appearance of multitudes of bodies agreeing in size and form with the inactive zoospores, but characterised by the presence of a contractile vesicle; the emergence and growth of these as active amœboid bodies and the appearance of sporangia. That the latter are certainly the product of the union of the amœboid bodies is clear from the result of other observations, but, as necessarily is the case in all massive cultivations, the evidence connecting the zoospores originally present with the amœboid bodies subsequently appearing remains imperfect. That the relation is one of identity is, no doubt, rendered probable by the fact that the excreta in the fresh condition never show any proportion of amœboid bodies or of still cellules capable of accounting for the enormous numbers subsequently present, unless an excessively rapid multiplicative division were assumed to take place from the scanty supply originally present. An assumption of this nature is, however, entirely devoid of any support from observation, as any division of the amœboid bodies previous to sporangic formation never appears to occur. On the other hand, we have the zoospores present in abundance from the outset, and capable of very rapid multiplication by division;[1] we find that it is impossible to distinguish between resting zoospores and bodies which pass on into an amœboid state; and we know that the zoospores merely differ from Amœbæ in the character of the protrusions which they emit, and therefore in the nature of their movements. Allowing the identity of the two forms, we have a ready means of accounting for the regularity and abundance of the crops of sporangia and the general ratio of these to the numbers of zoospores, while rejecting it we have no explanation to give of the appearance of the multitudinous development of amœboid bodies.

In the endeavour to obtain more positive evidence on this point, hundreds of cultivations on a small scale were carried out with more or less satisfactory results. In some cases there appeared to be no doubt that the zoospores originally present became converted into Amœbæ at a later period, but the difficulty of attaining to absolutely certain results appears to be almost insurmountable. In the first place, in order to render any such cultivation susceptible of continuous observation, it is necessary to introduce conditions which we have already found to exert a most prejudicial effect on the vitality of the zoospores, for the basis must necessarily be diluted with some fluid in order to render the organisms visible. A condition of fluidity of the basis, too, independent of any actually destructive effects, certainly influences the occurrence of developmental processes in other ways. The persistence of zoospore forms may be prolonged, and the appearance of amœboid ones be delayed by an excess of moisture as may readily be proved by experiment. So again an excess of fluidity in the medium seems to be one of the agencies capable of causing amœboid bodies present in it to assume the encysted condition in place of passing on to the normal sporangic development. Another great obstacle to the satisfactory decision of this question lies in the excessive and constant movement of the zoospores, which renders it impossible to secure any individual specimen for continuous observation over a prolonged period.

All that can be positively affirmed is that the amœboid bodies which replace the zoospores primarily present *appear* to be directly derived from the latter, and that the two forms seem merely to represent different developmental stages of one and the same organism, connected with one another by the intervention of an inactive stage.

The Amœbæ, when they first appear, are of very minute size, ranging from 5 μ to 7 μ and upwards in diameter when in a spherical condition (Pl. XVIII, fig. 1). In some cases a

---

[1] Processes of division have been observed to recur in one body twice in the course of an hour.

contractile vesicle is at once distinctly visible within them; in others such a structure can only be detected with extreme difficulty, and in still others it appears to be absent. The same inconstancy seems to prevail in regard to the presence of a nucleus and nucleolus. Generally, I believe, a clear nuclear area is visible at a comparatively early stage, but a differentiated nucleolus often does not appear until much later; when it does appear it is as a flattened circular disc in the nuclear area. The rate of growth in the Amœbæ and the size ultimately attained ere the occurrence of sporangic formation varies very much, apparently in accordance with the nature of the medium. In favorable cases it is wonderfully rapid, and where the growth is considerable, it is usually associated with further development of the nucleus (Pl. XVIII, fig. 10). A division of the nucleolus occurs, and the resultant bodies move somewhat apart, so that a pair of greenish discs replace the originally solitary one. Farther than this the nuclear area seems to become differentiated from the rest of the body-substance by a boundary layer, and a cross partition of similar nature passes inwards to separate the nucleoli (Pl. XVIII, fig. 15).

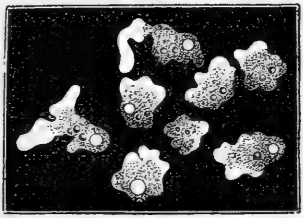


FIG. 11.—Cornuate Amœbæ × 180.

The characters of the movement also vary greatly in different specimens, and in one and the same specimen at different times. Sometimes it is of a free flowing character, the organism moving rapidly forward by means of successive protrusions of its substance. In other cases we find such movement alternating with a more sluggish action, in which the body presents an irregularly lobed or cornuate form, and only gives origin to limited extensions (Fig. 11). This condition frequently seems to coincide with defective nutrition, as the addition of fresh nutritive matter will often cause it to be exchanged for free progression. In still other cases again the body assumes a peculiar flattened scale-like condition, adhering by one surface to the glass of the slide, and moving forward with a slow gliding motion, accompanied with comparatively little change of form (Fig. 12). In such cases the free surface often shows curious linear markings due to the presence of longitudinal thickened ridges. Three distinct areas appear to be present in the body in this state; we have first a dense granular central portion constituting the substance of the ridges and the thicker portions; beyond this is a highly refractive area devoid of granules, and external to this again is a delicate tenuous layer of the protoplasm, often only distinguishable with difficulty from the surrounding medium. All the vital processes seem to be carried on in such cases with extreme slowness. The contractile vesicle dilates very gradually and often only undergoes imperfect obliteration on contraction, or it may remain absent for prolonged and uncertain intervals. In other cases it seems to be rigidly fixed in full dilatation. In appearing it sometimes is developed from a single centre, in other cases several minor vesicles appear and fuse into one as they increase in size. Granules of nutritive matter are ingested and frequently accumulate in spherical masses within vacuolar spaces filled with fluid. In other cases, however, they are irregularly diffused. When in full activity, a constant succession of fluctuating vacuoles is present in the body-substance. In some cases, and apparently connected with or preparatory to the resolution of the body into a collection of sporoid reproductive bodies—as it only occurs where they have ceased to move and have become aggregated in sporangoid masses—the large Amœbæ in place of showing at utmost two large nucleolar bodies as they normally do, contain from three to eight of smaller size (Pl XVIII, fig. 11). From the appearance and arrangement of these in different cases, there can be no doubt that the increased numbers are due to repeated binary division of the nucleoli originally present.

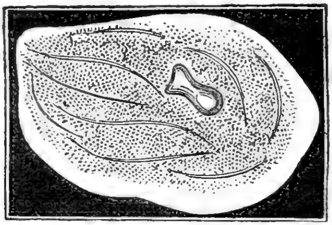


FIG. 12.—Amœba in epithelioid state with dividing nucleolus × 1000.

The size of the Amœbæ and nuclei varies so extremely in different cases and at different times that it is impossible to give any useful average measurements. They very frequently, when in an irregularly rounded condition, measure from 15 to 25 μ in diameter, with nucleoli, which, when paired, have a diameter of 3·5 μ, and when in larger numbers measure only half as much or under.

After continuing to progress through the medium for variable periods, the Amœbæ either cease to move, and remaining more or less isolated, become encysted, or becoming aggregated into masses give origin to sporangia. Where they are present in abundance, and where the conditions of the medium are unfavorable to sporangic development, the accumulation of encysted bodies on the surface often covers the medium with a fine greyish bloom, which, unless closely examined, may readily be ascribed to the presence of mycelial elements. The encysted bodies are either quite free or are associated in little groups and knots. As a rule, no further change appears to occur within them, and they remain unchanged for indefinite periods, ready to resume activity when favorable conditions again present themselves. In place of becoming encysted, however, we normally find thea Amœbæ, after some time, becoming more sluggish in their movements, and adhering to one another in pairs or groups of various sizes, the union becoming very intimate, and in some cases proceeding to such a degree of apparent fusion that we are only able to estimate the number of individual elements entering into the formation of a group by the number of nuclei or of rigidly dilated contractile vesicles which may persist (Fig. 13). This phenomenon is so far parallel to that occurring in the case of various Rhizopodous organisms, such as *Actinophrys sol*, &c., but the results following adhesion and union rather resemble those following the formation of Plasmodia in Myxomycetes, as the formation of the compound body is distinctly the antecedent to spore formation, the protoplasmic material becoming in greater part resolved into a mass of spores or reproductive cells, while a certain amount of it remains as an investing and intercellular

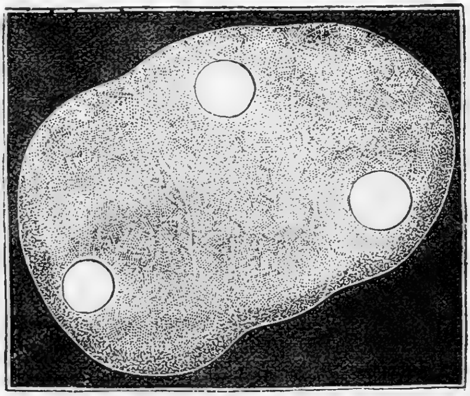


FIG. 13.—Compound body formed of three conjugate Amœbæ × 1000.

substance (Fig. 14). All the processes which have been just described can sometimes be observed to occur in slide cultivations beneath cover glasses, and capable of continuous observation; but under such circumstances they naturally never attain the magnitude and perfection exhibited under natural conditions where the sporangia are developed on an exposed basis of large bulk.

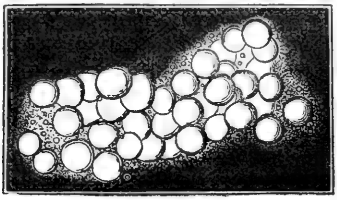


FIG. 14.—Amœbal mass breaking up into spores × 1000.

In such cases we may trace all stages of the formation of perfect sporangia from that in which we have mere irregular aggregations of closely adherent Amœbæ (Fig. 15), which on being detached and introduced into a new nutritive medium become resolved into their constituent Amœbæ (Fig. 16) by resumed activity of the latter, to that in which we have perfectly developed sporangia, with a distinct investing membrane, and even, in certain cases, an internal meshwork representing what may be regarded as a rudimentary capillitium. The degree to which an actual fusion of the constituents of the sporangic mass takes

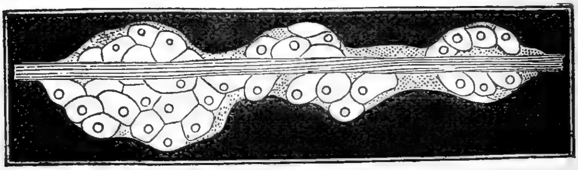


FIG. 15.—Irregular aggregate of Amœbæ × 180.

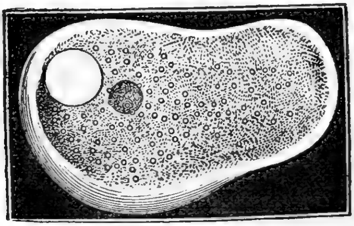


FIG. 16.—Amœba detached from rudimentary Sporangium × 1000.

place varies greatly in different cases and in different portions of one and the same specimen. Complete fusion often occurs in the basal portions of pedicillate sporangia, while the process is only partial higher up. In such cases while we find the stem consisting of a seemingly homogeneous mass, the head is generally more or less distinctly marked out into a series of irregular areas, which are in some cases so defined as to give the surface when viewed under a low microscopic power a somewhat granular or faintly nodulated aspect.

The appearances presented by the sporangic masses, where the constituent Amœbæ are yet distinctly recognisable, are very curious. The surface presents a strangely epithelioid appearance, due to the dense aggregation of irregular cells closely adapted to one another, so as to form a continuous layer, while on deeper focus we encounter sectional views of the interior, consisting of a dense mass of similar bodies (Pl. XVIII, fig. 3). We have a regular tissue formation due to aggregation and union of originally independent elements.

The sporangia vary greatly in size as well as in the extent to which a distinction between a stem and head is present. In many cases no stem formation takes place, and the spherical sporangium is merely attached at one or other point of its circumference, in others a pedicle of considerable length is present (Pl. XVIII, fig. 1). The heads may attain a diameter of 0·37 of a millimeter, and the pedicles a length of 0·25. When the pedicle is of any length it is usually dilated basally into a disc or into several root-like expansions, which embrace the body to which it is adherent. The sporangia are almost invariably situated on prominent projecting points of the basis, such as minute fragments of vegetable tissue, &c. When developing, they first appear as minute hyaline prominences or rods projecting on the surface of the basis. As their development advances they become dilated—the dilatation in the rod forms occurring terminally, and causing them to assume a capitate aspect—and at the same time an opalescence appears in the previously hyaline material. This increases in intensity and passes on to opacity, and the fresh mature sporangia are of a bright glistening whiteness, passing into various stages of yellow, buff, and amber, as drying sets in.

On examining many sporangia, even when the constituent Amœbæ are yet recognisable through more or less of their substance, the presence of a distinct investing membrane may be made out, and in all mature sporangia such a structure is invariably present. Owing, however, to variations in its structure under different circumstances, it is much more readily recognisable in some cases than in others, and may, indeed, sometimes readily escape detection in developing or recently formed sporangia, which have been kept in a very moist atmosphere; it is very soft, and quickly dissolves and disappears in water, although readily visible ere the addition of fluid, and especially so long as no pressure has been applied. In such cases, too, certain reagents, such as Liquor Iodi, readily demonstrate its existence. In older sporangia, which have undergone a certain amount of drying, it appears as a distinct, somewhat resistant, and very elastic membrane, of a yellowish colour. In structure it is finely molecular, and the external surface is covered with projecting organic corpuscles (Pl. XVIII, fig. 6). In the course of thorough desiccation again, it appears gradually to disintegrate and more or less completely disappear, leaving its contents exposed, and only adherent to one another by intercellular material. Its inner surface is sometimes distinctly mapped out by a series of prominent thickened ridges into polygonal areas corresponding with the formative Amœbæ (Fig. 17).

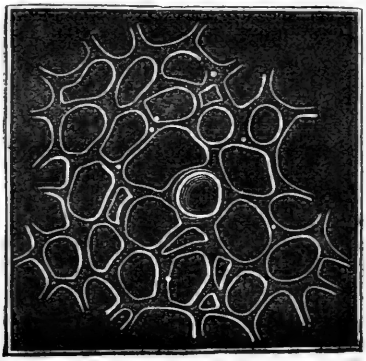


FIG 17.—Ridges and depressions on inner surface of Sporangial membrane × 1000.

After the sporangia have been, as it were, planned out by the aggregation and more or less intimate union of the Amœbæ, and the formation of an investing membrane, the process of spore formation normally sets in. When this is regularly carried out the bodies of the Amœbæ become resolved into masses of spherical spores, measuring from 5 to 9 μ in diameter. In cases where the fusion of the parent bodies seems to have been complete these are indiscriminately massed in the cavity of the sporangium embedded in an intercellular basis; where, on the other hand, the process has not gone so far they tend to adhere in groups of various sizes corresponding to individual Amœbæ, or to small groups of these. The intercellular material in recently developed sporangia is soft and seemingly more or less fluid, resembling the intercellular matter within Mucor sporangia. Like the material of the sporangial wall, however, it concretes or sets in drying, so as to appear in many preparations of partially dried sporangia in the form of a network, in the interspaces of which the spores are situated. The character and definition of this vary considerably in different instances, and in some cases it may be distinctly resolved into two series of meshes—a larger one, seemingly corresponding with the parent Amœbæ, and a smaller one with the individual spores (Figs. 18. 19). Both the sporangial wall and the intrasporangic network are, when fully developed, stained of a deep red brown by solutions of iodine, whilst the spores merely acquire a yellow tint. No blue colouration follows treatment with iodine and sulphuric acid, nor does any effervescence occur under the influence of acids.

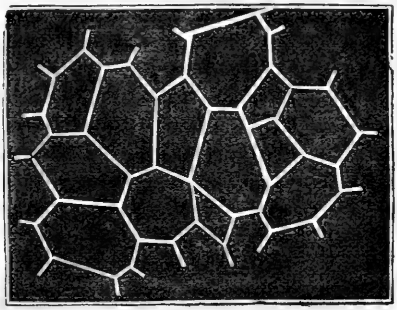


FIG. 18.—Large reticula of intercellular matrix × 1000.

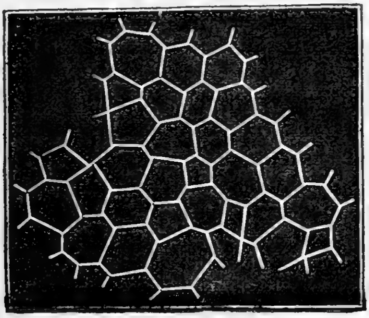


FIG. 19.—Fine reticula of matrix corresponding to spores × 1000.

The spores, as before mentioned, are, when first formed, of a spherical outline, or are, at all events, spherical when free, for, due to mutual pressure, they are frequently more or less polygonal while within the sporangium. The process of spore formation seems to be preceded by a disappearance of the nucleoli of the parent bodies, resulting, apparently, as some cases seem to show, from a process of repeated binary division (Pl. XVIII, fig. 11). As the sporangia mature and dry the spores lose their spherical form, a condensation of their substance seems to take place, and they become biconcave; when in this condition they closely resemble mammalian red blood-corpuscles, and, indeed, in many cases can hardly be distinguished when mingled with human blood. When in this state the margins measure about 2·7 μ and the central portions about 2·3 μ in thickness, the margin being of a faint greenish tint, and the centre almost colourless (Pl. XVIII, fig. 7). No evidence of the presence of a nucleus is, as a rule, present in such spores.

When a mature sporangium containing such biconcave spores is introduced into a suitable medium, the former very rapidly swell out and become spherical, and by their increased bulk exert a constantly increasing tension on the sporangial wall. The capsule ultimately ruptures at one or more points and contracts, forcing the spores out in streams and masses into the fluid (Pl. XVIII, fig. 2). Where the capsule has disintegrated and disappeared the process of swelling up is accompanied by curious writhing movements of the spore masses. A contractile vesicle soon makes its appearance in the spherical spores, which now, as a rule, show a clear central nuclear area surrounded by finely-clouded substance, and sometimes apparently containing a nucleolar particle of a greenish colour (Pl. XVIII, fig. 8 *a*). After the contractile vesicle has continued to pulsate for a short time the body begins to emit a delicate protrusion, and rapidly unfolds into a minute Amœbula, which crawls freely in the medium (Pl. XVIII, fig. 8 *b*, *c*, fig. 9). In many cases no evidence of any cyst is left behind, but in others, specially where the spores are derived from sporangia which have been subjected to prolonged desiccation, such a structure is present, appearing in the form of a delicate ring after the inmate has escaped. The spores then, as a rule, give origin to minute Amœbæ, but, in certain cases, in place of doing so, they appear to be resolved into flagellate zoospores, which swim off actively in the fluid.

The assumption of activity by the sporoids is manifestly influenced, both by the nature of the fluids into which they are introduced, and by the conditions to which they have previously been exposed. When introduced into ordinary pure or distilled water they remain unaltered for prolonged periods, and either fail entirely or in greater part to become active. A momentary exposure to the influence of boiling water, as by dropping the fluid on sporangia situated on a slide, does not prevent the subsequent development of the spores. The assumption of activity is retarded, but a certain number of the spores survive and subsequently give vent to Amœbæ. Prolonged boiling, however, is certainly fatal to them. They are capable of surviving a twenty-four hours' immersion in Liquor Iodi, remaining seemingly unaltered, and becoming active on the substitution of the reagent by a nutritive fluid. They can also survive immersion for several hours in 1 per cent. solutions of rectified spirit and of the pharmaceutical acetic acid. Mineral acids, even in very small proportions, appear to be fatal to them, and hydrochloric acid also immediately reduces any which are spherical to the biconcave form. The capacity for resisting various external influences is also regulated in some degree by the condition of the body. It is only the condensed biconcave spores which are capable of any decided resistance, those which are in the dilated spherical condition being much more susceptible to detriment.

Prolonged desiccation appears to influence the rate at which activity is developed, but certainly is not fatal. A careful series of experiments on this point showed that whilst Amœbulæ began to emerge from perfectly fresh spores within periods ranging from fifteen to twenty-five minutes, a gradual retardation of the process corresponding with different periods of desiccation manifested itself, so that, after a period of eighty-two days, emergence did not occur until within between five to twenty hours' exposure to favorable conditions.

When sporangia are introduced into preparations of fresh-boiled cow dung they rapidly disappear, and the cultivation within twenty-four hours, in favorable cases, shows an abundant new crop of sporangia. This process may be repeated again and again indefinitely so long as a fresh medium is supplied for each experiment; for, as in the case of the natural development, the soil appears to be exhausted in the process of producing a single crop. As a rule, in these cultivations we do not find a zoosporic stage represented, the spores at once giving origin to amœboid bodies, which, after having increased in size, become associated to form new sporangia. The crops of sporangia thus produced are, as a rule, peculiarly abundant and well developed as compared with natural ones, due, no doubt, to the comparative freedom which the organisms here enjoy from a struggle for existence. In some cases, however, in these artificial cultures we find a failure of development or a failure of sporangial formation, the surface becoming covered with a bloom of encysted Amœbæ.

Both in natural and artificial cultivations there is a distinct tendency to periodicity in reference to sporangial formation. In the notes regarding one case which were previously given, it is recorded that while at dawn the cultivation showed no traces of the presence of sporangia, an abundant crop of such bodies appeared within the course of the next few hours. This is merely an illustration of the fact that the development is regularly limited to the period between dawn and noon or at latest 1 p.m. If sporangia have not appeared by the latter hour they will not appear until the following morning. At first sight it appeared not improbable that light-conditions were the determinent of this phenomenon, but experiments proved that this was not so, for the development followed the same course even where all light was carefully and absolutely excluded.

The sporangia and spores described above are such as occur by far most regularly and may be regarded as the typical form of reproductive bodies in the organism, but certain other sporangial bodies occasionally accompany or replace them, which although differing in various particulars are, I believe, mere aberrant varieties determined by the coincidence of special conditions. In the first place, in place of those containing normal spores, only varying within the limits as to size and form ordinarily encountered, we also meet with sporangia which, in addition to normal spores, contain a greater or less proportion of irregular, unformed looking bodies (Fig. 20).

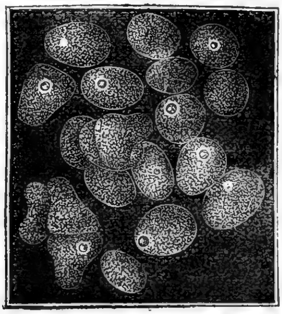


Fig. 20.—Abnormal forms of spores × 1000.

These, as a rule, are of larger size than the others, but are connected with them by a series of intermediate forms, and exhibit a precisely similar series of developmental changes in passing into a state of activity. Sporangia in which such bodies abound are frequently of an irregular form, and in some cases may assume a dendritic character, appearing in branched tufts which may attain a height of 1·5 mm. and a breadth of 2 mm. (Pl. XVIII, figs. 4, 5); in other cases either in association with these ill-formed spores or in normal sporangia, isolated encysted Amœbæ may be present, or bodies which resemble the framework of an Amœba containing sporoid bodies.

There is, however, a much more remarkable form of sporangium which appears to be interchangeable with the common one, sometimes almost entirely replacing it, sometimes occurring in various proportions along with it, and sometimes appearing in curious intermediate forms which combine the characters of both varieties in one and the same individual. The first occasion on which they were observed was in an artificial cultivation, consisting of a portion of recent, freshly boiled cow dung into which a normal sporangium from a previous cultivation had been introduced. Forty-eight hours after the cultivation had been set, the surface was found to be covered by a sprinkling of very minute hyaline sporangoid bodies situated on the projecting points of the medium. These on microscopic examination were found to consist of aggregations of large Amœbæ which in general were still readily separable and capable of resuming independent activity in the nutritive fluid into which they were introduced. On the following day the sporangia had increased in size and numbers, some of them being of a pearly-white colour, others pale yellow, and others of a bright warm Indian yellow. The white ones consisted of amœbal aggregates like those observed on the previous day; the pale yellow ones contained similar bodies, and a certain proportion of masses of minute oval or broadly fusiform cellules (Pl. XVIII, fig. 14); the Indian yellow sporangia contained enormous accumulations of such cellules and a few large Amœbæ. The sporangial membrane was very distinctly defined in some cases, and on its rupture masses of the cellules (Pl. XVIII, fig. 12) and large distinct Amœbæ were forced out into the fluid of the preparation. The cellules were, as before mentioned, broadly fusiform or oval in outline (Pl. XVIII, fig. 13 *b*). They were flattened, colourless, and contained a large refractive and apparently oily nucleolus of greenish yellow colour with a brilliant shining nucleolus within it. The cells measured on an average about $6{\cdot}2 \times 3{\cdot}7\ \mu$, and their oily nuclei $1{\cdot}8 \times 0{\cdot}9\ \mu$. In most cases when they first escaped from the sporangia they were aggregated into small lumps or groups by means of a gelatinous and very faintly molecular basis-substance which soon dissolved and disappeared in the nutritive fluid (Pl. XVIII, fig. 13 *a*).

The presence of sporangia containing similar cellules was recognised on several subsequent occasions. The sporangia in these cases varied in colour from clear pale yellow to full bright vermilion, a phenomenon dependent partly on the proportion of cellules present in them in relation to Amœbæ or normal spores, and partly on the proportion of oily matter around the nucleoli. In some cases this was hardly represented, in others it formed a large full-coloured globule, and in these the colour of the sporange was always highly developed. In some cases curious particoloured or piebald sporangia were present in which localised portions of the contents consisted respectively of cellules and of normal spores. The size of the cellules varied considerably in different instances, ranging from that previously given downwards to specimens measuring only 3 or $4 \times 2{\cdot}7\mu$; on other occasions in which there was no microscopic evidence of their presence, isolated masses of them were encountered among the Amœbæ and sporoids within normal sporangia or in those in which only an imperfect spore-formation had taken place.

When the masses of cellules are allowed to remain in a suitable nutritive fluid, the gelatinous investing substance in which they are embedded gradually dissolves and disappears leaving the individual cells free. These now show a slight gradual increase in size, the oily matter of the nucleus gradually disappears leaving the greenish nucleolar particle very conspicuous, and a minute contractile vesicle generally appears. The outline of the cell also becomes somewhat modified, for while one extremity retains its original pointed character, the other becomes somewhat rounded. Subsequently one or more flagellar filaments are protruded from the latter, and the body swims off as an active pear-shaped nucleated zoospore of minute size. The zoospores after continuing their active movements for some time, and in doing so frequently exhibiting very extensive amœboid changes of form, gradually cease to move, becoming at the same time more or less rounded, and finally creep off as minute Amœbæ (Pl. XVIII, fig. 16 *a, b, c, e, f*). Both zoospores and Amœbæ generally show the nucleolar particle originally present very distinctly. In other cases the flagellated zoosporic stage seems to be omitted and the cells, after undergoing a certain amount of increase in size, pass off at once as nucleated Amœbæ.

In one or two instances I have met with large active Amœbæ containing varying numbers of these cells within them, but whether this were a case of ingestion or of commencing development, could not be ascertained (Fig. 21). These cells certainly

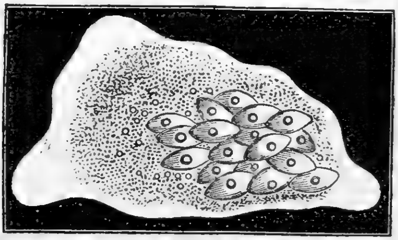


Fig. 21.—Large Amœba containing a mass of fusiform spores × 1000.

appear to resemble very closely, if not to be identical with those described by Cienkowski under the name of *Diplophrys stercorea,* as forming sporangioid aggregations on specimens of moist horse dung.[1] According to him, however, the sporangia were devoid of any investing membrane or matrix, both of which are unequivocally present in the present instance. The characters of the movements in the cells when in the active state is moreover different, and there does not seem to be any tendency to the formation of compound groups by adhesion of active cells as described by Cienkowski. Certainly, too, the cells here are unprovided with anything of the nature of a shell or differentiated external investing coat, which is regarded as probably present in Diplophrys.

Taking the facts that they are so closely related to the ordinary Amœbæ and spores of the media in which they occur; that they are included in many cases within the same sporangia with these bodies; that they appear to be developed in groups such as would naturally result from processes of division in Amœbæ, and that in the active state they present characters so similar to those of the zoospores and Amœbulæ developed from the spores of the common form of sporangia, I am inclined to regard these cells as merely a variety of reproductive bodies belonging to the same organism, and not as the representatives of a distinct species.

III.—*Development of Excretal Parasites in Abnormal Media.*

While in the excreta of cows and horses we find media which permit of the continued vitality and further development of the parasitic organisms which they contain whilst still within the body, it must not be supposed that they are peculiar in doing so. The excreta form the normal site for the reproduction of continuous series of generations external to the body, but other animal fluids apparently may more or less replace them in this respect. With regard to one at all events—blood—there can be no doubt. In comparing the appearances presented by the biconcave spores of normal sporangia with those of blood-corpuscles, it was accidentally ascertained that the spores in place of being destroyed by their transfer to the abnormal medium, appeared to find in it the conditions for further development. A series of special cultivations in isolated wax-cells was therefore carried out with the following results. When a drop of normal blood suspended from a cover-glass is sealed in a wax-cell,

---

[1] "Ueber einige Rhizopoden und verwandte Organismen," 'Archiv für mikrosk. Anat.,' Bd. xii, S. 44.

coagulation rapidly sets in, and with the contraction of the clot a clear peripheral ring of serum is generally formed into which white corpuscles emerge in varying numbers, retaining their activity for various periods up to twenty-four hours. In about four days the rouleaux have entirely broken up, leaving the corpuscles loose in the fluid. At the close of a period of a week the serous ring begins to become stained by the solution of the hæmoglobin, and shortly afterwards the colour of the central portion of the preparation begins to lose its brilliant scarlet and to acquire a carbuncle red hue. This change in colour becomes more pronounced, and as the staining of the peripheral portion advances, the entire drop assumes a uniform deep ruby colour. With the solution of the coagulum the white corpuscles which have been entangled in it, as well as those in the peripheral area which have not disintegrated, come conspicuously into view, appearing as shining, white, oily-looking globules among the surrounding deep red fluid; after this no further change occurs, and the preparation remains seemingly unaltered for months.

The phenomena in cases where a sporangium or spores have been introduced into the drop are very different. The following are the notes recorded regarding one set of experiments:—A drop of blood was inoculated with a couple of normal sporangia from a cultivation of cow dung, and sealed in a wax-cell. For some hours the specimen exhibited similar appearances to those of a pure blood specimen set at the same time for comparison; clear serum being freely expressed to form a peripheral zone into which white corpuscles emerged, and the colour of the clot remaining bright scarlet. Subsequently, however, a dark zone appeared around each of the sporangia, indicative of local deoxidation, a phenomenon frequently observed in ordinary slide preparations of sporangia in blood. On the following morning, twenty hours after the commencement of the experiment, the clot was of a dirty brownish colour and the serum was deeply stained. It contained an abundance of active, freely crawling amœboid bodies which, had it not been for the altered condition of the fluid and the fact of the sporangial inoculation, might have readily been taken for persistently active white blood-corpuscles, as in size, general appearance, and character of movement they were indistinguishable from such bodies. On the next day the clot and serum were somewhat darker coloured. The latter was full of active and still amœboid bodies of various sizes; some when spherical measuring from 12 to 15 μ in diameter. As a rule, they showed a single well-defined nucleolar particle, and some contained a pair of such bodies. They showed no signs of possessing a contractile vesicle. Some of them, in addition to the ordinary scattered granules, contained a more or less altered red blood-corpuscle in their interior. An abundance of still molecular matter was also present in the serum, but no active bacteria could be detected. On the next day the serum was full of circular cells, either motionless or still exhibiting slow form-change, while a few continued to progress slowly. The average diameter of the circular cells was about 15 μ, some of them contained three nucleoli. They appeared as bright, white, shining bodies in the yellow-stained serum. Their protoplasmic contents in some cases were aggregated into a central or lateral granular mass, leaving the rest of the body apparently occupied by a homogeneous fluid ; a few much larger bodies were present, attaining in some cases a diameter of 45μ, and of an even molecular substance. The still, amœbal bodies completely filled the field in many parts of the preparation, especially an the margins of the clot, were they formed continuous sheets and masses. A fresh drop of blood was now taken, inoculated from the previous one and sealed like it in a wax-cell. The initial phenomena in this were just those characteristic of normal blood. Twenty-four hours later, however, the clot had become of a dark red-brown colour, and the serum contained much molecular matter and numerous slowly moving amœboid bodies. On the subsequent day, the latter were again observed, the activity of movement being now more decided. They contained a dim nucleolus within a clear nuclear area, but were devoid of any contractile vesicle. Twenty-four hours later the serum was full of small Amœbæ, measuring about 10μ in diameter when circular, and provided with a distinct nucleolus. After another interval of twenty-four hours the clot was found to be almost everywhere surrounded by a bank of circular and slowly moving binucleolate Amœbæ, measuring when at rest about 15μ in diameter, and appearing as bright punched-out areas in the brownish serum.

A second transfer was now made as before, inoculation being effected by means of a needle which had, as formerly, been heated to redness, and allowed to cool immediately previous to the operation. In this case the results were of a similar nature to those in the previous experiment; only the appearance of the Amœbæ was somewhat retarded. Ultimately an enormous accumulation of still amœboid cells was formed as before. A third transfer was next carried out, but was followed by no development of Amœbæ, the basis merely rapidly breaking up and becoming full of molecular matter. The experiments as they stand, however, clearly show that the spores of the organism, although finding their natural medium in excretal matters, are perfectly capable of life and activity in other media. In some of the series of blood cultivations there certainly appeared to be a certain amount of spore formation, as subsequent to the appearance and cessation of activity in the Amœbæ, masses of much smaller spheres made their appearance among the amœbal aggregates, the individual cells of which measured from 4 to 6 μ in diameter. As no process of multiplication by division during activity was ever observed to take place, and as, at the same time, no evident diminution in the numbers of bodies developed in successive inoculations manifested itself, it seems, indeed, probable that the occurrence is a normal one. Cell cultivations in which boiled milk was substituted for blood failed to show any similar phenomena, the intense acidity developed in the medium subsequent to inoculation seeming to be fatal to the spores.

Numerous attempts were made to cultivate the sporangial spores and Amœbæ of cow dung in human excreta, but at first without any result. Like the similar bodies naturally present in the medium, they invariably appeared to be killed by the stage of acid fermentation. Even where the development of acidity was very limited, as in cases where the occurrence of *Oidium* was prevented by prolonged boiling, it was long before any positive results were obtained in dealing with fresh excretal matter, and the investigation had almost been given up, when, due to an accidental case of inoculation, it was ascertained that the case is very different when the medium has once entered on the alkaline stage of fermentation. Here, in place of being unfavorable to the vitality of the organism, the material appears rather to be specially adapted to it in some respects, although, at the same time, the normal cycle of developmental phenomena characterising it in its natural medium fails to occur with constancy. There is not the same strong tendency to the formation of regular sporangia, and the individual amœboid elements tend rather to retain an independent existence, attaining at the same time an abnormal magnitude; sporangial formation, however, is not always absent, although in most cases in which it occurs assuming an abnormal character.

The history of a case in which an imperfect development of sporangia occurred is as follows:—A portion of perfectly fresh normal human excreta was boiled for half an hour, and then set in a moist chamber. The material was almost neutral, and contained the usual microscopic constituents—*débris* of various sorts, an enormous accumulation of still bacterial matter, and a sprinkling of still circular Amœbæ possessing one or two distinct nucleolar particles. On the following day the material was unaltered in appearance. Its reaction was decidedly and permanently acid, and all the bacteria were still. Twenty-four hours later the acidity was less pronounced; at the close of forty-eight hours it had been replaced by strong alkalinity. The surface of the material was now covered with a creamy greyish-yellow layer of bacteria, which at once began to move actively in nutritive fluid. On the following day it was inoculated with one or two normal sporangia from a dried cultivation of cow dung, the condition of the sporangia being specially favorable to their ready transfer. Two days later the cultivation was again examined. The basis retained its highly alkaline reaction, but the bacterial rods had now been almost entirely reduced to series of spores (Fig. 3), so that the surface coating consisted of little save dense masses of brightly refractive granules. In the gelatinous matter of this coating numerous large Amœbæ were slowly crawling about. None of them at this time showed a contractile vesicle, but the majority possessed a distinct clear nuclear area containing two disc-shaped greenish nucleoli. In some cases the denser portion of the body was crowded with an amorphous mass of granules, and in others similar granules were aggregated into spheres contained in fluid vacuoles (Fig. 22). In appearance and measurements the granules within the Amœbæ were identical with the bacterial spores of the medium. The Amœbæ precisely resembled those frequently encountered in fresh human excreta, but in size considerably exceeded those normally developed in cow dung cultivations.

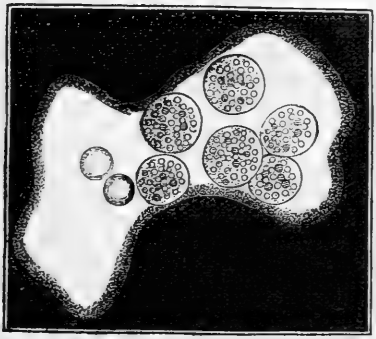


FIG. 22.—Large Amœba with digestive vacuoles × 1000.

On the following day the cultivation was found to be crowded with huge active Amœbæ, like those of the previous day. When first introduced into the nutritive fluid of the preparations they presented a peculiar tuberculate or irregularly cornuate outline, but they rapidly unfolded and crawled freely about. Their nucleoli varied greatly in size; in some cases the discs attained a diameter of 5·5 μ. Specimens of Amœbæ which had been reserved beneath a cover-glass had passed into the condition of dilatation and rigidity normal under such circumstances. They were circular, entirely or almost entirely motionless, the granular matter which they contained gathered into hard lumpy masses, and with a large sharply-defined clear vacuole, apparently a rigid contractile vesicle. Some of them showed a very instructive phenomenon. In such specimens vacuolar cavities containing spherical masses of bacterial spores were still present. The remarkable thing in reference to these was that in many instances a development of a new generation of active bacteria had occurred within them, active rods darting hither and thither in the peripheral fluid of the vacuoles, and knocking and turning about the persisting granular mass (Fig. 23). Interpreted in accordance

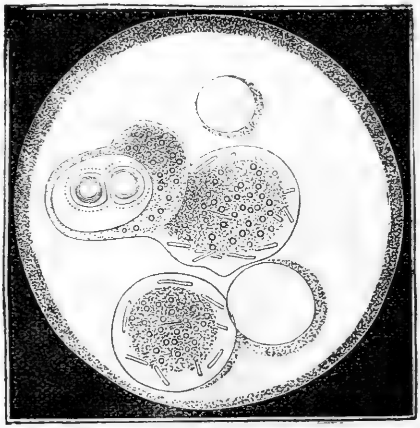


Fig. 23.—Development of Bacteria within digestive vacuoles of dying Amœba × 1000.

with current theories of disease, this phenomenon would indicate that the Amœbæ were dying, due to their infection with bacterial organisms; whereas, in fact, there can be no doubt that the presence of the latter was really the result of processes of development in the ingested spores occurring after the Amœbæ had been enfeebled or killed by the supervention of unfavorable conditions in the medium. The Amœbæ, as usual, were apparently slowly asphyxiated by insufficient access of air to the medium, and the bacterial elements within them now underwent development in place of digestion. They clearly had not invaded the amœbal body subsequent to its death, being confined solely to the digestive vacuoles of the interior, whilst the peripheral substance remained entirely free from them. Moreover, the Amœbæ, in certain instances, appeared to be enfeebled rather than actually dead, as faint changes in their form continued to manifest themselves with more or less distinctness.

On the following day the cultivation continued to swarm with huge Amœbæ. The body-substance was denser and less transparent than previously, and the nucleoli were very hard to distinguish, and in many cases indeed quite irrecognisable. In some cases they had begun to form masses of epithelioid tissue consisting of various numbers of more or less fused individuals. The figure on a former page (Fig. 13), illustrative of conjugate Amœbæ, was taken from this cultivation, and shows an example consisting of three individuals, each provided with a solitary large rigid vacuole. The Amœbæ in these masses were perfectly motionless. Twenty-four hours later the condition of the cultivation remained much as before. In still, dilated Amœbæ which had passed into a condition of rigor in a preparation of the previous day, the nucleus was in some instances very clearly defined. It was here seen to form a distinct bilocular capsule, which in the course of disintegration of the Amœbæ to which it belonged sometimes escaped entire into the fluid of the preparation (Pl. XVIII, fig. 15). Each of the cavities contained one sometimes two greenish discoid nucleoli of varying size. On the next day isolated and aggregate Amœbæ continued to be present in extreme abundance in the cultivation. They were now almost all characterised by the extreme indistinctness and very small size of their nucleoli, so that these bodies in many cases could not be detected even when specially sought for.

Two days later the aggregations of still Amœbæ had become so large in many cases as to form distinct irregular whitish masses visible to the unaided eye on the surface of the medium, and in many of these there were considerable numbers of smaller sporoid cells. Some of the large Amœbæ were in the flattened scale-like condition, and here the nucleus was almost or entirely invisible. A large, slowly acting contractile vesicle was, however, frequently present, which generally was formed by the fusion of several originally independent vacuoles which underwent fusion as they expanded. Some of the large adherent Amœbæ measured as much as $40 \times 37{\cdot}5\ \mu$ and upwards. The sporangioid bodies continued to increase in numbers and size, and in the course of the next three days were entirely converted into masses of spores, hardly a single large Amœbæ remaining recognisable. The spores were somewhat larger, as a rule, than those ordinarily present in the normal sporangia developed on cow dung, but they varied considerably in size in individual instances, ranging from 5 to 10 μ in diameter. Shortly after the sporangial masses were introduced into nutritive fluid, zoospores began to emerge from them. Most of these were of large size corresponding with that of the spores. They swam about actively in the fluid, and in many instances exhibited very free amœboid changes of form while doing so. In all their characters they were quite indistinguishable from similar bodies as encountered in fresh human excreta, and like them were frequently observed to multiply by division. Due to this and to the constant emergence of new individuals, the preparation in the course of a few hours was swarming with active zoospores. At this time a certain number of small active amœboid bodies was also present, which seemed in most cases to emerge directly from some of the larger spores. The preparation was reserved and again examined on the following day. The margins of the fluid still swarmed with active zoospores, some of them of very large size, but otherwise agreeing with their compeers in every respect. In many of them a contractile vesicle was clearly visible. Their movements varied greatly from time to time, free swimming being alternated with caudal adhesion, or with a crawling motion accomplished by means of amœboid protrusions of the body-substance.

The cultivation after this appeared to remain unchanged, no further development occurring, and the surface continuing covered with a thick layer of bacillar spores and of spore-cells derived from the Amœbæ. It remained throughout entirely free of any fungal mycelium.

In the majority of similar cultivations the results closely resembled those just described, but in one case, at all events, a development of well-formed normal sporangia took place, and in others various deviations in the form of arrested development occurred. The series, taken as a whole, appeared unequivocally to prove the identity of the organisms occurring in human and vaccine excreta, and also that the zoospores are merely a form which the reproductive bodies resulting from processes of division in the Amœbæ may assume, interchangeably with the common amœbal form directly developed in cultivations of sporangia in cow dung. In showing this they also afforded a ready explanation of the extreme frequency of the parasite in the human subject, for they indicated the presence of a constant source of readily transferable reproductive elements.

IV.—*Relation of the Excretal Parasites of the Lower Animals to those of the Human Subject.*

We have already seen that the vaccine excreta furnish the conditions for the continued existence and repeated reproductive multiplication of the parasites external to the host-body, crops of sporangia being apparently indefinitely produced so long as the spore-cells obtain access to the medium while in a recent state, if certain conditions of temperature and moisture be provided. Further, we have ascertained that the reproductive elements are capable of retaining their vitality for prolonged periods when in a dry state, and that they are then also capable of resisting influences which are fatal to them in activity, so that a constant supply is always at hand for introduction. These may of course obtain access to the body by various means. The transfer in the case of cattle probably occurs by means of fodder in which the sporangia are constantly liable to be present. In the case of the human subject it, no doubt, occurs in various ways, the great means of diffusion in all probability being the air. That the air is the chief agent by which the reproductive elements are diffused appears probable for several reasons. There can be no doubt as to the constant entrance of the reproductive bodies into the air, both as isolated spores and entire sporangia. The sporangia, when thoroughly dried are detached by the slightest contact from their points of attachment, and having been so, are so light as readily to be carried about by air-currents. One of the difficulties encountered in the study of dried sporangia is, in fact, dependent on their extreme lightness and the ease with which they are swept away by the air. This is not all, however; it is not only evident that a possibility for constantly recurring diffusion of the reproductive bodies by means of the air exists, but it also appears probable that when thus diffused they are more likely to undergo subsequent development than when diffused by the only other medium which can be supposed to play an influential part in the process—water. The more thoroughly dessicated the sporangia and spores are, the more are they capable of retaining their vitality under exposure to unfavorable conditions. When active, or when without being so, they have become softened and distended by immersion in passive fluids, they readily succumb to influences which, when dried, they are capable of resisting with impunity for considerable periods. Now, there appears to be little reason to doubt that in the acid gastric fluids we have such unfavorable media, likely to act prejudicially on the reproductive bodies entering the digestive canal unless specially protected. Active or softened elements will thus probably fail to reach a locality favouring their further development, while those in a desiccated condition will pass on unaffected to assume activity in the lower portions of the digestive tube.

In so far as the observations here recorded justify us in coming to a conclusion, the development of the parasite appears, as a rule, to follow a somewhat different course according as it takes place within or without a host-body. In media external to the body the spore-cells generally give direct origin to Amœbulæ, which in their turn produce a new generation of sporangia. Now, certainly, any true sporangial formation never occurs within the body, indeed, it is scarcely possible that it should occur seeing that the constant movements of the medium must mechanically tend to prevent the initial aggregation of the formative units. It is not so easy to determine to what extent any new spore formation takes place at all, or how far the entering spores normally assume an amœboid condition on emergence, or whether the zoosporic condition replaces the amœboid one as it does in certain cases external to the body. That they sometimes do give origin to Amœbæ, and that the latter, although failing to produce sporangia, may, in some cases, develop a new generation of reproductive elements, seems to be clear; but it remains undetermined how far this is a normal event. The question is, does an amœboid stage normally intervene between the entering spore and the zoosporic elements abounding in the lower portion of the intestinal tube? In other words, are the zoospores there the products of spores developed in Amœbæ derived from the extraneous reproductive elements, or are they directly derived from the latter? is the flagellate zoospore the normal form assumed by the reproductive elements within the body as the Amœba is external to it? This is a question which cannot, in the meantime, be definitely answered. There seems to be no doubt that zoospores, in certain circumstances, are developed as the normal product of the intra-intestinal Amœbæ; but this of course does not exclude the possibility of their coincident development from extraneous spores also.

It now remains to consider the relation which the presence of the parasite bears to cholera and other morbid conditions with which it appears to be specially associated. It may be asked why any special association should occur if the reproductive elements of the organism are so generally diffused and so constantly liable to be introduced into the digestive tube as they appear to be. The answer to this question appears to be as follows:—The special prevalence of the parasite in the excreta in cholera and other intestinal disorders seems to be determined by the abnormal characters of the intestinal contents.

With regard to this point it may be sufficient to recall the fact that the alkaline choleraic fluids may be readily demonstrated to be an efficient nutritive medium—a medium much more favorable to the parasite than the material of normal excreta is. They have frequently been employed as such in the study of the parasite as present in normal excreta, and again and again it has been observed that elements which in their natural medium were in an inactive and seemingly dying condition were rapidly roused to activity and multiplication under their influence. Leaving the chances of excessive or repeated introduction of extraneous reproductive elements entirely out of account, the rapidity with which multiplication by division may occur, under favorable circumstances, appears to be amply sufficient to account even for the excessive multitudes of zoospores present in certain specimens of choleraic excreta. As a matter of observation, it is undoubted that processes of division may recur at a rate of two per hour in the same zoospore, and a calculation of the numbers which may thus be developed under favorable circumstances, even within comparatively brief periods, renders it evident that the numbers of parasitic elements present, even where most excessive, do not necessitate the conclusion that the parent bodies originally introduced must have been very numerous.

Experiments on the artificial introduction of the sporangia into the bodies of healthy animals have never been followed by any special result. I have again and again caused a dog to swallow large numbers of sporangia in all stages of development and desiccation without the treatment producing the slightest appreciable effect, and on one occasion introduced a solution crowded with spores into the peritoneal cavity of a guinea pig with as little result. The presence of morbid conditions certainly determines the degree of development of the parasite, but the presence of the latter seems to be incapable of giving rise to disease. The result of these experiments is suggestive, inasmuch as it shows how closely parasitic organisms may be associated with disease without being causally related to it. In many cases in which experiments have been supposed to demonstrate the essential dependence of disease on parasitic organisms, the procedure has not, as in the present case, consisted in the introduction of these organisms *per se*, but in the introduction of morbid fluids or other materials containing them. For example, we find Lösch affirming the essential causation of certain dysenteric conditions to lie in the presence of his *Amœba coli*, because in one instance where he injected dysenteric excreta containing the parasite into the rectum of a dog, dysenteric lesions and a development of the parasite ensued. Now, there can be doubt that if in the present series of experiments morbid fluids containing the parasite had been employed in place of clean specimens of sporangia and spores, the results might have been very different. If a solution of choleraic or normal excreta containing the parasite had been substituted for the solution of the spores *per se*, in the experiment on the guinea pig, it may safely be affirmed that septicæmia leading to a fatal result would have followed; and it is very probable that had the parasitic elements in the excretal solution consisted of dried or encysted spores, we should have had a coincident development of the parasite parallel to that occurring in the blood cultivations previously described. Had this been so we should, following a line of argument similar that adopted in reference to the relation of *Amœba coli* to dysentery, have been led to conclude that the parasite was the cause of death.

The phenomena presented by the parasite whose life-history forms the subject of the foregoing pages, in the various stages of its development, render it somewhat difficult to determine to what group of organisms we ought properly to refer it. In any attempt at doing so, the question of its animal or vegetal nature need not occupy us, as it appears certainly to belong to that series of organisms which in the mean time, at all events, must be included in the Protista, the intermediate kingdom to which all doubtful organisms wanting in differentiated animal or vegetal characters are conveniently referred. There are two groups in this no-man's-land to which it shows certain points of affinity, appearing in some respects, indeed, to occupy an intermediate position between them. These are the *Monadinæ*, as they are termed by Cienkowski, or the *Protomonadinæ*, as they have been subsequently named by Hæckel, and the *Myxomycetes*, which are by some still regarded as an order of fungi. It appears to be related to the *Monadinæ* in the absence of any definite plasmodial stage interposed between the zoosporic and the sporangial one, and in the fact that individual units developed from single spores appear occasionally to proceed to spore formation. On the other hand, the complex nature of the sporangia, which are developed as the result of the close association and more or less complete fusion of distinct zoosporic elements, points to a close affinity to the Myxomycetes. In some cases, indeed, the fusion of the formative elements advances so far as practically to be equivalent to plasmodial formation, but the occurrence of such a phenomenon cannot be regarded as normal, the spores, as a rule, being developed in groups corresponding to individual units, and the fusion in any case being immediately antecedent to spore formation. In characters, too, the sporangia closely resemble those in certain forms of *Myxomycetes*. The organic granules developed in the walls closely resemble those characterising some myxomycete sporangia, and the ridging or reticulation of the inner surface of the membrane and the rudimentary capillitium clearly correspond to myxomycete structures. Taking all its characters into consideration, the organism appears rather to represent a rudimentary form of the myxomycete group, and it may, therefore, be conveniently distinguished by the name of *Protomyxomyces coprinarius.*

It has already been pointed out that the different developmental forms of the parasite exhibit a high degree of variability under the influence of variations in the external conditions to which they are exposed. Various forms of the zoospores are thus encountered, replacing one another in different media and in the same medium at different times. In some cases the flagellate zoospores show a distinct contractile vesicle and nucleolar point; in others any differentiation of such structures seems to be wanting. The degree and character of movement, the consistence, size, and outline of the body are also extremely inconstant; and a similar variability, although perhaps to a somewhat slighter extent, prevails in the amœboid stage. There is one very distinct form of the flagellate zoospores which in many respects is so unlike the common ones that it might readily be regarded as an indication of specific difference, were it not possible to observe its origin as a mere transition form. In this case the body is characterised by a peculiar spathulate flattened contour, and exhibits a peculiar type of movement, consisting in a hinge-like flexion of the posterior slender portion of the body on the anterior broader part. In some specimens of choleraic excreta, as was previously pointed out,[1] this variety almost entirely replaces the normal one, but its occurrence is not limited to such media, as it has more than once been observed to arise in cultivations of cow dung. Variation in the size of the spores in the same or in different sporangia is a phenomenon of constant occurrence, and one which runs through a wide range of development. As we have previously seen, moreover, there is some reason to believe that there are two distinct forms of spores, which may replace one another more or less completely under different circumstances, the commoner one being distinguished by its spherical or biconcave figure, the other by its smaller size, more or less fusiform outline, and well-marked nucleation.

So far as I have been able to ascertain, the occurrence within the digestive canal of the human subject in this part of India of zoospores or amœboid bodies belonging to any other developmental cycle than that which has been described above is very rare and quite exceptional. It is different, however, in the case of other animals in which the same parasitic forms occur. In many specimens of fresh vaccine excreta smaller numbers of various other organisms are also occasionally present. Some of these are unquestionably specifically distinct, and others, while not unequivocally so, still present certain characters requiring

---

[1] 'Seventh Annual Report of the Sanitary Commissioner with the Government of India,' Appendix B, p: 189.

that they should in the meantime be kept apart. Of the former class of bodies one of the most frequently present is apparently a species of Chlamydophrys, Cien.,[1] while as representatives of the latter we have various zoosporic forms characterised by the possession of a differentiated cell-wall, and by the fact that in the process of multiplication the line of division is longitudinal and not transverse to the original long axis of the body, and starts from the point of emergence of the flagellum (Fig. 24).

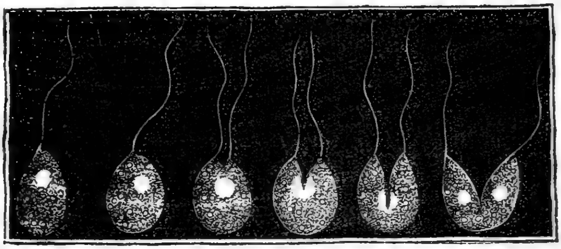


FIG. 24.—Multiplication of Zoospores by longitudinal division × 1000.

Another characteristic organism, occasionally present in considerable numbers, appears in the form of peculiar, somewhat crescentic, colourless cells, which closely resemble certain fungal conidia, and are frequently aggregated in linear series (Fig. 25).

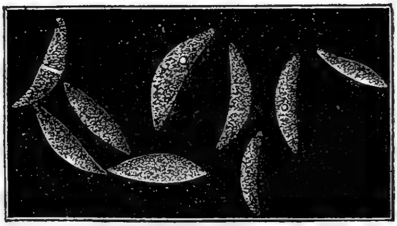


FIG. 25.—Fusiform cells from cow dung × 1000.

In some cases, too, a peculiar form of sporangioid structures makes its appearance either on the same basis with the characteristic sporangia, or apparently replacing them. In colour they vary considerably, in some cases being pale buff, in others salmon-coloured, and in others orange or red. They are always of relatively small size, of irregular outline, and unprovided with a pedicle (Fig. 26).

Their texture is firm, and they have a more or less distinctly

---

[1] "Ueber einige Rhizopoden und verwandte Organismen,' 'Archiv für mikrosk. Anat.,' Bd. xii, s. 39.

defined capsule. Within this, as a rule, we find a thin layer of granular matter surrounding a dense mass of minute circular sporoid bodies measuring about 1·8 μ in diameter. The development of these sporangia has not been followed out, so that their true nature remains uncertain.

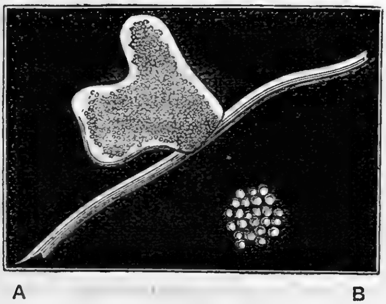


FIG. 26.—A, Sporangoid mass × 44. B, Sporules × 1000.

The principal conclusions which seem to be warranted as the result of these investigations have been already stated in the course of the narrative, but in concluding, it may be well to bring them together into a continuous series. They are as follows:

1. Special parasitic forms may be specially associated with particular forms of disease without holding any causal relation to them.

2. The monadic, amœbal and sporoid bodies, so abundant in many choleraic excreta, are all developmental forms of one species of parasite which I propose to call *Protomyxomyces coprinarius*.

3. This parasite appears to be closely related to the organisms included within the Protist groups of Protomonadinæ and Myxomycetes, and in certain respects seems to represent a connecting link between them.

4. It is not confined to choleraic or even to human excreta as a basis, and only attains its full development external to the bodies of the animals within which it occurs.

5. Its immature forms occur parasitically as normal inmates of the digestive canal in certain of the lower animals.

6. In the human subject, both in health and disease, they are very frequently present in varying numbers.

7. During health the number and activity are limited, due to repressive influences exerted by the normal intestinal contents as a medium.

8. Their excessive abundance in certain forms of disease is

due to abnormal conditions of the intestinal contents, permitting of the occurrence of processes of rapid multiplication.

9. Normal human excreta do not form a medium in which any farther development of the parasitic elements outside the host-body can occur.

10. On the contrary, the normal series of fermentative changes through which the excreta pass after exit from the body ensures the complete destruction of the parasitic elements.

11. No such destructive effect, however, is exerted by the changes occurring during the decomposition of the excreta in certain lower animals—specially cows and horses; and here the parasitic elements on their escape from the body undergo farther processes of development resulting in the production of reproductive bodies securing the continuance and diffusion of the species.

12. Such excretal matters, therefore, serve as a constant source whence parasitic elements may be transferred to the bodies of other animals.

13. Human excreta which have passed through the initial processes of decomposition, and which have thus become alkaline, allow of the continued existence and multiplication of elements of the parasite which may then obtain access to them, and may thus serve as a second centre of reproduction.

14. The introduction of the reproductive elements of the parasite into the human body is mainly effected through the medium of the air.

15. The introduction of the reproductive elements *per se* seems to be quite innocuous.

16. The special association of the parasite with intestinal disorders appears to be dependent on the abnormal condition of the intestinal contents allowing of the rapid multiplication of reproductive elements which may obtain access to them.

CALCUTTA; *November*, 1879.

RESEARCHES *upon the* DEVELOPMENT *of* STARCH-GRAINS.

By A. F. W. SCHIMPER. (Pl. XIX.)[1]

1. THE formation of starch-grains in chlorophyll-corpuscles was investigated first by Nägeli[2] and then by Sachs,[3] and the conclusions arrived at were the same in both cases. Both observers directed their attention principally to the leaves of flowering plants, but Nägeli further observed the green ground-tissue of the stems of certain Cacti and also the Characeæ. According to their accounts, which are essentially the same, the starch-grains are formed, either singly or several together, at various points in the chlorophyll-corpuscles; they then increase in size, and where several are present, they become flattened along the planes of contact. The chlorophyll-corpuscle also increases in size at first, but it subsequently diminishes, and it frequently disappears more or less completely.

I can confirm the results of these observers as regards the material which they used. This mode of the formation of starch appears to be universal in the mesophyll, and it occurs also in the green portions of the stems of many, but not of all phanerogamous plants. The mode of the formation of starch-grains in the stems of some plants differs considerably from that which has been described. In these cases the starch-grains do not originate at any indefinite points in the chlorophyll-corpuscles, but always just beneath their free surface (figs. 4, 6, 9). The thin layer of the chlorophyll-corpuscle which covers the starch-grains is soon ruptured, and the grains then project. They frequently appear to be quite superficial from the beginning.

If the chlorophyll-corpuscles are spherical and not flattened, starch-grains may originate at all points of the peripheral portion. If, however, and this is more frequently

---

[1] This paper was published in the 'Botanische Zeitung' for 1880, No. 52. The author had no opportunity of seeing the paper by Dehnecke on a similar subject, which was published earlier in the year, "Ueber nicht assimilirende Chlorophyllkörner" (Diss. Inaug. Bonn.,' 1880). Dehnecke shows that starch-grains may be formed in chlorophyll-corpuscles otherwise than by assimilation.

[2] 'Zeitsch. für wiss. Bot.,' Heft iii u. iv; 'Die Stärkekörner,' p. 398.

[3] "Ueb. den Einfluss des Lichtes auf die Bildung des Amylums in den Chlorophyllkörnern," 'Bot. Zeitg.,' 1862; "Ueb. die Auflösung und Wiederbildung des Amylums in den Chlorophyllkörnern bei wechselnder Beleuchtung," 'Bot. Zeitg.," 1864; 'Experimental Physiologie,' S. 320, f f.

the case, the chlorophyll-corpuscle is discoid, the localisation is greater, so that the formation of starch-grains, is confined to the equatorial zone. Chlorophyll-corpuscles of this kind often produce six or more starch-grains, which form a girdle in this plane, whereas the central portion and the flat surfaces of the corpuscles are free from them (figs. 4 and 5); occasionally a minute grain may be formed in the flat surface.

These peculiarities in the mode of development of the starch-grains from the chlorophyll-corpuscles, and in the mode of their nutrition, are intimately connected with certain peculiarities in their structure.

The starch-grains which originate in the interior of a chlorophyll-corpuscle and remain enclosed within it, have a concentric structure (for instance those formed in the cortical and medullary parenchyma of certain Cacti, such as *Cereus speciosissimus*); in most cases such starch-grains remain very small and exhibit no differentiation. The starch grains of *Vanilla planifolia*[1] (figs. 1—3), which are of this kind, deserve special mention. When mature they are spherical completely colourless compound grains, consisting of hundreds of small similar polyhedral grains; they closely resemble those of the endosperm of the Caryophylleæ and of the tuber of *Mirabilis Jalapa*. An investigation of their development shows that the small grains arise as minute points in the chlorophyll-corpuscles; they then increase in size, and become polyhedral in consequence of mutual pressure; the substance of the chlorophyll-corpuscle becomes gelatinous, decreases in size, and finally disappears.

Those starch-grains which originate in the second way, that is, in the peripheral portions of the chlorophyll-corpuscles, usually attain a much greater size. Those which occur in the stems of Begonia, Peperomia (*e. g. P. stenocarpa*), Pelargonium, *Oxalis Ortgiesii*, *Dieffenbachia Seguina*, *Costus Malortieanus*, and less markedly those which are present in the stem of the Potato, are among the largest and the most perfectly developed; they exhibit a very distinct differentiation of hilum and layers. Such starch-grains are all eccentric, and the side which has grown the most is, without exception, the one to which the chlorophyll-corpuscle is attached (figs. 8 *a*, 10 and 12). From this fact it clearly follows that the unequal growth of the two sides of the starch-grain is a consequence of an unequally distributed

---

[1] For this material I am indebted to the kindness of Prof. E. Morren of Liége.

nutrition. This conclusion is further supported by the fact that when the starch-grains come into contact with other chlorophyll-corpuscles, prominences are developed upon them at the points of contact (*Peperomia stenocarpa,* fig. 8; *Oxalis Ortgiesii, Dieffenbachia Seguina,* fig. 13).

The different stages in the development of the starch-grains, in so far as they are related to the chlorophyll-corpuscles, are essentially the following:—The starch-grains which are produced by flattened chlorophyll-corpuscles are at first wedge-shaped and flattened in the same planes as the chlorophyll-corpuscle; the side which is connected with the corpuscle is truncated, often somewhat concave or uneven, whereas the free end is rounded. When the formation of starch is very considerable, the chlorophyll-corpuscle gradually assumes a nearly isodiametric form; it diminishes in density, and subsequently also in size, until only a small residue of it is left, or it may disappear entirely. Simultaneously the starch-grain becomes thicker, and usually acquires an ovoid form. The growth of the starch-grain ceases with the disintegration of the chlorophyll-corpuscle. The process of development can be easily observed in *Peperomia stenocarpa* (figs. 6—8), and in *Oxalis Ortgiesii* (figs. 9 and 10); for observing the first formation the cortex of *Philodendron grandifolium* (figs. 4 and 5) may be recommended. The starch-grains which are formed in chlorophyll-corpuscles which are not flattened, are, so far as can be ascertained from the scanty observations, at first hemispherical, the flat surface being in contact with the chlorophyll-corpuscle.

In those chlorophyll-corpuscles which are capable of producing starch in all parts of their substance, the starch-grains may of course be formed near the surface and may sooner or later project freely. In this case, which is by no means rare in the mesophyll (*e. g.* Tradescantia, Begonia, &c.), the starch-grains must be excentric. I have, however, not succeeded as yet in finding grains of this kind exhibiting evident differentiation.

2. The observation of fresh sections, not too thin, of parts of plants which do not contain chlorophyll, shows that the starch-grains which are in process of development are not surrounded by ordinary protoplasm, but that they are conained in, or attached to, peculiar refrangible corpuscles which are usually spherical or spindle-shaped. These bodies are very unstable. So soon as the surrounding fluid penetrates into the cell they swell up considerably and then dissolve. Close observation has shown that prolonged

treatment, for days or weeks, with alcohol, causes these corpuscles to become smaller and more resistant. This effect is immediately produced when they are treated with a watery iodine solution, and this is the best means for examining them; they then become stained of a darker or lighter yellow colour, according to the concentration of the iodine solution. Millon's reagent colours them, when coagulated, a brick red, and nitric acid colours them yellow. These reactions indicate that the bodies in question consist of albuminous substances.

The investigation of the earliest stages shows that these corpuscles are present before the starch-grains, and that the starch-grains which are subsequently produced bear the same relation to these corpuscles as regards their development as the starch-grains which are developed in assimilating cells do to the chlorophyll-corpuscles which are present in those cells. The starch-grains may be produced at any points within these corpuscles, or their formation may be confined to the peripheral portion.

The starch-grains which are developed in the peripheral portion of these albuminous corpuscles, and which therefore have one side free at an early period, have an excentric structure, and the hilum lies near to the free end (figs. 19, 23, 36—40, 48), just as in the excentric starch-grains which are produced by chlorophyll-corpuscles. If the starch-grains come into contact with other of the corpuscles prominences of various forms are developed upon them. In the cases in which I have as yet investigated, the starch-grains which had been developed in the interior of one of these corpuscles were compound; the minute grains composing them rarely exhibited any perceptible differentiation, but when they did it was always concentric (figs. 24—29). These corpuscles, like the chlorophyll-corpuscles, become larger at first after the formation of the starch-grains, and this is usually accompanied by a diminished refrangibility; they then become smaller and gelatinous, and finally they altogether disappear.

The behaviour of these bodies indicates that they are the starch-forming organs in cells which do not assimilate, that is, that the conversion into starch of the assimilated substances which have been conveyed from other parts of the plant is effected by means of them.

In the cases in which a starch-grain is formed in the interior of one of these corpuscles its function is obvious. In those cases in which the starch-grain is formed in the peripheral portion of the corpuscle, and soon projects freely from it, the function of the corpuscle is indicated by the constant connection of the starch-grain with it, and by the fact that the portion of the grain which is in contact with it is the part which has grown the most rapidly. If the corpuscle were not a starch-former, it would only hinder the access to the starch-grain of plastic substances, and in that case the growth of the side of the grain in contact with the corpuscle would be less than that of the other.

I shall call these bodies in the following pages "starch-forming-corpuscles" (*Stärkebildner*).[1]

I will now give a short account of the development of these corpuscles and of the starch-grains in a few plants.

One of the most appropriate objects for observations of this kind is the epidermis of the stem and petiole of *Philodendron grandifolium* (figs. 14, 15). In young cells the nucleus, which is either parietal or suspended in the vacuole by threads of protoplasm, is seen to be surrounded by a considerable number of glistening spherical bodies, which much resemble the nucleoli. The development of these bodies is essentially the following:—The nucleus of a young cell is surrounded by a layer of dense protoplasm, which is at first uniformly thick, but which subsequently becomes uneven. The prominences, which are at first hemispherical, round themselves off to form the spherical bodies mentioned above, whilst the intermediate substance assumes the properties of ordinary protoplasm. This process is probably to be interpreted thus: that a substance which is at first uniformly distributed in the protoplasm investing the nucleus separates out, and collects around certain centres of attraction. These spherical bodies behave with reagents in the manner described above; they are the starch-forming-corpuscles. They develope numerous starch-grains close beneath their surface, which remain small, especially in the petiole, and often form a hollow sphere surrounding the central portion of the corpuscle. In this central position it appears that no starch-grains are formed. The duration of these starch-grains is limited; in the mature thick-walled cells of the stem

---

[1] The vesicles containing starch (*Brutbläschen*), which were discovered by Nägeli ('Zeitsch. f. Wiss. Bot.,' i, S. 149, iii, p. 109), are doubtless the same as our starch-forming-corpuscles. Trécul ('Ann. d. Sc. Nat.,' sér. 4, t. x, "Des formations vesiculaires dans les cellules végetales") observed and correctly drew these bodies in the endosperm of certain Caryophylleæ, Chenopodiaceæ, Gramineæ, &c. The numerous new observations which his paper contains have remained comparatively unknown in consequence of the extraordinary theories which he builds upon them, and of remarkable errors in matters of fact.

nothing can be seen of them, or of the corpuscles which have produced them.

The starch-forming-corpuscles of many plants have the same properties and are formed in the same way as those which have been described above, but they differ from them, more or less, in the mode in which they produce the starch-grains. In many instances the starch-grains are produced in the manner described above, but the number of them is smaller, and they attain a more considerable size. This is the case in the rhizome of *Amomum cardamomum,* which supplies excellent material for investigation.

The starch-grains in the rhizome of *A. cardamomum*[1] (figs. 16—20) are large and club-shaped when mature, and they exhibit distinct internal differentiation. The hilum is very excentric, and lies towards the thicker rounded end; the other end is truncate. Compound grains, consisting of two or three smaller ones, are not uncommon.

The starch-forming-corpuscles resemble those in the epidermis of Philodendron in respect of size, form, and mode of development, but they are paler and less stable. The starch-grains are formed in them just under the surface, or even on it, and are either solitary or two or three together. They are at first hemispherical, attached by the flat surface to the starch-forming corpuscles, which are flattened at the points of contact; they subsequently assume a club shape. The hilum always lies towards the free end of the grain. When two or three starch-grains are formed, compound grains are produced. The starch-forming-corpuscles become at first larger, and at the same time less dense; at a later stage, when the starch-grains have nearly attained their definitive size, the corpuscles can no longer be distinguished, or delicate gelatinous remains of them can be made out by the use of iodine solution. When the grains are mature it is impossible to detect any trace of the corpuscles.

The starch-forming-corpuscles in the rhizome of *Colocasia antiquorum* differ from those which have just been described only in that they give rise to starch-grains throughout their whole mass. The very numerous grains formed in a corpuscle cohere to form spuriously compound grains. This mode of the development of starch-grains is more fully described in the following example, in which I have studied it more closely.

The starch-grains in the endosperm of *Beta trigyna* (figs. 30—32) are large, spherical, or somewhat elongated,

---

[1] I obtained this from the Botanic Garden at Strassburg. As the plant was not in flower at the time I cannot vouch for its identity.

and they consist of innumerable minute polyhedral grains. In ripe seeds these minute grains have always become separate.

Immediately before the first formation of the starch-grains the starch-forming-corpuscles are spheres of rather larger size and lower refrangibility than those in the epidermis of *Philodendron grandifolium*. They are very numerous, and they are especially collected on the lateral walls of the cells. The mode of their development differs but little from that which has already been described in other cases. In the youngest cells the nucleus, which is usually suspended in the cavity of the cell, is surrounded by a very thick layer of peculiarly glistening protoplasm. This becomes paler, and a number of bright points become apparent in it; these enlarge into spheres, and the remainder assumes the appearances of ordinary finely granular protoplasm. Some spheres also make their appearance in the strands of protoplasm which connect the protoplasm around themselves with the parietal layer, and some appear also in this layer itself. The corpuscles enlarge, their refrangibility diminishing at the same time, and the protoplasm with the nucleus coalesce with the parietal layer.

The first starch-grains are apparently formed in the peripheral portion, but this has not yet been quite definitely ascertained. The corpuscle soon becomes turbid in consequence of the presence of a number of minute granules which increase in size and become starch-grains. The whole body increases considerably in size, and it may either retain its spherical form or become more or less elongated. The substance of the corpuscle diminishes, and finally disappears altogether, whilst the starch-grains fill up the whole space, and become polyhedral in consequence of mutual pressure. In this way the above-mentioned compound grains are produced, which must therefore be regarded as spuriously compound.

The starch-grains in the endosperm of *Melandryum macrocarpum* (figs. 24—29) are large, spherical, or ovoid, and consist of innumerable minute grains, which, as in Beta, separate as the seed ripens.

The starch-forming-corpuscles which produce them are moderately large, spherical, or spindle-shaped, and lie in the parietal protoplasm upon the nucleus. They are few in number. They differ from those which have been already described in that they are formed at various points in the protoplasm, which is, from the first, parietal. The process is in other respects essentially the same in all cases. The thin protoplasmic lining of the youngest cells is very dense and glistening; its surface is at first smooth, but it becomes uneven. The prominences become rounded off and form spheres or spindles, whilst the substance between them becomes ordinary protoplasm.

The formation of starch-grains begins very early, even before the complete differentiation of the starch-forming-corpuscles. It is indicated, as in Beta, by a turbidity in the corpuscle, and by its turning blue when treated with iodine. The grains become gradually larger and more numerous, and polyhedral in consequence of mutual pressure. The substance of the corpuscle diminishes and disappears.

The young white tubers, surrounded by leaves, and the roots of *Phajus grandifolius*, contain rather large starch-grains of a triangular, much flattened form, and of definite excentric structure (figs. 33—41).

The young starch-grains are attached by their posterior ends to rod-shaped bodies which lie parallel to the broadest sides of the starch-grains. These rods give the same reactions as the starch-forming-corpuscles, and the study of their development shows that they are bodies of this kind. When treated with water they become spherical vesicles and then disappear.

Bodies of this kind, which are not attached to starch-grains in older cells, are found in the epidermis, both of old tubers which have become green and in young ones, collected around the remarkably granular nucleus.[1] In very young cells they produce small starch-grains. I was able to study the development of these peculiar starch-forming-corpuscles, but the small amount of the material prevented me from making out all the details with certainty. In the youngest cells the nucleus is surrounded with a layer of dense glistening protoplasm, as in the epidermis of Philodendron. At a later stage numerous minute delicate spindles are found lying in the protoplasm which now presents its ordinary appearance. These spindles soon give rise to small starch-grains which disappear. The corpuscles enlarge and assume, even when the starch is being formed, a rod-shape.

Exactly the same apparently takes place in the root (figs. 33—36), where it is easy to observe the starch-forming-corpuscles before the appearance of the starch-grains and to follow the development of the latter. The corpuscles which are collected round the nucleus, and which are at first

---

[1] Gris, 'Ann. d. Sci. Nat.,' sér. 4, t. vii, pl. 8, fig. 4.

spindle shaped, form on their surface one starch-grain, or sometimes two or three, which is at first conical; when it has attained the thickness of the corpuscle, which has in the meantime become rod-shaped, it increases in size almost solely in a plane which is parallel to that of the corpuscle. It appears from this that not only does the unequal growth, which produces the excentric structure, depend upon the mode of nutrition of the grain, but also the unequal increase of its diameters which produces the flattened form. The layer of the corpuscle which immediately invests the grain is delicate, and is more or less swollen (fig. 41). The further behaviour of the corpuscles is similar to that described in other cases; they become less dense and stable, are reduced to a small swollen gelatinous residue, and finally disappear.

The formation of starch in the parenchyma of the young tubers (figs. 37—40) takes place in essentially the same way as in the root. I have not succeeded in observing the corpuscles in this case before the appearance of the starch-grains: the apices of the minute spindles already contained them. Both corpuscles and grains increase in size and become much larger than in the root.[1] Their further behaviour will be described in the following section:

The starch-grains in the rhizome of *Canna gigantea* (figs. 46—49) are very large, triangular, and flattened; they are excentric, and are either simple or partially or entirely compound, consisting of a few, seldom more than ten, small grains which are usually arranged in a row.

The starch-forming-corpuscles resemble those of *Amomum Cardamomum* in their development, and at first in their form also; they differ from them only in that they usually contain a tabular crystalloid, which is either octahedral or cubical, and which only becomes apparent on treatment with water. The first stages in the development of the starch-grains are the same as in Amomum; they are formed excentrically or even superficially in the corpuscle, and there may be one, two, or three; they have at first a rounded form which is somewhat flattened at the point of attachment.

The corpuscles now behave very differently from those of Amomum; they grow in one direction only and acquire an elongated form. The starch-grain grows, as in Phajus, in a plane which is parallel to that of the corpuscle which formed it, and the hilum lies towards the free end. The crystalloid lies in a projecting portion of the corpuscle.

The further behaviour of the corpuscles is the same as it

---

[1] This is well seen in sections which have been hardened in alcohol.

is in the preceding case. They become gradually less dense and resistant, and form only a delicate investment to the posterior ends of the starch-grains by the time that they have obtained one-half of their definite size.

In certain cells of the cortex[1] spindle-shaped bodies containing crystalloids are found, which are doubtless starch-forming-corpuscles which have produced no starch (fig. 54). In the external part of the cortex the corpuscles and the starch-grains are small, and always present the same appearance as the apical region in its earlier stages.

An account has now been given of all the various modes of the formation of starch which I have as yet observed. They may be conveniently tabulated as follows:

1. Starch-forming-corpuscles spherical.
    - *a.* They are formed only in the protoplasm which invests the nucleus:
        - (*a*) They form starch throughout their whole mass; Colocasia.
        - (β) They form starch only in their periphery; Philodendron, Amomum.
    - *b.* They are formed in the protoplasm surrounding the nucleus, but to some extent also in other parts:
        - (*a*) They form starch throughout their whole mass; *Beta trigyna.*
    - *c.* They are formed in all parts of the protoplasm:
        - (*a*) They form starch throughout their whole mass; Melandryum.
2. Starch-forming-corpuscles, spindle-shaped.
    - *a.* They are formed only in the protoplasm which invests the nucleus:
        - (β) They form starch only in their periphery; Phajus.
    - *c.* They are formed in all parts of the protoplasm:
        - (*a*) They form starch throughout their whole mass; Melandryum.
3. Starch-forming-corpuscles at first spherical, subsequently elongated.
    - *a.* They are formed only in the protoplasm which invests the nucleus:
        - (β) They form starch only in their periphery; *Canna gigantea.*

---

[1] This observation was made on *Canna discolor*, which in other respects resemble the species referred to above.

Other starch-grains which I have studied cannot be arranged under one or other of these types, inasmuch as I have not been able to make out all the details of their development. In many cases the development of the starch-forming-corpuscles was not observed, though it could be inferred from their position in the cell. In other cases it was impossible to see the first appearance of the starch-grains.

The following conform to the type of *Amomum Cardamomum,* namely, other Scitamineæ such as *Thalia setosa, Elettaria Cardamomum, Costus Malortieanus;* the Potato, so far as observations made on the cortical part of young potatoes go (the more central portions were too opaque in consequence of the presence of the starch grains); the rhizome of *Iris florentina,* in which the starch-forming-corpuscles have a peculiar granular appearance; the parenchyma of the pith of *Philodendron grandifolium.* In the following cases the same relation between starch-grain and starch-forming-corpuscles was observed, although I was unable to ascertain the mode of development of the latter; in the bulbils of *Ficaria ranunculoides;* in the cortical parenchyma of the rhizome of various species of Peperomia; in the cortical parenchyma of the scales of a Tydæa; in the tubers of *Dioscorea alata;* in the root of *Gunnera scabra.*

Silene inflata and *Lychnis dioica* belong to the Melandryum type.

The observations made on Phajus probably hold good with reference to the other allied Orchidaceous plants—Acanthephippium,[1] for instance.

The other species of Canna resemble *Canna gigantea,* and perhaps *Curcuma zedoaria* does also; the minuteness and indistinctness of the corpuscles in this plant made it impossible to observe them accurately; in their first formation and in their mode of development they resemble those of Amomum, and at a later period they appear to become elongated like those of Canna.

3. When we compare the starch-forming-corpuscles with other bodies contained in cells their resemblance to chlorophyll-corpuscles at once suggests itself. In their composition they appear to be essentially the same as the leucophyll-corpuscles[2] which are found in the more internal cells of etiolated stems, and which are quite colourless and very unstable. Further, there is a singular similarity in the mode of development; the mode of formation of the starch-

---

[1] Gris, loc. cit., p. 196.

[2] I prefer this term (which was suggested by Sachs) to the term "etiolin-corpuscles," for these corpuscles appear frequently to contain no etiolin.

forming-corpuscles in the endosperm of Melandryum agrees in all important points with the mode of development of the chlorophyll-corpuscles of many leaves, and their development in the epidermis of Philodendron has its perfect analogue in the formation of chlorophyll-corpuscles in many stems (*e.g.* of *Cereus speciosissimus*[1]), and in the leaf of *Vanilla planifolia.*[2] Again, the starch-forming-corpuscles, like the chlorophyll-corpuscles, produce starch-grains, although the origin of the grains is different in the two cases, inasmuch as in the latter they are the products of assimilation, whereas in the former they are formed from organic substances which had been assimilated elsewhere.

A distinct analogy appears in the relation in space of the starch-grains to the point of their formation; the same two types which we found in the chlorophyll-corpuscles recur in the starch-forming-corpuscles; and, further, as has already been pointed out, the behaviour of the starch-forming-corpuscles, after the formation of the starch-grains, is quite similar to that of the chlorophyll-corpuscles.

But these relations may be extended much further. In most cases the starch-forming-corpuscles may be actually converted into chlorophyll-corpuscles under the influence of light.

This conversion may take place normally and regularly in the development of a plant-organ; this is the case when the younger parts of the organ are protected from the light, either by a thick covering of leaves or by the soil, and are exposed to its influence at a later period (leaves of Iris, tubers of *Phajus grandifolius*).

In organs which remain, as a rule, permanently in the dark, the conversion of starch-forming-corpuscles into chlorophyll-corpuscles takes place as soon as they are exposed to light; in this way the false chlorophyll-corpuscles are produced which have been known, in the potato for instance, for so long.

Certain parts of some organs are exposed to light, whereas others are more or less protected from it; this is the case, for instance, in stems, the bases of which are buried in the soil (*e.g.* Peperomia, Begonia, &c.), and in thick opaque organs of which only the external cells are affected by light (*e.g. Philodendron grandifolium*). In such cases all possible intermediate stages between starch-forming-corpuscles and chlorophyll-corpuscles can be found.

This conversion always takes place in the same way; the

---

[1] From my own observation.

[2] Gris, loc. cit., p. 188.

starch-forming-corpuscles increase considerably in size, the contained starch-grains undergo partial or complete absorption, and at the same time a formation of pigment takes place.

I will content myself with briefly describing a few instances.

The conversion in question which accompanies the normal development of an organ appears to be of very general occurrence. The young leaves of *Iris florentina* contain no chlorophyll, but the parenchymatous cells which abut upon the fibrovascular bundles contain starch-grains which are attached to large starch-forming-corpuscles; these portions of the leaves subsequently become green in consequence of the conversion of the starch-forming-corpuscles into chlorophyll-corpuscles.

The behaviour of the starch-forming corpuscles in the tubers of *Phajus grandifolius* (figs. 42, 43) is very remarkable. The tuber is at first surrounded by a dense investment of leaves, but, in consequence of its growth, it gradually emerges and the leaves die off. The tuber is at first quite white, but it soon turns bright green after exposure to light. Close observation shows that the starch-forming-corpuscles increase considerably in size, that the contained starch-grains are partially absorbed, and that they develope into rod-shaped chlorophyll-corpuscles; bodies of this kind can be found especially well-developed in the bundle-sheaths of the upper parts of the tuber. Even when the starch-forming-corpuscle has been reduced to a small gelatinous residue, it becomes green in the manner described. In the external cells of the cortex only a partial conversion of the starch-forming-corpuscles takes place, and it is confined to that part of the corpuscle to which the starch-grain (which is in this case always very small) is attached; it becomes a somewhat elongated chlorophyll-corpuscle, the starch-grain undergoing partial or complete absorption, which remains attached to the portion which has remained unaltered. In this way very curious bodies are formed, which Gris[1] has already observed in Phajus and Acanthephippium.

In subterranean organs which are exposed to light the effect is the same. The outer cortical cells of the potato are especially instructive. The cells which lie immediately

---

[1] Gris, loc. cit., p. 195. I have never been able to observe the spherical bodies which he describes, in uninjured cells; but they always appear under the action of water.

beneath the cork, contain, according to Wiesner,[1] etiolin-corpuscles which become chlorophyll-corpuscles under the influence of light. These bodies, which are really starch-forming-corpuscles, and which, as in all other cases, produce no starch in the cortical cells, become converted, as far as I could observe, into very small and only slightly coloured chlorophyll-corpuscles, whereas those which are situated in the more internal cells and which contain starch-grains become converted into large and brightly coloured chlorophyll-corpuscles. When the contained starch-grains are very small they undergo complete absorption. In the still more internal parts of the tuber, where the starch-grains are very large, the starch-forming-corpuscles are reduced to a small gelatinous residue, they can naturally only become delicate ill-defined chlorophyll-corpuscles.

These facts can be very readily made out in the rhizome of Canna (figs. 50—53); here, in correlation with the form of the starch-forming-corpuscles, the chlorophyll-corpuscles are sickle- or spindle-shaped (spherical in the outer cells) and contain crystalloids.

The investigation of the following gave the same results; the rhizome of *Iris florentina,* of *Costus Malortieanus,* the scales of a Treviranía, the roots of *Gunnera scabra,* and of *Phajus grandifolius,* which resemble the tubers (fig. 45).

Not all starch-forming-corpuscles, however, are capable of being converted into chlorophyll-corpuscles, even when their whole development goes on in the presence of light (epidermis of Philodendron and Phajus, endosperm of Caryophylleæ).

It is evident, from what has been stated above, that there is a complete resemblance between starch-forming-corpuscles and leucophyll-corpuscles, and even with chlorophyll-corpuscles in their first stages of development; the question naturally suggests itself as to whether or not these corpuscles are identical. The only obvious difference between them is the former can produce starch-grains from assimilated substances, whereas the latter cannot produce any starch at all, so far as is at present known.

A more careful investigation shows, however, that even this difference is by no means constant; and that, on the contrary, these corpuscles completely resemble each other in this respect also.

It is well known that the mesophyll of etiolated plants, which have not yet exhausted their reserve materials, contains no starch, although it is present in quantity in their

---

[1] Wiesner, 'Oester. Bot. Zeitschr.,' 1877.

stems and petioles, and in the bundle-sheaths of their leaves. This starch, which is obviously not a product of assimilation, is produced by the leucophyll-corpuscles. Examples of this can be found in the Hyacinth (bundle-sheath), in the stem of *Begonia cucullata* (figs. 55, 56), and of *Oxalis Ortgiesii,* in the cortex of the stem of *Philodendron grandifolium.*

These leucophyll-corpuscles are very faintly tinged with yellow, if at all. They gave rise, to the cases observed, to starch-grains in their periphery, just like the chlorophyll-corpuscles which would have been produced there under ordinary circumstances. In those cases in which the starch-grains have a definite structure, as in the stem of *Begonia cucullata,* they are excentric, and the more developed side is the one which is in contact with the leucophyll-corpuscle; this naturally removes any doubt as to the physiological significance of the corpuscles.

The question now arises as to whether or not the property of forming starch out of assimilated materials is peculiar to the leucophyll-corpuscles and to the starch-forming-corpuscles, and is not possessed by the chlorophyll-corpuscles; and, further, whether this property is lost when these corpuscles become converted into chlorophyll-corpuscles.

In order to obtain an answer to this question a root-stock of *Tradescantia rubella* was kept in the dark until the large starch-grains which were present in the mesophyll had entirely disappeared;[1] it was then exposed for some time to light, which was sufficiently intense to effect the formation of normal chlorophyll-corpuscles, but not sufficiently intense to cause any formation of starch in consequence of assimilation.[2] The investigation of the axillary branches which were produced from it under these conditions (the apices of the branches were carefully removed before the commencement of the experiment) showed that there was no starch in the mesophyll, but that it was present in considerable quantity in the chlorophyll-corpuscles of the bundle-sheaths of the leaves and of the parenchyma of the stem.

These observations are not, as might appear at first sight, by any means adverse to Sach's theory, that the starch-grains found in chlorophyll-corpuscles are products of assimilation. On the contrary, they confirm this theory in certain points. The fact that the formation of starch in the mesophyll depends upon the same conditions as assimilation, whereas it

---

[1] Sach's method ('Exp. Phys.,' p. 322) for the detection of very small quantities of starch was made use of.

[2] That no assimilation took place was shown by the fact that the chlorophyll-corpuscles of the mesophyll produced no starch.

is independent of light in other parts of plants so long as there are reserve materials to draw upon, can only be explained by the assumption that starch can be formed in the chlorophyll-corpuscles of the mesophyll only by assimilation, and that in the other cases it has a different origin. The chlorophyll-corpuscles of the parenchyma of the stem and of the bundle-sheaths of the leaves can form starch, both by assimilation and by the conversion of assimilated substances conveyed to them from other parts. In other words, these chlorophyll-corpuscles combine the functions which are peculiar to chlorophyll-corpuscles with those which are performed by starch-forming-corpuscles. This assumption is further justified by the fact that the mesophyll is the principal seat of the assimilatory function, whilst the bundle-sheaths of leaves and the parenchyma of petioles and of stems are conducting tissues for starch; the parenchyma of stems is also to some extent a reservoir in which it is deposited.[1]

It is evident that the starch, which makes its appearance as the first visible product of assimilation, is not directly formed from carbon and water, but that a number of intermediate products must be formed.[2]

We may assume that the substances which are conveyed to the chlorophyll-corpuscles in question are nearly allied to, or even identical with, these intermediate products; hence the conversion into starch of the substances which have been formed in the chlorophyll-corpuscle itself, and of those which have been conveyed to it from other parts is really one and the same process.

The results of this investigation tend to show that there is no such great difference between assimilating and non-assimilating cells, as was thought to exist. In a cell which contains no chlorophyll there are certain organs which produce starch, and these organs are nothing more than imperfectly developed chlorophyll-corpuscles, which may develope into perfect chlorophyll-corpuscles under the influence of light. On the other hand, chlorophyll-corpuscles are not always merely assimilatory organs; they perform in the conducting tissues and in the reservoirs of material the same functions as the starch-forming-corpuscles in cells which do not assimilate; that is, they produce starch from assimilated substances which are conveyed to them from other parts of the plant.

---

[1] Sachs, 'Exp. Phys.,' p. 380 395 *et passim.*

[2] Sachs, 'Exp. Phys.' Pringsheim has recently discovered such an intermediate product, and has termed it hypochlorin ('Monatsbericht d. preuss. Akademie in Berlin,' 1879).

Upon the CAUSE *of the* STRIATION *of* VOLUNTARY MUSCULAR TISSUE. By JOHN BERRY HAYCRAFT, M.B., B.Sc., F.R.S.E., Senior Physiological Demonstrator in the University of Edinburgh. [Communicated to the Royal Society, of London, December 1st, 1880.]

THE structure of striated muscular tissue has occupied the attention of many histologists, and various, often antagonistic, have been the views held from time to time since Schwann first investigated this difficult subject.

I bring forward with much caution and hesitation any opinions of my own, nor should I venture thus far, did I not consider my views susceptible of direct proof, or disproof, not being matters of mere speculation, which may or may not be true, and which would tend, by their introduction to the literature of the subject, to make confusion worse confounded.

In this paper an attempt will be made to account for many of the observed structural phenomena of muscle on simple laws of geometrical optics, which will, if it be successful, reduce the subject to comparative simplicity. I shall commence by giving a sketch of the views of those physiologists who have especially written upon the structure of muscle. This must not be looked upon as a complete history, for I shall leave out entirely points which do not concern us here.

A short historical sketch of the views held upon the structure of striated muscle.—The writings of Mr. Bowman form the most important and brilliant contributions to the literature of this subject, and taking him as a landmark, it is convenient to speak of investigators before or after his time. Among the former Schwann, quoted by Müller ('Physiology,' translation by Baly, vol. ii, p. 878), describes the striated voluntary fibre, indicating its shape and size. The cross markings were observed by him, and, indeed, with one or two remarkable exceptions, by all the early observers (Lauth and Wagner, in Müller's 'Archiv für Anatomie und Physiologie, und Wissenschaftliche Medicin,' pp. 4 and 318 of the year 1835). Schwann, with Bauer, Krause, Müller, Home, Valentin, and Milne Edwards recognised the important fact that each fibre is composed of a number of threads or fibrillæ, packed side by side and joined together by a transparent tenacious fluid (Krause), and, moreover, that these threads or fibrillæ are cross striated, as is the fibre itself. Although Schultze describes the fibrillæ as being uniform filaments, he is alone in this opinion, most of his contemporaries recognising the beaded appearance.[1] The beaded thread was the cause of some dispute, for the question arose, Was it a linear series of globules or a moniliform filament? and the final settlement of this must, indeed, have been a matter of great difficulty to those older *savants*, when we consider the imperfect lenses at their disposal. Krause and others maintained the former view, while Schwann held that which subsequent investigators have shown to be the correct one. The fibrillæ, according to Schwann, present a very regular succession of bead-like enlargements, darker than the very short constrictions which lie between. Thus, before the time of Bowman, the following important facts had been made out, namely, that the fibre is composed of a bundle of beaded fibrillæ cemented together, and that the fibrillæ are cross striped, giving the whole fibre a like appearance of striation. Erroneous views had often, it is true, been advanced, but these had never received general acknowledgment. Mr. Skey ('Phil. Trans.' of 1837), for instance, considered the fibres to be tubes filled with a soluble gluten, the striæ surrounding and binding them together. Leeuwenhoek had a somewhat similar view of the construction of the cross striæ, and Prochaska considered them as depressions caused by the clasping of neighbouring capillaries and thready tissues.

Mr. Bowman communicated to the Royal Society, in 1840, a paper "On the Structure and Movements of Voluntary Muscle," in which he confirmed many of the opinions of his predecessors, adding, at the same time, much of what was fresh to our store of knowledge. He it was who first described the thin elastic membrane (sarcolemma) covering and ensheathing the fibres, showing how easily to demonstrate its existence, and giving figures of it, which have been copied into most modern histological works. The nuclei of the sarcolemma he also figured, but what most concerns us is his description of the cross striation. Mr. Bowman, I believe, first pointed out that not only can a fibre be split up longitudinally into fibrillæ along certain dark lines which may generally be seen, even in fresh preparations, but that it splits up transversely along the dark stripes. Each fibrilla may, therefore, be split up into tiny segments across the dark striæ. "On the whole, little doubt remains in my mind that the fibrillæ consist of a succession of solid segments or beads connected by intervals generally narrower, and I believe the beads to be light, and the intervals the dark spaces when the fibrilla is in exact focus." His idea of a fibre naturally follows from that just given of a fibrilla, and, quoting again from him,

---

[1] Consult a drawing by Allen Thomson in illustration of Dr. Martin Barry's paper on the "Structure of Muscular Fibrils," 'Phil. Mag.,' ser. 4, vol. 6, plate v, fig. 2.

we find "a fibre consists of sarcous elements (so he termed the little segments or beads) arranged and united together endways and sideways, so as to constitute in these directions respectively fibrillæ and discs, either of which may in certain cases be detached as such," and "the dark longitudinal striæ are shadows between fibrillæ, the dark transverse striæ shadows between discs."

It will be seen that in one particular Bowman disagreed with Schwann and the older writers, and at the same time with those of more recent date. According to him, the bead was light and the constriction dark, when the muscle was in exact focus, a description at variance with everyone. In the same paper he mentions this remarkable fact, that on altering the focus the stripes were reversed; he must have examined it—this bears in a most important way on our investigations, to be afterwards described—in the reverse focus of what it is ordinarily figured in. His view of the form, and the splitting of the fibre, was probably correct, for he described the cleavage as occurring in the narrow part, which appeared to him, focussing as he did, to be dark, and indeed it is often difficult to say which it is, whether dark or light, for, as we shall more particularly mention afterwards, the slightest alteration of the focus is sufficient to reverse the appearance of the fibre. Bowman, moreover, accounted for these light and dark parts of the fibrillæ, comparing a muscular filament to a glass rod with alternate swellings and depressions, which, when viewed with transmitted light, gives just the same appearance, and from a study of his paper, although it is here somewhat indefinite, I judge that he concluded the moniliform shape to be a cause of the striping.[1]

Now, this last-named and important discovery of Bowman's has, I believe, completely been lost sight of, for no mention of it can be found in any modern monograph nor in any systematic text-book that I have examined. The striking points in the paper and in the figures he gives, is the splitting up of the fibre into transverse discs and the demonstration of the sarcous elements as before quoted. This, together with the sarcolemma, every one connects with the name of Bowman. Modern investigators have worked mostly at the cross striping of muscle, and have found it more complicated than Bowman described, owing, no doubt, to the use of better glasses; while he explained the phenomenon as due simply to the shape of the fibres—believing, however, probably that it was due also to

---

[1] Bowman, nevertheless, seems to consider the dark stripe of a different structure from the light, not so much from the shading, but from the transverse cleavage. He is not quite definite here, but this is the impression I have gained from a careful perusal of his paper.

structural differences—modern investigators have introduced hypotheses to account for it, which entirely imply differences of structure along the filament. The reason of this is, if I may express an opinion, that his theory has been completely lost sight of, and that it was followed by the discovery of startling facts, which at first sight seemed to set it on one side.

In discussing the views of modern inquirers, I shall not, in all cases, consider them in the order of their priority, and illusion will not be made to much that has been written upon this subject, which, indeed, may safely be put on one side.

The light stripe—dark stripe of Bowman—has been shown by Dobie, Busk, and Huxley, to be traversed by a very fine dark band, or rather line "Querlinie" dividing it into two equal parts. We shall speak of this as Dobie's line, or the dark stripe in the centre of the light. (Fig. 1, D, woodcut.) Then, again, the dark stripe is traversed in its centre by a lighter band called Hensen's stripe.[1] (Fig. 1, H, woodcut.) Other bands border this stripe, but as they are certainly not to be seen in all specimens, however well prepared, and as we shall presently account for them, they need not trouble us here.

As early as the year 1839, Boeck showed that muscle refracts light doubly, which statement was, however, modified in 1857 by Brücke. The latter examined muscles prepared in alcohol by polarised light, and found that the dark stripe (dark in ordinarily non-polarised light) appeared luminous in the dark field of the microscope, and that the light stripes were dark when the Nicols were crossed. The dark stripes, therefore, appeared to be doubly refracting (anisotropous), and the light stripe singly refracting (isotropous), the fibre consisting of singly and doubly refracting discs alternating one with another. These observations he verified by an examination of the fibre with thin plates of selenite and mica. The views of Brücke have, in their turn, received considerable modifications which will be understood by reference to a diagram. Fig. 2 expresses very well the results of my own observations, which are in accordance, I find, with those of other observers. (See the 'Handbuch der Physiologie,' by Dr. L. Hermann, 1879, p. 20.) The black part of the diagram corresponds with the portion of the muscle which singly refracts light (isotropous), while the light shaded parts correspond with the anisotropous substance.

This diagram does not, it will at once be seen, correspond with the views held by Brücke, for the great mass of the light stripe, with Dobie's line in the centre of it is anisotropous, the

---

[1] This stripe was also described by Dobie in the 'Annals of Natural History for 1849,' and it may be called Dobie's light stripe.

dark band, as with Brücke, being anisotropous. The most recent view is, then, that both the light and the dark stripes doubly refract light, but that there are bands which lie between them and which are singly refracting. With the appearance which would partially warrant such a conclusion, I can entirely agree, but I shall endeavour to show hereafter how this may most satisfactorily be explained. It will readily be seen how Brücke's view, until quite recently accepted, would drive one to the conclusion that the light and dark stripes represent two different structures alternating in the length of the fibre, and this is corroborated by statements as to the action of staining agents on the tissues.

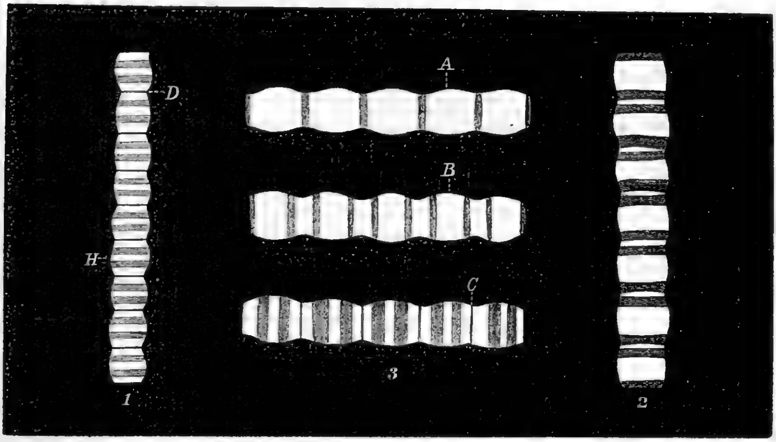


Fig. 1.—This represents diagramatically a fibre viewed with a very high power. The borders are wavy, and the cross stripes correspond with these irregularities. (D) marks the position of Dobie's lines placed in the centres of the depressions seen at the border. (H) represents Hensen's stripes or Dobie's light stripes placed on the summits of the ridges in the centres of the dark bands.

Fig. 2.—This shows the appearance of the fibre with crossed nicols. The shaded parts are seen on the slopes between the ridges and depressions. They are explained fully in the text.

Fig. 3.—A fibre is represented as seen with three positions of the lens. In (A) the lens is elevated, and the depressions appear dark. In (C) the lens is fully depressed, when the stripes are reversed, the depression being now light with Dobie's line in the centre, and the crests dark with Dobie's light stripe in the midst. In (B) the intermediate stage is seen.

Picric acid stains muscle very readily, but it is but faintly tinted by carmine, logwood, or eosine, although Ranvier, in his 'Traité Technique d'Histologie,' states that he has obtained very beautifully stained preparations of insects' muscle, when using Bœhmer's solution of logwood. According to this observer, the dark stripes as well as Dobie's lines are stained, while the rest of the fibre remains colourless. Klein, in his 'Atlas of Histology,' figures the sarcous matter of the dark band clearly tinted, while that of the light stripe is absolutely colourless. The statement will not be far wrong, that every one at the present time considers the dark and light stripes as representing two different structures, distinct one from another in their physical properties, for the dark stripe is spoken of as possessing a higher refracting power than the light, and chemically, for their compositions have already been hinted at by more than one observer. The dark stripes are looked upon by most as the true contracting part of the fibre, and they are termed the sarcous discs, or "Muskelprismen," "Hauptsubstanz," or masses of disdiaclasts, and the light stripes as merely connecting matter (Zwischensubstanz), or "Muskelkästchenflüssigkeit." Dobie's line—more especially from the dipping down and attachment of the sarcolemma in insects' muscle at this point—has been looked upon (Krause, 'Allgemeine und Microscopische Anatomie,' section "Muskel System," pp. 80—90) as a delicate transverse membrane. This view has received the assent of such microscopists as Klein and Ranvier, but not of Wagener ('Jahresberichte der Anatomie und Physiologie,' Hofmann and Schwalbe) and Rutherford ('Text-book of Physiology,' p. 128), who describe Dobie's line as consisting of a row of dots. Engelmann, indeed, describes a row of dots on either side of this line.

Krause would have us believe that the fibre is divided by these membranes into a linear series of little boxes, each box or casket "Müskelkästchen" containing a dark stripe with (as the membrane lies in the centre of the light stripe) one half of that on either side. Merkel ('Lehrbuch der Gewebelehre,' Stuttgart, 1877, p. 83), to make the Müskelkästchen self-containing, affirms that the membrane of Krause is double. As to the stripe of Hensen, this is by very many looked upon as still another structure lying in the centre of the dark stripe; it is in many fibres very clearly to be made out, its border being well defined, and in stained preparations (logwood) it has decidedly a lighter tint than the rest of the stripe. Still, some (Krause) look upon it as an indication of the highly refracting power of the dark stripe, comparing the appearance with the light centre of an oil globule. The other cross striæ, of which there are many described by some observers, but none at all universally accepted, are, as a rule, considered as indicating further complications in the muscle fibre; indeed, the Müskelkästchen, by most advanced microscopists, although not $\frac{1}{10000}$ of an inch in length, consists of some ten or twelve different parts. We may postpone, I think, indefinitely the consideration of these details.

While there is great unity as to the appearance of a fibre during a state of rest, the changes which the fibre undergoes when passing into the contracted condition are not at all understood. Not only does one fail to find among histologists agreement as to the changes in appearance, but the interpretations of these are as numerous as the investigators themselves. All are agreed that, during contraction, the fibre as a whole shortens and thickens, but the changes in form which the cross striæ undergo are not understood so well.

Klein, in his 'Atlas of Histology,' maintains the broadening of both stripes transversely, the dark stripe becoming thinner in the long axis, and the bright stripe more opaque. Ranvier ('Traité Technique d'Histologie,' p. 489) states that the only points one can conscientiously observe in the contraction of a living fibre are, that a knot or bulging forms, in which the dark bands approximate, being only separated by Dobie's line. This led him to believe that the dark bands are the true contracting part of the fibre. Ranvier worked especially with osmic acid, fixing the fibres when at rest and during contraction. W. Krause ('Allgemeine und Mikroscopische Anatomie,' p. 92) describes the contraction as follows:—The thickness (in the length of the fibre) of the dark stripe or an isotropous substance remains the same as far as can be seen, while the thickness of the isotropous substance "Zwischensubstanz" becomes less. From this he argues that the substance of the clear stripe, which he considers as fluid "Muskelkästchenflüssigkeit," passes between the little elements of the dark stripe, causing their lateral separation, and therefore broadening and shortening the fibre. Engelmann ("Neue Untersuchungen über die Microskopischen Vorgänge bei der Muskelcontraction," in 'Pfluger's Archiv,' Band xviii) is certain that the light stripe during complete contraction becomes darker than the dark stripe, and that there is a period, as naturally follows from this observation, when the fibre is quite unstriated. The stripes are, in fact, reversed, the bright one becoming the darker, and *vice versâ.* Both stripes narrow, but especially the bright one. Engelmann advances a theory to account for this, holding that the cause of contraction is the passage of fluid from the isotropous clear stripe into the anisotropous substance; the former shrinks and the latter swells. Most startling is the view of Merkel ('Hofmann und Schwalbe,' vol. i, p. 116), who believes that the dark stripe shifts its position, arranging itself by Dobie's line, while the light stripe passes to the centre.

It is, as will readily be admitted, somewhat difficult to know what to believe, for there is such entire disagreement among physiologists as to simple facts, to say nothing of any conclusions which may be drawn from them. Thinking that there must be some simple clue which would solve the whole problem, I commenced to work at the subject in the summer of 1878. At the onset the clue was discovered, and the substance of the present paper was written by the end of that year, before I had read for the first time the paper of Mr. Bowman's, in the 'Transactions' of this Society. My astonishment was indeed great to find in it the first glimmerings of my own opinions, for although the subject had then been worked out but in the rough, and he had a much simpler problem to deal with, yet undoubtedly he held the same views in the main. My obvious course was, therefore, entirely to rewrite my paper, making every acknowledgment to his already published work. Mr. Bowman considered, as far as I can make out, that the light stripe was to be compared with the cement seen in longitudinal fibrillation between the fibrillæ, yet he looked upon the striæ as being due to the shape of the fibre. From the history of the subject, which has just been given, it will be seen that all observers are not agreed as to the actual appearances of a striped fibre, and especially the changes which occur during contraction, and we hold that they have fallen into great and unwarrantable error in the conclusions (these, indeed, are all contradictory) drawn from these appearances. A fibre has been observed in the field of the microscope, which is marked transversely, as already described, and all modern investigators have concluded that the transverse bands mark the positions of discs (seen on edge) of tissue of different refractive indices and chemical composition, alternating in the long axis of the fibre. This is, however, purely an assumption which in no way follows.

We can also account for all these cross markings in a way which involves no theory, and requires for its appreciation but a knowledge of most elementary geometrical optics.

If a small fragment of muscle be teazed out in water, salt solution, or almost any other fluid, and examined in the ordinary way with a power of 300 diameters or more, the important fact may be made out which is the basis of all these future observations, that the borders of the fibres are not smooth, but undulate, presenting wavy margins (Fig. 1).

In the fresh unstained preparation there is a halo around the edge of the fibre which masks this appearance, yet by carefully adjusting the mirror so as to obtain oblique light, or by searching for a fibre partly in the shade of another, the crenulated border may be made out; in the case of insects' muscle this is, however, always easy to demonstrate, for the fibres are much coarser, indeed, the appearance has been often figured in the works, even of recent histologists. If the preparation be stained by any of the ordinary dyes, perhaps most readily by picro-carmine, the border is in all cases very distinct, and the regularly sinuous margin is unmistakable. Now, what is the significance of the wavy outline? It is, as will readily be understood, that the fibre is ampullated, the wavy outline being but the optical expression of such a figure. A muscular fibre is, then, not a smooth cylinder, but is like the turned leg of a chair, or like the transversely ribbed neck of a common water bottle in shape. If the fibre be broken up into fibrillæ, which is very easy, after maceration in alcohol, these are seen to have just the same characters; indeed, a small bundle of fibrils is most convenient for study. It may be well to remark that the ultimate fibrillæ often show but little cross marking, and appear almost filamentous; that is, however, only due to their small size; a good lens will bring out both points.

The above described appearances may be observed in all the varieties of muscle that I have as yet examined, *e. g.* those obtained from man, the dog, cat, rabbit, guinea-pig, mouse, frog, mussel, crab, bee, wasp, Dytiscus, Hydrophilus, common house-fly, &c., &c.

The transverse stripings of the fibre are related to and correspond with the inequalities of the surface (Fig. 1). The little elevations at the borders correspond, of course, to the little ridges which run round the fibre, while the dips at the borders are the optical expressions of little valleys running between them. In the ordinary position the dark stripe marks the position of the ridge, and the light stripe lies in the little valleys, as will be seen on reference to fig. 1. Then, again, Dobie's line (Krause's membrane), which is a faint dark band in the very centre of the bright stripe, runs along the bottom of the valleys (D in the diagram), and Hensen's stripe in the centre of the dark band, lies on the exact summit of the ridges. (H, fig. 1.)

This position of the stripes in a normal muscular fibre is the invariable rule, and the idea at once suggested itself, *may not the shape of the fibre itself cause the cross stripings?*

Any student of natural philosophy would at once affirm that a structureless fibre of such a shape must be cross striped, and a glance at the neck of the ribbed water bottle on the table will elicit the same answer from any one.

The question we must now determine is, are the appearances seen in the fibre just the same in all their details, as would be produced by a piece of glass, or any other homogeneous transparent substance of the same shape?

Before, however, entering into theoretical grounds, it may be as well to give a full description of what is actually to be seen, for this has yet not been stated.

With a structure of complicated figure, such as the one we are considering, it is obvious that there is no one focus in which it may be described. There is one pretty definite focus for a single speck or thin film, but even when examining a simple cylinder, it is evident that when the borders of it are clear and distinct, the upper surface is slightly out of focus. We shall see, that in the case of the muscle, although there is one position of the lens when the parts are very distinctly seen, and in which they have mostly been described, yet that on slightly altering the focus, the appearance is changed. These changes we must carefully study.

For this purpose we may select the large muscles of the thigh of a rabbit; stretch them ever so little upon a piece of wood, and place them for some days in 50 per cent. alcohol. A high power is required for their examination; I have been in the habit of using a $\frac{1}{24}$ inch of Gundlach, a very perfect lens; a $\frac{1}{10}$ inch will, however, do. A small bundle of fibrils should be selected in preference to a whole fibre for examination.

On focussing it becomes at once apparent that on varying the adjustment ever so little, you may bring into focus the tops of the ridges or the bottoms of the valleys which lie between them. Now this slight alteration is sufficient entirely to change the optical appearances.

First raise the lens until the fibre be out of focus and is only to be seen as a dim streak running across the field, then bring it down until its form and the cross markings are distinctly to be seen (the border is now not quite distinct on a level with the horizontal axis of the fibre). In this position alternating light and dark bands are made out, but no vestiges of Hensen's stripes or Dobie's lines. (Fig. 3, A.) The dark band corresponds with the valley and the light one to the ridge, or crest. This was the focus in which Bowman described his preparations as far as I can gather from the paper. If the lens be now lowered ever so little, the stripes are reversed, a most curious point, which was noticed by Bowman, but afterwards lost sight of. The dark band now corresponds with the ridge, and the bright band with the valley. (Fig. 3, C.) This is the focussing in which it is usually described, and in this position Dobie's line and Hensen's stripe are to be seen as a rule in uncontracted fibres.

Between these two positions of the lens there is generally a well-marked intermediate one, which is depicted in Fig. 3, B. The crests and valleys are both bright and equally so, although the slightest movement of the fine adjuster will make either one or the other the darker; on the slopes, as it were, there are, however, narrow shaded bands, which are shown in Fig. 3, B. The fibre is now quite clear and distinct, and the longitudinal fibrillation is now best made out—if it can be seen at all—and yet there is no sign of either Hensen's or Dobie's stripes.

These being the observed appearances (and they may be verified without much trouble), we will calculate theoretically the appearances which a homogeneous fibre of such a shape should present when examined by transmitted light, so as to see whether our observed effects tally with what may be theoretically calculated. Parallel rays of light pass upwards through the fibre, and in their course are altered in direction (see Fig. 4). The substance of the fibre being of higher refrangibility than the fluid in which it is mounted, the thicker parts which correspond to the ridges will act like converging lenses, causing the rays of light to come to a focus (A A′ A″), diverging again. The thinner parts (the valleys) will, on the other hand, act as diverging lenses, causing the rays to spread out, as may be seen on reference to the diagram. Now it is evident that when the objective is arranged to focus those rays which have passed through the fibre and converge over the ridges, at that same position the rays above the valleys will be diverging (see Fig. 4). This will produce a difference in the appearance, for the converging rays will give a bright band, while the position of those rays which diverge will appear darker. Alter the focus by screwing the lens up or down, and, provided the fibre can still be seen, this state of matters will be reversed; for after converging, the rays above, the position of the ridges will now be diverging, while at the same time those over the valley will be converging and will appear bright.

The condition seen in Fig. 3, *b*, which is intermediate between the low and high focussed picture of the fibre, would be obtained by shifting the lens half way between these two positions. Hensen's stripe is no doubt due to rays passing through the centre of the ridges suffering little refraction in their course, and thus causing a brightness. Dobie's line might, of course, be the reverse of this, no rays at this point coming to the eye of the observer; but we shall speak of this more hereafter, when we shall show that there is some reason for suspecting at this point a distinct structure.

Although it is indispensable to account theoretically for these appearances, yet to most persons a simple demonstration will carry more conviction than any proof deduced from the laws of optics, however well they be understood. Instead of showing "what should be," we will study "what is."

For this purpose we will imitate as nearly as possible the figure of a muscular fibre on a small scale, and it shall be made out of a substance of uniform consistence throughout. What appearances shall we see on microscopic examination? This has been accomplished in the following manner: A glass rod is

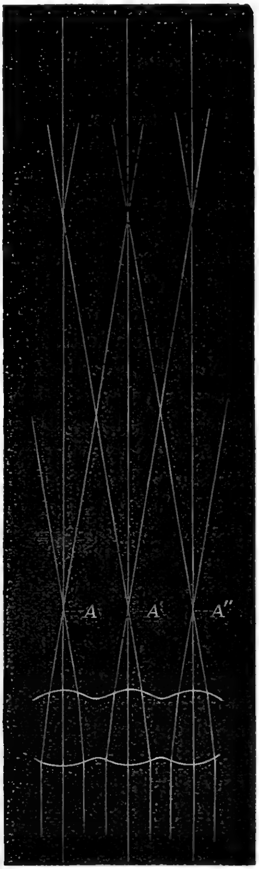


Fig. 4.—This shows the passage. The convex parts converge the rays to focus A' A'' A''', after which they diverge. The lens shifted up or down (vertically) over the ridges, and depressions will focus on the retina alternately, converging and diverging rays.

heated in a spirit-lamp and plunged into a bottle of Canada balsam; it is then withdrawn, and a little drop of the balsam is allowed to fall on a glass slide, or a thread of it may be laid out on the surface of the glass. Before the drop or thread has solidified it is indented with the milled head of a fine screw, and examined with a power of from twenty to fifty diameters, when cross shadings are to be observed. These are seen, moreover, to correspond with the surface impressions, and not only so, but they are reversed on altering the focus. Hensen's stripe is generally very well seen. The most beautiful and convincing object to study in this connection is a scale of the Lepisma. They are oval in shape, transparent, and single refractile throughout, and beautifully ribbed in their length, these ribbings or groovings being indeed so fine that a power of at least 500 diameters will be required to make out those points to be here described. You would think on looking at one of these scales that a piece of muscle was flattened out before you on the field: no rough balsam model, but a perfect illustration taken from the back of a tiny insect.

The appearances it is needless to describe, for they are, almost to the minutest detail, those of a muscular fibre. The bright and dark stripe interchanging with every alteration of focus, Hensen's stripe, and Dobie's line (Krause's membrane) are all to be seen. In the case of the Lepisma scale the line of Dobie is in the centre of a bright band, which is broader than the dark band with Hensen's stripe. This is, of course, the other way in the case of the muscular fibre.

We see, therefore, that a muscular fibre presents just those appearances which a transparent body of uniform texture and of similar shape would possess. However conclusive these proofs

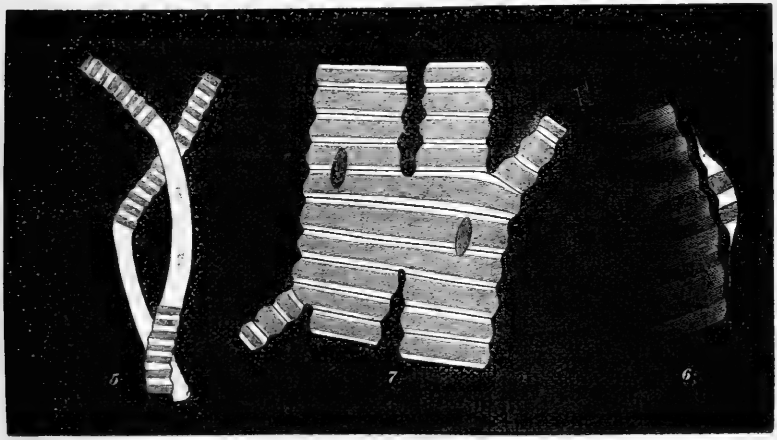


FIG. 5.—Muscular fibre described by Messrs. Geddes and Beddard.
FIG. 6.—On the right a nucleus is moulded on the fibre and is striped, the stripes corresponding to the depressions on its surface.
FIG. 7.—This shows the muscular fibres of the heart.

may have been, it is well to collect all evidence possible to show that these markings are nothing more than optical effects, to which end a very testing experiment was suggested to me by Professor Tait. It is evident that if these cross bands are seen when parallel, or nearly parallel, rays of light are passing through the fibre, by using converging or diverging rays the appearance will be altered, and it will be possible by careful adjustment of a lens to cause a total reversal of the striping. If a fibre be carefully focussed and a strong biconcave diverging lens be placed between the stage of the microscope and the mirror, and carefully moved about with the fingers, it will be possible entirely to alter the fibre, causing a total reversal of the cross bands. On withdrawing the lens, of course the fibre resumes its normal appearance. I may mention that several lenses were tried before one was found which would in at all a satisfactory manner show this phenomenon; when successful the experiment is very striking.

In opposition to my view is the one generally accepted, namely, that the cross stripings are produced by differences along the fibre of chemical composition, and refrangibility.

Now, suppose that there were along the fibre two alternating structures, A and B. Let A represent the bright stripe and B the dark stripe. If A has a higher or lower refractive index than B, it is evident that although they were immersed in any number of fluids of refrangibility varying from the lowest to the highest, yet A would always be distinguishable from B, and the striping would always be apparent. Then, again, by placing the fibres in fluids of indices near to that either of A or B, the most striking would be the contrast. If, however, the fibre were homogeneous throughout, the striping being merely due to the form, then if the fluid and the fibre have the same refractive index all striping will disappear. On Professor Tait's suggestion, I tried a series of fluids formed by mixing, in various proportions, alcohol, whose refractive index is low, with oil of cassia, which is high. In this way I have prepared specimens showing almost no cross striæ, the fibre appearing uniform until after most careful examination.

Dr. Klein has since shown me some muscular fibres of an insect. They were quite smooth and cylindrical, and *were unstriated.* In these specimens there were, on very close examination, cross lines separated by comparatively wide intervals. It is possible that they represented Dobie's lines.[1]

---

[1] More recently my friends, Messrs. Geddes and Beddard have demonstrated a very curious condition in the muscular fibres of the Echinus, which my views entirely explain. They noticed that in the same fibre some parts were cross striped, while in parts no striation was to be seen. Hearing of

But it may well be asked, What about the action of staining agents, such as logwood, which is stated to tint the dark stripe and Dobie's line? Does this not show a difference of structure along the fibres?

Once having the clue it will be understood that just as the unstained fibre will modify and change the direction of rays passing through it, so will also a stained fibre produce what are apparently modifications of the staining effect. It is generally stated that the dark band and Dobie's line are stained by logwood and carmine, while the bright bands remain unaffected; also that Hensen's stripe in the centre of the dark stripe is stained only to a slight degree; whence it follows that if staining action is to be the criterion, this stripe differs in structure from the dark stripe.

We, however, affirm that the whole fibre is stained, and equally stained throughout. The bright band is undoubtedly stained, although it appears not of the deep blue of the dark stripe when coloured by logwood; and this conclusion is drawn not only from an examination of my own specimens, but also from some of great beauty shown to me by Dr. Klein. Why the bright band does not appear of so dark a blue is, that the apparent shading of the latter is added to the blue tint, producing a depth of colour. The most conclusive proof of this is, that one can often reverse the colouring on readjusting the focus, and that Hensen's stripe or the bright part of the dark stripe is only of a faint light blue, like that of the bright stripe.

Picric acid stains muscle very readily, and colours it throughout. The fibre to the naked eye is yellow and uniformly so, but when examined by the microscope, alternating yellow and shaded yellow bands are to be observed, which reverse their position on changing the focus. With a high focus—when the crests are bright in the unstained preparation—they are of a bright yellow, while the valleys are of a deeper yellow tint.

To show the effects which a fibre of this shape can produce when transmitting monochromatic light, nothing can be more conclusive than the following experiment:—A slip of coloured blue glass is held obliquely between the reflector and the stage of the microscope, so that blue rays pass through the fibre. It does not appear of a uniform tint, but beautiful blue stripes are seen corresponding with the crests and valleys, and varying with alterations of focus. If a piece of red glass be substituted for the blue slip, red cross stripes are seen in corresponding places.

---

my explanation of the markings, they re-examined their specimens (which I have also seen), and found that when the striæ were visible there, and only there, the fibre was ampullated. (See Fig. 5.)

For this experiment the fresh fibres of insects' muscle should be examined, for, with fine mammalian muscle, the light is not so good, owing to the higher power required. This experiment has been introduced here with the description of stained muscle, not that it can be strictly compared with an ordinary staining process, but simply to show what an influence the fibre's shape must have upon the tinting, supposing, as we do, that this is in reality uniform.

An investigation such as this is beset with many difficulties and fallacies, and I may mention one which befel me in this stage of my work.

I had stained a few muscular fibres of a rabbit with picrocarmine, and on examination, what was my surprise to find that in some of them the light stripes (valleys) were most brilliantly stained with carmine. I was long puzzled at this, when it was last discovered that the picro-carmine had dried somewhat on the preparation, and the carmine had mechanically precipitated along the valleys, filling them up. At the end of one or two fibres this precipitation had partially peeled off, showing undoubtedly the true nature of the phenomenon.

I have in my possession very beautiful alcoholic preparations stained with logwood. At first sight, from a study of many of the fibres, one would be led to believe that the bright stripe is wholly unstained, while the dark stripes are of a beautiful violet. A careful examination, however, reveals the fact that such fibres are broken up transversely, looking like piles of coins, a very common occurrence, especially in preparations that have been long mounted. The coins, lying close to one another, with narrow chinks between, of course revealed transverse unstained tracts, which could well be mistaken for the bright stripe.

More interest and discussion has hitherto accrued to the action of muscle on polarised light than to the effects of staining reagents. We have seen that much difference of opinion exists; Brücke has maintained that not only is the dark stripe (ridge), as all are agreed, doubly refracting, but that the whole of the light stripe is isotropous. I myself was led to modify this, discovering that on careful focussing with a fibre not at all sheared in its length, the central part of the light stripe was undoubtedly anisotropous. This I have afterwards seen figured, as before mentioned, in Hermann's 'Physiology,' and have introduced the diagram into Fig. 2. It is a point of some practical difficulty to mark exactly the positions of the cross bands while turning the analyser, and thus changing the character of the field. This difficulty has been overcome completely by a suggestion of Professor Tait's, who has helped me much in this part of the work. Very fine emery powder should be sprinkled over the preparation before covering it; for then, on examination, numberless little black specks will be seen in the field. A cross band of a fibre is selected for examination which is exactly opposite one of these little specks, then when you rotate you can definitely affirm, having the little black speck for your guide, what change has occurred.

Rabbits' muscles are very satisfactory objects for examination, as they do not cleave across at all readily. The adductor muscles of the leg should be excised, slightly stretched on a piece of wood, and placed in 50 per cent. alcohol until they split readily into fibrils. They may then be mounted in any ordinary fluid, a pinch of emery powder having been sprinkled over the preparation before covering.

It is necessary to use a power of 800 or 1000 diameters in the investigation of mammalian muscle, while in the case of the insect one of 300 diameters is quite enough.

In the living and dead muscular fibre the whole of its substance is doubly refracting. The observations of some modern observers entirely agree with my own, in that with crossed Nicols the crests (dark bands) and the centres of the valleys (bright stripes) appear bright and therefore refract light doubly, and that there are two dark bands on the slopes between them (see diagram, p. 5, Fig. 2). It does not follow, however, that these two dark bands represent tracts of isotropous substance. This is the point at issue. The dark lines between the valleys and ridges which appear when the Nicols are crossed have been interpreted as marking the positions of cross bands of singly refracting substance, but this is a fault of reasoning. If the fibre were smooth and cylindrical it would then follow, but the fibre is not, as we have already insisted. These bands lie just on the sloped parts of the fibre; those sections, in fact, which are oblique to the passing rays; and the explanation is now quite easy, for the extraordinary ray passing through the fibre is naturally deflected at these parts, and does not reach the eye of the observer. Hence the body appears not to transmit them at all at these parts.

It is not difficult to explain the discrepancies between Brücke's description of the bright stripe and my own.

It is essential to be very scrupulous in the selection of a fibre for examination. It must not be at all twisted, nor sheared in the slightest degree, for then the cross stripes are not at right angles to the long axis, and as their width is several times their thickness (in the length) overlapping will to some extent occur. This will certainly lead to very confusing results, and the bright centre of the bright stripe (valley) may well be overlooked, Moreover, the fibre should be slightly stretched, and as small as possible.

It has also previously been mentioned that in many preparations the fibres split up transversely in a most regular manner, and, unless the cover-glass be pressed upon, the little discs remain in position with narrow chinks between them. These chinks will be filled with the isotropous fluid used for mounting, which will lead to very anomalous appearances, and which may perhaps help to account for some of Brücke's statements. These fallacies may be avoided by a study of the fresh fibres of insects' muscle. Dytiscus and hydrophilus muscle has received a large share of the attention of histologists, but that from the wasp or blue-bottle fly is quite as good. A leg should be pulled from the trunk of a blue-bottle fly, and this again forcibly separated at the middle joint. A piece of muscle will project from one of the segments, which may be cut off and examined in a drop of fluid expressed from the thorax of the fly. The polariscopic effects may then be made clearly out in the still contracting fibres. I have tested all these points by a careful examination of insects' fibres with thin plates of selenite and mica. This method is not so satisfactory, nor do the differences of colour seem to give such reliable evidence as may be obtained by the crossed Nicols alone.

The fibre during contraction.—Living insects' muscle may be examined and the changes observed when the waves of contraction pass along the fibre, or, perhaps better still, they may be fixed with osmic acid. The muscles from the leg of an insect are rapidly separated out on a slide, and a drop of weak osmic acid added, which kills the fibres instantaneously, fixing them in the position that they happen to be in. On examination one generally finds fibres which in part of their course are contracted, and in other parts relaxed, when the differences in appearance may readily be studied. It may here be observed that the fibres bulge at the contracted part, so that if the surfaces be examined the focus of the microscope must be accommodated.

The cross stripes are nearer one to another and correspond, as before, with the ridges and valleys seen at the margin, which are much more prominent and bolder in outline.

In the contracted fibre the striping is practically the same as in the stretched condition. The contracted fibre exhibits just the same reversing of stripes on alteration of focus, and Dobie's line and Hensen's stripe can both be seen in the same positions as in the uncontracted muscle, provided the fibre is suitably placed for examination, and not sheared in its length. We must entirely deny the common statement, first introduced, we believe, by Engelmann, that in the contracted state the bright band becomes the darker. If good specimens of insects' muscle be examined, which have been treated with osmic acid, and if the fibre be not sheared, the valley is always bright in the ordinary or deeper focus. I have verified this point in very many cases. Passing along a fibre from the relaxed end to a part where the contraction is fullest, the appearances vary in degree, but not in kind. The main features are in both cases the same, but the stripes are now narrower, and often it is not so easy to see Dobie's and Hensen's stripes. It follows, from the statement of Engelmann's, "the bright stripes become darker than the dim," as he himself notices that at one point, or phase, in the contraction, no striping is to be made out. We agree with Ranvier that this is not true; indeed, it would be impossible for a muscular fibre with its configuration not to be marked across its length.

This subject will call up to the mind of every working histologist appearances which he must have met with in other fields of research. Many tissues naturally, or after clumsy manipulation, present ampullations which always coexist with cross striæ. The fibres of the crystalline lens are wavy in outline, and when many of them are bound together and seen on edge with the wavy outline towards the eye of the observer, cross bands are seen which in chance preparations (especially those of the frog's lens) simulate muscle in a wonderful manner. Ordinary non-striped muscle, which may be so well seen in the frog's bladder, is often faintly ampullated, especially, perhaps, in chloride-of-gold preparations. Cross stripes may also here be seen. The fibres of Tomes, when a section of softened tooth is teazed, are pulled out of the dentinal tubules, and, being of a soft and somewhat elastic nature, on breaking they become often very beautifully ampullated, and it would be impossible to distinguish them from muscular fibrillæ. In the class of practical histology, on more than one occasion students have asked me the meaning of beautiful cross shadings seen on nerve fibres; a slight ampullation, which fully accounted for it, was always found.

Many more instances of a like nature will be recalled in the experience of every one; it is needless to enumerate further.

In the winter of 1879-80, while examining fibres of the muscles of a newly-born child, a very curious discovery was made. A nucleus adhering to the sarcolemma was seen beautifully striped. It was not in close apposition to the fibre, a very narrow chink intervening. On focussing with great care, it was seen that the cross bands upon it corresponded with those of the adjoining fibre, a dark one, however, for a light one, and *vice versâ* (fig. 6). Now, the curious point was that the nucleus had evidently been impressed by the fibre, moulded upon it, as it were, and on being pulled apart had presented a perfect cast of the surface. One would hardly believe in sarcous elements here. Last summer (1880) my friend Mr. Priestley communicated to me a similar and independent observation of his own, as a contribution towards the maintenance of my views upon the formation of the stripes.

The position that we have reached is this:—A muscular fibre presents such cross markings, varying with shifting the lens up or down, as a filament of homogeneous structure and similar shape. I have shown this experimentally, and have illustrated with simple experiments, which it is in the power of any one to test. This being the case, I have searched to find if there be reason to assert any want of uniformity along the fibre, using various methods of staining. This I have failed to do, and have shown that the views commonly held are to be explained simply by the shapes of the fibres. As to the action of muscle on polarised light, I saw reason to dissent from the views of Brücke, and subsequently found my own in accordance with those of other recent observers. I differ from them in the explanation I offer of the two dark bands seen with crossed Nicols, for here, again, the shape of the fibre explains their presence without looking for any special structure.

So far we are led to consider the fibre as made up of many ampullated fibrils, packed side by side, forming an ampullated fibre, *these fibrils being uniform throughout*, and joined together by some cementing material, the nature of which we will not hazard. The only point which would suggest a definite structure along the fibril is the attachment of the sarcolemma in insects' muscle to Dobie's lines. There is no doubt that this membrane dips down and seems prolonged into Dobie's lines in a most beautiful and regular manner. The significance of this is very obscure, and is quite beyond me. There are many possibilities. It may be, although there is no proof of it, that a membrane exists here continuous with the sarcolemma; it may be that there is nothing but some cementing substance more soluble in alcohol than the sarcous matter; it may be that there is a little minor crest at this point to which the sarcolemma is attached. This little crest I have certainly seen in some fibres, and it has already been figured by more than one writer, yet in others, whose outlines are wonderfully distinct, no trace of it is to be made out. The fibres can hardly be said to break across in the line of Dobie; all that can definitely be affirmed is that they cleave in the thinnest part, or the light stripe. The investigation of this point is one of great difficulty, owing to the haze around the broken points, and I can never make up my mind to any definite statement. This transverse cleavage is not, of course, a point of very much weight, as the fibre would naturally tend to split across in or near Dobie's lines, as here it is thinnest.

The striping of muscle can be easily explained, as shown before, which leads us to our final statement. A fibril is structureless throughout its entire length, except that, perhaps, there may be membranes, or lines of fission, or layers of cement at the positions of the lines of Dobie; this we leave an open question. In using the word "structureless," I must not be misunderstood; structureless membranes and tissues are fast losing their place in histology, and once simple protoplasm is now most complex. What I infer is that the stripes do not mark the positions of alternating layers of different structure, the presence of which are ordinarily maintained. The complicated Muskelkästchen of the Germans does not exist.

The muscular tissue of the heart presents many peculiarities which it is needless here to enumerate, for the cross striping alone concerns us. All those cross bands which have been described in ordinary voluntary muscle may here also be seen, and they are placed in the same relations with the turned surface of the fibre. The dark stripe corresponding to the crests, or ridges, the light bands to the depressions between them. (Fig. 7.) Dobie's lines may be made out with great ease, and as there is no sarcolemma here, they may be accounted for also purely from the shape of the fibres. I have often thought that Dobie's lines marked the positions of tiny ridges in the valleys, but this is a point more difficult to decide perhaps than in the case of the skeletal muscles. Transverse cleavage takes place here also in the thinner part of the fibre, namely, in the bright stripe, but whether or not exactly in Dobie's line I have not yet definitely made out.

A curious appearance, often presented by insects' muscle, and sometimes also by that of the mammalia, has been described and figured by Mr. Schäfer. A paper descriptive of these he communicated to the Royal Society of London (1873), which came out later on in the 'Transactions' of this Society, and his observations are published also in the eighth edition of Quain's 'Anatomy.' These have been almost entirely overlooked by French and German physiologists, yet in many English laboratories his observations have been verified, and his conclusions taught.

They are well illustrated in a representation of the muscular fibre of a Dytiscus, which may be seen in Quain's 'Anatomy.' The dark stripes are traversed longitudinally by dark rods, which end at both extremities in little knobs. These knobs lie in the borderland between the bright and dark stripes. The only point which I would add to his figure is this, that the knobs are joined across the clear stripes or valleys by lines, just as they are so joined across the dark stripes, although the lens must be depressed ever so little to make this out. These lines are, in fact, nothing more or less than the longitudinal striæ described many years ago as lying between the fibrillæ of which the fibre is composed, these little knobs lying in their course. This can, perhaps, most conclusively be made out in the following way:—Allow a piece of insect's muscle to remain in a drop of water for some hours (which will vary with the temperature) until it has partially putrefied. Then cover and examine, when many of the fibres will have separated towards their ends into fibrillæ. One can then distinctly trace the chinks between the separated fibrillæ as being continuous with the striæ, on which the knobs are still seen in the centre of the fibre. I think that the following is a feasible explanation of these knob-like enlargements of the cementing substance seen as longitudinal striæ. These knobs occur, as will be beautifully seen on referring to the woodcut in Quain, on the slopes between the valleys and the ridges. The cementing substance dips down here with the fibre itself, and if there be the slightest lateral obliquity it will appear larger. You see the cementing matter on edge, and differing as it does from the muscle-substance in refrangibility, a distortion occurs, giving rise not to a dark line as on the surface, but to a dark knob. This is, in fact, but an optical delusion, for the striæ are quite uniform, and were the fibræ cylindrical would appear so. This may be proved by the fact that very often if the rays of light from the reflector are oblique, but one set of dots appears, which shift over to the other side on twisting the mirror. By shifting the preparation about, or by twisting the tube of the microscope obliquely, the dots disappear from one part of the fibre to appear in another, showing that it is but an optical effect, and that no structure here exists.

Before concluding I must gratefully acknowledge much help and sympathy which I have received in this investigation.

To Professor Tait I have gone when in any difficulty, for an observer in a case such as this must have the aid of an experienced physicist, otherwise grievous error is but courted. To him, as has been seen in the text, I owe many suggestions, and he has kindly entirely looked over my paper. Dr. Klein has shown me great kindness in carefully examining my preparations from the histological point of view, and as has before been mentioned, in showing me preparations to corroborate my views. My thanks are due to my friend Mr. Priestley for many useful hints, especially concerning the literature of the subject.

Addendum.—An assertion has been lately made which if true would be entirely fatal to my views. It was, that in the fresh condition the fibres are cross striped, but at the same time their borders are quite smooth. That this is erroneous is easy of demonstration with any fresh fibre, but especially with that of an insect. It is true that often from the manipulation, or their own contraction, they are twisted, when the convex border will on a careless inspection appear as a distinct line. The stripes at this point are not at all distinctly seen, but yet can always be made out, as well as the crenulated border. Indeed, this has been figured by more than one observer (Krause), and it was in the fresh muscle of the crab that I first observed it.

Dr. Klein informs me that there is no doubt as to the transitional stage in the contraction of a muscle described by Engelmann. It will be remembered that this is intermediate between the contracted and relaxed part, and that here the fibre is non-striated. Dr. Klein from a study of one of Englemann's preparations was able to give the true explanation of this. The part which was non-striated was perfectly smooth in outline, passing at either end into the striped and crenulated fibre. Probably the intermediate part is a piece of the fibre stretched by its contraction, the two ends being fixed.

On the RELATION *of* MICRO-ORGANISMS *to* DISEASE. BY JOSEPH LISTER, F.R.S., Professor of Clinical Surgery in King's College, London.[1]

THE relation of micro-organisms to disease is a subject of vast extent and importance. If we compare the present state of knowledge regarding it with that of twenty years ago, we are astonished at the progress which has been made in the interval. At that time bacteria were little more than scientific curiosities; whether they were animal or vegetable few people knew or cared, but most regarded them as animals on account of the active movements which they often exhibited. That they were causes of putrefaction, or other fermentative changes, was a thing scarcely thought of; and the notion that they had special relations to disease would have been regarded as the wildest of speculations. Now, however, a mass of information has been accumulated regarding all these points, of which it would be hopeless for me to attempt to give even a brief sketch in the time at my disposal, and all that I can do is to present to the pathlological section a few examples illustrating the progress which is being made in this department of research.

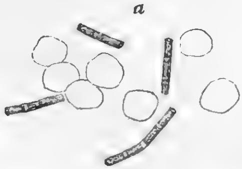
First, I will mention some examples of the labours of Dr. Koch, of Wollstein, in Germany. Though a hard-worked general practitioner, Koch has contrived to devote an immense amount of time and energy to his investigations, and by a combination of well-planned experiments, ingenious methods of staining bacteria out of proportion to the tissues among which they lie, a beautiful adaptation of optical principles to render the coloured objects discernible by the human eye, and, further, by a most successful application of micro-photography, he has succeeded in demonstrating the presence of these minute organisms in a manner never before attained.

The *Bacillus anthracis* is now universally recognised among pathologists as the cause of splenic fever, so fatal among cattle in this and other countries, and capable of being communicated to various other animals, and, among the rest, to the human species, as has been lately illustrated by the so-called woolsorters' disease, in the North of England. The *Bacillus anthracis* is a large form of bacte-

---

[1] An address delivered before the Pathological Section of the British Medical Association at Cambridge, August 12th, 1880.

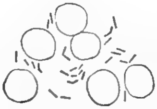
rium, as is shown at *a* in the accompanying woodcut. It is



there represented magnified 700 diameters, along with red blood-corpuscles of a mouse, and the rods of which it is composed are seen to be in diameter nearly one fourth of that of the red corpuscles. Koch's method of staining the sections shows in the most beautiful manner that these bacilli are not only present in the spleen and some other organs, but that they people the blood in the minute vessels of all parts. Koch has thus added to our conviction that the bacillus is the cause of the symptoms, seeing that, as he remarks, it is impossible to suppose that an organism can develop in such enormous numbers at the expense of the vital fluid without exerting a serious influence upon the system.

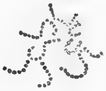
But the most striking and important results of Koch's methods of investigation are those which relate to organisms of much smaller dimensions. He found that, if putrid liquid is injected under the skin of a mouse, the animal may die in the course of a short time, as the result of the chemically toxic effects of the products of putrefaction absorbed into the circulation; but, if it survive this primary disorder, it may succumb in the course of about two days to blood disease. If the point of a lancet be dipped into the blood of the heart of a mouse which has died in this way, and a scratch be made in the skin of a healthy mouse with the envenomed instrument, the second mouse dies with similar symptoms to those of the first, the poison being absolutely certain in its virulent operation; and the same thing may be continued indefinitely through any series of animals. If now sections of the tissues be made and stained, and examined by Koch's procedures, it is found that the entire blood of the diseased animal is peopled with bacteria, resembling those of the *Bacillus anthracis* in the enormous multitudes in which they are produced, and also in their rod-like form, but differing from them in being exquisitely minute and delicate, as is shown at *b* (drawn on the same scale as *a*, as is indicated by the accompanying outlines of red corpuscles), where it is seen that the diameter can only be represented by a slender streak not one eighth of the diameter of the *Bacillus anthracis*, and such as, before the introduction of Koch's method, would have escaped notice altogether. Now, this disease is totally distinct from pyæmia, being not accompanied with multiple abscesses or embolism; and thus it has been shown by Koch that septicæmia may exist as a deadly blood disease, caused by the development of micro-organisms, equally distinct from pyæmia and from the chemically toxic effects of septic products.

b



On some occasions, as the result of the introduction of putrid fluid under the mouse's skin, Koch found, besides septicæmia, a local affection of the seat of inoculation, in the form of spreading gangrene; and, on investigating the part, he discovered in it, exactly corresponding with the extent of the local affection, another organism very differently formed from that of the septicæmia, viz. a micrococcus, consisting of minute spherical granules arranged in linear series, like strings of exquisitely minute beads, as represented at *c* in the woodcut. Believing that this locally developing organism must be the cause of the gangrene, he tried to separate it from the bacillus of the septicæmia, and succeeded through an accidental observation of great interest. Having till that time employed the house mouse in his experiments, he happened to try the inoculation of a field mouse. This animal, though so closely allied, proved not susceptible of the septicæmia. The bacillus of that disease was unable to grow in the blood of the field mouse, but the micrococcus of the gangrene could develop among its tissues. The new organism was thus obtained in an isolated form, and, when now inoculated into the house mouse, produced in that animal gangrene pure and simple, extending for an indefinite period among its tissues.

c



Thus the animal body, which had previously been an obscure field of labour in this department, in which the pathologist did little more than grope in the dark, was converted by Koch into a pure cultivating apparatus, in which the growth and effects of the micro-organisms of various infective diseases could be studied with the utmost simplicity and precision.

One more example I must take from Koch's work. On one occasion, as the result of inoculating putrid liquid into a rabbit, he observed a spreading inflammation having all the clinical characters of erysipelas; and, on examining stained sections of the part, he discovered another exquisitely delicate bacillus resembling the micrococcus of the gangrene in being local in its development, while its exact correspondence in extent with that of the disease led fairly to the conclusion that it constituted the *materies morbi.*[1]

I will next refer to a disease occasioned by a micro-organism discovered by the eminent pathologist, Professor Toussaint, of Toulouse, whom I am proud to see present in this Section to-day. This disease has been somewhat inappropriately termed *Cholera des poules,* or fowl cholera, for it is not attended with diarrhœa or any other of the symptoms of cholera; but, as it happened to be extremely destructive among the poultry-yards of Paris at the same time that an epidemic of cholera was raging in the city, the disorder that prevailed among the fowls was also given the name of cholera. The lesions by which it is chiefly characterised are great swelling of the chains of lymphatic glands in the vicinity of the trachea, pericarditis accompanied with great effusion, and congestion, and it may be ulceration, of the mucous membrane of the duodenum. It is a blood disease, and highly infectious. If some of the blood of a chicken that has died of it be mixed with the oats with which healthy chickens are fed, a considerable proportion, perhaps four out of six, are affected and die; and similar results are produced by mixing the intestinal excreta of diseased fowls with the food. It is an interesting question how the virus thus administered enters the circulation. The invariable affection of the lymphatic glands of the throat suggests to M. Toussaint the idea that some accidental abrasion of the epithelium in the mouth or pharynx is probably the channel; and this view is confirmed by the fact that a similar affection of the lymphatic glands, together with other symptoms of the disease, is produced by inoculating the chicken in the mouth; and fur-

---

[1] See 'Untersuchungen über die Ætiologie der Wundinfectionskrankheiten,' von Robert Dr. Koch, Leipzig, 1878. A translation has just been issued by the Sydenham Society.

ther, by the circumstance that such chickens as fail to take the disease when fed with the infected food are liable to it when inoculated, implying that it was merely some accidental circumstance which secured their previous immunity. This disease has been made the subject of special investigation by M. Pasteur. He found that the micro-organism could be readily cultivated outside the body of the fowl. It was, indeed, somewhat particular as regards the fluid in which it would grow; thus yeast-water, in which the *Bacillus anthracis* grows readily, proved an unsuitable medium for this organism, but it grew luxuriantly in chicken broth, and, indeed, in infusion of other kinds of meat; but chicken broth proved peculiarly convenient for the purpose. M. Pasteur has been so kind as to send me some tubes in which the organism has been cultivated, and a drop of the liquid has been placed under a microscope on the table. It will be seen that the organism is a minute form of bacterium, oval-shaped, tending to multiplication by transverse constriction, and very frequently seen in pairs, and occasionally in chains. Its transverse diameter is from 1·50,000th to 1·25,000th of an inch, so that it resembles very closely the *Bacterium lactis*. The woodcut *d* represents a camera lucida sketch of the organism sent by M. Pasteur. So far as I am aware, this is the first time this bacterium has been shown in this country. Now, it was found by Pasteur that the organism could be produced in chicken broth in any number of successive cultivations, and to the last retain its full virulence, so that, if a healthy chicken be inoculated with it, the fatal disease was produced as surely as by inoculation with the blood of a fowl that had died of the complaint. This was pretty conclusive evidence that the organism was the cause of the disease, and that it constituted the true infective element; because any other material that might be supposed to accompany it in the blood of the diseased animal must have been got rid of by the successive cultivations in chicken broth.

d

The growth of the organism occasions no putrefaction in the liquid; so that this is a good example of a bacterium which is most destructive as a disease, but which is at the same time entirely destitute of septic property, in the primitive sense of that term as equivalent to putrefactive. After the bacterium has grown for a certain time in a given portion of chicken broth, it ceases to develop further; and when this is the case, although the broth has lost only a very small proportion of its substance by weight, and although, as aforesaid, it has not undergone putrefaction, and still constitutes an excellent pabulum for ordinary forms of bacteria, the bacterium of the fowl-cholera, though introduced from some new source, is incapable of growing in it. This fact seems highly suggestive of an analogy with the effects of vaccination, or those of an attack of measles or scarlatina in securing immunity from the disease for the future. Here we have a certain medium invaded by a virus capable of self-multiplication, as is the case with those diseases in the animal body; the medium itself little affected chemically by the growth of the virus within it, but nevertheless rendered unfit for the development of that virus for the future. But something more than the suggestion of analogy with vaccination has been effected by M. Pasteur. By cultivating this bacterium in a particular manner, which he has not yet published, he enfeebles the organism, as he believes, and produces such an alteration in it that, when inoculated into a healthy fowl, it produces only a modified, and no longer fatal form of complaint, but the bird is thereby rendered secure against taking the ordinary form of the disease. It has been really vaccinated, if we adopt M. Pasteur's extension of the term vaccination to other similar cases; for just as we speak of an iron milestone, we may, if we please, apply the term vaccination to the use of a virus other than the vaccine obtained from a heifer. But though the vaccination with the modified bacteria of the fowl-cholera does not occasion the fatal disease, it produces pretty severe local effects. If inoculated on the breast of the fowl, it causes a limited gangrene of the pectoral muscle, the affected part falling off in due time as a dry slough. Through the great kindness of M. Pasteur, I have now the opportunity of showing to the Section a hen which has been treated in this way. You observe a slough on the breast of the bird about as large as a penny piece; it is dry, and obviously old. The fowl has been some days in my possession subsequently to its journey from Paris; but though more than enough time has elapsed since the inoculation to have caused its death, had the dieease been in the ordinary form, it is, you see, in good health, bright and active, and it both eats and sleeps well.[1]

---

[1] M. Pasteur's researches on this subject are related in the 'Comptes Rendus de l'Académie des Sciences,' February, April, and May, 1880.

I will now return to the *Bacillus anthracis,* with regard to which I shall have again to refer to the labours of M. Toussaint. First, however, I must allude to the work of some of my own countrymen. In March, 1878, an experiment was made at the Brown Institution, at the suggestion of Dr. Burdon Sanderson, of inoculating a calf with the blood of a guinea-pig which had died of splenic fever, which is exceedingly fatal to rodentia. The result was that the calf took the disease, but in a mild form, and recovered from it; and a similar fact was observed in two heifers treated in the same way.[1]

This line of inquiry has since been followed up by Dr. Sanderson's successor at the Brown Institution, Dr. Greenfield, with a view of ascertaining whether the milder form of the disease in cattle, resulting from inoculation with the blood of rodentia affected with it, confers upon the cattle immunity from the complaint in its fatal form; or, to use again M. Pasteur's expression, whether the cattle have been vaccinated with reference to anthrax. And I have great pleasure in being able to inform the Section, by Dr. Greenfield's permission, that the question has been answered in the affirmative; and that one bovine animal, inoculated seven months ago with virus from a rodent, has proved itself, on repeated inoculations, entirely incapable of contracting splenic fever, remaining free from either constitutional or local manifestations of it.

And now to return to M. Toussaint, who has made observations with regard to this same subject of vaccination against anthrax fraught with the very deepest interest. The question arises with regard to effective vaccination, using the term in Pasteur's general sense: Is it essential that micro-organisms should develop in the blood of the animal in which immunity from further attacks of the disease is to be secured? Or is it possible that the necessary influence upon the system may be exerted by merely chemical products of the growth of that organism in some other medium? With the view of approaching the solution of this question, M. Toussaint has performed experiment of injecting into the blood of healthy sheep blood taken from an animal affected with splenic fever, but deprived of the *Bacillus anthracis.* Taking blood from a sheep just on the point of death, when the bacillus has presumably produced all its possible effect upon the vital fluid, M. Toussaint proceeds to deprive it of the living bacillus in either of two

---

[1] See "Report on Experiments on Anthrax," by Dr. Sanderson ('Journal of the Royal Agricultural Society of England,' vol. xvi, s.s., part i).

ways—by filtration, or by destroying the vitality of the organism. The former he effects by mixing the blood with three or four parts of water, and then passing it through about twelve layers of ordinary filter-paper. The bacillus, in consequence of its large dimensions, is entirely retained by this form of filter, as is proved by the fact that the filtrate no longer gives rise to the organism in a cultivating liquid or in a living animal. Nevertheless, if injected in considerable quantity into the circulation of a healthy sheep, it produces a true vaccinating influence, that is to say, secures immunity from splenic fever. But, what is further extremely interesting, in order that this change in the constitution of the sheep may be brought about, the lapse of a certain time is essential. If a vaccinated sheep be inoculated with anthrax within a few days of the operation, it will die of splenic fever; but if from twelve to fifteen days be allowed to elapse, complete immunity is found to have been produced. Similar results followed from the injection of anthrax blood treated by Mr. Toussaint's other method, which consists of maintaining it for a considerable time at a temperature of 55° C. (131° F.), which has the effect of killing the bacillus; after which one half per cent. of carbolic acid is added, to prevent putrefaction of the liquid. The blood treated in this way having been proved to be free from living bacilli by negative results of an experiment upon a rodent, about four cubic *centimètres* are injected into the venous system of a sheep, with the effect of producing the same protective influence against splenic fever as is ensured by the filtered blood. These experiments are still in progress; but M. Toussaint informs me that he has already ascertained the existence of immunity against anthrax for three months and a half in both sheep and dog treated in this way.

I need hardly remark on the surpassing importance of researches such as these. No one can say but that, if the British Medical Association should meet at Cambridge again ten years hence, some one may be able to record the discovery of the appropriate vaccine for measles, scarlet fever, and other acute specific diseases of the human subject. But even should nothing more be effected than what seems to be already on the point of attainment, the means of securing poultry from death by fowl-cholera, and cattle from the terrible destructive splenic fever, it must be admitted that we have an instance of a most valuable result from the much-reviled vivisection.

I have yet one more example to give of researches in this domain of pathology; and this also has reference to the *Bacillus anthracis*. The investigator in this instance is Dr. Buchner, assistant physican in Munich. It is well known that the *Bacillus anthracis* is morphologically identical with an organism frequently met with in infusion of hay, which may be termed hay-bacillus. Such being the case, it occurred to Dr. Buchner that they might be merely one and the same organism modified by circumstances. For my own part, I am quite prepared to hear of such modifying influence being exerted upon bacteria, having made the observation several years ago that, when the *Bacterium lactis* had been cultivated for some time in unboiled urine, it proved but a feeble lactic ferment when introduced again into milk. Its power of producing the lactic fermentation had been impaired by residence in the new medium. In the case before us, indeed, the physiological difference between the two organisms seems, at first sight, so great as to forbid the idea of anything other than a specific difference. The *Bacillus anthracis* refuses to grow in hay-infusion, in which the hay-bacillus thrives with the utmost luxuriance; and conversely, the hay-bacillus is utterly incapable of growing in the blood of a living animal, whether introduced in small or in large quantities. The hay-bacillus is remarkable for its power of resistance to high temperatures, which is not the case with the *Bacillus anthracis*. The latter is destroyed by a very slight acidity of the liquid of cultivation, or by any considerable degree of alkalinity, whereas the former survives under such conditions. Both will grow in diluted extract of meat, but their mode of growth differs greatly. The hay-bacillus multiplies rapidly, and forms a dry and wrinkled skin upon the surface, while the *Bacillus anthracis* produces a delicate cloud at the bottom of the vessel, increasing slowly. Nothing daunted by these apparently essential differences, Dr. Buchner has laboured with indomitable perseverance, by means of experiments carried on in Professor Nägeli's laboratory, to solve the double problem of changing the *Bacillus anthracis* into hay-bacillus, and the converse. Having devised an ingenious apparatus by which a large reservoir of pure cultivating liquid was placed in communication with a cultivating vessel, so that any cultivation could be drawn off by simply turning a stop-cock, and further cultivating liquid supplied to the organisms remaining in the vessel by a mere inclination of the apparatus, Buchner proceeded to cultivate the isolated *Bacillus anthracis* in extract of meat for several hundred successive generations. As an early result of these experiments, he found that the bacillus lost its power of producing disease in an animal inoculated with it. Up to this point he is confirmed by Dr. Greenfield, who has found that, when the *Bacillus anthracis* is cultivated in aqeous humour, after about six generations it loses its infective property. Then as Buchner's experiments proceeded the appearance of the growing organism was found to undergo gradual modification. Instead of a cloud at the bottom of the vessel, a scum began to make its appearance—at first greasy-looking and easily broken up—constituting, so far as appearances went, an intermediate form between the two organisms; and in course of time the scum became drier and firmer, and at length the modified *Bacillus anthracis* was found to be capable of growing in an acid hay infusion, and to present in every respect the characters of the hay-bacillus. The converse feat of changing the hay-bacillus into the *Bacillus anthracis* proved very much more difficult. A great number of ingenious devices were adopted by Buchner, who was, nevertheless, continually baffled, till at last he attained success in the following manner:—Having obtained the blood of a healthy animal under antiseptic precautions, and defibrinated it also antiseptically, and having arranged his apparatus so that the pure defibrinated blood, which was to be the cultivating medium, should be kept in constant movement, so as to continually break up the scum, and also keep the red corpuscles in perpetual motion so as to convey oxygen to all parts of the liquid—in this way imitating, to a certain extent, the conditions of growth of the *Bacillus anthracis* outside the animal body, within which the hay-bacillus could not be got by any means to develope—he proceded to cultivate through numerous successive generations. A transitional form soon made its appearance; but the change advanced only to a limited degree, so that further progress by this method became hopeless. The modified form hitherto obtained failed entirely to grow when injected into the blood of an animal. On the contrary, it was in a short time completely eliminated from the system, just like the ordinary hay-bacillus. It had, however, been observed by Buchner that spores had never been formed by the bacillus growing in the defibrinated blood; and it occurred to him that, perhaps, if it were transferred to extract of meat, and induced to form spores there, the modified organism might yet grow in the blood of a living animal. The carryiug out of this idea was crowned with success; and, by injecting various different quantities of the liquid containing the organism into different individuals, Buchner at length succeeded, both in the mouse and in the rabbit, in developing true *Bacillus anthracis* out of the progeny of the hay-bacillus. When large quantities were introduced, the animals died rapidly from the merely chemical toxic effects of the injected liquid; but, in some instances in which a smaller amount was injected, after the period for these primary effects had passed, a fatal disease supervened—attended, as in anthrax, with great swelling of the spleen, the blood of which was found peopled, as in that affection, with newly formed bacilli; and the spleens affected in this way were found to communicate anthrax to healthy animals, just like those of animals which have died of ordinary splenic fever.[1]

Supposing these results to be trustworthy (and the record of them bears the stamp of authenticity) I need scarcely point out to a meeting like the present their transcendant importance as bearing upon the origin of infective diseases, and their modifications as exhibited in epidemics.

I trust that these examples may suffice to convey some idea of the work now going on with reference to the relations of micro-organisms to disease.

Since the address was delivered M. Pasteur has published the method by which he produces the "attenuation" of the virus, or in other words, the enfeeblement of the organism of fowl-cholera which fits it for securing immunity from the fatal form of the disease. This method consists in cultivating the organism, pure and unmixed, in chicken broth, to which access of air is permitted while dust is excluded, and simply allowing some months to elapse before it is employed. If the period does not amount to more than about two months, the organism retains its virulence little abated, but if the period is extended to three or four months it is found that animals inoculated with the organism take the disease, but have it in a milder form, and a considerable proportion recover; and if the time is made still greater, as, for exemple, eight months, the organism has so far lost its potency that though chickens inoculated with it still go through an attack of the disease all recover. If the period is sufficiently prolonged, there comes a time when the organism is found to have lost its vitality altogether, so that it will no longer give rise to new development when introduced into fresh cultivating liquid.

In considering by what agency this enfeeblement of the organism and ultimate extinction of its vitality was brought

---

[1] See 'Ueber die experimentelle Erzeugung des Milzbrandcontagiums aus den Heupilzen,' von Hans Buchner, München, 1880.

about under the circumstances referred to, it occurred to M. Pasteur that it might perhaps be the oxygen of the air admitted to the vessels. Oxygen is essential to the growth of the organism, but it might, as M. Pasteur thought, be, nevertheless, in long-continued action upon it, a cause of weakness. With a view of testing this idea he instituted cultivations of the bacterium in broth contained in tubes partially filled with the liquid, that is to say, containing a certain proportion of their volume of air, but sealed hermetically. The result was a growth of the organism, indicated by turbidity of the clear fluid, attaining a degree proportioned to the amount of air present in the tube, but soon coming to an end when that air was exhausted, so that the little organism, no longer growing throughout the liquid, fell to the bottom of the vessel, leaving the fluid again clear. The organism having now exhausted all the free oxygen, was from this time forth presumably protected from the influence of that element, and, in exact accordance with M. Pasteur's theory, it was found that no matter how long these closed tubes were kept, the organism retained not only its vitality, but its full virulence, as tested by inoculation of healthy chickens.[1]

These facts are certainly fraught with the deepest interest, and the medical world must for ever remain deeply indebted to M. Pasteur for eliciting them. Doubts may, however, be entertained regarding the interpretation of the phenomena. Thus Dr. Greenfield, whose own researches have had special reference to the modifying influence exerted upon bacteria by the medium in which they grow, has thrown out the suggestion that the enfeeblement of the organisms of fowl cholera grown with free access of air may be due to alterations in the fluid which they inhabit rather than to the effect of oxygen upon them. When free access of oxygen is permitted, the organism, he contends, will continue to grow till all the material suitable for its nutrition is exhausted, and as the nutriment becomes defective the progeny will be feeble. At the same time this exhaustive development of the organism will be attended by the full measure of possible alteration in the quality of the liquid which the growth of the organism can effect, and this alteration will naturally involve the production of substances which may exert a prejudical influence upon the organism itself. On the other hand, the bacterium, when growing in a sealed tube with limited supply of oxygen, has its development brought to a stand by the exhaustion of that gas, while the organism is

---

[1] See 'Comptes Rendus,' 26th Oct., 1880.

in full vigour and in a fluid but slightly changed from its original wholesome condition. It thus remains like a vigorous seed, ready to start into energetic growth when the conditions for its germination are supplied.[1] The essential difference between the two views may be stated shortly thus: M. Pasteur regards oxygen as a slow poison of the bacterium; Dr. Greenfield seeks for the slow poison in the products of the fermentative agency of the organism.

The time which has passed since the delivery of this address, has brought out facts which have led M. Toussaint to take a different view of the nature of the liquid used in his "vaccinations" against anthrax above referred to. In a letter which he had the kindness to write to me on the subject, he informs me that on two different occasions injections of anthrax blood treated by one of his methods has led to the death of the animal from anthrax; and in one instance, a similar injection induced a local affection which appeared to have the characters of malignant pustule. He has hence been led to the conclusion that the diseased blood treated by his methods, instead of being (as he at first believed) free from the living bacillus, contained the organism in an "attenuated" form.

Thus it would appear that the observations of Pasteur, Toussaint, and Greenfield, agree in ascribing the "vaccinating" influence to a modified form of the disease concerned.

At the same time some other observations have been made which tend to justify the original line of inquiry pursued by Toussaint. Chauveau has found that if ewes inoculated with anthrax in the last months of gestation recover from the disease, not only are the mothers no longer susceptible, but the lambs enjoy similar immunity.[2] Further, it has been ascertained by others, including Dr. Greenfield, that the blood and tissues of the fœtus of an animal dying of anthrax contain no bacilli, while those of the mother swarm with them. Putting these two observations together we are led to the inference that while the integrity of the placental vessels prevents the bacilli from entering the fœtal circulation, the fœtus is so dosed with soluble products of the development of the bacilli in the maternal blood as to be rendered proof against the disease.

---

[1] See "The Brown Lectures," by W. S. Greenfield, &c.; Lecture II, 'The Lancet,' Jan. 1st, 1881.

[2] See Dr. Greenfield's 1st "Brown Lecture," 'The Lancet,' 18th Dec., 1880.

OBSERVATIONS *and* REFLECTIONS *on the* APPENDAGES *and on the* NERVOUS SYSTEM *of* APUS CANCRIFORMIS. By E. RAY LANKESTER, M.A., F.R.S., Jodrell Professor of Zoology in University College, London. (With Plate XX.)

APUS CANCRIFORMIS is in many respects one of the most important of the Crustacea. Not only is it of exceptionally large size for one of the Entomostraca, and therefore suited to anatomical investigation, but it possesses peculiarities of organisation which mark it out (together with its immediate congeners, the Phyllopoda) as an archaic form, probably standing nearer to the extinct ancestors of the Crustacea than any other living members of the group.

The almost world-wide distribution of the genus Apus (Greenland, Tasmania, New Zealand, Australia, Europe, North America, West Indies, Affghanistan, China), and its fresh-water habit, tend to confirm the conclusion as to its high antiquity.

But it is not only for such reasons that Apus has claims on our special attention. Its great abundance in certain localities renders it especially suitable for study as a type or standard of the organisation of the lower Crustacea, and it is on this account especially to be desired that an accurate account of its structure should be accessible. Strange as it appears, it is yet the fact that such an account does not exist, and that recent writers of authority have given divergent and erroneous accounts of such prominent features in the structure of Apus as the antennæ, jaws, and thoracic appendages.

In 1841 E. G. Zaddach published a memoir entitled 'De Apodis cancriformis anatome et historiâ evolutionis,' which is a most carefully worked out and admirably illustrated monograph, worthy to be placed alongside the similar studies of Arthropod anatomy, which were produced at about the same period by George Newport.

Zaddach's most valuable observations refer to the internal anatomy and to the development of Apus from the egg, but the problems relative to the morphology of the appendages and the reciprocal relationships of the great groups of Arthropoda which now occupy attention had not in 1841 come into prominence, and accordingly we do not find his observations upon the various appendages altogether equal in value to the rest of his work.

Since the date of Zaddach's monograph the only writers whom it is necessary to cite who have dealt with the structure of Apus are: Grube ("Bemerkungen über die Phyllopoden"), in the 'Archiv f. Naturg.,' 1853; Baird, in his 'British Entomostraca,' Ray Soc., 1850; Claus, in a paper published in the 'Gottingen Abhandlungen,' vol. xviii, 1873, entitled "Zur Kentniss des Baues und der Entwicklung von Branchipus stagnalis und Apus cancriformis;" Huxley, in his 'Anatomy of Invertebrate Animals,' 1877, p. 281; Gerstaecker, in Bronn's 'Classen und Ordnungen des Thierreichs,' "Crustacea," p. 860, &c., plates xxx, xxxii, 1879; and again, Claus, in the last edition (now in course of publication) of his 'Handbuch der Zoologie,' p. 527, 1880.

Having made a careful examination of the appendages of *Apus cancriformis,* of which I have received numerous specimens from the neighbourhood of Munich, from Prag, and from Padua, through the kindness of Professor von Siebold, of Professor Fricz, and of Professor Pavesi, respectively, as also of a specimen of *Apus Dukii* from Affghanistan, which I owe to the kindness of Surgeon-Major Day,[1] I was surprised to find that neither Zaddach nor any one of the authors above cited gives a full account of these structures. Moreover, I found my results to be at variance on one or more important points with the statements of each of my predecessors, who also differ from one another as to the interpretation of some portions of the series of limbs.

The most complete set of figures is that given by Professor Gerstaecker of the appendages of *Apus productus,* which are incorporated with reproductions of the figures of Zaddach, Claus, and Brauer,[2] in the plates of his valuable treatise on "Crustacea" (Bronn's 'Thierreich').

These original drawings, and the interpretation put upon them in the explanation to the plate, appear to me to be so inaccurate that I think that my sketches, reproduced in the plate (Plate XX) accompanying this paper, and the following observations, may be found of value as a contribution towards a true history of the "Krebsartige Kieferfuss" of Schaeffer.

Description of the Appendages.

The modified limbs or lateral appendages of Apus may be divided into four series, according to their position, viz.—1.

---

[1] This form, recently described by Mr. Day, is perhaps the *A. Himalayanus* cited in Gerstaecker's list.

[3] 'Sitzungsber. der Akad.,' Wien, Bd. lxix.

Two pairs in front of the mouth—"præoral appendages." 2. Three pairs of jaw-legs, more especially related to the purposes of the mouth as jaws, and immediately following it—"oral appendages." 3. Eleven pairs of variously modified but essentially locomotor legs, placed one on each of the eleven body segments, in front of and inclusive of the segment carrying the genital apertures—"thoracic appendages." 4. Fifty-two pairs of locomotor legs, gradually diminishing in size, and posterior to the genital apertures (less numerous in incompletely-grown specimens)—"abdominal appendages." They are more numerous than the annulations of the integument of this region of the body, which are only seventeen in number, but they correspond in number with the ganglionic enlargements of the nerve cord.[1] There are four annulations of the body posterior to these appendages, and devoid of a continuation of the nervous axis, as well as of appendages. A fifth post-pedal segment is perforated by the anus, and carries a pair of jointed setose appendages, which may or may not be equivalents of the legs of the anterior region.

The two pairs of appendages in front of the mouth.—Both these pairs of appendages are of small size—relatively very much smaller than they are in the newly-hatched larva. The second pair is much smaller than the first, although in the larva the reverse relation obtains. Moreover, the second pair, like the first, are simple filamentous tactile organs; although in the larva they are powerful biramose swimming legs. In the adult Apus, therefore, not only with reference to their homology with the corresponding appendages of the lobster, but also in view of their function, these two pairs of appendages are entitled to the names antennules (1st) and antennæ (2nd) respectively.

It appears to be desirable, in order to arrive at true conclusions with regard to the homologies of the limbs of the Arthropoda, to abandon altogether the use of such terms as "antenna," "mandible," and "maxillipede," as homological categories, and to apply them merely as descriptive terms proper to the particular case under examination. In the consideration of homologies the appendages should be regarded simply as first, second, third, and so forth, without the introduction of terms calculated by their reference to function to prejudice the argument as to homology. The first appendage of an Arthropod A. may be homologous with (or homogenous with) the first appendage or with the

---

[1] The first four post-genital body-rings carry five pairs of legs, the next four carry ten pairs of legs, the next four thirteen, the last five leg-bearing body-rings carry twenty four, as nearly as can be estimated.

second or third of another Arthropod B., and so on; but ambiguity is inevitably introduced if we attempt to indicate this homology by the use of such terms as antennule and antenna, to be applied in both cases alike, for in such cases as the parasitic Copepoda, the various Arachnida, and the living and fossil branchiate scorpions (Merostomata), these descriptive terms, and others like them, are found to be absolutely contrary to fact in their implications, and involve also debatable assumptions in reference to ancestral primitive forms.

The *first pair* of appendages of *Apus cancriformis* may, therefore, be described as functional antennæ. Each consists of two segments, separated by a joint or soft ring of the chitinous cuticle, which allows the bending of one joint upon the other; but there does not appear to be any muscular band entering the appendage in the adult Apus. A variable number (two to four) of setæ[1] are set upon the free end of the appendage.

The *second pair* of appendages is also antenniform. Each consists of a single filament, the base of which is attached to the under surface of the head, not far from the first. The filament is strongly curved but possesses no joint,[2] nor does any muscular fibre penetrate its axis. It tapers towards its free extremity which is setiform. Its total length is about one third of that of the first pair.

In the adult *Apus cancriformis* and in *Apus Dukii,* from Affghanistan (? *A. Himalayanus*), this second pair of præoral appendages, although reduced to a rudimentary condition, is always present, so far as my observations go. I have found them always present in full-grown specimens of *Apus cancriformis* from Munich, from Prag, and from Padua.

Their existence in the adult has recently been denied. Zaddach states that they are generally absent in *A. cancriformis,* but were found by him in two cases; Huxley states that he was unable to find them in *Apus glacialis* examined

---

[1] The setæ of Crustacea are in distinction from the bristles of Chætopods, which are also often called "setæ," superficial prolongations of the continuous chitinous cuticle which is produced by the epidermis, and are not, as are the bristles of Chætopoda, formed in open or closed follicles each as the cuticular excrescence of one specialised cell of the epiderm. It would be well to distinguish the follicle-formed setæ of Chætopoda as "chætæ" or "chætomes."

[2] A comparison with Zaddach's and Claus's figures of Apus larvæ leads to the conclusion that the part of the larval second antenna which thus survives is the base and short *proximal* ramus (neither exopodite nor endopodite); all beyond this forming the larger part of the larval antenna ppears to atrophy completely.

by him, whilst Claus, whose statements have the very greatest weight, both on account of his extended investigation of the morphology of the Crustacea and of his special observations on the development of Apus and Branchipus, brings forward the total absence of the second pair of præoral appendages in Apus as a special characteristic of the family Apusidæ. He says, in the fourth edition of his 'Grundzüge der Zoologie,' 1880, p. 527, "Die Tastantennen sind kurz zweigliedrige Fädchen, die hintern, welche bei der Larve einen zweiästigen Ruderarm bilden, fallen ganz aus." A similar statement occurs on p. 523. Gerstaecker offers no original observation on the subject, nor does he figure either the first or second pair of appendages of the *Apus productus*, which he has illustrated in his Pl. xxx, but confines himself to quoting the statement of Zaddach.

The complete suppression of a pair of appendages is a matter of some importance, and in this particular case the presence or absence of the pair in question has a special interest in relation to the condition of that part of the nervous system by which they should be supplied. The fact is that, though rudimentary, the second pair of præoral appendages (so-called antennæ) is present in the adult Apus of at least two species.

Abdominal appendages: appendages behind the generative apertures.—It will be most convenient to pass next to the description of this group of appendages, since they appear to present the least specialised form of the whole series.

It has been customary to regard the higher Podophthalmata as a standard for the morphology of Crustacean appendages, and to interpret the parts seen in other Crustacea by reference to them, and to apply to those parts the analysis indicated in the terms protopodite (including coxopodite and basipodite), endopodite, exopodite, and epipodite. When, however, the question is looked at from the point of view of evolutional morphology, there appears to be no ground for expecting that the analysis applicable to the special adaptations of the Podophthalmata should also be applicable to the lower Crustacea, and it will be best to consider the parts of the appendages of Apus merely in reference to one another and to the appendages of closely allied forms, deferring for the present the consideration of the homologies or agreements of these parts with those of widely separated forms.

Taking the first post-genital appendage of the right side (seventeenth of the whole series), we find it to be a leaf-like plate attached by one end to the body, so that its flat surface is vertical to the ventral surface of the animal, and at right angles to the antero-posterior axis. It is provided with numerous lamelliform processes. The median portion may be spoken of as the axis or corm, whilst the processes may be called "phyllites" or "apophyses," those ranged along the ventral or neural border of the corm being called "endites," and those given off from the dorsal border being called "exites." There are six endites, and two exites much larger than any of the endites, whilst in front of (distal to) the two large exites is a less strongly marked outgrowth of the corm, which it will be convenient to call the "sub-apical lobe." The corm is devoid of segmentation or jointing,[1] its chitinous cuticle forming one continuous investment to it; and moreover, no muscles are inserted into the corm in such a way as to cause it to bend upon itself, and so call into existence functional, if not structural joints.[2]

The cuticle is thin at the base of the corm, where it becomes continuous with that of the ventral surface of the body, and the movement upon the joint so constituted is provided for by powerful muscles, which enter the appendage and are inserted into its walls.

Of the six *endites* the proximal is somewhat isolated and pushed towards the middle line. Its surface is beset with powerful setæ, and it clearly has the function of assisting, by means of apposition to its fellow of the opposite side, in seizing and moving particles which may be introduced into the mouth. It is a jaw process, and may be spoken of as the "*gnathobase*." It is a fact of no little significance that a gnathobase is developed on every one of the sixty-three postoral appendages of Apus, especially when we remember that a similar feature is characteristic of Limulus, the most archaic representative of the Arachnida.

The gnathobase of the appendage under consideration is provided (as is the rule throughout the whole series) with powerful special muscles inserted into the corm (see figure), but it is not possible to define any arthrodial thinning of the cuticle which marks it off from the corm as a distinct segment.

---

[1] Zaddach describes the corm as three-jointed, and Huxley states that in *Apus glacialus* it consists of coxopodite, basipodite, and ischiopodite. I cannot find any evidence of these joints in the specimens studied by me in any of the appendages excepting the first two of the thoracic series.

[2] Such functional joints not indicated by any thinning of the chitinous cuticle to form an arthrodial membrane appear to exist at the bases of many of the phyllites in limbs from various parts of the body, and are indicated by the insertions of the muscles in the appendages of various crustacean Nauplii, as well as in those of the remarkable Rotifer, Pedalion.

On account of its possessing muscles capable of moving it upon the corm, the gnathobase may be distinguished from such outgrowths of the corm as are devoid of muscles or not jointed at their base. Such outgrowths or apophyses probably are phylogenetically antecedent to outgrowths capable of flexion, or actually jointed at their base; accordingly, the simpler early condition may be indicated by the term "lobe," whilst the later flexible condition is indicated by the term "arthrite."

Mere lobes tend in various regions of the arthropod body to become arthrites, *e. g.* the spines on the abdominal carapace of Limulus, or, again, the carapace of some Entomostraca and the wings of insects.

Further, an arthrite may be monarthrous, or, as we shall see exemplified in other appendages of Apus, may become polyarthrous.

The four endites distal to the gnathobase are oval, leaf-like bodies, of which the proximal is somewhat broader than the others, and is, by exception in this and other appendages of the post-genital region, devoid of a special muscular slip, and therefore in the condition of a lobe instead of an arthrite. The three apophyses distal to it (3, 4, 5) are true arthrites, each being connected at its base with a muscular slip, which unites with its fellows to form a powerful muscle traversing the corm and inserted into the body wall. The arrangement of setæ on these endites is characteristic, and is best understood by reference to the figures.

The *distal endite* (6) is much larger than the preceding, and is attached at the point, which is practically the apex of the corm. It spreads both ventrally and dorsally from its point of attachment, so that a dorsal process may be distinguished from a ventral. It is provided with a muscle distinct from that of the other endites which traverses the corm.

Next in order we come to the *sub-apical lobe,* which may be regarded either as the termination of the axis of the corm or as the distal lobe of its external or dorsal border. In the appendage under description (the first post-genital), the subapical lobe is exceptionally large, but it is here as always a lobe—devoid of muscular supply, and not in any way jointed.

Passing midward along the dorsal border of the limb, we come to the largest of all the outgrowths of the corm—the *second exite* (counting the proximal as the first) the *flabellum* or *fan.*

It has been usual with authors to speak of this exite as a branchia, and to distinguish it from the next exite as the "lamelliform branchia," whilst the latter is termed the "bottle-shaped branchia." But the fact is, that it is possible with justice to attribute a respiratory function to any such broad lamelliform structure, although it may have other important functions. The flabellum is moved by three muscular slips arising from the corm, and is first of all a swimming or fanning plate. It is more highly developed as an "arthrite" than any other of the outgrowths of the corm. It is somewhat triangular in shape, attached by one angle to the corm, and has a setose margin.

The *proximal* or *first exite* is not really vesicular although frequently described as being so. It is somewhat thicker than the flabellum—oval in shape, with a very narrow and short pedicle, by which it is connected with the corm. It is devoid of setæ, and not provided with any muscles. It must be regarded as a "lobe" (in the sense above defined) rather than as an "arthrite." Throughout the series of limbs where present this proximal exite has much the same shape, it is always without setæ and is invariably devoid of muscular connections, although strongly emarginated and attached by but a slender neck to the corm. It can therefore have no locomotor function, and is, in virtue of negative qualities, *the* branchial outgrowth. On account of its passive character, as contrasted with the active fanning flabellum, it may be called the *bract* (bractea—a weather-cock).

In form the abdominal appendages which follow the first post-genital pair agree closely with the latter in all respects, except in the reduction of the relative size of the subapical lobe.

In size they undergo a gradual and very great reduction, so that the proportionate size of the last of the series is represented by the small fig. 13, of Pl. XX. The whole appendage is little bigger than the gnathobase of the first post-genital limb.

Gerstaecker's figure of the thirtieth truncal appendage in *Apus productus* (his Pl. xxx, fig. 10) appears to me to be inaccurate. These minute processes are easily mutilated in removing them from the body, and such has probably been the case with the appendage figured by him. The "flabellum" appears to have been broken away in Gerstaecker's specimen, and what he marks as flabellum (*br.*) is in reality the subapical lobe.

Abnormal appendage.—Any abnormality in a Crustacean appendage is of interest, as showing possible directions of new development or recurrence of ancestral conditions of form. In Pl. XX, fig. 12, an abnormal specimen of the fortieth appendage, right side, is drawn. The abnormality consists in the development of a second flabellum nearer the base of the corm than the first, and this is accompanied by an almost complete reduction of the bract. The additional flabellum is much smaller than the normal one, but has a similar form, and is supplied with a muscular slip.

Thoracic appendages, or appendages at and in front of the genital apertures and behind the specialised oral appendages.—It will be most convenient to examine these appendages by proceeding from behind forwards.

Oostegopods.—The pair which are attached to the segment in which the genital ducts open in female specimens of Apus are, as is well known, modified so as to form receptacles for the eggs. In the males, as shown by Brauer ('Wiener Sitzungsber,' vol. lxix), the appendages of this segment are in nowise different from those of the segments immediately in front of them.

The oostegopods, or brooding-legs (as the eleventh pair of the thoracic appendages may be called), present the following peculiarities as compared with the appendages in the region posterior to them. The gnathobase and four succeeding endites are normal (Pl. XX, fig. 10), and resemble those of the next posterior appendage (Pl. XX, fig. 11), but the sixth endite is greatly modified. It is expanded and confluent with the subapical lobe (*p*). The subapical lobe is widely produced in the form of a hemispherical cup. On to this expansion the flabellum (*Fl*) fits as a lid—an emarginated aperture being formed posteriorly to its short peduncle by the notching of its border (Pl. XX, fig. 10, *or*).

The great development of the subapical lobe and modification of the flabellum seems to have entailed an atrophy of the bract (*Br*), which exists in a rudimentary filamentous condition (compare the abnormal appendage drawn in fig. 12).

The general form of this appendage has been described by many previous observers, and the relations of its parts to those of neighbouring appendages correctly pointed out. The correspondence of the immovable, cup-like portion, developed from the axis or corm, with the conjoined sixth endite and subapical lobe (which is relatively large in the next following appendage posterior to it, fig. 11), has not, however, been insisted upon. Zaddach correctly identifies the movable lid with the flabellum, and figures the limb in an immature condition with the rudimentary bract present. He states that usually the rudimentary bract is absent in the full-grown condition, but I have, on the contrary, never failed to find it. Claus (loc. cit.) likewise figures this appendage from a larval Apus, and correctly identifies its component parts and the rudimentary bract which does not enter into the composition of the egg box. Huxley also (loc. cit.) describes the adult oostegopod, but without mentioning the rudimentary bract; he, however, identifies the movable lid as the flabellum (his "exopodite"), and the fixed cup-like portion as an outgrowth of the axis (his "endopodite"). Gerstaecker, however, having missed the rudimentary bract (which may or may not be present in the *Apus productus* figured by him), proceeds to identify the fixed portion of the egg box with the flabellum (his "lamelliform branchia"), and the movable lid with the bract (his vesicular (blasenförmiger) branchia). Gerstaecker's being the latest study and identification of these organs, it is of some importance to point out emphatically that the movable lid is not the bract, but the flabellum, whilst the bract exists in *Apus cancriformis* in a rudimentary condition in the adult appendage, as exhibited in Pl. XX, fig. 10.

I have not had an opportunity of examining, in the living state, the exact disposition of the muscles and the mechanism of the egg box, but I must point out that the circular muscles described by Zaddach have no existence. The modified flabellum alone has a muscular supply, which does not differ from that of the other flabella.

Appendages next in front of the oostegopods.—A marked difference in the proportions of the outgrowths of the limbs in front of the oostegopods is observed, as compared with those posterior to those specialised appendages.

In Pl. XX, fig. 9, the ninth of the thoracic (prægenital) series of foot-like appendages is represented. Whilst the gnathobase (endite 1) and the four next endites (2, 3, 4, 5) present no peculiarity for notice, the sixth endite is remarkable for its firmly chitinised walls, its denticulate ventral margin and its ex-axial lobe, which projects beyond the point at which the endite is articulated to the axis, and works in a notch of that portion of the limb.

The subapical lobe (*p*) is well developed and acutely angular. The flabellum is more elongated than in the abdominal (post-genital) appendages, being developed on either side of its point of attachment to the axis, instead of being quadrate or oblong. The two regions of the flabellum may conveniently be spoken of as "the distal lobe" (that apexwards in relation to the muscular attachment), and "the proximal lobe" (that basewards, in relation to the same attachment).

It is important to note this development, since in some Phyllopods and other Crustacea it is very greatly exaggerated, and the flabellum practically divided into two moieties (see woodcut, fig. 1, *XIX*, *XIV*, and *VI*, *F d*).

The muscles distributed to the endites and the flabellum are more strongly developed in the præ-genital than in the post-genital appendages of the locomotor series, as may be seen by comparing Pl. XX, fig. 9, with fig. 11. Whilst in the post-genital appendages endite 2 ceases to have a distinct muscular slip supplied to it, this endite, like all the others, has in the prægenital series a muscular slip from the great muscle of the axis, which is distributed to the five distal endites. The sixth endite has, in addition, a separate muscle, which runs parallel with the common muscle of the endites, and the first endite or gnathobase has also its own special muscles. The muscular supply of the sixth and first endites is the same in all the thoracic and abdominal appendages, excepting the first two of the thoracic series (two anterior thoracic feet of authors).

The bract is in all cases devoid of any muscular supply—a fact which is not without importance in the determination of its homologue in other Crustacea.

The appendages in front of the ninth of the thoracic series have a form closely similar to that drawn in Pl. XX, fig. 9, the endites becoming slightly more elongated in form, and the axis also elongated, so as to separate the endites from one another in those nearest the first two thoracic feet.

The first two thoracic feet require special description.

Second thoracic foot.—This appendage is drawn in Pl. XX, fig. 8. The most important feature which it presents, as distinguished from those behind it which have been already considered, is the jointing of the corm or axis. The corm is divided into two segments or pieces by a soft fold of its chitinous cuticle, and muscles are attached to the distal segment so as to move it upon the basal segment. To the basal segment (Ax^1) are attached the two proximal endites (en^1 and en^2), whilst the four distal endites, the flabellum and the bract, are attached to the distal segment (Ax^2). The endites are much elongated and notched at their margins, retaining, however, a flat or lamelliform character. The terminal endite (en^6) is strongly denticulate and has its exaxial process markedly developed, whilst the notch in the axis into which this process fits is peculiarly constructed, as may be best understood from the figure (Pl. XX, fig. 8, *p* 2), and from the similar structure more definitely developed in the thoracic appendage (fig. 7 *a*.). I am not able to suggest what may be the significance of this lobe and notch, which has not been hitherto noticed by those who have described Apus, but it in some degree suggests a comparison with the peculiarly modified lobes on the first thoracic foot of the male *Limnetis brachyurus* (as described by Grube, Wiegmann's 'Archiv,' 1853).

The sub-apical lobe is relatively small, the flabellum and bract similar to those of hinder appendages.

First thoracic foot.—This appendage is more strikingly modified than any one of the locomotor series, excepting the oostegopods, the remarkable feature being the elongation and jointing of the four middle endites in the form of filamentous flagella. The axis or corm of this appendage is divided into four successive segments, which are marked in the figure respectively Ax^1, Ax^2, Ax^3, Ax^4 (Pl. XX, fig. 7). These segments are movable upon one another, the chitinous cuticle being soft so as to form an "arthrodial membrane" between the successive joints. This is the only one of the truncal series of appendages (those following the oral appendages) which presents four joints to the corm, whilst the second thoracic foot is the only other appendage of the truncal series which presents a joint in the corm at all and in it only two segments exist. A different structure is assigned by Professor Huxley to the corresponding limb and to the thoracic limbs generally of *Apus glacialis*, but it seems possible that the coxopodite, basipodite, ischiopodite and the endopodite with four joints recognised by him in that animal, are due to an interpretation of parts identical with those of *Apus cancriformis*, which differs from the interpretation here given. I must refer the reader for his views to the description and figures given on pp. 280, 281, of the 'Anatomy of Invertebrate Animals.'

The proximal or basal segment of the corm of the first thoracic limb of *Apus cancriformis* supports one endite, the gnathobase (En^1), which is quadrangular in outline and provided with strong setæ as in the other thoracic limbs. The second segment (Ax^2) supports a single endite, which is short and of filamentous form, with ten joints or annulations of the chitinous cuticle. These annulations in the present and other filamentous endites are not complete, but are developed only at the sides of the filament, leaving a continuous band of non-annulated chitinous cuticle mesially between the two series of half-rings; and moreover, the half-rings do not always coincide with one another in position, that is, are not exactly opposite to one another.

The third segment of the corm supports on its ventral margin one filamentous endite which has forty annulations or thereabouts. On its dorsal margin this segment gives attachment to the flabellum and the bract.

The fourth segment of the corm carries one long endite with fifty annulations (*end*^{4}) on its ventral margin, and terminally is produced into a non-movable process from which two endites arise, one (*end*^{5}) very long and filamentous with eighty annulations, the other (*end*^{6}) minute and boat-shaped, resembling the terminal endite (*end*^{6}) of other thoracic limbs. The peculiar shape of this terminal endite and the notch of the corm into which its exaxial process fits is shown in Pl. XX, fig. 7*a*.

It is at once obvious that in the first thoracic limb we have a very special differentiation of the endites in respect of their filamentous jointed form and of the corm in respect of its four movable segments. In both these features the second thoracic foot is intermediate between the first foot and those which follow posteriorly.

The first thoracic foot at once suggests comparison with some of the appendages of the Podophthalmatous Crustacea, as also with the limbs of Nauplius, but the discussion of the homologies of its parts with those of the limbs of other Crustacea, must be deferred to a subsequent section of this memoir.

The oral appendages.—The appendages immediately following the mouth constitute a natural group, which are distinguished by their small size and peculiar structure. I recognise three such pairs of appendages, a first (nearest the mouth), the mandibles (Pl. XX, fig. 3); a second, the maxillæ (figs. 4, 4 *a*, 5); a third, the maxillipedes (fig. 6). Very different interpretations have been placed upon these parts by different writers. Zaddach regarded the two pieces of the maxilla (figs. 4 and 5) as two distinct maxillæ—a first and second—in which view, with the curious difference that he transposes erroneously the natural position of the pieces, Gerstaecker follows him.

The maxillipede is described by Zaddach as a rudimentary thoracic foot—a view which is no doubt perfectly correct, though, on account of its relation to the oral series, it is more convenient to indicate it by the term maxillipede.

Grube agrees with Zaddach in regarding the two pieces of the maxilla as two distinct maxillæ, and the maxillipede as a rudimentary foot. Claus appears to have been the first to assign the two pieces of the maxilla to one appendage—a single maxilla—but causes some difficulty in arriving at a conclusion as to what he means by denoting the maxillipede (rudimentary foot of Zaddach and Grube) as the "second maxilla," without any explanation as to the difference between *his* second maxilla and Zaddach's. Huxley agrees with Claus in his statement, and characterises the second maxilla (my maxillipede) as foliaceous and almost rudimentary.

Mandibles.—These are figured in Pl. XX, figs. 3, 3 *a.* They appear to consist (according to Claus's observations on their development) of the basal portion of the corm of the larval appendage, which had at one time a form similar to that of the second præoral pair of appendages (woodcut fig. 1, *XVIII*).

Maxillæ.—The maxillæ (right and left) consist each of two plate-like pieces fitted closely together, one behind the other. The piece furthest from the mouth (which was described by Zaddach as the second maxilla and by Gerstaecker, through an oversight, as the first maxilla) seems to correspond to the gnathobase or proximal endite (*en*^{1}) of a thoracic foot. It is provided with numerous stout setæ (Pl. XX, fig. 5). The piece nearer the mouth (Zaddach's first maxilla, Gerstaecker's second) is in the form of a half-arch of chitinous substance, with a broad surface for the attachment of the other piece. It is provided with very numerous delicate setæ, which are arranged in the manner shown in the figures (Pl. XX, fig. 4), showing the aboral surface, and fig. 4 *a*, the adoral surface. The exact relation of the arch of the maxilla (as we may term the last described piece) to the gnathobase is not quite clear from the account given by Claus of the development, but it is quite certain that it is only a part of the same appendage as the latter.

Maxillipedes.—These appendages are of a very remarkable character. They have been universally recognised as *rudimentary,* not only on account of their small size (that drawn in Pl. XX, fig. 6, is on the same scale as that adopted for all the appendages), but also from the fact that they do not act as jaws on account of the wide separation of the right appendage from the left—whilst at the same time they are not fitted to be of much value as locomotor organs. They have not hitherto been correctly figured, the drawings of Zaddach and Grube being on a very small scale, whilst the drawing of Gerstaecker is out of all proportion in its exaggeration of the size relatively to that of other appendages, whilst the shape is incorrectly given.

A curious feature about these appendages is, that they resemble *bits* of a thoracic foot rather than a whole thoracic foot in a rudimentary condition. The corm or axis is utterly evanescent, a mere ridge (Pl. XX, fig. 6, *ax*). To this are attached two very well developed processes, one of which (*Br*) is clearly enough the bract, being identical in every respect with the bracts of the post-genital thoracic limbs. The other process is an oval chitinous plate, with long marginal setæ (en^1); it may possibly represent the flabellum, but more probably one of the endites, perhaps endite 1 (the gnathobase). There is no means of deciding this point, for Claus gives but a very slight allusion to the early condition of this appendage in his account of the development of Apus.

A highly important fact relative to this appendage was pointed out by Zaddach. Whilst a separate ganglion-pair of the ventral nerve-cord supplies the mandibles with nerves, and another distinct ganglion pair supplies the maxillæ, and another supplies the first thoracic foot, and a distinct ganglion pair is present for each of the succeeding pairs of thoracic feet, this pair of appendages *has no ganglion pair appropriated to it, but receives its nerves from the longitudinal cord* connecting the maxilla's ganglion pair with that of the first thoracic foot (woodcut, fig. 2, *V mp.*). It is important to observe that there is no evidence here of the fusion of two ganglion pairs and the consequent supply of two pairs of appendages from one and the same ganglion, but the nerves to the maxillipedes come off from the longitudinal commissure and not from a ganglion at all. In fact, we have evidence of the *total disappearance* of a ganglion pair corresponding to these rudimentary limbs.

Any facts tending to establish the occurrence of intercalation or of excalation of a segment (as represented by such structures as nerve ganglion-pairs and appendages) in the series building up the body of a Crustacean, Insect, or Arachnid is of great interest, since, if it be once admitted that a segment may disappear, or that a new segment may be introduced, the attempt to derive the series of segments and appendages of an Insect from those of a Crustacean or of an Arachnid, or of all three from a common ancestral form, loses all the difficulty which is encountered when the disappearance of segments is admitted as occurring *only* in the extreme posterior region, or only in that and the extreme anterior region of the series.

The maxillipedes of Apus appear to be rudimentary structures in the sense of structures which are degenerating and evanescent, not in the sense of structures which retain a primæval simplicity of form. In the course of the ancestral development of some other Phyllopoda (not in all), the corresponding appendages have entirely disappeared without leaving a trace behind them. It is well to bear in mind that were the maxillipedes of Apus a little further advanced in degeneration, so as to be devoid of the bract, and consist, like the "chilaria"[1] (metastoma) of Limulus, of simple setose chitinous plates, we should have no justification for calling them "appendages" at all, and they might very well, since no separate ganglion exists for them in the nerve-chain, although they receive each a nerve from the ventral nerve-cords, be regarded as median processes simulating rudimentary appendages. Such a view is very generally adopted with regard to processes termed "paragnathi" and "metastoma," and with regard to the "chilaria" of Limulus which last, like the maxillipedes of Apus, receive each a special nerve from the latero-ventral cords (woodcut, fig. 3, *chi*).

---

[1] This useful term is introduced by Prof. Owen in his account of Limulus, 'Trans. Linnæan Soc.,' 1873.

Tabular enumeration of the Appendages of Apus cancriformis.

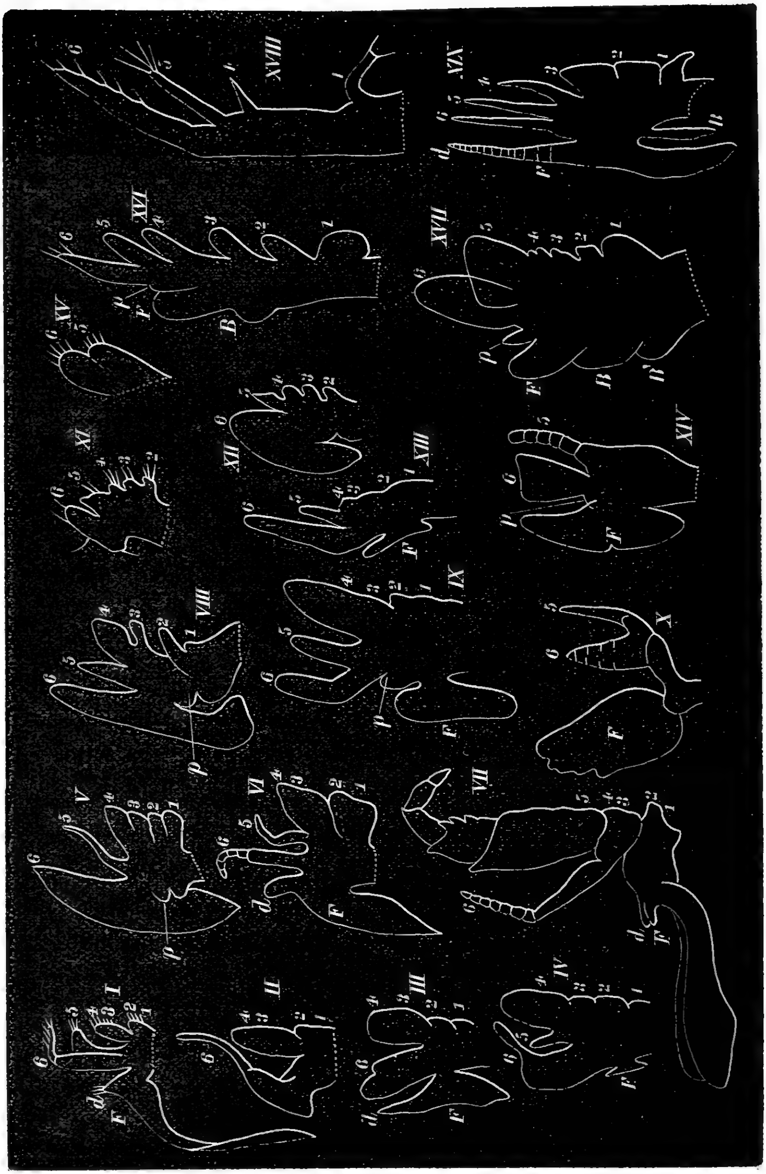
		Name adopted.		*Synonyms.*
Præoral appendages.		I, First antenna		Antennule: tactile antenna. First thoracic foot of Zaddach.
		II, Second antenna		Antenna: rowing-antenna. Second thoracic foot of Zaddach. Absent according to Claus.
		MOUTH		
Oral appendages.		III, Mandible		
		IV, Maxilla		First and second maxillæ of Zaddach, Grube, and Gerstaecker.
		V, Maxillipede		Second maxilla of Claus; third thoracic foot of Zaddach; rudimentary foot of Grube and Gerstaecker.
Truncal appendages.	Prægenital (Thoracic).	VI, 1st thoracic foot	(Eleven corresponding annulations of the body.)	Leg-like foot.
		VII, 2nd ,,		Swimming foot.
		VIII, 3rd ,,		,, ,,
		IX, 4th ,,		,, ,,
		X, 5th ,,		,, ,,
		XI, 6th ,,		,, ,,
		XII, 7th ,,		,, ,,
		XIII, 8th ,,		,, ,,
		XIV, 9th ,,		,, ,,
		XV, 10th ,,		,, ,,
		XVI, 11th ,,		Oostegopod in female, or brooding foot (Mutterfuss of Schaeffer).
	Postgenital (abdominal).	XVII, 1st abdominal foot	(Only seventeen corresponding annulations of the body.)	
		⋮		
		LXVIII, 52nd abdominal foot.		

N.B.—The carapace is not attached to the segments posterior to that bearing the maxillipedes.

The last body-segment (thirty-third from the attachment of the carapace) has a pair of furcal processes, and is perforated by the anus.

The four body-segments in front of it carry no feet, the next two in front (28th and 29th of the segments of the trunk) have together as many as twelve pairs.

Fig. 1.


I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
XVII
XVIII
XIX
1
2
3
4
5
6
F
d
p
B
B'

Explanation of Woodcut, Fig. 1.

In all the figures the endites are numbered from the basal one forward, as 1, 2, 3, 4, 5, 6. The proximal endites are often rudimentary or connate. In *II* and *III* endite 5 is suppressed, as becomes obvious on comparison with *IV*. *F* is the flabellum of the Apus limb, or its equivalent (*epipodite*); *p*, the sub-apical lobe; *B*, the bract; *B'*, a second bract in *XVII;* 5, the fifth endite or *endopodite;* 6, the sixth endite or *exopodite.*

I. First maxillipede of *Carcinus mænas* (after Boas, in 'Studier over Decapodernes Slœgtskabsforhold,' Kopenhagen, 1880).

II. Ditto of *Pagurus tuberculatus* (after Boas).

III. Ditto of Callianassa (after Boas).

IV. Ditto of Caridina (after Boas).

V. Second maxilla of *Astacus fluviatilis.*

VI. First maxillipede of ditto.

VII. Third maxillipede of ditto. 1, 2, rudimentary endites of the coxopodite; 3, 4, of the basipodite; 5, the ischiopodite or basal segment of the polyarthrous endopodite.

VIII. Second maxilla of Sergestes (after Claus).

IX. First maxillipede of ditto (after Claus).

X. Third abdominal foot of Palinurus (female), after Boas.

XI. Second maxilla of Zoea of Palæmonetes (after Boas).

XII. The same at the Mysis stage.

XIII. First maxillipede of same at the Mysis stage (compare with *XVI*).

XIV. Abdominal foot of Nebalia (after Claus), compare with *III* and with *X.*

XV. First rudiment of thoracic foot of Branchipus (after Claus).

XVI. Thoracic foot of larval Apus (after Claus).

X*VII.* Thoracic foot of *Branchipus Josephinæ,* after Grube.

XVIII. Second præoral appendage (second Nauplius' limb) of larval Apus.

XIX. Second thoracic foot of male Limnetis (after Grube).

Comparison of the appendages of Apus cancriformis with those of other Phyllopoda and of the Nauplius larva.—The appendages of the other species of Apus which have been described differ from those of *A. cancriformis* as to the number present in the post-genital or abdominal region. The length and the number of the joints in the filamentous endites of the first thoracic feet appear also to afford distinctive specific characters.

In other Phyllopoda the truncal appendages conform very closely to the plan of structure exhibited in Apus, presenting a corm with six endites, often a subapical lobe, and two exites. *Branchipus Josephinæ* of Grube (woodcut, fig. 1, *XVII*) is remarkable for possessing a third exite.

In the genera Branchipus, Artemia, Limnetis, Limnadia, and Estheria, the truncal appendages present special modifications of the constituent parts not shown in any part of the series in Apus, although the corresponding 'apophyses' are readily identified. One of the most remarkable modifications is seen in Limnetis, where the distal lobe of the flabellum becomes polyarthrous (woodcut, fig. 1, *XIX*, *d*). The interest of this modification consists in the fact that it lends some support to the identification of the flabellum of the Phyllopoda with the polyarthrous ramus termed 'exopodite' in the Decapoda. On other grounds I am led to doubt the correctness of this identification, as explained below. The fourth, fifth, and sixth endites in the same appendage of Limnetis are elongated, whilst the first, second, and third are short and jaw-like. The tendency of the flabellum to elongate itself in the direction parallel with the long axis of the corm is remarkable, and tends to its separation into a distal and a proximal lobe.

It is an important fact, which space does not allow me to further illustrate here, that the number of apophyses (both endites and exites), with the exceptional presence of an additional exite, is constant in the truncal appendages of all the Phyllopoda.

The præoral and oral appendages are very variously modified.

In order to determine further the homologies of the parts of the Crustacean limb it becomes important to ascertain what relation the parts of the foliaceous truncal appendages of the Phyllopoda have to the typical biramose appendages of a Nauplius.

The investigations of Zaddach, and more especially of Claus, on the development of Apus furnish data for this determination. The limbs present in the Nauplius larva of Apus and Branchipus persist in their primitive form at a time when the thoracic appendages have attained to a size nearly equal to theirs, and the comparison of the first and second thoracic foot (woodcut, fig. 1, *XVI*) with the biramose second pair of antennæ (same figure, *XVIII*) leaves little room for doubt as to the homologous parts. The Nauplius biramose appendage is devoid of flabellum and of bract; it has, in fact, no exites. Its terminal segment (outer ramus or exopodite) corresponds to the terminal endite of the thoracic foot (the sixth), and its inner ramus (endopodite) corresponds to the next endite of the thoracic foot or the fifth. Rudiments of two other endites are present in the second pair of Nauplian appendages of Apus.

The identification thus indicated is not capable of any more convincing proof than that afforded by the general similarity of relations in the two appendages compared; and when the extreme fluidity (if the expression may be used) or the Crustacean limb is borne in mind, and the undeniable tendency of one limb to become a pseudomorph of another, attaining *similar* form by identical modelling of parts *not* identical in origin, we have to be very careful in insisting upon conclusions arrived at by giving value to simple correspondence in form.

At the same time it appears most probable, and has been admitted by Claus and others, that the Nauplius appendages of Phyllopoda are devoid of flabellum and bract. Hence we may formulate the conclusion that the exopodite and endopodite of the Nauplius limb are homologous with the sixth and the fifth endites respectively of the thoracic limbs, and, similarly, that the flabellum is not homologous with the exopodite.

But when we come to examine the Nauplius of Decapoda (Penæus, &c.) we find that the exopodite of the second pair of Nauplian appendages becomes the short exopodite of the adult antennule, whilst the endopodite becomes the polyarthrous filament. Accordingly, if the exopodite and endopodite of the antenna of such a Decapod as Astacus are correctly identified with the parts called exopodite and endopodite (external and internal ramus) in the postoral series, the flabellum cannot be homologous with the exopodite of the maxillipedes and maxillæ. Such an homology might, however, be maintained by abandoning the current identification of the parts of the præoral and postoral appendages of Astacus. With regard to the antennules or first præorals of Astacus, there seems to be but little to justify the homology implied in calling its two rami respectively exopodite and endopodite, since in no Nauplius is this appendage biramose. Its distal segment in the Nauplius phase may with probability be identified with the external ramus (endite 6) of the antenna, but its inner ramus appears in Penæus at a late period, and may or may not be the equivalent of endite 5, the internal ramus of the antenna.

The drawing *XVI* in the woodcut, fig. 1, represents the form of all the thoracic appendages in the developing Apus (a younger condition is drawn in X*V*). It is interesting to note the extreme modification which the apophyses subsequently undergo in the case of the first thoracic foot. The flabellum and the bract expand, but the terminal endite becomes almost rudimentary and claw-like, whilst endite 5 grows out as a long polyarthrous filament, and endite 4 attains nearly the same length; endites 3 and 2 become shorter polyarthrous filaments, and endite 1 becomes the jaw process. On a first glance at the adult limb (Pl. XX, fig. 7) one would suppose that the long endites 5 and 4 were the parts homologous with the external and internal ramus respectively of the Nauplius limb; but the actual fact is that the reduced claw-like endite 6 is the homologue of the exopodite of the Nauplius antenna.

As has been pointed out above, the biramose antenna of the young Apus survives in the adult as a minute filamentous appendage, in which both endites 6 and 5 have atrophied, and only a portion of the axis and the small endite 1 (see woodcut, *XVIII*) persist.

The homologies of the parts of the appendages of some Malacostraca with those of Apus.—The attempt to establish a close correspondence between the apophyses of the appendages of the Phyllopoda and the Podophthalmata has its justification in the existence of the annectant form Nebalia, and of the Nauplius larval forms of some of the latter group. The foliaceous maxillæ and maxillipedes of such Decapods as Astacus also suggest a correspondence with the foliaceous truncal feet of Phyllopoda, which can be established in detail on close comparison.

If we take the second maxilla of Astacus to begin with (woodcut, *V*) we find it to consist of an unjointed corm with six endites, comparable to the six endites of the abdominal feet of Apus (Pl. XX, fig. 11).

The terminal endite is enlarged, and has a distal and a proximal lobe, as is more or less the case in the corresponding endite of the Apus limb. Two rudimentary exites (*p* in the woodcut) only are present, and in the adult are fused with the proximal lobe of the enlarged endite. In the Zoea phase of many Decapoda the enlarged endite is free in this region (woodcut, *VIII*), and in some cases the resemblance to a Phyllopod foot with suppressed flabellum and bract is very strong (woodcut, *IX*).

Passing to the next appendage of Astacus—the first maxillipede (woodcut, *VI*)—we can count the six endites as in the second maxilla, the sixth being greatly changed in form; instead of being a simple crescentic plate, it now has the shape of a rod, and is polyarthrous. But this change is no greater than that presented by the transition from the polyarthrous endites of the first thoracic limb of Apus to the lamelliform endites of the second and third limbs of that animal. A large exite is now present (*F*) with a small distal (*d*), and larger proximal lobe. It corresponds with the flabellum of the Apus limb and cannot be compared to the bract, owing to the fact that in this and subsequent appendages it has a muscular connection at its base and is richly setose on the surface, whereas, the bract of Apus is always devoid of muscular connection, and on its surface free from setæ. In fact, the bract of the limb of Apus appears to correspond, if with anything, in the limbs of Astacus and other Decapods, with branchial villi or lamellæ, and not with any of the large divisions of the limb. The corresponding maxillipede of a young Sergestes (*IX*) and of Palæmonetes (*XIII*) at the mysis-stage of development exhibit points of agreement with the Apus limb, which confirm the identifications just made in the case of Astacus. In Sergestes a subapical lobe (*p*) is present, whilst the resemblance of the Palæmonetes limb (*XIII*) to the young thoracic foot of Apus (*XVI*) is extraordinarily close. The flabellum in the two cases and the terminal endites 6 and 5 have practically identical form and relations respectively.

If we now take the third maxillipede of Astacus (*VII*) there is no difficulty in recognising the corresponding parts in it and the various appendages already noticed. In this appendage we have fully and clearly developed the apophyses which are, uniformly by modern writers, termed internal ramus or endopodite (5, *VII*); external ramus or exopodite (6, *VII*), and accessory ramus or epipodite (*F, VII*).

Accordingly it is now obvious that the endopodite of the Astacus maxillipede is the homologue of the endite 5 of the Apus limb, its exopodite is homologous with endite 6 of the Apus limb, and its epipodite is homologous with the flabellum of the Apus limb. This identification agrees with that arrived at in regard to the endopodite and exopodite of the antenna of Astacus as compared to the Apus limb through the intermediary of the Nauplius biramose appendage, and accordingly, it may be convenient to speak of the flabellum of the Phyllopod limb as the epipodite, the terminal endite as the exopodite, and the fifth endite as the endopodite. The two basal joints of the Astacus maxillipede constitute the corm, and are usually termed the protopodite collectively, the proximal joint being the "coxopodite" (*VII* 1, 2), and the distal the "basipodite" (*VII* 3, 4). These two pieces agree precisely in their relations to endites and exites with the two segments of the corm of the second thoracic foot of Apus (Pl. XX, fig. 8, $Ax,^1$ Ax^2); on the other hand, the first thoracic foot of Apus (Pl. XX, fig. 7), shows each of the two pieces of the axis or corm in a further state of division. The four pieces of the corm of this limb have no similarly fourfold representative in the protopodite of any Decapod; and the two proximal *together* represent the coxopodite, and the two distal *together* represent the basipodite of the typical Decapod limb.

The homologies thus arrived at do not agree with those put forward by Professor Huxley in his 'Anatomy of Invertebrated Animals,' 1877, p. 281, since he identifies the flabellum of the Apus limb with the exopodite of Decapods, and regards the bract of Apus as representing the epipodite. Further, he considers a part of the corm or protopodite of the first thoracic foot of Apus as the endopodite, and the endites as merely secondary processes.

The careful examination, however, of the structure of the appendages of Apus leads, I think, when combined with the examination of the numerous modifications of the oral appendages of Decapoda, which recent writers have minutely described, to the conclusions which I have advanced above.

There are some further points of interest arising from the comparison of the walking and swimming feet of Malacostraca with the abdominal feet of Apus.

In those Decapoda in which, at an early period of their development, the maxillipedes and even the representatives of the ambulatory appendages resemble in form the biramose Nauplius limb (Sergestes, Penæus, and Schizopods) we obtain data for identifying the exopodite of the antenna with the exopodite of the postoral series, and similarly for identification of the endopodites. And thus by referring again to the Nauplius antenna of Apus and the assumed homologies of its part with the foliaceous limbs of the truncal region of that animal, we arrive by another method than that adopted when the comparison of the second maxilla of Decapods with the abdominal feet of Apus is made the starting point, at an identical determination of the homologies of the parts. The persistent ramus of the ambulatory limb of the Decapoda is the fifth endite of the Apus limb, its coxopodite and basipodite are the corm of the Apus limb, the sixth endite is suppressed as it so nearly is in the first thoracic foot of Apus—the epipodite is the flabellum, and the branchial plume, where present, probably represents the bract of the Phyllopod's appendage.

The relations of the thoracic swimming feet of Nebalia, and the abdominal swimming feet of Palinurus to the Apus limb—as they appear to me—are indicated by the letters affixed to those swimming feet in the woodcut (*XIV* and *X*).

A remarkably close agreement in form is seen when the first maxillipede of Callianassa (*III*) is compared with the lamelliform foot of Nebalia (*XIV*). But upon studying a series of first maxillipedes it becomes probable that the internal ramus (5) of Nebalia's swimming foot is *not* the same apophysis as that which forms the internal ramus of Callianassa's first maxillipede. For as shown by a comparison of the first maxillipedes of Carcinus (*I*) and of Caridina (*IV*), an apophysis (number 5) which is already much reduced in Caridina has altogether disappeared, both in Callianassa and in Pagurus (*II*).

This simulation of identical constitution by the thoracic foot of Nebalia and the maxillipede of Callianassa is an example of that 'pseudomorphism' which renders the determination of homologies of the parts of the Crustacean appendage in different regions and in different genera so difficult a problem.

The first maxillipede of Pagurus (woodcut, fig. 1, *II*) presents a feature which is of importance in relation to the determination of the parts present in the second maxilla of Astacus and allied forms. The second maxilla in the latter is destitute of any developed representative of the flabellum (epipodite); on the other hand, the first maxillipede *usually* in these Decapoda has a well developed flabellum. Pagurus, however, presents a first maxillipede, which, *like the second maxilla* of other Decapoda, is without any flabellum, and in connection with that absence we find a tendency of the sixth endite (exopodite) to become broad and flabelliform, as it is in the second maxilla. In Caridina (*IV*), a very small flabellum is present on the first maxillipede, and by its presence confirms the identification of parts adopted in regard to the first maxillipede of Pagurus.

THE CENTRAL NERVOUS SYSTEM OF APUS.

The remarkable condition of the central nervous system of Apus has been described and figured with great care by Zaddach, but whether his observations have been considered untrustworthy, or from whatever cause, no notice has been taken by recent writers of the very significant and exceptional disposition of the ganglia in the anterior region of the body which he brought to light. There is every reason for accepting the accuracy of Zaddach's observations, not only on account of the care which he appears to have given to this investigation, but because Grube described in 1853 (loc. cit.) a very similar but not quite identical disposition of the ganglia in *Limnetis brachyurus*.

In the Crustacea generally, the two pairs of præoral appendages receive their nerves from the præoral ganglion, which supplies also the eyes and the integument of the præoral region. On this account the two pairs of præoral appendages have been looked upon as being aboriginally appendages of the region in front of the mouth and in various other Arthropoda (*e.g.* the Arachnida and Limulus) the attempt has again and again been made to determine whether the first pair of appendages which are present in a given case are to be considered homologous with one of the præoral pairs of appendages of the Crustacea by an examination of the nerve-supply—it being held that if an appendage received its nerves from the præoral ganglion it would necessarily be the homologue of one of the similarly innervated præoral appendages of the Crustacea.

Anton Dohrn, for example, in discussing the affinities of Limulus, says: " Bei allen Krustern empfangen nämlich die beiden vorderen Extremitätenpaare ihre Nerven aus dem oberen Schlundganglion." And so firmly fixed is this conception with regard to the nerve supply of the two pairs of præoral appendages in Crustacea, that Dohrn has made it a subject of special remark that in certain Nauplius larvæ the first pair only (the antennules) receive their nerves from the præoral ganglion, whilst the second receive their nerves from the lateral cords, which pass posteriorly to form the ventral ganglionated cord.

Claus, who described this difference in the mode of nerve supply of the two præoral appendages of larval Phyllopoda, is led by it to a remarkable conclusion as to the nature of the two pairs of præoral appendages of Crustacea. He holds ('Grundzuge der Zoologie,' 3rd edition, p. 518) that *because* in these Nauplii the first pair of antennæ are supplied by the cerebral or præoral ganglion they are the equivalents of the antennæ or tactile appendages of the cephalic lobe of Chætopoda (Annelides); whilst the second pair of Nauplius appendages, or second pair of antennæ, are to be regarded as equivalents of postoral segmental appendages of Chætopoda, *because* they receive their nerves from the lateral cords and not from the præoral ganglion.

I have long been of the opinion which Prof. Claus appears to hold, that the appendages of the Arthropoda are homologous (or, to use a more distinctive term, " homogenous ") with the appendages of the Chætopoda, and on this account I consider it a proper step in classification to associate the Chætopoda with the Arthropoda and Rotifera in one large phylum, the Appendiculata (see "Notes on Embryology and Classification," this Journal, 1876, and Preface to the English translation of Gegenbaur's 'Elements of Comparative Anatomy').

At the same time I have not been led to conclude, as Prof. Claus does, that only one pair of the Crustacean antennæ are to be regarded as primarily postoral in position, and as representing the appendages of two originally postoral somites, but I think it probable that *both* antennæ are in this case, and that in the Crustacean there is no representative of the "antennæ" or tactile processes of the cephalic lobe of Chætopoda. Whilst this appears to me probable in regard to the Crustacea, it yet seems to me very possible that the antennæ of Peripatus and of Hexapod and Myriapod insects may represent true processes of the cephalic lobe or prostomium, as seen in Chætopoda.

In 1873, in the 'Annals and Mag. of Natural History' for May, p. 336, I said: "Much more likely, it seems, is the explanation that the oral aperture shifts position, and that the ophthalmic segment alone in Arthropoda represents the prostomium, the antennary and antennular segments being aboriginally metastomial, and only prostomial by later *adaptational shifting of the oral aperture*." I was led to take this view by a consideration of the relations of the mouth to the appendages in Nauplius, and by the facts of the constitution of the head and its appendages in the Chætopoda.

Prof. Claus' removal of the second pair of antennæ from the præstomial to the metastomial region is based on a solid embryological fact as to the innervation of that appendage in the Nauplius, but it appears to me that the facts ascertained by Zaddach and Grube as to the structure of the nervous system and the supply of the appendages in certain adult Phyllopods—facts which were unknown to me in 1873—entirely confirm and establish on a firm basis the view that, in the Crustacea at least, the two præoral pairs of appendages are primitively postoral, and are neither of them related to the primitive prostomium or cephalic lobe, viz. the region innervated by the primitive cephalic ganglion, which ganglion I will venture, for want of a better name, to call the 'archi-cerebrum' of the Appendiculata.

The condition of things described and figured by Zaddach in *Apus cancriformis* is represented in the woodcut, fig. 2. The nerves to both pairs of antennæ are seen to arise, *not* from the quadrangular archi-cerebrum, but from the lateral cords at some distance from it, which are continued posteriorly to form the so-called 'ventral' nerve ganglion chain. A similar but less considerable separation of the nerves to the first pair of antennæ from the cerebral ganglion is described and figured by Grube in Limnetis ('Archiv für Naturgeschichte,' 1853).

In some very important respects the central nervous system of *Apus* appears to be more archaic and more nearly like that of the Chætopod annelids than is that of any other Crustaceans.

Recent researches on Planarians, Nemertines, Chætopoda, and Mollusca, point to the conclusion that the central nervous organs in all bilaterally symmetrical Cœlomata are primarily (or after a certain stage of evolution) a pair of *lateral* cords, consisting of nerve ganglion cells and nerve fibres, which are at first united to one another across the middle line only by commissural fibres. The more abundant nerve cells in the prostomial portions of these primitive nerve-cords form a pair of archi-cerebral ganglia, which unite with one another to constitute the primitive præstomial ganglion or archi-cerebrum. Meanwhile other ganglia or concentrations of nerve cells are formed upon the portions of the lateral cords lying behind the mouth, and these apparently correspond in number to the segments (if segments exist), which are differentiated in the metastomial axis. These metastomial ganglia may become highly specialised by the localising in them of all nerve cells, whilst the intermediate parts of the cords remain as simple bands of nerve fibres (Leeches, some Chætopods, most Arthropods, most Mollusca), or nerve cells may still occur in quantity between the ganglia (Peripatus, some Chætopods, Chitons). A distinct method of further change consists in the approximation to one another in the middle line of the two cords with their ganglia. This approximation may be slight, when it indicates an archaic condition (Peripatus, Serpula, &c.), or it may be so fully carried out that the ganglia, though rarely the cords themselves, are fused to one another in the middle line.

Apus is remarkable, as the woodcut (fig. 2) shows, for the wide divergence of its nerve cords in the first part of their course behind the mouth, and in this disposition confirms the conclusion (to which the isolation of its archi-cerebrum leads) as to its nervous system being preserved in a primæval or archaic condition.

It appears that, just as there is a tendency in the primarily lateral nerve ganglia of bilaterally symmetrical animals to move towards one another and fuse in the middle line of the body, so also there is another and similar tendency for primarily distinct and isolated ganglia to travel either backwards or forwards (usually forwards) along the lateral cords, *and*

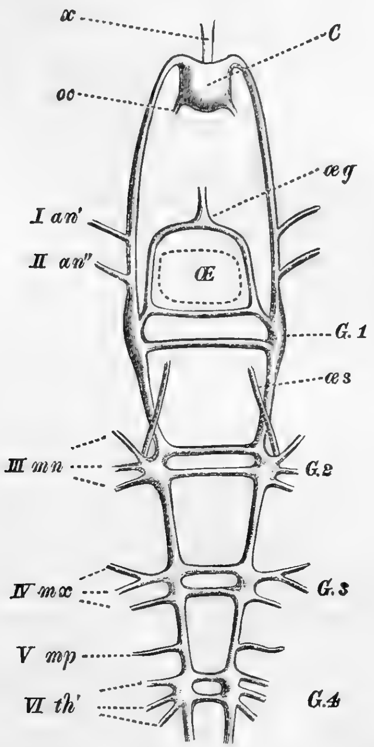


Fig. 2.—Diagram of the anterior portion of the nervous system of *Apus cancriformis* seen from below (constructed from Zaddach's figures).

C. Archi-cerebrum.
Œ. Œsophagus.
G. 1. First post-cerebral ganglion.
G. 2. Second post-cerebral ganglion (ganglion of the mandibular segment).
G. 3. Third post-cerebral ganglion (ganglion of the maxillary segment).
G. 4. Fourth post-cerebral ganglion (ganglion of the first thoracic segment).
I an'. Nerve to the first antenna.
II an''. Nerve to the second antenna.
III mn. Nerves to the mandible.
IV mx. Nerves to the maxilla.
V mp. Nerve to the maxillipede.
VI th'. Nerves to the first thoracic appendage.
x. Frontal nerves.
oc. Nerve to the left eye.
œg. Œsophageal ganglion impar.
œs. Posterior œsophageal nerve.

to fuse with neighbouring ganglia of the same side. This tendency is most obviously exhibited in the Mollusca, and in those animals affects more especially the part which has interest in connection with the morphology of Apus, namely, the adjacent præoral and postoral regions.

It is now well established that the archi-cerebrum (the primitive præstomial ganglion) of Gasteropods may, as it were, attract to itself ganglia developed originally at a distance from it, which were connected to it by nerve-cords, and may be described as running up these cords to fuse with the archi-cerebrum, and thus to constitute a complex cerebral ganglion, containing altogether new morphological elements. Such a process of complicating the præœsophageal ganglion pair is seen most obviously (to mention only one example) in the Nudibranchiata (Gegenbaur, English translation, p. 348), and a due appreciation of the possibilities of shifting and fusion of primarily distinct ganglia thus opened out affords the key to the very varied structure of the ganglion groups of the cephalous Mollusca.

Whilst, then, we distinguish the original ganglion pair of the præstomial region as the archi-cerebrum—it is well to designate by a distinct term the composite ganglion, which may result from the fusion with it of other ganglia—it may be called a syn-cerebrum.[1]

In the Chætopoda, the præ-œsophageal ganglion appears always to remain a pure *archi-cerebrum.* But in the Crustacea (and possibly all other Arthropoda, though there is a case to be considered for Peripatus and for the Hexapoda and Myriapoda, on the supposition that their antennæ are *not* the equivalents of Crustacean antennæ but of the processes of the cephalic lobe of Chætopoda) the præœsophageal ganglion is a *syn-cerebrum* consisting of the archi-cerebrum and of the ganglion masses appropriate to the first and second pair of appendages which were originally postoral, but have assumed a præoral position whilst carrying their ganglion masses up to the archi-cerebrum to fuse with it. This is true of all Crustacea, excepting Apus and possibly some other Phyllopods, and in possessing a widely isolated and pure archi-cerebrum (woodcut, fig. 2, C) Apus stands forward in a very marked position.

The only other case amongst adult Arthropods, in which it appears with certainty that the so-called cerebral ganglion is a pure archi-cerebrum, is that of Limulus.[2] Whilst some

---

[1] I am aware of the objection which may be taken to the compounding in these terms of Greek prefixes with Latin substantives, but I see no alternative.

[2] Balfour has shown that in spiders the ganglion of the cheliceræ is in

investigators have traced the nerves of the *two* first appendages of Limulus to the cerebral ganglion, and others have decided that only one (the first) pair derive their nerves from this ganglion—it appears from the admirable researches of M. Alphonse Milne-Edwards, that *none* of the appendages receive their nerves from the cerebral ganglion, but that it exists as a distinct emarginated mass of nervous matter, giving off nerves *only* to the simple and compound eyes and the frontal integument, as shown in the woodcut, fig. 3.

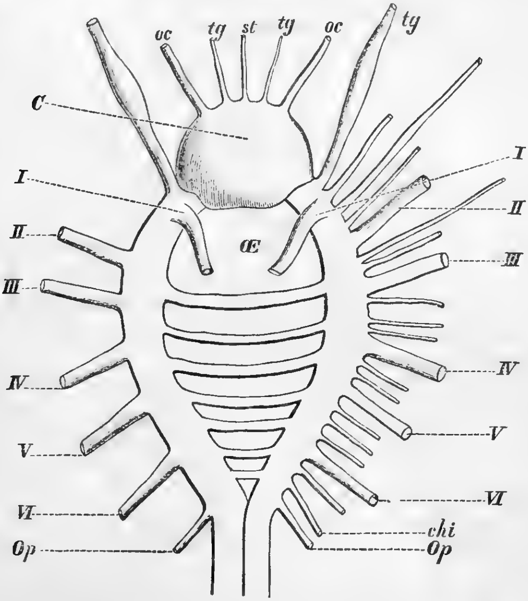


Fig. 3.—Diagram of the anterior portion of the nervous system of *Limulus polyphemus*, seen from below (schematized after Alphonse Milne-Edwards).

Œ. Position of œsophagus.
C. Archi-cerebrum.
oc. Nerves to the compound eyes.
tg. Frontal tegumentary nerves.
st. Nerve to the stemmata.
chi. Nerve to the chilaria (left side).
Op. Nerve to the operculum (left side).
I. Nerves of the cheliceræ or first pair of appendages.
II to *VI.* Nerves of the second, third, fourth, fifth, and sixth pairs of appendages.

An attempt has already been made to investigate the

---

development postoral and distinct from the archi-cerebrum. This Journal, April, 1880.

intimate structure of the cerebral ganglion of Arthropods (*e.g.* of Astacus, of Blatta, and of Limulus). It appears to me that it will be of very great importance in this analysis to determine first of all the characteristic structure of the archi-cerebrum of Chætopoda, of Apus, and of Limulus, and then to advance to the composite syn-cerebra of the higher Crustacea, when it may be possible to distinguish as distinct morphological elements the nucleus furnished by the archi-cerebrum and the later accretions of primarily postoral ganglia. Possibly such a method of analysis may help in deciding whether the Hexapods' and Myriapods' antenna is a true outgrowth of the præstomial region (like the tactile processes of Chætopods) or a postoral appendage which has moved forwards.

Returning again to the woodcut diagram of the anterior portion of the nerve-cords of Apus (fig. 2), it will be observed that there are some other remarkable features besides the pure archi-cerebrum and the widely separated position of the lateral nerve-cords.

The nerves to the first and to the second antennæ come off from the lateral cord just in front of the elongated ganglionic swelling (*G* 1), which is joined to its fellow of the opposite side by two transverse commissures, as are the more posterior ganglia. A secondary œsophageal ring surrounding the dotted area of the œsophagus (*Œ*) is given off from the anterior of these transverse commissures, which are both post-œsophageal, and accordingly determine the ganglia (*G* 1) as post-œsophageal.

The question arises whether these elongated ganglia represent the fused ganglia of the antenna and antennæ. Possibly they do; in any case it is an altogether remarkable fact that there exists in Apus a pair of ganglia *behind* the œsophagus *in front* of the pair which are appropriate to the mandibles.

There is no such arrangement known in any other Crustacean excepting in Limnetis, where, according to Grube, there are actually *two* distinct pairs of ganglia in this position, the interior of which supplies the second pair of antennæ with nerves, whilst the first pair of antennæ are supplied from the lateral cords at the base of the cerebrum. On account of the small size of Limnetis some doubt may be entertained as to the complete accuracy of Grube's observations, but they tend very strongly to establish the general accuracy of those of Zaddach upon Apus[1].

---

[1] I have not been able in my specimens of Apus, on account of their state of preservation, to make dissections of the nervous system; I should

It is quite possible that in Apus, owing to the diminutive size and rudimentary character of both pairs of antennæ, the ganglia appropriate to them have not fused with any others, but have entirely disappeared from the lateral cords. This possibility is forced upon the attention by the fact that the nerve to the maxillipedes comes off on each side from the lateral cord and has no ganglionic enlargement related to it (*V mp*). The mandibles and the maxillæ have, on the other hand, each a perfectly distinct, widely separate pair of ganglia (*G* 2 and *G* 3), the pairs being connected each by two transverse commissural strands. When the thoracic region is reached the nerve-cords approximate more closely to the middle line, but a distinct ganglion pair is developed for each pair of thoracic feet, of which the first only is seen in the figure (VI *th'*). Posteriorly, the ganglia are very closely set upon the cords, but, according to the statement of Zaddach, there is a distinct ganglion for each of the feet, even to the smallest of the abdominal (post-genital) series.

The facts as to the nervous system of Apus which I have above cited from Zaddach appear to deserve very serious attention, since they tend to show that the nervous system of Crustacea consists primarily of an archi-cerebrum and of two lateral cords, and that on each cord is a ganglion corresponding to each appendage; that the cords tend to meet one another in the middle line; that when appendages become rudimentary (*e.g.* the maxillipedes) their ganglia may disappear; that the first postoral ganglion pair is not in all cases that of the mandibles; and finally, that the nerves of the two first pairs of appendages are, in certain thoroughly typical Crustacea, given off from the lateral cords at a point far removed from the archi-cerebrum.

Whether the ganglion (*G* 1) has any relation to the nerves of the first and second antennæ might possibly be determined by a microscopical examination of the roots of those nerves.

In the course of the foregoing pages I have alluded to some points of resemblance between Apus and Limulus, the most important being the jaw-processes at the base of all the ambulatory limbs, and the presence of a pure archi-cerebrum in both genera.

I should wish, however, to guard against the inference that I consider any close affinity to obtain between Apus and Limulus. Apus appears to me to be an archaic Crustacean, and Limulus an archaic Arachnidan (not merely

therefore be very grateful to any naturalist who would send to me by post perfectly fresh living examples of Apus.

"related to" the Arachnida, but definitely to be classed in that group). The points in which they agree are probably points in which they both approach the common ancestor of the Arachnida and Crustacea—but each of them presents clearly and definitely the dominant and distinctive structural attributes of its own class or branch of the pedigree of those Appendiculata which have appendages modified to serve as jaws—namely, the Arthropods or, better, Gnathopods.

NOTES AND MEMORANDA.

Claus on Intracellular Digestion in Hydrozoa.—Professor Claus, of Vienna, has written to us to point out that in 1874 he had published observations on the amœboid movements of the endoderm cells of the stomach tube of Siphonophora (Monophyes), and had concluded from them, and from the presence of foreign bodies within the cell-substance, that food particles might be taken in by this same method. In his paper on Halistemma ('Zool. Arbeiten aus der Wiener Institut,' p. 37), published in 1878, he says, "I have already elsewhere given an account ('Schrift. Zool. Inhalts,' Wien, 1874, pp. 30, 31) of the amœboid processes and of the movements which the ciliated stomach-cells of the polyps exhibit during life, which fully explain how it comes about that foreign bodies, such as exploded thread-cells, are so frequently found in their protoplasm. Probably, also, the observations of C. Vogt ('Siphonophoren von Nizze,' 1854, p. 102) upon the entry of small indigo particles into the vacuoles of the cells are to be explained in this way, although no doubt the vacuoles were erroneously interpreted by him as shallow cavities or open gland-sacs."

Professor Claus appears, therefore, to have been the first observer to suggest, as the result of direct observation of a Hydrozoon, that its endoderm cells were capable of ingesting solid food-particles.

Kent's Manual of the Infusoria.—Mr. Saville Kent's treatise on the Ciliate and Flagellate Protozoa, now in course of publication, is a carefully executed and valuable work of reference. The existing English books on the subject are quite out of date, and there are not complete systematic treatises on the subject in other languages.

Hence the naturalists (who, we are glad to know, are as numerous as ever) who occupy themselves with the special study of microscopic organisms have no standard of reference, no trustworthy guide either to enable them to assign a name to the forms which they observe or to satisfy themselves that a novelty has come before them. Mr. Kent's manual will, by its very full and complete series of illustrative plates and the clear systematic descriptions which he has drawn up, efficiently supply the want we have indicated, so far as the ciliate and flagellate forms are concerned.

Mr. Kent's work also embraces the general anatomy and physiology of the ciliate and flagellate Protozoa, in dealing with which he shows himself to be thoroughly acquainted with the most recent literature, and also a skilful exponent and able original observer. Mr. Kent still maintains James-Clark's view as to the close affinity of the Flagellata and the Sponges, and although not altogether an acceptable theory, it serves in Mr. Kent's hands to give a special interest to the various forms and colonies of the Flagellate Protozoa.

We are glad to hear that a volume on the 'British Rotifera,' by Dr. C. T. Hudson, of Manilla Hall, Bristol, who will always be remembered, not only for his numerous contributions to our knowledge of this group, but especially for his discovery and clear illustration of the most important member of the group, namely, *Pedalion,* is in course of preparation for the Ray Society. Dr. Hudson will have the advantage of making use of all Mr. Gosse's beautiful drawings of Rotifera which that veteran student of microscopic organisms has placed at his disposal.

MEMOIRS.

On the LYMPHATIC SYSTEM *of the* SKIN *and* MUCOUS MEMBRANES. By E. KLEIN, M.D., F.R.S. (With Plates XXI and XXII.)

(From the Report of the Medical Officer of the Local Government Board for 1879.)

IN No. III (New Series) of the Reports of the Medical Officer of the Privy Council and Local Government Board, I have given an extensive summary of an anatomical investigation of the lymphatic system of the serous membranes and the lung, carried on during 1871 and 1872, with special reference to the process of chronic infective inflammation and tuberculosis.

These investigations having been published in an extended form, with the sanction of the then Medical Officer, and by the aid of the Royal Society, as 'The Anatomy of the Lymphatic System.' Part I, "The Serous Membranes," 1873; part II, "The lung," 1875.

With the permission of the late Dr. Seaton and the present Medical Officer, I have been able to resume these investigations and to extend them, during the past two years, to the lymphatic system of the skin and mucous membranes, and on these I propose to report in the following pages.

I.—THE LYMPHATICS OF THE SKIN OF MAN AND MAMMALS.

1. In accordance with the observations of Teichmann, published in his classical work: 'Das Saugadersystem vom anatomischen Standpunkte,' Leipzig, 1861; also I. Neumann, in his recent investigations of the lymphatics of the skin ('Zur Kenntniss der Lymphgefässe der Haut,' &c., Vienna, 1873), distinguishes the superficial from the deep-seated lymphatics, both forming a plexus, *i.e.* the superficial and the deep plexus. The former is denser and its vessels finer than in the latter. G. and F. Hoggan ('Proceedings of the

Royal Society,' No. 182, p. 289[1]) describe the lymphatics of the mammalian skin as consisting of a plexus of "subepidermic" and one of "subhypodermic" vessels; both are connected by the "dermic" vessels which form horizontal and vertical sets.

All these authors agree that the superficial vessels are capillaries, while the deep ones are the efferent vessels possessed of valves, and G. and F. Hoggan consider also the "dermic" vessels as possessed of valves.

Both Teichmann and Neumann noticed lymphatics in the papillæ of the corium; they are branches of the superficial plexus. According to the former the papillæ of the hand and foot contain each a lymphatic vessel terminating in a cæcal extremity, but always less superficially than the blood capillaries. Neumann saw in all well-formed papillæ either a single lymphatic terminating in a cæcal extremity or a loop.

G. and F. Hoggan deny the existence of these papillary lymphatics, but this is probably owing to their having studied only the skin of mammals, which generally do not possess well-formed papillæ. In well injected specimens of mammalian skin, however, indications of papillary lymphatics are undoubtedly present (*see* below).

2. The lymphatic vessels referred to here and in the following as the deep efferent vessels are distinct from, and much finer than the vessels which, being the collecting vessels of large cutaneous districts, run between the skin and the subjacent organs, as fasciæ, tendons, &c. These tubes are possessed of a comparatively thick wall, composed, besides the lining endothelium, of an elastic intima, a circular muscular media, and a thin connective-tissue adventitia. In this respect they conspicuously differ from the lymphatics belonging to the skin, whose wall is merely a single layer of endothelial plates, either more or less elongated with straight or sinuous outlines, as in the deep and larger, or sinuous in shape, as in the superficial smaller vessels.

The collecting vessels possess, as shown by Biesiadecki, heir own system of blood-vessels, consisting of an arterial and venous branch, and a network of capillaries.

A. Dogiel quite recently ('Archiv f. Mikr. Anatom.,' vol. xvii, p. 335) demonstrated the existence of a network of blood capillaries surrounding the large efferent (collecting)

---

[1] I have not been able to find where the full paper of these authors is printed, and I am therefore limited to the "Abstract" published in the 'Proceedings.' Through the kindness of the authors I have learned quite recently (May, 1881) that their paper has been published in the 'Journal de l'Anatomie et Physiologie,' by Robin and Pouchet, Jan. and Feb., 1879.

vessels underneath the skin of the ear lobe and of the hind extremity of the rat, in the mesentery of the cat and the dog.

3. In studying the lymphatics of the skin, it is necessary, as we shall show by-and-bye, to consider them in their relation to the various organs of which the skin consists, for they, viz. the lymphatics, as well as the blood-vessels, as shown by Tomsa, stand in a special relation to those organs.

We shall then consider them as: A. The lymphatics of the connective-tissue matrix of the skin; B. those of the fat tissue; C. those of the sweat glands; and D. those of the hair-follicles, sebaceous glands and arrectores pili.

Such an attempt of distinction has been made also by Neumann, but, as I shall show, what this observer described as the lymphatics of the fat tissue, hair-follicles, sebaceous glands, and sweat glands, are merely the lymphatics of the surrounding connective tissue, and do not in strictness bear any other relation to those organs than that of efferent vessels. The lymphatics that properly belong to those organs have not been known to Neumann or to his predecessors.

The assertions of G. and F. Hoggan as to the non-existence of lymphatics in the fat tissue, hair-follicles, sebaceous glands, arrectores pili and sweat glands have, like most negative results, only a relative value, inasmuch as they show, what, however, did not require any special proof, viz. that whilst it is comparatively easy to demonstrate the lymphatics of the connective-tissue ground substance of the skin, those of the fat tissue and the glandular structures require special methods and attention.

A.—*The Lymphatics of the Connective-tissue Ground Substance.*

4. These are the lymphatics that have been known to all observers, and have been specially studied through injections with various colouring matters by Teichmann, Neumann, and others. They have been mentioned on the preceding page. Teichmann as well as Neumann (and also G. and F. Hoggan) consider the above lymphatics as the ultimate vessels and as not possessing any direct connection with the surrounding connective tissue. That they should have arrived at such a conclusion with reference to the connective tissue need not surprise us, seeing that they were not able to demonstrate the ultimate vessels belonging to the fat tissue or the glandular structures of the skin.

5. The methods used by Teichmann and Neumann seem to be altogether unfavorable for the demonstration of the

ultimate lymphatics. This is the method followed by Neumann (l. c., p. 8); "The method that I used was the modified method of Hyrtl-Teichmann; the epidermis having been removed by maceration in a mixture of alcohol, acetic acid, and water, and also the rest of the tissue having become macerated, an incision half to one line deep is made with a pointed cataract needle, into which a fine tubule is fixed, and the injection through a fine brass syringe is proceeded with. . . . ." As injection material Neumann used (l. c., p. 9) carmin-ammonia in conjunction with glycerine, and carbonate of lead rubbed down in a little glycerine. On p. 10 he informs us that to prevent mistaking the lymphatics for blood-vessels he injected the latter with Berlin blue. But, to our profound astonishment, we find in the beautiful plates accompanying his memoir, unexpectedly all lymphatics represented as if injected with Berlin blue, although this pigment, according to his statement, is reversed solely for the injection of the blood-vessels. These latter are represented (in the plates) as if injected with carmin-ammonia; but, according to his statement, this is the material which he used for the injection of the lymphatics.

After this, and after his mentioning (l. c., p. 9) that it is advisable to stretch the skin that is to be injected over the left finger during each injection, we cannot expect that Neumann should have been able to trace the ultimate rootlets of the lymphatics.

6. The method which I follow is the same in the case of the skin and mucous membranes; it is this: I always use the material as fresh as possible; my most successful specimens were obtained from skin and mucous membranes prepared immediately after death. This applies equally to the structures of man and animals. To use material that had been previously macerated in alcohol and acetic acid, as in the case of Neumann's material, and to expect that the injection would reveal the finest rootlets, appears to me too sanguine.

Human skin is best; that of the dog and rabbit is also good. Of the former the scalp, the skin of the face, of the lips, of the palm of the hand and that of the sole of the foot, are those parts that yielded the best preparations.

The method of preparation is that of injection by "puncture." This is always performed on the skin *in sitû,* not on skin cut out previously and stretched over the finger (!) as is done by Neumann.

7. The injection material used is Brücke's Berlin blue in a solution of two to four per cent., or asphalt dissolved in

benzole on the plan of A. Budge ('Archiv f. Mikr. Anat.,' Band xiv., p. 70). This latter is an excellent material for injecting the finest rootlets of the lymphatics, for it flows with great ease and rapidity and has no influence, hardening or otherwise, on the tissue. And in these two respects it is greatly preferable to any other injection matter. Berlin blue or nitrate of silver possess both, especially the latter, a hardening influence, and cannot be expected therefore to flow as easily into the most distant and finest rootlets as the asphalt-benzole solution. The most successful injections of the finest rootlets in the connective tissue, fat tissue, hair-follicles, and glandular organs have been obtained by this last-named reagent. I must, however, add that in some cases, even with the asphalt-benzole solution only, the lymphatics of the superficial and deeper plexuses became injected, the injection matter not having penetrated into the ultimate rootlets, although the former were widely distended.

The solution is made thus: a very concentrated solution of asphalt in benzole is prepared, and before using it it is mixed with ½ to 1 of its volume of benzole. Asphalt in chloroform, as originally employed in C. Ludwig's laboratory, is not so advantageous, and still less asphalt in turpentine.

8. The injection is carried out in this manner:—A fine steel canula, as fine as it can be got, filled with the injection material, is gradually pushed in a very oblique direction, as horizontal as possible, into the *corium itself*. Injections into the deep subcutaneous tissue do not yield good results, at any rate even in the most successful injections one does not generally obtain much more than an injection of the deep big lymphatics.

An ordinary hypodermic syringe filled with the injection matter, and fitted into the canula, is then gently and gradually emptied into the canula. In a successful injection the skin becomes very gradually thicker and swells up, without becoming bulged out; the swelling spreads gradually and uniformly into the periphery. *The mouths of the hair-follicles become very conspicuous as depressions* owing to the parts between them gradually projecting beyond the general surface. And this is one of the best signs of a successful injection. Continuing the injection the skin becomes still more swollen, and the circumference of the injection also increases accordingly.

Owing to the opacity of the epidermis it is not possible even with a lens to see the lymphatics themselves, the skin presenting a uniform colour, brownish black if asphalt is used, blue with Berlin blue.

The intensity of the colour and the swelling up of the injected skin is always greater in the former, *i.e.* asphalt, than in the latter case, *i.e.* Berlin blue. But of course this depends on the amount of the injected material and on the ease with which it spreads in the skin, for if a relatively large quantity is injected which does not spread into a comparatively large area, the skin will be deeply coloured and it becomes very thick, owing to the lymphatics being greatly distended by, and filled with the injection matter.

In the description of the appearances of the lymphatics, to be given below, we shall refer only to such preparations in which the injection has been effected with great ease, the skin and mucous membranes having become readily injected, and slightly but distinctly thicker than in the uninjected state; the lymphatics are then found in a moderate state of distension.

In the case of the mucous membranes where the lymphatics are easily recognised in the injected organs with the unaided eye, the successful progress of the injection becomes soon apparent for the vessels become suddenly filled over large areas.

9. The skin and mucous membranes after the injection are hardened in dilute spirit: three parts of methylated spirit to one part of water; or in a mixture of two parts of $\frac{1}{6}$ per cent. watery solution of chromic acid and one part of methylated alcohol; or in $\frac{1}{6}$ to $\frac{1}{4}$ per cent. watery solution of chromic acid. When sufficiently hard, sections are made, stained, and mounted in the usual manner.

There are, however, certain disadvantages connected with the use of the asphalt-benzole. In the first place it is not so easily managed as watery solutions, and this of course applies to all other similar materials dissolved in turpentine, chloroform, oil, &c.; the cleaning of the syringes, canulas, tubes, and instruments require a deal of time and attention.

In the second place the difficulties of mounting and preserving the sections of the injected skin are considerable. These can be of course mounted and preserved in glycerine without any difficulty, but the section lacks in transparency and in smoothness of appearance of the injected vessels. The sections cannot be preserved in Canada balsam or dammar varnish on account of the solubility of the asphalt in balsam, in turpentine, and chloroform.

I have succeeded, however, to preserve specimens in a transparent state for several months in this way: the sections, after staining, are treated in the usual way for mounting in balsam or dammar, viz. they are placed in absolute alcohol, and then in oil of cloves, where they obtain the desired transparency. The section having been lifted out of the oil of cloves and the excess of the latter having been allowed to run off, is mounted in glycerine jelly. For the first month or two the lymphatics retain the asphalt in its original (black) state, but this becoming partly dissolved the vessels appear then filled with a paler (yellowish-brown) material. After several months the material still more fades into a transparent yellow, but the vessels remain nevertheless distinct.

In isolated cases I have been able to preserve some of the asphalt injection, of course only in a faint brown colour, in the lymphatics of the skin of the new-born child, even in sections mounted in Canada balsam solution.

10. It is hardly necessary specially to add that in the examination of sections, both through the injected skin and mucous membranes, only such parts have been selected that are at some distance from the point of "puncture," so that artificial extravasations may be excluded.

In the description of the lymphatics of the connective-tissue ground substance of the skin we shall take first those of man.

11. In a vertical section through the skin, *e.g.* of the hand or foot, scalp, or of the face, whose lymphatics appear well injected, most of these present themselves cut transversely or obliquely This would of course indicate that most of them possess a more or less horizontal direction, *i.e.* parallel with the surface. There are, however, connecting vessels to be seen in all layers which run for a shorter or longer distance a longitudinal course. *From the papillary body down to the layer of the adipose tissue the connective-tissue of the skin in a vertical section appears uniformly pervaded by these lymphatics,* except of course where it is interrupted by the presence of the hair-follicles, sebaceous glands, and sweat glands; a distinction into a superficial and deep stratum, as maintained by the other observers, cannot be well maintained.

12. J. Neumann, in his above-quoted memoir, gives in fig. 1, plate i, an illustration of continuous plexuses of lymphatics in a *vertical* section through the skin of the finger of the new-born. Such appearances, viz. a continuous plexus of lymphatics in a *vertical* section, I have not met with in any preparation of the skin in either man or mammal, and I doubt whether Neumann's figure is drawn from an

actual vertical section, or is perhaps merely a diagrammatic representation. The section requires to be very oblique, in order to show only approximately so many longitudinal vessels as are here drawn. No vessel is shown in this figure in transverse section, whereas most lymphatics from Neumann's own description follow a horizontal course.

13. As regards the size of the vessels seen in one vertical section we find, as a general rule, that the majority of the vessels belonging to the deepest layer of the corium, that is, the layer containing the hair-bulbs and sweat glands, are larger than those of the middle layer; those of the latter, again, smaller than those of the most superficial layer, *i.e.* of the papillary body, bearing in mind that we are here speaking merely of their relative numerical proportion and of preparations whose lymphatics have been distended to a medium degree.

14. The papillæ, especially were they are well developed, as in the fingers, scalp, &c., contain, as already described by Neumann, lymphatics either apparently terminating in pointed or cæcal extremities, or as a single or even double loop; and I have seen papillæ in the finger of a new-born child containing complex loops of lymphatics, that is, a *special network of their own.* Sappey mentions a similar relation with regard to lymphatics of the papillæ. In those parts of the skin of the new-born that do not contain well-developed papillæ, the lymphatics are indicated by short prolongations of the most superficial lymphatics.

The best view of the lymphatics of the papillæ is obtained in a thick vertical section.

15. Making a series of horizontal sections from the papillary layer down into the adipose tissue, we find *in each section a beautiful and more or less uniform plexus of lymphatics,* the vessels varying very much in size. The plexuses are of course interrupted by the presence of the hair-follicles and glands. Corresponding to the above-named appearances in the vertical section, viz. that most lymphatics run a course parallel to the surface, we find them in a horizontal section chiefly longitudinal; only few vessels are seen cut transversely or obliquely, that is, those running vertically or obliquely. In all strata we meet with vessels which appear to terminate *in the tissue.* Of these are at once to be omitted those which on focussing can be seen to be cut away obliquely, that is, are running to the next upper or lower stratum; but there always remain a good many vessels of which this cannot be said, and which really terminate in the tissue; they are generally running out into a narrow

cleft or channel, or terminate with a cæcal extremity, as is the case in some papillæ (*see* below). As a general rule we find the majority of the vessels of the most superficial stratum larger than those of the middle layers, and these again smaller than those of the deeper sections. I cannot agree with Teichmann, Neumann, and others, that the lymphatics are arranged as a superficial and deep plexus, than that those of the former are finer and their plexus denser than that of the deeper. I maintain, on the contrary, that throughout the thickness of the skin there are horizontal plexuses of lymphatics, of course with oblique or even vertical connecting branches; that the vessels of the superficial layers are larger than those of the middle layer; and that there is no absolute difference in the density of the plexuses, for the vessels are tolerably densely arranged in all layers, but relatively less so in the deeper strata, owing to the interposition of the sweat glands and the hair bulbs.

16. As regards the shape and nature of these vessels they appear more or less tubular, but many of them, and this refers to all layers, including the papillary stratum, are irregular, since they show isolated saccular dilatations, or appear through a succession of them, more or less varicose. In all layers very fine vessels are seen to join tubes many times larger, and one and the same vessel, if followed for a distance, may be seen to change its calibre. In these respects the lymphatics comport themselves like those of other organs. Following in a horizontal section under a higher power the outline of some vessels, especially of those that run out freely into the tissue, it, viz. the outline, will be found not quite smooth, but showing from place to place minute discontinuities and irregularities, which give the vessel a more or less crenate appearance. This has been observed also by Schenk; its significance will be explained later on.

All lymphatics are lined with a single layer of elongated endothelial plates, and in this respect they do not differ from other lymphatics. Besides this endothelium there is no other tissue which can be called their own, except the trabeculæ of the ground substance forming their outer support.

17. Teichmann, as well as Neumann, maintain that only the lymphatics of the deep subcutaneous plexus are possessed of valves.

G. and F. Hoggan ascribe such also to their "dermic" vessels, that is, to those extending as horizontal and vertical sets of vessels through the whole thickness of the skin.

I agree in this respect with G. and F. Hoggan, inasmuch as I find that there are lymphatics with valves in all layers of the skin.

18. Continuing the horizontal sections below the subcutaneous tissue, *i.e.* into the adipose tissue, we find that the connective-tissue septa between the lobules of the latter, which in the new-born child are very well marked and regulary arranged, contain *very numerous lymphatics*; these in well-injected specimens appear as plexuses of densely placed vessels and clefts, situated between the lamellæ constituting those septa. They will be later on described as the interlobular lymphatics of the adipose tissue. The vessels are smaller than those of the subcutaneous tissue, and they pass into the plexus of large lymphatics situated underneath the adipose tissue.

19. A very interesting observation that I made in the skin of a child that had died during, or very soon after birth, was this:—The skin was covered, as usual, with a thick layer of the secretion of the sebaceous glands, but in vertical and horizontal sections through the hardened skin lymphatic vessels in all different layers could be seen, *containing in their interior small and large clumps of what was undoubtedly the same sebum as that on the free surface.* In the large lymphatics of the depth this was especially very easily ascertained.

We shall presently have to return to this fact, and it will then become clear how this sebum found its way into the lymphatics, and of how great importance this is in the understanding of the absorption from the external surface by the lymphatics of the skin. At present we wish to point out that the mouths of the hair-follicles were greatly distended by, and filled with this sebum.

20. The lymphatics of the skin of the dog are similar in their arrangement to those of man, and for this reason we shall be able to be brief in their description, especially since we can refer the reader to the figures 1, 2, 3, 5 of the Plates accompanying this report. These illustrations and their explanation printed at the end of this paper give an accurate and sufficiently clear idea of the distribution and nature of these lymphatics. It will be thus seen that the lymphatics, even of the most superficial layer (Fig. 2), are large vessels, some of which present all appearances associated with valves in lymphatics, viz. saccular dilatations and corresponding constrictions. The vessels of this superficial layer are very densely placed, and therefore of short extension. Owing to the arrangement of the hairs in groups of four to six or more hair-follicles of unequal thickness,

the plexuses of lymphatics are not uniform, being limited to the connective tissue separating or surrounding these groups.

The septa of the adipose tissue possess fewer interlobular lymphatics than in man.

21. In what connection do the above lymphatics stand to the blood vessels? Both in the skin of man and mammals, but more especially in the former, I find in all strata of the corium, notably in the middle strata, some lymphatic vessels entering a more or less intimate relation with the blood-vessels; this consists in the following:—(*a*) We find a blood-vessel, either an arterial or venous branch, ascending to, or descending respectively from the surface of the corium accompanied for a longer or shorter distance on one or both sides by one or two lymphatic vessels; or (*b*) the blood vessel is crossed by one or two or even more small lymphatics in a very oblique direction, and the latter appear at this point as if belonging to the sheath of the former. Around the larger arterial branches we find numerous smaller and larger lymphatics which, in some instances, form a more or less complete sheath around the blood-vessel, perivascular lymphatics.

22. In what relation do the lymphatics stand to the general connective-tissue matrix, or with other words, what is the nature of the lymphatic rootlets? Brücke, Ludwig, v. Recklinghausen were the first who maintained the origin of the lymphatics in the clefts of the connective tissue.

These clefts, situated between the bundles or groups of bundles of the connective-tissue, have been injected in many organs naturally and artificially, and have been shown to be connected with the lymphatics. Without regarding it necessary to enter into a detailed historical review of this question, I will only mention the researches of v. Recklinghausen, Key, and Retzius, of Sikorsky, myself, Arnold, Sachs, Küttner, and others, by which it has been conclusively established that the interfascicular spaces, *i.e.* the clefts and channels between the bundles and groups of bundles of connective-tissue fibrils, containing in a semi-fluid interstitial albuminous substance the flattened connective-tissue cells, are the paths through which the normal current of plasma, irrigating the connective tissue, proceeds, and that through this current fluid as well as formed material is carried into the lymphatics. How is this, then, effected? How are the interfascicular clefts and spaces connected with the lymphatics?

As I have pointed out in my ‘Anatomy of the Lymphatic

System,' in agreement with v. Recklinghausen, Sikorsky, and others, the lymphatic capillaries connected on the one hand with the plexus of lymphatics, and originating on the other hand in the tissue, open everywhere freely into the interfascicular spaces, their lumen communicating freely with the latter.[1] This accounts for the endothelial wall being here perforated by numerous holes or stomata. The identical condition is met with in the intertubular lymphatics of the testis (v. Mihalkovitch) and in the connective tissue of the membranes of the brain and spinal cord (Key and Retzius), where lymphatics are lined with perforated endothelium, the holes leading into the spaces within the connective-tissue bundles. But there is also another indirect mode of connection of the lymphatic with the interfascicular spaces, and that is the mode which no doubt is the common one, and which occurs in the lymphatics in all strata; it is this: the albuminous cement substance between the endothelial cells forming the wall of the lymphatic is continuous with the similar substance acting as cement between the connective-tissue bundles. This substance being soft and semi-fluid does not present any obstacle to the passage of either fluid or formed matter from outside, *i.e.* from the interfascicular spaces into the lymphatic vessel.

This mode of connection is the one by pseudostomata in contradistinction to the former or direct one by real stomata.

This relation plays an important part in the absorption of chyle globules by the chyle vessels of the intestinal villi (Watney), and the same relation also exists between the lymphatics and the epithelium of the surface of the mucous membranes and glands (Sikorsky, Klein, Arnold, Thoma, Küttner, and others), and the endothelium of the surface of the serous membranes, as I have shown in my 'Anatomy of the Lymphatics.'

The assertions of Neumann and of G. and F. Hoggan that the lymphatic vessels are closed tubes not in communication with the surrounding connective tissue refer only to their respective preparations; they are not applicable to what I find in my preparations in which the lymphatics are successfully injected with an asphalt-benzole solution. Both in the skin of the dog, pig, rabbit, and especially in that of man, I find clear proofs of the free opening of lymphatic capillaries into the interfascicular spaces (*see* Fig. 6). But also in some instances of Berlin-blue injections I have

---

[1] The above-named crenate and irregular outline of some of the lymphatics is due to this fact, viz. that their endothelium is perforated, and their lumen opening into the spaces of the surrounding connective tissue.

traced most decidedly the Berlin blue from the interfascicular spaces into lymphatic tubes, as is represented in Fig. 8. Schenk, likewise, maintained such a connection.

Very interesting and decisive proofs of this connection I obtained from preparations of the case of the new-born child above mentioned, whose lymphatic trunks contained clumps of sebum. Here I found in many parts of the skin *smaller and larger drops and clumps of sebum contained in the interfascicular spaces,* being either unstained or stained brown with the asphalt-benzole. In some places the interfascicular spaces were filled with the sebum particles, and we had then a *good natural injection of the interfascicular spaces. They could be easily traced into the lymphatic vessels.*

B.—*The Lymphatics of the Adipose Tissue.*

23. The adipose tissue is very richly supplied with lymphatics, and the investigation of these forms one of the important points of this inquiry. Teichmann knew of no lymphatics in the fat-tissue, and also G. and F. Hoggan deny the existence of such. Neumann mentions in his monography above quoted (l.c., p. 25, cap. III, fig. 5) that the lymphatics form wide meshes which surround the individual lobules of the fat tissue, but he did not succeed to trace lymphatics between the fat-cells, *i.e.* into the interior of the lobules. Those vessels, seen and described by Neumann, are, as I have already pointed out, merely lymphatics belonging to the connective-tissue septa between the lobules of the fat-tissue, and for this reason they may be described as the *interlobular lymphatics.* Comparing Neumann's drawings with my own, I am led to conclude that the former saw very much fewer interlobular lymphatics than I. I have also drawn attention to the difference in numbers of these interlobular lymphatics in the fat-tissue of the human skin and in that of mammals, they being greatly more numerous in the former than in the latter.

The interlobular lymphatics take up everywhere *fine lymphatic sinuses and clefts which are traceable between each two fat-cells.* In specimens successfully injected with Berlin blue, and especially in those with asphalt-benzole, these *intralobular* or *intercellular* lymphatics are shown with great distinctness. I will particularly draw the attention of the reader to Figs. 1, 3, and Fig. 5, illustrating the ultimate lymphatics in the fat tissue of the skin of the dog, and Fig. 4, showing the same in man.[1]

---

[1] The asphalt-benzole, in a successful injection of the fat tissue, penetrates generally a good distance into the lobule, but the different lobules

24. Several important questions present themselves in connection with these intralobular or intercellular lymphatics: (*a*) what is their shape and minute arrangement? (*b*) what is the nature of their wall? and (*c*) what is the relation of the latter to the fat-cells themselves.

As regards their minute arrangement it is that of a dense network whose meshes contain each one fat-cell. It is more difficult to ascertain their precise shape. At first sight these intercellular lymphatics appear as thin narrow canals, but on closer inspection it will be found that many are in reality sinus-like, surrounding the fat-cells for a larger or smaller section of their circumference, and therefore possessing the shape of a portion of a spheroid. There are, however, some of them that are tubular, or approximately so, their optical section being either circular or more or less oval.

In specimens of fat-tissue that had been successfully injected with asphalt-benzole, it will be found that the injection material indicating the intercellular lymphatics is distinctly contained in a well-defined space, there being a delicate but distinct membrane limiting it. But it is exceedingly difficult to ascertain what this membrane is, viz. whether an endothelial membrane representing the proper wall of the lymphatic, or whether it is merely the membranous connective tissue between the fat-cells. I am inclined to think the latter is the correct interpretation. In very thin and well stained sections, especially in teased ones, it is possible to distinguish, besides the fat-cells and blood-capillaries, a honeycombed matrix of connective tissue, which consists of flattened, nucleated connective-tissue cells, attached to a plexus of very thin fibre bundles derived from the interlobular septa.

The successful injections make it highly probable that each of the above intercellular lymphatics is bounded by the fat-cell on the one side, and the intercellular honeycombed connective tissue on the other. Thus an analogy would be established between the ultimate lymphatics in the adipose tissue of the skin and those in the nerve trunks (Key and Retzius, 'Studien in d. Anatom d. Nervensystems,' &c.), in the latter the lymphatics between the neighbouring nerve fibres being separated by the endoneural connective tissue.

I need hardly add here of how great importance in phy-

---

vary greatly in this respect, for I have seen in the same section, both of human and dog's skin, lobules, the greater part of which was injected, besides others in which only the peripheral portions showed the lymphatic injection.

siological and pathological respects this intimate relation between the adipose tissue of the skin and the absorbents is, since that tissue plays a very prominent part in the economy of the body in health and disease.

In my 'Anatomy of the Lymphatics,' Part I, Chapter II, I have shown that in its formation the fat tissue of the serous membranes has an intimate relation to the ultimate lymphatics of those parts, the fat-cells being formed from connective-tissue cells (Flemming) situated in the rootlets of the lymphatics, *i. e.* in the lymph canalicular system; what I have now described of the anatomical distribution of the intercellular lymphatics in the fat tissue of the skin bears this out in a very striking degree.

C.—*The Lymphatics of the Sweat Glands.*

25. The sweat glands possess also their own minute lymphatics. These are very distinct in preparations of the human skin successfully injected with asphalt-benzole; they are taken up by the lymph-vessels of the surrounding tissue described above, *i. e.* those belonging to the plexus of lymphatics of the connective-tissue ground substance. In Fig. 6 are seen the *lymph-clefts* situated between the coils of the sweat-gland tube and the connective tissue separating the former. The relation of the lymph-clefts to the coils of the gland tube on the one hand and to the connective tissue on the other appears to be the same as that described of the fat tissue, viz. the lymph-clefts do not possess their own proper wall of endothelium. Also along the duct of the sweat gland, while passing through the corium, lymph-clefts may be traced for a longer or shorter distance, as is shown in horizontal sections in which the ducts are cut transversely, or nearly so. The above lymph-clefts surround sometimes half or even more of the circumference of the duct, so that this latter appears almost invaginated by the lymphatic.

D.—*The Lymphatics of the Hair-Follicles and Sebaceous Glands.*

26. The hair-follicles possess, according to Teichmann, G. and F. Hoggan, no lymphatics; Neumann describes lymphatic vessels which surround the hair-follicles as the proper vessels of these latter. But I recognise in his description and illustration (l.c., fig. 5, plate iii), merely lymphatics of the general connective-tissue ground substance.

After a great many negative results I have succeeded, in a few instances, in the human scalp as well as in that of the dog, to inject the ultimate lymphatics of the hair-follicles. Both in the asphalt-benzole injections, but especially in those with Berlin blue, I have ascertained that the lymphatic vessels and interfascicular lymph-spaces of the connective tissue surrounding the hair-follicle bear an intimate and, I need hardly add, important relation to the latter.

The fibrous coat of the hair-follicle, that is, the hair-sac proper, contains, both in the human hair as well as in that of the dog, more or less continuous lymph-channels, which are in communication with the plexus of the lymphatic vessels of the corium, and with the interfascicular lymph-spaces around. In a longitudinal section these lymph-channels are well seen in close neighbourhood to the outer root-sheath of the hair. In transverse sections it is ascertained that the lymphatics of the hair-sac *surround like a sinus the outer root-sheath. See* Fig. 9.

In these last two figures, which represent hair-follicles of the scalp of the dog, whose lymphatics had been injected with Berlin blue, it is seen with great distinctness that the injection material is contained not only *in the lymphatic channels and spaces of the hair-sac and its surroundings,* but that it *penetrates also into the root-sheath.* The injection material passes directly from the lymph-sinus around the root-sheath *into the interstitial or cement substance between the epithelial cells,* and what is still more remarkable and interesting, the injection material having penetrated up to the inner root-sheath accumulates there *in a distinct layer or space between this and the outer root-sheath and also between the former and the hair itself.* It is very probable that it reaches this latter in the same way as the former, viz. through the cement substance of its (*i.e.* the inner root-sheaths) scales. In the above case of the skin of the new-born child, whose lymphatics contained smaller and larger clumps of sebum, there existed most distinct signs of this latter substance being accumulated between the hair itself and the inner root-sheaths; further, between this and the outer root-sheath minute bright sebum particles were to be met with in the cement substance between the epithelial cells of the outer root-sheath, and were seen accumulated in the lymph-channels of the hair-sac. This is, then, clear evidence that the paths followed by the above Berlin-blue injection are the real ways of absorption, that is, are not produced artificially, since in the case of the sebum-absorption above referred to, it cannot be denied that the absorption had taken place during life.

The anatomical connection of the lymphatics of the connective tissue with the interstitial substance between the epithelial cells of the root-sheath is in perfect agreement with what is now well established for the mucous membranes, glands, serous membranes, &c. And I would again refer in this respect to the researches of Arnold, Thoma, myself, Küttner, Sikorsky, Watney, and others.

Just as I have shown it for the epithelium lining the bronchi and alveoli of the lung, and for the endothelium covering the serous membranes, so also in the case of the stratified epithelium forming the outer root-sheath of the hair we find in the interstitial cement substance branched, nucleated connective-tissue cells anastomosing with the connective-tissue cells of the surrounding hair-sac.

That this connection of the lymphatics of the hair-sac with the space between the hair and the inner root-sheath is of the utmost physiological and pathological interest is obvious. There can be no doubt that, in the absorption of fluid and formed matter from the outer skin, the lymphatics of the hair-follicles play a conspicuous part, and the practical experience of the efficiency of medicaments, especially those suspended in fatty matter rubbed into the skin, by which they are well pushed into the depth of the hair-follicles, receives hereby its theoretical explanation.

R. Fleischer, Erlangen, 1877, and quite recently in 'Virchow's Archiv,' vol. 79, p. 458, still persists in questioning the positive results obtained by Lassar ('Virchow's Archiv,' vol. 67, p. 1), as to the passage of oily matters rubbed into the intact skin of a rabbit, through the absorbents into the internal organs, for he (viz. Fleischer) maintains that such passage does not take place except through the injured skin.

My results above described clearly show that the anatomical facts are in favour of Lassar's assertions.

27. Whether a similar intimate connection exists between the lymphatics around the sweat gland (both duct and coiled tube) and the interstitial cement substance of its epithelium and the inner cavity of the tube, is very probable, but I have not been fortunate in showing this. Inunctions with pigment suspended in fatty matter would, I have little doubt, yield positive results.

28. As regards the lymphatics around the sebaceous glands and the muscle of the hair, I am able to record positive results, against the negative ones of Teichmann and

G. and F. Hoggan. Neumann's assertions as to the existence of lymphatics of these organs are of the same value as those referring to his lymphatics of the fat tissue and the hair-follicles, viz. they belong merely to the surrounding connective tissue.

In preparations of the human scalp, the lymphatics of which had been successfully injected with asphalt-benzole, the alveoli of the sebaceous glands are seen surrounded for a larger or smaller part of their circumference by *lymphatic spaces and sinuses,* which are connected both with lymphatic vessels and with the interfascicular lymph-spaces of the connective tissue.

Elongated lymphatic clefts are also seen between the bundles of the arrector muscle, similar to those to be observed in the unstriped muscle-tissue generally.

II.—THE LYMPHATICS OF THE SKIN OF THE FROG.

29. Owing to the great abundance of glands in the skin of the frog, and owing also to the importance in the process of absorption of this organ, I have investigated also its lymphatics. But there were very great difficulties to overcome. What I intended was to demonstrate by a good injection the arrangement and distribution of the lymphatics. The method of puncture was here out of question, for it was impossible for me to obtain a canula of the necessary fineness. It occurred to me that Budge's method of forcing by pressure (of air, water, or mercury) the injection material into the lymphatics as practised by him in the case of the lymphatics of the cartilage and bone (l. c.) might be productive of good results. My expectations were soon realised. The method I followed was this: in a decapitated frog I cut off the anterior extremities, and enucleate the trunk from its skin, after severing the septa between the cutaneous lymph-sacs of the chest, abdomen, and dorsum; I then place a ligature round the root of the trunk, and cut this latter off close to the ligature. I have now a preparation that consists of the entire lower extremities and the empty skin of the abdomen, chest, and dorsum. This quasi-bag is tied round the mouth of a small funnel, specially provided there with a groove, and the tube of the funnel is fixed in a retort holder, so that the preparation is hanging downwards. The tube is a long glass tube, and is connected with the injection bottle containing the injection fluid. This is pressed into the funnel by a mercury pump,

A pressure of two to two and a half inches being applied, and a 2 to 4 per cent. solution of Brücke's Berlin blue being used, it is found that soon the injection forces its way into the cutaneous lymph-sacs of the lower extremities, without, however, penetrating into the lymphatics of the skin itself. But on the inside of the thigh, next the median line and in the neighbourhood of the anus, *i. e.* in those parts which are in the common frog conspicuous by their thickness, owing to warty prominences of the cutaneous gland-sacs, a beautiful injection appears, which gradually increases in breadth and intensity, but does not penetrate anywhere into the lymphatics of the ordinary thin skin of the surrounding parts. Asphalt-benzole yielded no results.

30. Looking at the injected skin with the unaided eye there appears a beautiful mosaic or network of blue lines, each of its meshes corresponding to a wart. Under a lens, or, still better, under a low power of the microscope, it is ascertained that what with the unaided eye appears as a single line of the mosaic is a dense plexus of lymphatics.

There is a deep plexus of large vessels connected with a much denser plexus of fine vessels belonging to the superficial part of the skin. These latter vessels are of various sizes and of a very irregular outline. Fig. 7 shows them very well as seen under a higher power. The plexus of the finer vessels is interrupted by the cutaneous glands, as is noticed in Fig. 7. But there exist special lymphatics surrounding a smaller or greater section of the circumference of the gland-sacs; these latter, viz. the gland-sacs, are shown only in outline in this figure.

The lymphatics destined for the glands are apparently terminating with a cæcal extremity; and there are also other vessels of the general plexus which appear to terminate in a cæcal manner in the connective tissue of the skin.

III.—The Lymphatics of the Conjunctiva.

31. According to Schmidt ('Die Lymphfollikel d. Bindehaut' Wien, 1871), the lymphatics of the conjunctiva form a superficial and deep network connected with one another by many short branches. The vessels of the superficial network are very fine, and some of them terminate in the tissue with a pointed or cæcal extremity. The vessels of the deep plexus are possessed of valves. The superficial network is densest at the limbus, and less dense in the conjunctiva fornicis than in the conjunctiva palpebræ.

I have investigated the lymphatics of the eyelids of the new-born child and of the rabbit.

The best preparations were also here obtained with the asphalt-benzole injection.

32. The lymphatics of the skin are to be distinguished from those of the conjunctiva palpebræ.

Injecting into the subcutaneous tissue of the eyelid, it will be seen that the lymphatics of the skin become injected up to the outer free margin; the injection does not apparently pass into the lymphatics of the conjunctiva.

In making sections through such an eyelid it will be found that the distribution of the lymphatics of the corium differs in no way from those of other parts; the plexus of the subcutaneous lymphatics is conspicuous by the size of the vessels and by their large saccular dilatations; this plexus takes up lymphatic vessels situated between the bundles of the sphincter orbicularis. These vessels form a plexus, and are possessed of valves. They extend close to the dense fibrous tissue which forms the tarsal plate.

In vertical sections through the eyelid there are seen occasionally, but rarely, fine lymphatic vessels *passing through the tarsal plate,* so as to join the lymphatics of the conjunctiva palpebræ.

The chief communication is, however, established at the margin of the eyelid, where the lymphatic vessels of the corium anastomose with the vessels belonging to the conjunctiva palpebræ proper, situated behind the tarsal plate, *i. e.* between the layer of the Meibomian glands and the epithelium covering the free surface of the conjunctiva.

These results are in perfect agreement with those obtained by E. Fuchs, in 'Medic. Centralblatt,' 1878, N. 28.

33. To obtain a good view of the lymphatics of the conjunctiva palpebræ and fornicis the canula must be inserted into the proper tissue of the conjunctiva palpebræ.

When successful, the inspection of the surface with a lens reveals an exceedingly dense network of lymphatics situated very near the surface. In the human conjunctiva palpebræ these vessels are very fine, and the plexus extends to the free margin of the eyelid. The arrangement of the vessels is very characteristic, for about the first third, that is, the one nearest the free margin, they form a dense network with small and polyhedral meshes; in the second third the meshes become slightly elongated, but in a more or less oblique direction, the vessels assuming a tendency to run towards the angle of the eye; and in the last third, that is, the one nearest the fornix, the meshes become still more elongated, and have an almost horizontal position, the vessels following a course very nearly parallel to the margin of the lid. They join everywhere the plexus of the lymphatics of the fornix. These latter appear as a plexus of fine vessels, situated superficially, and one of large deep vessels; most vessels of both plexuses follow a course more or less parallel to the margin of the lid, *i. e.* longitudinal towards the angle of the eye, as mentioned above.

In horizontal and vertical sections through the eyelid the lymphatic vessels of the conjunctiva palpabræ extend from near the epithelium through the whole thickness of the conjunctiva.

The layer next the Meibomian glands contains large vessels (Colasanti) with valves, and these may be considered as the deep lymphatics.

Fuchs also injected lymphatic sinuses around the alveoli of the Meibomian gland. .

In the plexus of the superficial lymphatics of the fornix and also in that of the conjunctiva palpebræ nearest the fornix, there are found some vessels possessed of valves and corresponding saccular dilatations, but most of them are without valves.

Injecting in the same manner the conjunctiva of the rabbit it will be observed that in the conjunctiva palpebræ the blood capillaries and veins often become injected. Viewed with a lens or under the microscope the deep lymphatics with valves are also well seen.

The lymphatics of the conjunctiva fornicis come out with great distinctness, both those of the superficial parts as well as those of the depth.

The above fact of the blood capillaries and veins becoming injected would lead one at once to say that the passage of the injection matter into the blood-vessels is due to an injury of the blood-vessels by the canula. But, on careful examination, I failed to detect this; but, on the other hand, I have found what appears to be a direct anastomosis of minute veins with lymphatics.[1] This is, however, a point of so great an importance that, definitely to express an opinion, it would require a greater number of observations and a more minute examination than I have been able to make.

I have good reason to believe that there exists an indirect communication between the blood-capillaries of the conjunctiva palpebræ of the rabbit and its lymphatics through the lymph-canalicular system (v. Recklinghausen).

[1] V. Recklinghausen observed a similar passage of the injection matter from the lymphatics into the blood vessels. Waldeyer considers it due to an artificial rupture.

That the anastomosis of the lymph-canalicular system with the blood-capillaries appears here so patent and extensive may be owing to abnormally delicate capillaries.

It is possible that in the rabbit the interstitial cement substance of the endothelial wall of the capillaries of the conjunctiva palpebræ is more liable to give way, and to allow the injection material to pass from the lymphatic rootlets into the blood-capillary.

IV.—The Lymphatics of the Oral Cavity and Pharynx.

35. Teichmann (l. c.), and subsequent writers quoting his work, describe the lymphatics of the oral cavity and pharynx as being arranged similar to those of the skin, as a superficial plexus of fine vessels and a deep one composed of large vessels with valves. The superficial plexus is the denser of the two, and is in connection with the cæcal lymphatics belonging to the papillæ.

The observations which I propose to describe here refer to the lymphatics of the lips, buccal mucous membrane, the palate and pharynx, and the tongue; they were obtained by injecting into the mucous membrane, as near as possible to the surface of these different regions, asphalt-benzole or Berlin blue, in precisely the same manner as was described on a former page in connection with the skin.

The results of these injections confirm, in a general way, the observations of Teichmann, but extend them in some special details; the relation of the lymphatics of the connective-tissue ground substance to the epithelium of the surface, the lymphatics of the glands, and the muscular bundles, are points which, in Teichmann's work, have not been sufficiently considered, but which, as I shall be able to show, deserve special attention.

As in the case of the skin, so also in that of the mucous membranes, it is necessary to consider the lymphatics as forming different groups, such as the lymphatics of the ground substance of the mucous membrane; those of the fat-tissue, where such is present; those of the glands, mucous and serous; and, finally, those of the muscular tissue.

The Lymphatics of the Ground Substance.

36. In a successful injection into the mucous membrane of the lips of the mouth it will be found that there appears a very dense and beautiful network of fine lymphatics,

situated close to the surface. When examined under a lense it will be seen that the network is very dense, and that the vessels are either straight or slightly wavy and irregular. A few larger vessels with distinct valves may be seen in the depth; the direction of the course of the efferent trunks is away from the free margin of the lip, in the parts near the middle line, straight towards the gums, at the side towards the mucous membrane of the angle of the mouth.

When injecting into the part situated between the cutis and mucous membrane of the lip, it will be found that the injection material passes much more frequently into the lymphatics of the mucous membrane than into those of the cutis. But in every injection into the cutis of the one side of the lip the injection does not readily pass beyond the middle line, that is, from one side into the other. Injecting, however, the lymphatics of the mucous membrane, no such demarcation between the two sides can be observed.

37. The injection of the buccal mucous membrane and of that of the palate and pharynx shows also—these organs being examined in the fresh state in the bird's-eye view—a beautiful and dense network of fine lymphatics, the meshes being of a more or less uniform polyhedral shape.

38. In the tongue, however, the aspect of the lymphatics, as seen in the bird's-eye view of the fresh organ with the unaided eye or with a lens, greatly differs from the above organs, owing, of course, to the presence of the papillæ. On the dorsal part of the tongue there is found a network of fine lymphatics, whose meshes correspond to the papillæ; they are naturally larger for the fungiform than for the filiform papillæ.

The lymphatics of the tissue of the papillæ themselves are not visible under these circumstances.

Owing to the peculiar change of the surface at the margin of the tongue, viz. the papillæ becoming arranged in more or less distinct transverse rows, the shape of the network of the lymphatics undergoes a corresponding variation, that is, its meshes become elongated in a transverse direction.

At the margin of the tongue we notice numerous efferent vessels passing from the dorsum on to the lower smooth surface; they are more or less parallel with one another.

At the base of the tongue the arrangement of the lymphatics changes from a uniform network of fine vessels into one with elongated meshes. There is an exquisitely by dense network of very fine vessels surrounding each circumvallate papilla.

In man and in animals I have always found a plexus of large and fine lymphatics running from the dorsum of the tongue towards the base of the epiglottis. The vessels anastomose under acute angles, and increase in size as they approach the epiglottis; their valves are very conspicuous.

Sappey already injected the dense network of the lymphatics of the mucous membrane of the gums and soft palate and tongue.

39. Examining a successfully injected and hardened lip, palate, or mucous membrane of pharynx in vertical and horizontal sections under the microscope, it will be seen that the lymphatics are present in and extend through all parts of the mucous membrane more or less uniformly from the epithelium of the surface down to the deep tissue that contains the glands, fat, muscle, &c. The vessels are connected everywhere in plexuses, but there are in the superficial parts a good many vessels that appear to terminate freely in the tissue (see below).

Many vessels run in a horizontal direction, and they can be, therefore, better seen in horizontal than in vertical sections. Those belonging to the superficial parts of the mucous membrane are fine as compared with those of the deeper parts, and they run a more or less straight or wavy course. *Everywhere the papillæ possess their own lymphatics in the form of single or compound loops connected with the superficial lymphatics.* They penetrate more than half or two thirds into the tissue of the papillæ. These papillary lymphatics are best seen in the human lip and soft palate, owing to the very great length of the papillæ, but they are present also in those of other parts in man, dog, and rabbit. The lymphatics of the deeper parts are distinctly possessed of valves; such valves are not easily seen in the more superficially seated lymphatics, although in some isolated instances there are indications of their presence in the shape of saccular dilatations of the vessel.

The wall of the lymphatics of the superficial layers is irregular in its outline, being apparently possessed of more or less numerous pointed processes (see below).

The lymphatics of the loose submucous tissue are considerably larger and possessed of valves; they communicate freely with the plexus of the large lymphatics of the muscles and the fat tissue.

40. The relation between the lymphatics of the mucous membrane and the interfascicular lymph-spaces of the connective-tissue ground substance is a very intimate one, and is exactly the same as that described in the connective tissue of the skin, and it is, therefore, unnecessary to again minutely

describe this relation, and I will merely refer the reader to Fig. 12. The above irregular outline of the wall of some of the lymphatics is due to the lumen of the vessels being in open communication with the interfascicular spaces of the surrounding tissue.

41. An important and intimate relation exists between the most superficial lymphatics and the epithelium of the surface.

This relation is of the same nature as the one I have shown to exist between the epithelium constituting the outer root-sheath of the hair-follicles and the surrounding lymphatics, viz. *a direct passage of injection material from the lymphatics*—both the lymphatic vessels and sinuses as well as the interfascicular lymph-spaces—*into the interstitial or cement substance between the epithelial cells.* That there exists a direct connection between the lymph-spaces of the most superficial layer of the mucosa, including the papillæ, and the interstitial cement-substance of the epithelium, can be ascertained in every thin section of a well-prepared mucous membrane; it will be found, on careful examination, that the spaces between the fine connective-tissue bundles —some of which correspond, as is shown in injections, to lymphatic vessels—are distinctly prolonged into the interstitial substance of the epithelium. This is still more conspicuous in slight inflammatory conditions, when the lymph-spaces are enlarged and the interstitial substance is much increased in amount. The branched cells situated in the interstitial substance of the epithelium, mentioned on former occasions, are also here in connection with the connective-tissue cells situated in those lymph-spaces.

In Fig. 11 is represented a horizontal section through the most superficial parts of the mucous membrane of the lip of a rabbit, and it is noticed here that the interstitial substance of the epithelium, which includes here as well as in many other mammals *pigment granules* and *pigmented* branched cells, contains the injection matter (Berlin blue), and that this passes directly into the interfascicular lymph-spaces and the most superficial lymphatic vessels.

The very same condition exists everywhere in the oral mucous membrane and pharynx as regards the interstitial substance of the stratified epithelium of the free surface and the superficial lymphatics—a condition which, I need hardly say, is of great physiological importance. This relation can be studied in sections of simple uninjected preparations as well as in successfully injected specimens, and it is borne but by the observation of Toma and Arnold and others as regards the relation existing between the interstitial substance of the epithelium of the mucous membrane of the frog's mouth with the tissue of the latter.

42. In vertical sections through the injected tongue we see, under the microscope, a very rich plexus of lymphatics situated in the thin mucous membrane; these vessels are very irregular in size and outline, and some of them possess valves. Their course is chiefly parallel to the surface, and both in longitudinal as well as in transverse sections through the tongue a great many vessels are seen cut longitudinally.

Sappey describes fine vessels which enter the papillæ and then form a network. I can fully confirm this, for I find that each papilla filiformis and fungiformis (which, as is well known, are again possessed of minute secondary papillæ) of the human tongue contains its own network of lymphatics. *From the network we see lymphatic vessels ascending the secondary papillæ* and either terminating in a cæcal extremity or in a loop.

In animals (dog, rabbit) this is in so far different, as the filiform papillæ are mostly simple, not compound, but in the fungiform papillæ the same relations exist as in those of the human tongue.

43. The papillæ circumvallatæ of the human tongue contain a very great number of lymphatics. They are all fine vessels connected into a network, which does not show any special richness at the base where the taste-goblets are found; on the contrary, the vessels are if anything less numerous at that part than at the rest of the "papilla."

In the papilla foliata of the rabbit each of the ridges, which constitutes this organ, and as is well known, corresponds to the "papilla" of a circumvallate papilla drawn out into a fold, contains at its basis rows of taste-goblets; we find here in so far a variation of the above arrangement of the lymphatics, as from the superficial network there generally *ascends in the centre of each of the folds a cleft-like or sinuous lymphatic* which terminates in a cæcal extremity near the top of the fold, but does not reach as high as the blood-vessels. In some folds small branchlets may be found to come off at the basis from the central vessel; they then ascend towards the taste-goblets; it is not possible to ascertain whether they stand in any definite relation to the latter.

44. At the root of the human tongue, where the mucous membrane contains masses of adenoid tissue, either diffuse or as more or less well-defined lymph-follicles, this arrangement of the lymphatics in so far suffers an alteration as their

plexuses become as it were interrupted by the lymph-follicles.

These latter appear surrounded by saccular lymphatics, but also amongst the diffuse masses of adenoid tissue may be seen lymphatic vessels.

In both instances the endothelial wall of the lymphatic is *directly continuous with the reticulum of the surrounding adenoid tissue,* in the same manner as I described it both of the serous membranes and the bronchial walls in Parts I and II of my 'Anatomy of the Lymphatic System.'

45. The lymphatic vessels of the deeper parts of the tongue, that is, of the connective tissue separating the muscle-bundles of the tongue, are exceedingly numerous, very large, possessed of valves, and anastomosing into a rich plexus. Their efferent trunks run in company with the large blood-vessels, forming around these a beautiful plexus.

The arterial branches of the muscular tissue of the tongue, as well as those entering the mucous membrane, are generally accompanied by one or two lymphatic vessels; in some instances these latter appear like sinuses, *invaginating the whole or the greater part of the circumference of the blood-vessel.*

46. The arrangement of the lymphatics of the fat-tissue, in the lip, in the submucous tissue of the soft palate, especially at the base of the tongue, being in all respects identical with that described of the fat-tissue of the skin, does not require any special description; the connection of the lymphatic vessels situated in the connective-tissue septa between larger or smaller groups of fat-cells with their ultimate radicals between the individual fat-cells is very distinct in parts, where the lymphatics have been successfully injected. The root of the tongue of the child, rabbit, and dog have yielded the best preparations.

47. The mucous and serous glands in the root of the tongue, as well as the mucous glands in other parts of the oral cavity and pharynx, possess their own lymphatics. The tongue, after its lymphatics had been injected with Berlin blue, if hardened and the sections obtained from it stained in picro-carmine, yields very beautiful specimens. There can be no doubt whatever, that just as in the case of the sweat glands of the skin, so also in that of the mucous and serous glands, *the lymphatic vessels of the surrounding connective tissue anastomose with sinuous-like clefts surrounding more or less of the circumference of the gland-tubes or alveoli.* These lymph-clefts are situated between the membrana

propria of the alveoli and the thin connective-tissue lamellæ separating these latter.

In Fig. 13 these interalveolar lymph-clefts of a portion of a serous gland in the root of the tongue of the rabbit are well shown.

Both the ducts of the mucous and serous glands possess their own lymphatics; they appear as lymph-spaces extending along the duct for a longer or shorter distance, and in many cases either completely invaginating it, or only partially so, as in Figs. 14 and 15.

48. The relation of the lymphatics to the striped muscle-tissue, where it occurs in the mucous membrane itself, *e.g.* in the lip, palate, and tongue, is a very intimate one.

We have already mentioned the large lymphatic vessels present in the connective-tissue septa between the bundles of the muscle-tissue; they are connected into a plexus whose vessels run chiefly in a direction parallel to the long axis of the bundles. These vessels may be appropriately called the *interfascicular* lymphatics. Just as is the case with the fat-tissue, so also here, the ultimate lymphatic radicles are lymph-spaces between the individual muscle-fibres. Each muscle-fibre of a bundle is surrounded by a lymph-space (*see* Fig. 16.), and this latter is consequently contained between the sarcolemma of the muscle-fibre and the connective tissue separating the individual muscle-fibres, *i.e.* the endomysium. These lymph-spaces may justly be termed the *intrafascicular* lymph-clefts. The lymphatics, therefore, existing in the bundles of the striped muscle-fibres are identical with these of the nerve-bundles as described by Key and Retzius.

49. We have finally to mention the existence of continuous lymph-spaces in the sheath of the nerve-bundles and the capsule of the ganglia, existing in the connective tissue both between the lobules of the glands and between the bundles of the muscle-fibres in the root of the tongue. These spaces correspond to the perineural lymph-spaces described by Key and Retzius of the cerebro-spinal nerves in general.

The DEVELOPMENT *of the* WATER-GLANDS *in the* LEAF *of* SAXIFRAGA CRUSTATA. By WALTER GARDINER, Natural Science Scholar of Clare College, Cambridge. With Plate XXIII. (From the Botanical Laboratory, Cambridge.

THE leaves of *Saxifraga crustata*—oblong obovate, with a crenate margin—are arranged in a rosette on the axis, the internodes of which are hardly at all developed.

In the centre of each of the little crenate lobes with which the leaf is fringed is seen a pit or depression, covered with a white incrustation, which appears to spread from the pit, covering the entire lobe, and even extending over other parts of the leaf as well. The incrustation consists, apparently, of calcic carbonate.

If a transparent preparation be made of the leaf (fig. 1) it is seen that the peripheral terminations of the fibro-vascular bundles which ramify through the mesophyll present the appearance of a number of dilatations. Each such dilatation is placed immediately under the bottom of the depression of each lobe, and constitutes what is known as the water-gland.

The mature glands of this plant were first described by Unger,[1] but, so far as I am aware, their development has not been investigated, and it is the object of the following paper to deal with this latter question. Since the development of the leaf and its tissues are necessarily very closely connected with that of the glands, it seems best to consider first the development of the leaf as a whole, and then that of the glands.

Development of the leaf.—The *punctum vegetationis* of *Saxifraga crustata* is a hemispherical mass of meristematic cells, the external layer of which is differentiated as a dermatogen (fig. 2). The first rudiment of the leaf appears as a lateral outgrowth of this primary meristem, covered, of course, by dermatogen. At first the rudimentary leaf enlarges by rapid apical growth, and thus the first or terminal lobe is formed; with its formation apical growth ceases. The further elongation of the leaf takes place by the activity of a zone of meristematic cells at its base. Hence, its subsequent growth is basipetal, *i. e.* the youngest lobes are nearest the base. But although the growth of the leaf, as a

---

[1] Beit. 3, 'Physiol. d. Pflzn.' viii. For the literature of the subject, see De Bary. 'Vergl. Anatomie,' pp. 113, 389.

whole, is basipetal, each pair of lateral lobes arise and grow from the basal meristematic zone in an apical manner, so that their development is quite similar to that of the primary lobe (fig. 3). Whilst the apical growth of the terminal lobe is still proceeding a strand of cells is differentiated in the median line of the leaf; they are small and elongated, their nuclei are very conspicuous and elongated in form, their protoplasm is granular and stains deeply with hæmatoxylin, and their cell walls are very delicate. The differentiation proceeds from the base towards the apex, although it may be said to be almost simultaneous. This strand of cells is evidently a procambium.

At the same time that this differentiation has been going on in the leaf, strands of cells have become differentiated in the stem, just behind the *punctum vegetationis*, in such a way that the apex of one of these strands of the stem is on a level with the base of the leaf, and by a differentiation in the few intervening cells the procambium of the stem comes to be continuous with that of the leaf.

Like the primary lobe, the lateral lobes consist at first of meristem covered by a dermatogen. Here, too, the procambium becomes differentiated as strands of similar cells, exactly resembling those first described. The procambium of a lobe may be directly continuous with that of the main leaf-bundle, or may join with one or more strands from contiguous lobes before so doing. The differentiation of primary meristem into procambium, in fact, does not, as a rule, follow any definite course, but starts at particular centres and in various ways, thus giving rise to the reticulate distribution of the fibro-vascular bundles in the fully-developed leaf.

The next step is the conversion of the procambium into permanent tissue. A single row of spirally thickened protoxylem cells makes its appearance in the centre of the median procambium strand. The further conversion of the meristematic into permanent tissue takes place centrifugally.

There is a definite but slight increase in the number of the elements of the main fibro-vascular bundle from the apex towards the base. The zone of meristem cells at the base of the leaf is traversed by the primary fibro-vascular bundle, and as the lateral bundles are differentiated they become continuous at their proximal extremities with the peripheral portion of the primary fibro-vascular bundle, which is still of the nature of procambium. The fact that the conversion of procambium into permanent tissue takes place in a centrifugal manner allows of cell fusion so long as any procambium remains, and enables points of junction to be established. The last-formed fibro-vascular bundles become connected in the same manner with the main lateral branches.

An examination of the thickest part of the midrib, as seen in a transparent preparation of the leaf, enables one to make out very clearly the comparative ages of the constituent elements of which the whole bundle is composed. The vessels in which the turns of the spiral thickenings are approximated most closely can always be traced to the last-formed or youngest lobes, whereas those vessels in which they are most widely separated belong to the primary fibro-vascular bundle, and between these two extremes there is every possible gradation. This also proves conclusively that the vessels are capable of growth after the formation of the spiral thickening. The xylem and phloem are arranged on the collateral type, and the bundle is surrounded by a well-developed endodermis.

Development of the water-gland.—Having thus followed out the differentation of the tissues of the leaf, we may proceed to follow out the development of the water-glands. And since what is true of one gland is true of all, it will suffice to study the development of the first-formed or terminal gland. This can be most satisfactorily done by a series of longitudinal sections of the leaf in various stages of growth (fig 2).

As before stated the rudimentary leaf consists of undifferentiated meristematic cells covered by a dermatogen. When the differentiation of the procambium bundles has taken place, the meristematic ground-tissue cells at its apex divide, forming a mass of closely packed polygonal cells with delicate cell walls and very conspicuous nuclei. This is the first indication of what will hereafter be the gland. The further conversion of the primary meristem into gland-tissue takes place from the point of its first occurrence, towards the upper side of the leaf, finally ending beneath the dermatogen. At the inner extremity of the gland towards the fibro-vascular bundle, and at the lower third of its under surface (*i.e.* the surface corresponding to the lower side of the leaf) certain cells are conspicuous as becoming more elongated and fusiform than the rest and as subsequently presenting reticulate thickenings on their walls. They are joined end to end, and eventually become continuous with the spiral vessels of the fibro-vascular bundle. These cells present a series of intermediate forms between reticulate cells and spiral vessels. At the same time changes have been going on in the primary ground-tissue, for those cells immediately next the gland have become differentiated in a regular and definite manner so as to form a sheath of one or at most two layers of cells, entirely surrounding the gland with the exception of that portion of it which is covered by epidermis. This sheath is continuous with the endodermis of the bundles of the leaf.

Development of the water-pore.—One, two, or rarely three, of the cells of the dermatogen, where it is in immediate relation to the outer end of the gland, increase in size. Each is distinguished by a large and distinct nucleus, and each is the mother-cell of a water-pore (fig. 4).

The nucleus of this cell divides and a wall is formed, dividing the cell into two parts. The two cells thus produced separate from one another, leaving a hole or opening between them. This is the water-pore or water-stoma. It thus consists of two guard cells, which when fully developed contain chlorophyll. There is a difference in the time of formation of a water-pore as contrasted with that of the ordinary stomata. Thus the water-pores are fully developed before any trace can be detected of the ordinary stomata. This difference in point of time is exhibited in a still more striking manner by *Crassula coccinea*, in which the water-pores may be seen completely formed, when the cells of the epidermis are just beginning to divide to form stomata in the peculiar and complicated manner so characteristic of the Crassulacæ. And not only does the water-pore differ from the stoma in its time of development, but also in size, shape, and mode of development (figs. 8 and 9). It is slightly larger than the stoma. Its contour is rounded as compared with the elliptical shape of the ordinary stomata, the breadth being greater in the former than in the latter case. The actual pore or opening of the stoma is, however, larger than the water-pore.

The water-pore is thus produced by the simple division into two equal halves of a cell of the dermatogen, which has become larger than its neighbours. This is well shown by fig. 4, which is a surface view. But the ordinary stoma is formed in a more complicated manner. One of the cells of the dermatogen divides. Of the two cells thus produced one becomes the mother-cell of the stoma. It increases in size, and after previous division of the nucleus, a wall makes its appearance in nearly every case at right angles to the plane of the first division, and thus forms the two guard-cells of the stoma. The second cell of the two formed in the first instance becomes somewhat displaced in the course of subsequent growth. It never seems to grow as large as the adjoining epidermal cells, and may always be recognised in the fully developed condition as interfering with the arrangement of the four or five epidermal cells which would otherwise surround the stoma in a symmetrical manner (figs. 9 and 10).

Gland hairs.—At the same time that the mother-cells of the water-pores are making themselves apparent by their increased size, outgrowths have arisen on the surfaces of numerous dermatogen cells. These are the rudiments of the small knob-like hairs which are borne in the mature leaf on the sides of the depression, which, as has already been mentioned, marks the position of the gland. They are especially numerous on the outer side of the lobe, and when fully developed are almost perfectly spherical, with very thick and very highly refractive cell walls (fig. 5). Cell-division though not cell-growth of the water-gland tissue practically ceases with the division of the mother-cell of the water-pore; but in the leaf, cell-division still goes on, ceasing only with the formation of stomata. As a consequence of this, depressions are produced over the glands, the sides of which are fringed by the delicate highly refractive hairs.

Structure of the mature gland.—By far the larger portion of the gland-tissue is made up of polygonal cells, slightly longer than broad, closely fitting one to the other, with no intercellular spaces. The cell-walls are thin and the cells themselves are much smaller than those of the surrounding ground tissue. The protoplasm is very granular. At first it fills the cells entirely, with the nucleus in the centre. When mature a central vacuole makes its appearance, and the protoplasm then forms a thick layer, which closely invests the cell wall; the nucleus is either imbedded in the protoplasm, or it is suspended in the vacuole by strands of protoplasm, its position being no longer central but eccentric (see fig. 7). The whole gland is roughly pear-shaped, broad towards the surface of the leaf, tapering inwards towards the fibro-vascular bundle (figs. 5 and 6). It is invested by a sheath of cells continuous with the endodermis of the fibro-vascular bundle, except where it is in contact with the epidermis. The endodermis cells are long and cylindrical with moderately thick walls. They contain numerous chlorophyll granules. External to this is the mesophyll of the leaf. Free communication between the gland tissue and the external mesophyll is afforded by means of the one or more water-pores, the development and structure of which we have previously studied.

Mode of action of the gland.—Since the aim of this paper is to regard the gland solely from a histological standpoint, the mode of action will be only glanced at in a very cursory manner. In the daytime, when the temperature is high and transpiration very vigorous, the secretion of drops of water does not take place, and even if water be secreted by the gland cells it is evaporated as fast as formed. But at night, when the temperature falls, causing decrease of transpiration, the walls of the vessels and even the vessels themselves become gorged with water, and every facility for secretion by the gland is offered. Then drops exude through the water-pores, subsequently filling the pit. Now the portion of the lobe which intervenes between the gland and the edge of the leaf slopes downwards and outwards, and it is on this side especially on which the hairs surrounding the pit are most abundant. As a consequence, when an excess of water is secreted and the pit overflows, the water will tend to collect on the margin of each lobe. The water, charged as it is with carbonic acid, holds in solution a quantity of calcic carbonate. As the water evaporates and the carbonic acid gas comes off, the chalk is precipitated, and that which is deposited in the vicinity of the pit, tends to aggregate around the hairs and becomes thus firmly held and prevented from falling into the pit and stopping up the water-pores. In spite of this special provision, however, the older glands frequently become inefficient on account of being choked, the pit becoming completely filled with the very large concretions of chalk formed.

The whole phenomenon may be easily seen by placing a bell jar over a vigorous plant. Since the air becomes saturated with aqueous vapour, transpiration is reduced and large drops of water are secreted. On removing the jar, the water rapidly evaporates and a deposit of chalk is formed.

Glands in general.—As far as I have had an opportunity of examining water-glands, it appears that the gland of *Saxifraga crustata* is the most highly differentiated of all as regards the special provision made for the deposition of the calcic carbonate, the distinct differentiation of the gland tissue, the well-marked endodermis, the extreme granularity of the protoplasm, and the activity of function.

Next come the glands of the Crassulas, where there are neither hairs nor endodermis. The protoplasm is not nearly so granular, neither is the activity of function so great.

Between the glands of the Saxifragaceæ and Crassulaceæ and those occurring in the rest of the vegetable kingdom, there appears to be a great gap. We look in vain for the well-differentiated gland and the conspicuous difference between the gland-cells and the adjacent parenchyma. But the water-pore long preserves its individuality, although in many cases it seems probable that its function is taken on by an ordinary stoma, of which, indeed, even in its most differentiated form, it is but a modification.

It is interesting to note that the diminution of gland-tissue is often accompanied by an increase of fibro-vascular tissue, which appear to replace it. Comparing, for instance, *Saxifraga crustata* and *Crassula arborescens,* the reticulated cells at the base of the gland are much more numerous in the latter than in the former, and this seems to be a general tendency in the less highly differentiated glands.

As to the position of the glands, it seems to be a rule that they occur on the margin of the leaf. The exceptions to this statement are afforded by *Crassula cordifolia, Crassula arborescens,* and *Crassula portulacea,* where they occur distributed over the surface. In leaves with an entire margin, they usually occur only at the apex, *i. e.* the extremity of the main fibro-vascular bundles, as in species of *Azalea, Myosotis.* In leaves whose margins are cut or indented there may be a gland at the apex of each tooth, as in *Primula sinensis, Fuchsia globosa, Alchemilla vulgaris,* between two teeth, in the indentation, as in *Crassula spathulata,* or even on the sides of the tooth, as in *Senecio petasitis.*

In species of *Saxifraga* they appear to occur only on the upper side of the leaf. In *Crassula lactea* and *Crassula coccinea* on the upper and under margin, and in *Sedum Sieboldii* only on the under side.

The number of water-pores in relation with each gland varies very much. In *Saxifraga crustata* 2—3, *Bryophyllum calycinum* 5—6, *Crassula coccinea* 1—3, *Crassula spathulata* 15—20, *Crassula lactea* 15—18, *Primula sinensis* 1—2, *Hordeum vulgare* 1—2.

In by far the larger number of plants the activity and life of the water-gland and structures analogous to it appear to be co-existent with that of the leaf as a whole. In certain cases, however, *e.g.* species of *Musa, Richardia,* and grasses, in which the gland is borne at the apex of the leaf, the apex soon withers and the gland becomes destroyed. Thus, in very young barley plants (*Hordeum vulgare*) large drops of water may be seen hanging on the apex of each leaf, even though the temperature of the surrounding air be as much as 74° Fahr. As they grow older the secretion stops, and later on each leaf-apex withers up and dies.

The DEVELOPMENT *of the* SPERMATOZOA. Part II, HELIX *and* RANA. By J. E. BLOMFIELD, B.A., Oxon. (With Plates XXIV and XXV.)

THE following observations on the development of the spermatozoa in Rana and Helix were made as sequels to a paper published in this Journal "On the Development of the Spermatozoa in the Earth-worm." The observations have extended over more than a year in each case, and by the kindness of Prof. Lankester I have been allowed to work in the Zoological Laboratory of University College, to whom, for this kindness and for his advice, my best thanks are due.

A. **Helix.**—I was led to make these observations in consequence of finding that in the development of the spermatozoa of Lumbricus a portion of the protoplasm was left behind to serve as a support for the developing spermatozoa. I looked to see if this "blastophoral cell," perhaps of theoretical interest, occurred in other forms, and taking, by chance, Helix, I found that the spermatozoa were held together at their heads in bundles by darkly granular cells, which, similar in function to the blastophoral corpuscles in the worm, differed from them in the possession of a nucleus (fig. 23).

To determine the relation of this cell to the spermatozoa it was necessary to work through its development, and I continued my observations on Helix, now and then making preparations from other Gasteropoda in which the process seemed to be carried on in a similar way.

If the ovotestis of Helix be opened about the end of summer, in September, and the contents examined, the first and last stages of the series will be seen, the first consisting of spherical cells with relatively large nuclei, in which a well-marked intranuclear network is generally visible; and the last, of mature, or nearly mature, spermatozoa, united by their heads, in most cases, to an irregularly shaped cell, which is noticeable for its dark blue-black granules after treatment with osmic acid.

I say in most cases, because this cell is not present in every bundle, but its absence, I believe, may be accounted for by its having fallen off during the slight teasing necessary to spread the contents of the testis on the slide, or by the fact that the bundles of spermatozoa are cast off, leaving the cell behind.

The mature spermatozoa of Helix exhibit a small pear-shaped head, which stains readily with picrocarmine, and a long, slightly flattened tail. Besides the very early stage of the nucleated spermatospore,[1] the first commencement of the formation of the polyplast is evident in cells with two or three nuclei and a corresponding slight segmentation of the enveloping protoplasm. In this state the testis is found during the winter when the animal is torpid, and no further growth takes place till the following spring (Plate XXIV, figs. 1—6).

If the contents of the ovotestis be examined in the spring, in the months of May or June, the spermatospores will be found to have advanced, most of them, to the stage of polyplasts of the ordinary mulberry-like form, consisting of pear-shaped spermatoblasts, one of which can be distinguished from the rest by the granular nature of the plasma around it and by the larger size of the nucleus (fig. 9 *b, c*). This nucleus also, under the action of picrocarmine, takes up a darker hue than that of the surrounding spermatoblasts. It is not so large as the nucleus of the original cell (spermatospore).

This cell is always on the side of the polyplast, next to the wall of the ampulla of the gland, and it is the first appearance of the blastophoral cell, which, from the time of its appearance, undergoes no further division, but remains inactive while the other spermatoblasts continue their process of multiplication more or less supported by it.

During the process of formation of the spermatoblasts the nuclear division appears to take place with no mathematical regularity. The nuclei do not divide at the same time, so that an uneven number of spermatoblasts is as common as an even. In the early stages the cell with three nuclei is as common as that with two or four. It is possible that one of the nuclei of the three-celled form ceases to undergo further change and remains as the blastophoral cell, while the others continue their development; but, as there are no granules or other characteristic marks to distinguish this body before the stage of eight or ten spermatoblasts, it is impossible to say whether this is the case or not.

To see the remainder of the process of the development of the spermatozoa, the contents of a generative gland must be taken about the beginning of August. This appears to consist, as in the earth-worm, in an elongation of the soft

---

[1] With reference to these and other terms, see my former paper, this Journal, January, 1880, and also the list of terms at the end of the present paper.

viscid protoplasm of the spermatoblast; but the nucleus does not undergo any corresponding increase in length, it is only reduced in size as division proceeds, and finally assumes its mature pyriform shape.

Before any sign of commencing elongation of the plasma is visible, the nucleus appears at the pole of the spermatoblast, next to the blastophoral cell; and soon after this, from the opposite pole, there may be seen a fine whip of protoplasm, which consists of a proximal short, stiffer portion, terminating in a slight knob, and a distal, very fine lashlike part, which requires a good light to make its presence recognisable.

This filament is visible at the extremity of the spermatoblast for some time, but appears to be absent in the mature spermatozoon. It seems as if it served as a guide to the elongating plasma, and became itself swallowed up in the process as it nears completion.

This elongation of the plasma seems to be a kind of flowing down of the semi-viscid substance, for sometimes the greater part of it is found as an irregular or roughly spherical drop collected at the distal end of the spermatoblast; at other times it elongates, as a whole, with various swellings in droplike beads at the distal end, giving it a monilliform appearance.

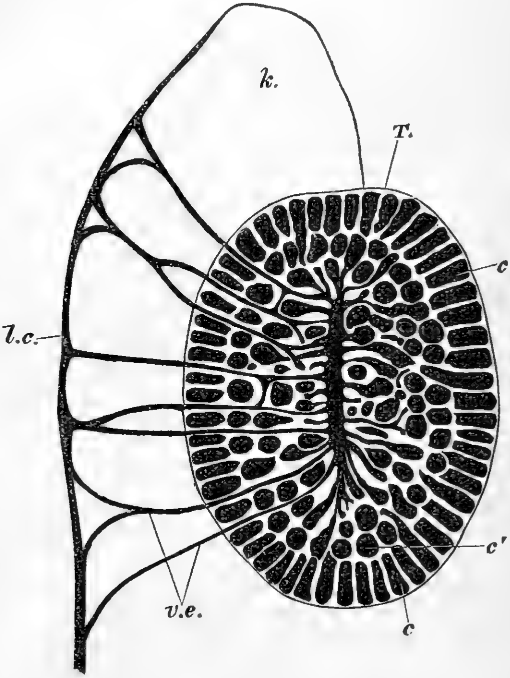
Division of the nucleus appears to continue after the external plasma has commenced to "drop," as the mass is not always divided below in correspondence with the nuclear heads; it is not uncommon to find two or three heads connected with a common mass of protoplasm, from the distal end of which there is commonly to be seen a corresponding number of lashes.

As regards the fate of the blastophoral cell after the spermatozoa have left it, I have no observations to offer; but if one may judge by what occurs in other divisions of the animal kingdom, it is extremely probable that it atrophies.

Rana.—*Structure of the testis and arrangement of the vasa efferentia.*—Though the frog is such a common animal, and its anatomy so perpetually studied, there is not, so far as I am aware, a figure or concise description of the testis and its efferent ducts in this language. I therefore append a diagrammatic woodcut of the right testis (T.) and a portion of the right kidney (*k.*) with the following description:

Spengel, in his "Urogenital System of Amphibia," published in 'Semper's Arbeiten,' vol. iii, gives an account of the testis and its ducts as found in Rana, and from this my account is drawn, confirmed by my own observation as far as that has gone.

The testis consists of a series of short tubes, which for want of a better name I have called Crypts (c.), which will be seen in a horizontal or transverse section to be arranged radially around the circumference of the gland. In the centre is an irregular sinus, into which these crypts open, but not in a radial direction; before doing so, there is a considerable winding and twisting of each crypt (c.′). From this sinus, with various communications and branchings which constitute the intratesticular network of Spengel, the vasa efferentia (*v. e.*) run through the parenchyma of the gland to emerge at the mediad border slightly on the posterior surface. They unite together on the way, and sometimes give off short blind processes. This is the extratesticular network, all the branches of which unite in a canal which is found on the mediad border of the kidney (*l. c.*). This is Spengel's "lang canal," which runs along the mediad posterior, or dorsal surface of the kidney.



When isolated this canal presents a varicose appearance, and from each swelling may be seen a short tube running on the dorsal surface of the kidney towards the ureter, but it soon becomes lost in the substance of the kidney. The time at which to observe these canals and the whole course of the semen is in the spawning time, when by squeezing the gland the vessels become injected with spermatozoa and are conspicuous by their pure white colour.

The urinary tubules into which these canals open, were determined by Spengel to be the collecting tubes or last part which runs transversely across to join the ureter, and so convey the semen to the exterior.

There was no difference discernible in a tube which contained spermatozoa from that which had none.

Development of the Spermatozoa.—The development of the spermatozoa of Rana will be most conveniently described by taking the structure and contents of the testis at four different periods of the year, and from these compiling a complete history of the changes undergone.

If the testis be examined any time from about the end of August or the beginning of September till the spawning time in the spring, it will be found to be full of bundles of spermatozoa arranged radially around the circumference of a testicular crypt (Plate XXV, fig. 1).

If the contents of a testis be received and teased slightly in a drop of salt solution, and then exposed to the vapour of osmic acid and stained, these bundles will be seen to have taken up the staining fluid for the greater part of their length, which represents the nuclear part of the spermatozoon, while at one extremity of the bundle they taper away, generally ending in a slight knot, stained yellow by the picrocarmine, which represents the tail, and is at this stage, when observed fresh, in a state of vibration, like that of the mature spermatozoon. At the other extremity of the bundle is, in the majority of specimens, a mass of a non-stained substance, not granular, or very slightly so, which contains a spherical or ovoidal nucleus, and it was this body which, from its similarity to the blastophoral cell of Lumbricus and Helix, first induced me to follow in detail the history of the development of the spermatozoa (Plate XXV, figs. 2—6).

This body is not found attached to every bundle, but, as the subsequent history will show, it is extremely natural that it should not always be present, as it is left behind when the spermatozoa are shed, and in many cases it would be detached during the slight teasing which is necessary to separate the bundles from each other; the adhesion decreases as age increases.

The bundles of spermatozoa do not lie against the true wall of the testicular crypt; at all times there is to be made out quadrate or roughly spherical cells which represent the testicular epithelium, and more particularly is this the case if the testis be taken about the month of December, when the cells have begun to grow slightly and form a very distinct epithelium between the bundles and crypt wall. (This is shown in fig. 1.) Between these epithelial cells are others of a supporting nature, generally semilunar in shape and darkly stained, which are the interstitial cells. They are particularly well seen when the epithelium is looked down on in a section which has passed longitudinally along a crypt in the right plane, or in sections of the testis of a frog which is not yet sexually mature. Fig. 1 *a* shows two of these cells from a young testis.

The next stage is to be found in the early summer after the spawning time is over, when the testis is empty of the bundles of the spermatozoa, and consequently rather shrunk in size.

In a teased preparation of this stage are found a few unbroken bundles and many free spermatozoa; but besides these there are peculiar spindle-shaped or irregular cells, which have no definite nucleus, but what seems to be the remains of a broken up nucleus, viz. two, three, or more spherical stained spots, varying in size and in position. Frequently the surface of the cell is marked with striæ in the longitudinal direction, and these are due, I believe, to the adhesion of the spermatozoa round this body, representing the blastophoral cell, which as they slide off elongate and mark the cell which supported them (figs. 9—21).

Another very noticeable feature about these cells is the number of vacuoles.

These cells are, I believe, the breaking down blastophoral corpuscles, which, after being drawn out by the sliding off of the bundles, and having served their purpose, are thrown off and undergo degeneration.

In a section of the testis in the lumen of the crypt, intermingled with free spermatozoa and breaking up bundles, are seen the spindle-shaped cells just described, and on the periphery of the crypt the testicular epithelium, as seen in fig. 1, *spp.*, where it is beginning to undergo changes of growth; cells are found with several nuclei, often eight, and in many places the epithelium does not consist of a single layer of cells but two, formed by the transverse division of a cell.

In places the degenerated blastophoral corpuscles are seen projecting radially towards the lumen.

The next stage for description is that of the sperm-polyplasts, which is reached about the end of July or the beginning of August. The testis at this stage is considerably swollen and very vascular, showing that growth is rapidly going on. The minute blood-vessels are so full of blood that if the surface be examined under a simple lens the terminations of the crypts are marked out as hexagonal areæ by the blood-vessels. In a section (fig. 22) it is seen that the crypts contain large multinuclear masses which almost obliterate the lumen of the tube. They are in various states of progress, the more mature forms projecting as pyriform masses into the crypt, and they are more or less held together by the interstitial cells, so that in making a teased preparation it is exceedingly difficult to free them from one another without breaking them up.

Another important fact about them is that they are hollow vesicles formed of a single layer of cells, and it is towards the centre of the cavity that the spermatoblasts will elongate to form the spermatozoa. The elongation in this case is different to that in Helix, it is centripetal, while that in Helix is centrifugal. At first it seemed a puzzle how from these hollow vesicles the spermatozoa could come to be arranged in bundles, each bundle connected with a nucleus and cell, for at first there was no evidence of a nucleus.

But on examining the sperm-polyplasts in fresh salt solution, and staining with magenta or picrocarmine, keeping the mass under observation while the staining is going on, certain nuclei were seen to take up the staining fluid more rapidly than the rest, two or three to each polyplast, before the others showed more than a faint tinge, while the intermediate mass of plasma swelled up with the water of the solution, and became mapped into areæ corresponding to the spermatoblasts (fig. 23). On more careful examination it was found that these nuclei were superficial to the rest, and were surrounded by a granular protoplasm; and in their general character and colouring seemed to show their connection with the blastophoral nucleus, which is described with the next stage.

In sections stained with hæmatoxylin an interesting difference of the nuclei of different polyplasts is observed, represented in figs. 27, 28, 29. This appears to be due to changes in the nuclear network, which seems to break up into an irregular mass before the nucleus forms the head of the spermatozoon. The interstitial cells still hold the masses together. About the middle of August the next stage is reached, the hollow vesicle begins to contain developing spermatozoa, which appear to be formed by elongation of the nucleus to form the head and of the protoplasm to form the tail; and when this has progressed some way, so that the head is about half its future length, *the vesicle splits and the spermatoblasts fall back,* several in connection with one of the superficial nuclei referred to before, on to the wall of the crypt, and assume a radial position. After this the only further change which takes place is the growth in length of the nuclear head till it has attained the size of the mature spermatozoon.

In one section (fig. 31), taken at the right period, a crypt will be found to contain vesicles not yet split, others in the act of splitting (figs. 36, 37, 38), and the spermatozoa arranged more or less radially with reference to a blastophoral cell, and others again which are in the act of being applied to the testicular wall. In this process of sinking back on the parietes of the crypt it seems that the interstitial cells play an important part, from their being connected with the fibres forming the wall as guides to determine the application of the bundle to the wall.

Recapitulation.—The history of spermatogenesis in the frog is then as follows:—Starting with one of the spermatospores which line the testicular crypts and form the testis epithelium, we find that after spawning is over it commences to grow in preparation for the next year's stock by division of its nucleus. This process continues until there is formed a hollow body, spherical when freed from the pressure of neighbouring polyplasts, which is the sperm polyplast. The exact mode of the formation of this hollow sphere I am not able to state. Each of the spermatoblasts of this polyplast becomes a spermatozoon, the tail is formed from the plasma by elongation towards the centre of the sphere and the head in a similar or perhaps more complicated fashion. In forming this body all the nuclei are not concerned; certain of them are left behind, superficial to the rest, and by proper means can be brought distinctly into view. About the end of the summer the spermatoblasts, which are very little different from mature spermatozoa, arrange themselves in bundles round one of these more superficial nuclei, and become with them applied to the wall of the testicular crypt, forming a series of bundles arranged radially round the wall of the crypt, with their tails projecting into the lumen, supported by their heads on the superficial cells, which have now become blastophoral corpuscles.

When the time comes for their being shed the bundles are thrown off and break up, leaving the blastophoral cells behind, which afterwards atrophy by degeneration and breaking up of the nucleus, and are thrown away.

General Considerations and previous Literature relative to Spermatogenesis.—The point on which I wish to lay stress in the previous observations is the existence of a blastophoral cell, which, from the cases I have taken by chance for study, and the figures in the papers of other writers, seems to be of very general distribution. The question naturally arises, What is its morphological significance?

When I found the body in the earth-worm Professor Lankester told me of a suggestion which had been made to him by Professor Van Beneden, of Liège, viz. that it corresponded to the ovum whilst the spermatoblasts correspond to the polar vesicles or directive corpuscles which are thrown off from the ovum previous to fertilization. These bodies, of very general occurrence, are hard to explain. Balfour (this Journal, vol. xviii, p. 123) says, "I would suggest that in the formation of these polar cells, part of the constituents of the germinal vesicle which are requisite for its functions as a complete and independent nucleus, are removed to make room for the supply of the necessary parts to it again by the spermatic nucleus," and it may be that as the polar cells represent the elimination of a male element from a cell whose future destiny is female, so the formation of the blastophoral cell represents the same ridding of the female element from a cell whose destiny is male.

After the publication of a paper "On the Development of the Spermatozoa of Lumbricus" (this Journal, April, 1880), I received a paper from Mr. Minot, published in the 'American Naturalist' for February, 1880, in which, from theoretical considerations, he arrived at the conclusion that some such structure as the body alluded to above must be found in the development of the spermatozoa, and records Semper's "Observations on the Development of Spermatozoa in Elasmobranchs" in support of his view, in which Semper describes a part of the original cell which is left behind as "Mutterkern," though it is evidently the same as our blastophoral cell.

The considerations offered above are, of course, pure speculations, and it is quite possible that these blastophoral cells have no morphological significance, but function as bodies for the support and nutrition of the young spermatozoa, and, having performed this duty, undergo degeneration and disappear.

We have seen that, in Rana, from one original cell more than one blastophoral cell is developed, and this seems to be paralleled by what takes place in Insects, as fig. 25, Plate XXIV, from Dytiscus, and figs. 26 and 27 from Pieris, will show. The exact origin of these cells in Insects I have not determined, but at the first glance they seem comparable to a large extent to the " superficial nuclei " of Rana.

The literature on the development of the spermatozoa is very copious, more particularly that which treats of the development in Mammals, as may be seen by reference to the papers of von la Valette St. George in the 'Archiv für Mikr. Anatomie.'

I do not propose to consider every individual publication, but to mention those who have put forward plans of spermatogenesis capable of application to the whole, or at any rate large, divisions of the animal kingdom; but before doing this I may briefly state what my own views on the subject are.

A cell whose future destiny is male (spermatospore) commences to undergo changes which fit it for its new function, and by a process of multiplication gives rise to many fertilising elements. The first steps are division and multiplication of the nucleus, and a corresponding constriction of the surrounding plasma, till a multicellular mulberry-like mass (sperm-polyplast) is produced, which may be solid or hollow, consisting of young spermatozoa or spermatoblasts. During this process some portion or portions of the original cell cease to undergo further change and remain behind to support and nourish the developing spermatozoa (blastophoral cell).

The nucleus of the spermatoblast forms the head of the spermatozoon, and the tail is formed by the centripetal or centrifugal elongation of the plasma. When fully formed the spermatozoa are supported on the blastophoral cells till required and then shed off, leaving their supports to atrophy and decay.

How far this view holds good for the Mammalia I am not able to say precisely, but the abundance of figures confirming the above account in papers treating on this subject, justifies the idea (compare the account below of Meyer's paper) that spermatogenesis will be found to be essentially the same in that class.

Many of the figures show the blastophoral cell under various names, and represent stages which seem in most respects comparable to those found in the spermatogenesis of Helix.

Kölliker ('Zeit. für Wissen. Zool.,' Bd. vii, p. 201) seems to have been the first to give a plan of spermatogenesis. He gives figures of developmental stages from the bull, pigeon, frog, and carp; at the same time noting that the account holds good for all animals.

He believed that the spermatozoa were developed by a direct metamorphosis of the nuclei of the cells of the testis. This change takes place in what he calls "Bläschen," which are probably nothing more, as Meyer suggests, than polyplasts modified by the fluid (Müller's) with which he treated them. He conceived that the tail as well as the body originated from the nucleus.

He very shortly describes the process in the frog, but with the exception of the figures, which show well the bundles of spermatozoa united by the blastophoral cells, there is little agreement between my account and his.

V. la Valette St. George has, in his fifth communication, "On the Development of the Spermatozoa," to Max Schultze's 'Archiv,' Bd. xv, p. 261, given an account of spermatogenesis in general.

The paper commences with an exhaustive *résumè* of previous observations and papers published in connection with the subject, and then gives an account of the process as he has observed it in many Mammalia—bull, ram, stallion, rabbit, &c., and ends with a summary which embodies his ideas of spermatogenesis based on his former papers. He recognises in the testis tubule two kinds of cells. Of the first, he says, "Peculiarly like young ovarian cells they are destined to multiply as Ursamenzellen or Spermatogonia; in a similar manner by division and by transformation of their descendants (the Spermatocytes) they are destined to give rise to the spermatosomes (Samenkörperchen). They produce a mass of cells which either by an arrangement of the peripheral cells develop a special cover—Keimkugeln, Samenkugeln, Spermatocysten (Insects and Amphibia), or remains coverless—Samenknospen, Samensprossen, Spermatogemmæ, whilst the protoplasm which belongs to each cell is more or less segmented. In many cases one of the cells resulting from the division or its nucleus is preserved at the foot of the Spermatogemmæ."

"The second kind of cell which I call follicle cells are bound together into a tissue which, while it embeds the Spermatogonia, also covers and protects the Speratomgemmæ in their multiplication by division."

From this it will be seen that he recognises the cell which is formed at the base of the Spermatogemmæ as being part of the original cell which has been left behind in the growth and multiplication of the others, but he does not appear to recognise the similarity between this cell and those which are found in the Spermatogemmæ or Samenkugeln of the frog which is formed in the same way, and has a similar function.

In Semper's "Monograph on the Urogenital System in Elasmobranchs" ('Semper's Arbeiten,' Bd. ii), he gives an account of the development of the spermatozoa. The section of a mature ampulla, with its bundles of spermatozoa, is very like that of the frog; each bundle is connected with a nucleated mass which rests on the wall of the ampulla, and which he calls, from its protective function, "Deckzelle," though he recognises it as being the remains of the Mutterzelle. He traces the fate of these bodies after the spermatozoa are shed, and finds that they undergo fatty degeneration, the nuclei fall together and are only held together by a granular detritus, while the ampulla itself collapses. This he compares to the formation of the corpus luteum in the ovary. In Elasmobranchs there is a continuous development of spermatozoa in the testis from within out, so that we get degenerated ampullæ, full ampullæ, ampullæ with spermatozoa developing, and the formation of ampullæ in the same testis, not coming as in Rana in successive crops from the same ampulla. The spermatozoa do not develop as in Rana from vesicles (hollow polyplasts), but on a plan more like that of the snail. He calls the young immature spermatozoa spermatoblasts, and says that he was unable to make out the origin of the tail.

Klein, in his 'Atlas of Histology,' gives an account of spermatogenesis based on researches on man, rabbit, mouse, &c. In the contents of the seminal tubules he recognises two kinds of cells, the inner and outer seminal cells. The latter present two kinds according to the state of the nucleus; in one kind the nucleus is finely granular, in the other it is devoid of a limiting membrane, and has rods or filaments twisted in many directions in its interior. Nearer the lumen of the tubule are the inner seminal cells, which are seldom limited to one or two layers. They do not directly touch each other, but are joined by an interstitial substance. These cells are polyhedral from mutual compression, and their nuclei are similar to the second type of the outer seminal cells, that is, they contain rods or filaments variously arranged. Their nuclear rods vary in shape and arrangement, which he considers indicative of division and multiplication. Nearer still to the lumen of the tube the cells are loosely connected, and they may be seen dividing each into two daughter nuclei. These small cells, the daughter cells, undergo changes leading to the formation of the spermatozoa, and for these he uses Sertoli's term spermatoblasts. The first change is seen in the nucleus, which becomes finely granular and assumes a membrane. This kind of nucleus he calls the resting nucleus. At the same time it moves to one pole of the cell, which is itself elongated, and constitutes a "granular mass," separated from the nucleus by a "clear bag." When the young spermatozoa are in this state they assume a definite arrangement, and become placed in fan-shaped groups along the tubule, with the handle of the fan sunk among the seminal cells; their further progress consists in elongation. He then goes on to mention the views of Ebner and Neumann, who consider that the groups of spermatozoa are formed in a single cell which consists of a base, which has (Ebner) or has not (Neumann) a nucleus next the membrana propria, a peduncle, and a broad mass at the end of the peduncle in which the spermatozoa are produced.

He regards the head, as well as the "middle piece," as formed from the nucleus.

In Rollett's 'Untersuchungen aus dem Institut für Phys. und Hist.' for 1871, there is a paper by v. Ebner, in which he gives an account of the development of the spermatozoa in the rat and mouse, referring to other mammals, and a series of drawings illustrating that process in the first-named animal.

He divides the processes into eight stages, which I will not enumerate, but try to epitomize his account. The fundamental idea in which he differs from other writers is in the existence of a "Keimnetz," in which the spermatozoa are developed. This consists of a welded mass of cells next the tunica propria of the tubule in which two kinds of nuclei are discerned, the one pale, nucleolated, with distinct outline; the other granular, dark, with an indistinct outline. On taking a superficial view of this layer it has the appearance of a network. From it processes project towards the lumen of the tubule which, when first formed, are nothing but plasma; then nuclear hardenings commence at its inner extremity, which soon unmistakably assume the appearance of spermatozoa heads, in this case being pointed at one end, which end is directed towards the periphery. The tails are formed from the plasma. At the base of this process, which he calls a spermatoblast, there is often a nucleus, which can be distinguished by its slightly irregular shape, and from its being elongated in the direction of the spermatoblast, but he gives no account of the origin or fate of this nucleus.

The spaces between the processes of the "Keimnetz" are filled up with cells of various sizes and in various states of multiplication. These he regards not as in any way concerned with the formation of the spermatozoa, but only present to assist the growth by supply of nutritive material to the young spermatozoa; and he traces their origin to their having wandered from the lymph spaces of the testis. As regards their fate, he believes that they form the coagulated particles, or "Eiweisskugeln," which are to be found in the lumen of the tubule of a ripe testis. Sometimes these bodies contain crumpled-up semilunar nuclei.

It is obvious from this that part of the "Keimnetz" corresponds to my testicular epithelium, the outer seminal cells of Klein, and the "Ursamenzellen" of other writers; moreover, the two kinds of cells which Klein mentions as being in an active or passive condition, according to the condition of the nucleus, are here clearly indicated.

As regards his spermatoblast, he says that, as far as his observations went, it arose without any division of a nucleus, but in this I believe he will be found to be mistaken. And it is my opinion that the nucleus at the base, and the nuclei, as he says, newly formed at the other end, are due to a division of one cell.

As regards the cells which lie in the meshes of the network, and which, he thinks, are only of use for the nourishment of the spermatozoa, the opinion of almost every other writer is that they are concerned in the formation of the spermatozoa and represent various stages of the process. These are the inner seminal cells of Klein, the Spermatogemme and Samenknospen of v. la Valette St. George, and the idea that they form the "Eiweisskugeln," and similar bodies found in the lumina of the tubules, is untenable.

I think these bodies will be found to owe their origin to the breaking up of blastophoral cells which, as in the frog, after the spermatozoa are ripe and have dropped from their supports, themselves are thrown off and undergo fatty degeneration.

It may be mentioned that he considers the middle piece to arise from the same consolidation of plasma as the nucleus.

In the 'Archiv für Mikr. Anatomie,' vol. xviii, p. 233, Prof. Flemming has a paper in continuation of his researches on cells and nuclei, in which he gives an account of the formation of the spermatozoa in salamander. The interesting point for our purposes is that which refers to the formation of the head of the spermatozoon, not, as has been supposed since the observations subsequent to Kölliker's paper appeared, from the whole nucleus, but from that part of it which he calls the "chromatin." This collects into a spirally folded mass, which becomes more closely folded as it increases in length, till the whole head may be seen coiled up in the nucleus. The origin of the tail he traces to the cell plasma. These observations agree with those of Kölliker if we substitute spermatozoon head for the whole spermatozoon.

I have not worked with sufficient detail to determine the exact origin of the spermatozoon head, but I must confess that my ideas agreed with those of previous observers that the whole nucleus took part in the formation of this structure, though, since the publication of Flemming's paper, I should not like to say that it was so, and can only hope to make some observations on its exact origin in the Mammalia. In the earth-worm it is not possible to distinguish in the nucleus "chromatin" from other constituents, and hence the head of the spermatozoon cannot be traced in this case to such an element.

The most recent utterance on this subject is by Meyer, in the 'Memoirs of the St. Petersburg Academy,' tome xxxii, 1880, and as it contains many figures confirmatory of my views, I purpose to give a slightly more lengthy account of it.

His observations were made on the dog, cat, rat, mouse, bear, rabbit, and guinea pig. In a testicular tubule next the wall are found the Ursamenzellen, which consist of two kinds of cells, one small, often darkly granular, which he calls follicle cells, the other larger, with large nucleus, each containing a nucleolus, the nucleus itself being surrounded by a clear plasma.

These would be the outer seminal cells of Klein, or what I have called the testicular epithelium and its interstitial cells.

The first change which an Ursamenzelle undergoes is division. This takes place in a tangential direction, and the cell which is directed towards the lumen of the tube takes on the characters of a spermatocyte, which consists in the nucleus and plasma becoming darker and more granular (cf. his Taf. ii, fig. 94). The spermatocytes thus formed by division from the Ursamenzellen gradually increase in size and assume an oval form. The next stage consists in a multiplication of this nucleus, apparently by its breaking up and a reappearance of the pieces in two or three places, producing a corresponding number of nuclei. By a repetition of this process the Spermatogemme is produced (figs. 9—29, Taf. i). These bodies he regards as fundamentally a collection of several cells, and in support of this view adduces the fact that a single spermatocyte may run its course to a mature spermatozoon without presenting this form, never possessing more than one nucleus. These bodies have a radial arrangement in the testicular tubule and an elongated shape from the pressure of the surrounding cells, but they are generally spherical when isolated and floating free in a liquid.

The last stage is the change of the nucleus to form the head and the division of the plasma of the Spermatogemme into tongue-like processes which form the tail. The first change varies according to the animal examined, that is, to the shape of the spermatozoon head. He regards the middle piece as derived not from the nucleus, but from the plasma. The rest of the plasma which has taken no part in the formation of the spermatozoa remains behind for a time to support them, but finally undergoes a kind of fatty degeneration.

He recognises the existence of v. Ebner's spermatoblasts under the name of "Samenähren," and says that "intermediate forms may be seen from the spermatocyte to the mature 'Samenähren' connected with the Ursamenzelle, to which they owe their origin, by a process of plasma (figs. 35, 36, 38, 40)."

He disagrees with v. la Valette St. George in thinking that the interstitial cells do not multiply at the same time as the Ursamenzellen, and form layers round the spermatocytes and Samensprossen as the last named observer does.

From this account it will appear that his Samenähren are the bases of v. Ebner's spermatoblasts, and correspond to the cell which is left at the foot of the Spermatogemme described by v. la Valette St. George.

The foregoing papers of which I have given an account are few out of many, but they embody the results of those which I have not mentioned, and taking into consideration the facts and drawings given in them it seems possible to reconcile to a large extent the different accounts of spermatogenesis in mammals in the following short *résumé*, omitting as far as possible the particular terms used by each author. The wall of the testis is lined on the inside with a testicular epithelium, in which the true testis cells are supported by interstitial cells. One of these testis cells divides tangentially, giving rise to two cells which are held together by a common plasma; the cell next the lumen of the tube grows and the nucleus multiplies, giving rise to several (8—12, according to v. Ebner) nuclei embedded in a plasma; or, according to Meyer, the cell and its nucleus may proceed to form a single spermatozoon without multiplication. These nuclei form the heads of the spermatozoa, and the plasma the tails. When nearly mature the young spermatozoa are supported on the plasma, but when they are ripe they are cast off from it and enter the lumen of the tube. After the spermatozoa have left it this body itself is thrown off with its nucleus, which has remained at its base, and undergoes fatty degeneration, being found in the lumen of the tube in this state.

For convenience I append a list of synonyms which are used by various authors in describing the process of spermatogenesis.

1. Spermatospore = Spermatogone (St. George, Meyer).
1*a*. Spermatocyte, intermediate form (St. George).
2. Sperm-polyblast = Bläschen (Kölliker), consisting of the following:
3. Spermatoblasts = Spermatoblasts of Semper, Klein, Sertoli, or Samensprossen or Spermatogemme (St. George, Meyer).
4. Mature spermatozoa, united into bundles by blastophoral cells (spermatoblasts, v. Ebner). Samenähren (Meyer).
5. Spermblastophor = Deckzellen of Semper.
6. Testicular epithelium = Ursamenzellen (St. George).
7. Interstitial cells = Follikelzellen (St. George, Meyer).

On the EARLY DEVELOPMENT *of the* ANTERIOR PART *of the* WOLFFIAN DUCT *and* BODY *in the* CHICK, *together with some* REMARKS *on the* EXCRETORY SYSTEM *of the* VERTEBRATA.

By ADAM SEDGWICK, M.A., Fellow of Trinity College, Cambridge.

With Plate XXVI.

THE following paper is divided into two parts. The first part contains an account of observations on the development of the Wolffian duct and anterior Wolffian tubules in the chick, being supplementary to my paper on the "Kidney of the Chick."[1] The second part is devoted to a discussion of the vertebrate excretory system in general.

I. *Early Development of the Wolffian Duct and Anterior Wolffian Tubules in the Chick.*

The first trace of the Wolffian duct is visible in an embryo with eight protovertebræ as a slight projection from the intermediate cell mass towards the epiblast in the region of the 7th and 8th protovertebræ. The projection also extends back behind the region of the protovertebræ for a short distance. In a chick with nine or ten protovertebræ a similar condition is found, *i.e.* a projection from the intermediate cell mass towards the epiblast in the region of the 7th, 8th, 9th, and 10th protovertebræ, and for a short distance behind the region of the protovertebræ.

In a chick with ten protovertebræ the projection is beginning to show signs of separation from the intermediate cell mass at certain points. The appearance presented by the rudiment of the Wolffian duct in the 10th segment of a chick with ten segments is shown in fig. 1.

In a chick with eleven protovertebræ the rudiment of the Wolffian duct is still present as a projection from the intermediate cell mass in the region of the 7th, 8th, 9th, 10th, and 11th protovertebræ; but behind the region of the protovertebræ it has grown back for a short distance between the epiblast and mesoblast as an irregular cord of cells not connected to the peritoneal epithelium. A partial separation of the Wolffian duct from the intermediate cell mass is now effected in the region of the 7th to the 10th protovertebræ. This separation is not, however, complete; but *the Wolffian duct remains connected*

---

[1] "Development of the Kidney in its relation to the Wolffian Body in the Chick," 'Quart. Journ. Mic. Sci.,' vol. xx.

to the peritoneal epithelium at certain intervals by short cords of cells.

In a chick with twelve protovertebræ the separation of the Wolffian duct from the intermediate cell mass in the region of the 7th to the 11th protovertebræ inclusive is as complete as it ever will be, *i.e.* it has separated for the greater part of its length, but remains attached to the peritoneal epithelium at certain points, by cords of cells (fig. 2) derived from the cells of the intermediate cell mass connecting the rudiment of the Wolffian duct with the peritoneal epithelium. These cords of cells are the commencing Wolffian tubules of the anterior part of the Wolffian body, and are more numerous than the segments in which they are placed. Behind the region of the protovertebræ in a chick of this age (twelve protovertebræ), the Wolffian duct has grown back as an irregular cord of cells (fig. 6), independent of the intermediate cell mass, for a short distance, thus repeating the feature of the last and succeeding stages in this particular. In the region of the last (12th) protovertebra, however, the cord of cells constituting the Wolffian duct at this stage is now continuous with the intermediate cell mass at certain intervals. Comparing the sections through the 12th segment of this stage with those just behind the 11th protovertebra of the previous stage, it is seen that the Wolffian duct has enlarged, and by a downgrowth of cells from it, with which probably is connected an upgrowth from the intermediate cell mass, has become in certain places connected with the intermediate cell mass. These secondary connections constitute the commencing tubules of this part of the Wolffian body.

In a chick with thirteen protovertebræ an advance precisely similar to that characterising the previous stage has taken place, *i.e.* the Wolffian duct has become connected with the intermediate cell mass in the 13th segment (fig. 7), and behind this point is free from adjacent structures.

In a chick with fourteen or fifteen protovertebræ the process of development remains the same. So that in a chick with fifteen segments the following is the condition of the Wolffian duct:—It extends from the 7th to the 15th segment as a solid cord of cells, connected at intervals with the peritoneal epithelium by the commencing Wolffian tubules; behind the 15th segment it extends for a short distance as a free cord. The further development differs from that just recorded in this important particular; the duct does not become connected with the intermediate cell mass of the newly-formed last segment, but remains separate for a considerable interval of time (till towards the end of the third day) from it. In other words, the formation of the Wolffian tubules and their connection with the Wolffian duct is deferred behind the 15th segment.

To sum up the developmental changes above recorded, the Wolffian duct arises as a continuous ridge of cells projecting from the intermediate cell mass towards the epiblast in the region of the 7th to 11th protovertebræ inclusive. This ridge separates from the intermediate cell mass from before backwards, remaining, however, connected with it at intervals by the rudimentary Wolffian tubules. Meanwhile, from the hind end of it there grows back a cord of cells independent at first of the adjacent structures, but immediately on the formation of the hinder segments becoming connected with the intermediate cell mass of each segment in turn. This happens as far back as the 15th segment; behind this point it grows back as a solid cord, which does not become connected with the intermediate cell mass until the tubules of the Wolffian body have made considerable advance in their development.

Figs. 1—7 are meant to illustrate the above method of development. Figs. 1—5 are from the 10th segment of chicks, with ten, twelve, thirteen, and fourteen protovertebræ respectively. They are all taken through points where the Wolffian duct remains attached to the peritoneal epithelium, *i.e.* through a rudimentary tubule, excepting fig. 4, which is from a section close to fig. 3, and shows the condition of things in one of the intervals between the points of continuity.

Fig. 6 is taken from a section just behind the last segment of a chick with twelve segments, and shows the complete independence of the Wolffian duct.

Fig. 7 is from the 13th segment of a chick with thirteen segments, *i.e.* from the same region as fig. 6, and it shows the connection which has become established between the Wolffian duct and the intermediate cell mass by a mutual growth of these structures.

Fig. 8 is from the 16th segment of a chick with twenty-two protovertebræ, and is illustrative of the fact derived from an inspection of all the sections of the segment, that the Wolffian duct is independent of the peritoneal epithelium. From the 15th segment the Wolffian duct grows back independently to the cloaca, into which it eventually opens, and a lumen appears in it from before backwards.

In fig. 11, taken from a chick at the end of the third day, it is still distinct from the now considerably developed Wolffian tubule (*w.t.*).

For purposes of description I shall divide the Wolffian body into three regions—(1) The part found within the limits of the 7th—11th segments inclusive; (2) the part found within the

12th—15th segments inclusive; (3) that found behind the 15th segment.

In a previous paper[1] I have described at some length the early development of the Wolffian body behind the 16th segment, and I have there shown that that part may be divided into two parts, each characterised by a peculiarity in the early development. In this paper I shall make but little reference to the development of the Wolffian body in this region, confining myself almost entirely to that part lying within the area of the 7th to the 15th segments inclusive.

Development of Wolffian Tubules in region of 7th—11th Segments.

The Wolffian tubules and Wolffian duct in this region attain but a slight development. They may almost be said to have reached their highest point at the stage with fourteen protovertebræ, the only difference in later stages being the development of a lumen in them. The lumen in the tubule may acquire an opening into the Wolffian duct in some cases. In this case the string of cells seen in fig. 5 becomes very short, and the Wolffian duct appears as a narrow groove in the peritoneal epithelium. This state of things is usually found in chicks with from nineteen to thirty-two protovertebræ.

The Wolffian duct in this region exhibits great variations in calibre, and in later stages parts of it appear to atrophy, and isolated portions are found connected with rudimentary tubules. An enlarged section of the Wolffian duct in front is nearly always found as Gasser[2] has described. The duct and tubules in this region appear entirely to atrophy in chicks with more than thirty-five protovertebræ.

I have not thought it worth while to preserve figures of the duct and tubules in this region of the Wolffian body after their first appearance, as the arrangement just described may be easily observed in sections of an embryo chick of the third day.

The interest in the development of this region lies in the fact of the *continuity of development* of the Wolffian tubules and Wolffian duct. It has always appeared to me astonishing that the Wolffian duct developed as a continuous ridge from the intermediate cell mass, which, from our knowledge of Elasmobranch development, may be called the peritoneal epithelium, should entirely separate from it and then secondarily become connected with it by the tubules of the Wolffian body. My investigations, which have been made with some care on a large number of

---

[1] Loc. cit.

[2] Loc. cit.

chicks of all ages from nine to thirty protovertebræ, have entirely convinced me that the usual statements on this point are not true, and show to my mind most conclusively that the duct and tubules of the Wolffian body in the region in question do develop in continuity, precisely as do the duct and peritoneal openings of the head-kidney in most Ichthyopsidan types.

The number of rudimentary tubules in each segment of this region I have not determined precisely. They occur as often as not between the segments, and there seems to be about two for each segment. In the seventh segment I have never seen more than one.

Before proceeding to give an account of the further development in the next region, I will briefly refer to the points in which my observations differ from those of previous observers on the development of the Wolffian duct.

Gasser's account[1] of the development of the Wolffian duct is the most recent and exact. In his valuable paper will be found a complete account of the literature of the subject, to which I need not further refer.

"The first trace of it which he finds is visible in an embryo with eight protovertebræ as a slight projection from the intermediate cell mass towards the epiblast in the region of the three hindermost protovertebræ. In the next stage with eleven protovertebræ, the solid rudiment of the duct extends from the 5th to the 11th protovertebræ; from the 8th to the 11th protovertebræ it lies between the mesoblast and epiblast, and is quite distinct from both, and Dr. Gasser distinctly states that in its growth backwards from the 8th protovertebræ the Wolffian duct never comes into continuity with the adjacent layers. In the region of the 5th protovertebræ, where the duct, &c., was originally continuous with the mesoblast, it has now become free, but is still attached in the region of the 6th to the 8th. In an embryo with fourteen protovertebræ the duct extends from the 4th to the 14th, and is now free between epiblast and mesoblast for its whole extent."

The points in which the preceding account differs from that of Dr. Gasser's briefly are:

1. The position of the continuous ridge of the Wolffian duct.
2. The subsequent complete isolation of the duct in the region of the ridge.
3. The independence of the backward growth of the duct in the 12th to the 15th segment.

I have never seen any trace of the Wolffian duct in front of the 7th segment, and in all the chicks I have examined I find

---

[1] 'Arch. für Mic. Anat.,' vol. xiv.

that the continuous ridge extends from the 7th to the 11th segments.

With regard to Gasser's statement of the complete isolation of the duct in the anterior region from the intermediate cell mass, I can only say that my observations point to an entirely different conclusion.

Thirdly, I differ with him in his statement that the duct in the growth back from the attached extremity does not come into relation with adjacent structures.

As stated above, it seems to me that for the space of four segments the small cord of cells which grows back from the hind end of the ridge, does almost immediately become connected with the intermediate cell mass.

Development of the Wolffian Duct and Body from the 12*th*—15*th Segment.*

I now pass to the most interesting point which has turned up in my investigations on the excretory system of the chick.

In a paper by Mr. Balfour and myself in the 'Quart. Journ. of Micr. Science,' vol. xix, describing the development of what we believed to be a rudimentary head-kidney in the chick, we drew attention to a structure which so closely resembled the glomerulus[1] of the head-kidney of the Ichthyopsida that we identified it as an homologous structure.

Gasser[2] has also independently discovered and similarly identified this structure.

In the paper just referred to no attempt was made to trace the development of this glomerulus, but it was merely described as it appeared at the time of its greatest development.

The following description is taken from that paper:

"In the chick the glomerulus is paired, and consists of a vascular outgrowth or ridge projecting into the body cavity on each side at the root of the mesentery. It extends from the anterior end of the Wolffian body to the point where the foremost opening of the head-kidney commences. We have found it at a period slightly earlier than that of the first development of the head-kidney....In the interior of this body is seen a stroma with numerous vascular channels and blood-corpuscles, and a vascular connection is apparently becoming established, if it is not so already, between the glomerulus and the aorta. The stalk connecting the glomerulus with the attachment of the

---

[1] I have already given a preliminary account of the development of this structure in the 'Proc. Cambridge Phil. Soc.,' May 3, 1880.

[2] 'Sitzungsberichte der Gesellschaft zur Bedförd d. gesam. Naturwiss.,' No. 5, 1879.

mesentery varies in thickness in different sections, but we believe that the glomerulus is continued unbroken throughout the very considerable region through which it extends. This point is, however, difficult to make sure of, owing to the facility with which the glomerulus breaks away. At the stage we are describing no true Malpighian bodies are present in the part of the Wolffian body on the same level with the anterior end of the glomerulus, but the Wolffian body merely consists of the Wolffian duct. At the level of the posterior part of the glomerulus this is no longer the case, but here a regular series of primary Malpighian bodies is present, and the glomerulus of the head-kidney may frequently be seen in the same section as a Malpighian body. In most sections the two bodies appear quite disconnected, but in those sections in which the glomerulus of the Malpighian body comes into view it is seen to be derived from the same formation as the glomerulus of the head-kidney."

The point which is left in doubt in the above description, viz. as to whether the glomerulus constitutes a continuous structure, is at once decided by a study of its development.

I may here state that it is not a continuous structure, but consists of a series of external glomeruli, each of which corresponds and is continuous with the glomeruli of the Malpighian bodies found in this part of the trunk.

The first development of the Wolffian tubules in the region under consideration has already been described. They appear as outgrowths from the Wolffian duct meeting outgrowths from the intermediate cell mass immediately on the formation of the segment in which they are placed; so that in a chick with fifteen protovertebræ the Wolffian duct is connected with the intermediate cell mass by a certain number of cell cords in the 12th, 13th, 14th, and 15th segments.

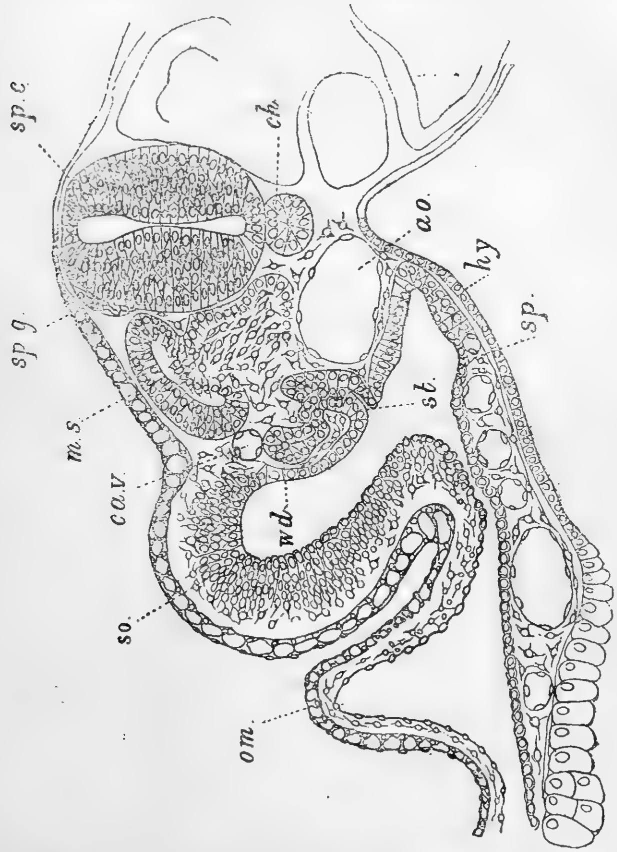
The duct and cords, which have at first rather an irregular outline, soon become well-defined compact structures.

Fig. 12, taken from the 12th segment of an embryo with twenty-two segments, represents the condition of things at this age.

The Wolffian tubules in this region are derived from two distinct structures—(1) the outgrowth from the Wolffian duct; (2) part of the intermediate cell mass.

The intermediate cell mass is at first continuous with the peritoneal epithelium in every section; but, as described in a previous paper, this connection soon becomes lost at certain points (fig. 9), and maintained at others (fig. 10). Figs. 9 and 10 are contiguous sections through the 15th segment of a chick with twenty-two segments, showing this point. At these points, where the continuity is retained, a peritoneal funnel is subsequently formed by the development of a lumen extending from the body cavity into the intermediate cell mass.

The features of the stage of development now reached are well known; it is that of the S-shaped cords of cells which have been so often described. In the adjoining woodcut is represented part of one of these S-shaped strings, showing clearly the above



Transverse Section through the Trunk of a Duck Embryo with about twenty-four Mesoblastic Somites.

am. amnion; *so.* somatopleure; *sp.* splanchnopleure; *wd.* Wolffian duct; *st.* segmental tube; *ca.v.* cardinal vein; *ms.* muscle-plate; *sp.g.* spinal ganglion; *sp.c.* spinal cord; *ch.* notochord; *ao.* aorta; *hy.* hypoblast.

features of a tubule, &c., viz.—(1) the Wolffian duct, in which a lumen has appeared; (2) the outgrowth from it to the intermediate cell mass forming the upper limb of the S; (3) the intermediate cell mass with the commencing lumen from the body cavity.

In the next section the intermediate cell mass is not connected to the peritoneal epithelium.

In chicks of gradually increasing number of protovertebræ this cavity in the intermediate cell mass gradually becomes more marked (figs. 13, 14), and extends into that part of it immediately behind the peritoneal connection (fig. 15).

Figs. 13, 14, and 15 are three successive sections through the 13th segment of a chick with about thirty segments, showing the features of a tubule at this stage.

The Wolffian duct is connected with the lower end of the intermediate cell mass in all the three sections. A distinct lumen has appeared in the intermediate cell mass which opens into the body cavity in front (figs. 13 and 14), but is separate from the body cavity in the hindermost section (fig. 15).

Comparing these figures with figs. 9 and 10 it is seen that fig. 13 or 14 corresponds to fig. 9 in the fact of the continuity between the intermediate cell mass and peritoneal epithelium; while fig. 15 corresponds to fig. 10, in both the continuity having been lost. The difference between them consists in the presence of a distinct lumen in the older series, opening into the body cavity, and continued behind into the part of the intermediate cell mass which has separated from the peritoneal epithelium. This part, marked *i. c. m.* in fig. 15, will in the next stage become converted into that part of the tubule in which a Malpighian body is developed, while the anterior part, which is open to the body cavity, will widen out considerably, and give rise to a wide peritoneal funnel.

In fig. 11 is represented a section through a developing Wolffian tubule in the hinder part of the Wolffian body. The tubule (*w.* t^1.) in this section precisely resembles the part of the tubule (*i. c. m.*) represented in fig. 15. Supposing the anterior part of *w.* t^1. were open to the body cavity it would almost be a repetition of the anterior tubule, save in the fact that it is not yet united to the Wolffian duct. But the hinder tubule (fig. 11) does not develop until after the intermediate cell mass has separated from the peritoneal epithelium, *i. e.* subsequent to the obliteration of the rudiment of the peritoneal funnel.

Not only do the Wolffian tubules in the region of the 12th to 15th segments develop a lumen while still continuous with the peritoneal epithelium, but further, a glomerulus appears in them while still open to the body cavity; and this glomerulus not only appears in the hinder part of the tubule (fig. 15) which has separated from the peritoneal epithelium, but also in the anterior part (figs. 13 and 14) where it is open to the body cavity. This is at once clear on inspection of figs. 16, 17, 18. These figures are taken from the 13th segment of a chick with thirty-four protovertebræ. There was a section not figured between fig. 17 and 18, otherwise the sections are successive, fig. 16 being the anterior.

In fig. 16 is seen the commencement of the peritoneal funnel as a bay lying between the Wolffian duct and mesentery.

In fig. 17, a glomerulus (*gl.*) has appeared projecting into this bay. In the next section, not figured, the bay was almost closed up by an approximation of its edges, while in fig. 18 the bay is completely shut off from the body cavity, and we have a section of a true Malpighian body with its contained glomerulus.

Fig. 18 clearly corresponds to fig. 15 of the previous stage, while fig. 17 corresponds to fig. 14, the difference being that a distinct cellular projection (*gl.*) has appeared at the point where the projection of cells from the Wolffian duct joins the intermediate cell mass.

I have given a diagram (fig. 22) representing an ideal longitudinal dorso-ventral section through two of these Wolffian tubules at this stage. This diagram has been made from a study of many embryos showing the development of the external glomerulus.

The open peritoneal funnel is represented at *p. f.*, the arrow pointing into it. Through it is projecting the anterior part of the glomerulus (*gl.*), that part which I shall call the external glomerulus. A transverse section through this part would give the appearance represented in fig. 17.

Into the closed hinder part of the tubule (*mb.*) is projecting the hinder part of the glomerulus (*i. gl.*), which I shall call the internal glomerulus. It was not possible to represent satisfactorily in this diagram the Wolffian duct, which, obviously from its position in transverse section, would not be seen in a longitudinal section passing through the attachment of the glomerulus.

In fig. 23 is represented somewhat diagrammatically a transverse section through a chick with thirty-three protovertebræ, *i. e.* from a slightly younger embyro than that from which figs. 16—18 were taken, in which the cord of cells connecting the Wolffian duct with the cavity of the glomerulus had acquired a distinct lumen, the cavity of the Wolffian duct being here distinctly continuous with that of the bay in which is placed the rudimentary external glomerulus, and so with the body cavity. At subsequent stages this part of the tubule appears to persist, but only in a rudimentary fashion.

The next stage which I propose to describe was found in a chick in which thirty-six protovertebræ could be counted, but possibly there were more.

The glomerulus has grown immensely (figs. 19, 20, 21), and has now acquired the peculiar histological features which characterise it at the time of its greatest development, and which have already been described in a former paper.

Anteriorly the bay has widened out considerably (fig. 19), and the glomerulus (*e. gl.*) projects directly into the body cavity. Posteriorly the bay remains deep (figs. 20, 21), and the glomerulus almost completely fills it and projects beyond it into the body cavity. In sections behind fig. 21 there was seen a fairly well-developed internal glomerulus.

The edges of the bay are gathering round the glomerulus preparatory to fusing with it, and so closing up the peritoneal funnel and dividing the glomerulus completely into two parts, the internal vascular tissues of which, however, are continuous.

In this stage the epithelial covering of the external glomerulus (*e. gl.*) was distinctly, as in the previous stage, continued behind directly into that covering the posterior internal glomerulus.

When, however, the peritoneal funnel closes by the completion of the process commencing in figs. 20 and 21, this epithelial continuity is lost, and we have the final stage of the glomerulus, the last which I have observed, in which the separation above described is complete, so that in this stage, which is that of the greatest development of the external glomerulus, and corresponds with the commencing formation of the head-kidney, the glomerulus belonging to one tubule is divided into three parts.

(1) An anterior[1] part projecting into the body cavity. This corresponds to a further development of fig. 19.

(2) A middle part, continuous with (1), also projecting freely into the body cavity, but also connected by vascular structures with an internal glomerulus. This part is figured in fig. 26, and corresponds to a further development of the part from which fig. 20 and 21 were taken.

(3) A posterior part, in which there is no external glomerulus, but merely an internal one belonging to a true Malpighian body of the mesonephros, which I have not thought it necessary to figure in this or the previous stage. It is a further development of fig. 18. This stage, which may be observed about the middle of the fourth day of incubation, brings to a close my observations on this extraordinary structure. It appears that in the chick the stage just described is that of the greatest development of the external glomerulus. In the duck, however, I have often met with it even larger and more developed, and

---

[1] Fig. E, Pl. II, in the paper on the "Head-Kidney of the Chick," 'Quart. Journ. Mic. Sci.,' vol. xix.

it appears to me after its separation from the internal glomerulus to get an independent growth, and while the latter is undergoing atrophy to become larger and extend itself posteriorly, so as almost to overlap the external glomerulus of the next tubule.

With regard to the number of the external glomeruli in the chick and the exact limits of their occurrence, the following is briefly what I have been able to make out in a chick with thirty protovertebræ:

In the 11th segment there are two rudimentary tubules running from the Wolffian duct to the peritoneal epithelium. At the point of attachment of these there is a small rudiment of the external glomerulus, visible for only one section in each case.

In the 12th segment there is at the beginning a Wolffian tubule and a well-marked external glomerulus extending through three sections. At the hind end of the 12th segment and beginning of the 13th there is an external glomerulus for three sections continued into part of the segmental tube behind, in which an internal glomerulus will subsequently be developed.

In the 13th segment there is an external glomerulus for three sections.

In the 14th segment there are two segmental tubes with developing external glomeruli.

In the 15th segment no external glomeruli appear to be developed, the segmental tubes being already separated from the peritoneal epithelium.

In later stages only the three or four hindermost of the above external glomeruli appear to develop further. The anterior glomeruli soon atrophy with the adjoining tubules and duct.

In the duck a much greater number become developed, and they may be seen in the anterior segments after their respective tubules have entirely atrophied.

The bearing of the developmental processes above recorded on any hypothesis as to the phylogenetic history of the vertebrate excretory system I propose to examine in the second part of this paper (pp. 460—462; 464).

Part II. *A Discussion of the Vertebrate Excretory System in General.*

The most peculiar feature of the excretory system of the vertebrata is the presence of three more or less distinct parts, the pronephros, the mesonephros, and the metanephros or kidney proper. In the following pages my object will be to explain the relation of these parts, more especially those of the pronephros and mesonephros, and to show that they have arisen as differentiations of a primitively uniform structure.

For this purpose it is necessary briefly to recapitulate the more important features in the development which have a bearing on my argument.

Segmental Duct and Pronephros.

The first part of the excretory system to make its appearance is always a duct. This duct has received various names, but its homology in different forms is undisputed. I shall call it the segmental duct.

In the chick the segmental duct is commonly known as the Wolffian duct.

All the Ichthyopsida whose development is known, with the exception of Elasmobranchs, possess a structure called the head-kidney or pronephros. The pronephros when present always develops in continuity with the anterior end of the segmental duct.

In the Amphibian the segmental duct arises as a groove of the parietal peritoneum, just ventral to the place where the body cavity is connected with the cavities of the muscle plates. This groove, which arises first of all anteriorly just behind the branchial region, is continued for a certain distance backward. It soon, however, becomes constricted into a canal lying between the ectoderm and parietal peritoneum. This constriction has been described as taking place in the following manner:—It first appears in the middle region of the groove, giving rise to a canal opening into the body cavity in front and behind. It then is continued backwards until the groove is completely converted into a canal behind, which soon acquires an opening into the cloaca. Anteriorly the wide opening meanwhile is divided up into two,[1] three,[2] or four[3] openings, according to the species.

The canal immediately behind the last of these openings becomes coiled and placed on the same level but ventral to the openings. The part of the body cavity into which the openings of the segmental duct pass widens out, a vascular projection—the glomerulus—from the dorsal inner wall is formed, extending uninterruptedly from opposite the anterior opening of the segmental duct to as far back as the posterior. The dilated section of the body cavity in which the glomerulus lies, and into which the segmental duct opens, is partially separated from the rest of the body cavity. The whole structure, including openings of duct, ventral coiled part of duct, glomerulus, and dilated part of body cavity, is known as the pronephros. The number of open-

[1] Urodela. [2] Anura. [3] Cæcilia.

ings from the segmental duct into the body cavity corresponds with the number of segments through which the pronephros extends.[1]

With its excretory system in this condition the young Amphibian is hatched. Fundamentally the head-kidney retains the above structure, increasing only in size until it begins to atrophy, an occurrence which takes place on the development of the mesonephros.

This method of development of the segmental duct and pronephros is fundamentally repeated in other animals which possess a pronephros.

About the marsipobranch development very little is known. Fürbringer (loc. cit.), quoting W. Müller and his own observations, makes the following statements for Petromyzon:—In the earliest stage which has been observed there was present at about the level of the heart a groove in the parietal peritoneum, which leads behind into a duct, which eventually, by a backward growth, reaches the cloaca and opens into it. The anterior groove or opening of the duct soon becomes divided up into four openings.

In the young Ammocætes there is present a pronephros made up of a complicated coiled duct and four or five openings into the body cavity, opposite which is placed a vascular glomerulus; the whole structure extends over four or five segments.[2] The pronephros atrophies in the adult.

In Myxine nothing is known of the development, but in the adult a pronephros has been described, which, however, is not functional in old individuals (adult ?), as in them it has lost its connection with the backward continuation of the segmental duct.

It[3] consists of the segmental duct, which gives off dorsally a number of diverticula, in which are found glomeruli, and ventrally a number of coiled canals, which open apparently into the pericardial cavity.

The fully-formed pronephros of Petromyzon then resembles in structure very closely that of Amphibia, while the pronephros of Myxine differs in certain important points.

The Teleostei possess a pronephros, which persists as a large organ in the adult. It develops in connection with the seg-

---

[1] Fürbringer, 'Morph. Jahrbuch,' Bd. 3, p. 5.

[2] Scott, in a recent paper ('Morph. Jahrbuch,' vol. viii), states that the segmental duct in Petromyzon, develops as a solid cord of cells from the somatic mesoblast, which subsequently becomes hollow. The peritoneal openings of the head-kidney are developed as outgrowths from the anterior end of this duct to the body cavity.

[3] 'Jenaische Zeitschrift,' vol. vii, 1873.

mental duct precisely as does the pronephros in Amphibia. The only difference between the two is that in Teleostei the segmental duct has never more than one anterior opening, and the part of the body cavity into which it opens, and in which the glomerulus lies, is completely constricted off from the rest of the body cavity, and comes to resemble exactly an enormous Malpighian body.[1]

I may here sum up the common features characterising the ontogeny of the pronephros and its duct (segmental duct) in all the forms of the Ichthyopsida in which the development is at all known:

1. The segmental duct arises first as a ridge from the parietal peritoneum. This ridge usually contains a diverticulum from the body cavity, and is continuously constricted off to form a duct.[2]

2. Except anteriorly, where the constriction only takes place at intervals, leaving the openings of the pronephros (except in Teleostei, where there is only one opening).

3. These openings correspond in number with the segments which the pronephros occupies.[3]

4. A vascular structure, called glomerulus, is formed, projecting on each side of the aorta into a specialised dilatation of the anterior part of the body cavity. Myxine forms a peculiar exception to this otherwise universal fact.

5. This dilated part of the body cavity may become partially or completely separated off to form a capsule, into which the glomerulus projects and the anterior end of the segmental duct opens.

6. The pronephros in all those Ichthyopsida in which it is found attains a functional development, but is usually only active during a period intervening between the hatching and the attainment of full maturity, *i. e.* it only functions in the larva.

In Elasmobranchs, which do not, so far as is known, possess a pronephros, the segmental duct arises as a solid ridge from the somatic layer of the intermediate cell mass in the anterior region of the trunk. From this ridge there grows back a column of cells to the cloaca. On the development of a lumen the segmental duct, with its peritoneal opening, is established. The duct develops quite independently of adjacent structure behind

---

[1] There is a functional head-kidney in adult Ganoids. It appears to be formed on the Teleostean type (vide Balfour, 'Comp., Embryology, vol. 2, p. 51).

[2] In Petromyzon, Scott (see note, p. 445) states that this duct arises as a solid rod of cells, which secondarily becomes connected with the body-cavity epithelium, to form the pronephric funnels. This account, in my opinion, needs confirmation.

[3] 'Fürb.,' p. 5, p. 42.

the point of its original attachment, and does not unite with the segmental tubes till considerably after its first development.

The difference in the development of the segmental duct in the forms possessing a pronephros and in Elasmobranchs is only one of degree.

In both cases it at first arises as a projection, either solid or containing a diverticulum from the body cavity, from the parietal peritoneum just ventral to the muscle plates; but in the one case this groove has a greater longitudinal extension than in the other. In all probability the hinder part of the segmental duct is in all cases formed by an independent growth from the hind end of this groove.

Amongst the Amniota the chick is the type in which the development of the segmental duct has been most carefully examined.

In the chick it arises as in Amphibia as a projection (solid in the chick) from the parietal mesoderm just ventral to the muscle plates; and the extent of the ridge is the space occupied by five segments.

This ridge is constricted off at intervals from the intermediate cell mass, but remains attached at certain points. The hind end of the duct is formed by a growth back from the hind end of this ridge, which takes place independently of adjacent structures.

The question now presents itself: are these structures at the anterior end of the segmental duct in the chick, which so closely resemble in development the openings of the Ichthyopsidan head-kidney, homologous with that head-kidney?

To a consideration of this question I shall return.

Mesonephros.

The mesonephros obtains a large development in all the groups of the Vertebrata; but it does not persist as an excretory organ in the adult of the Amniota.

It develops in three very markedly distinct ways.

The first of these characterises the Elasmobranchii.

The second the Amphibia, Teleostei, Ganoidei, Marsipobranchii.

The third the Amniota.

The Development of Mesonephros in Elasmobranchii.

The segmental tubes of Elasmobranchii were originally described by Balfour as arising as solid diverticula of the peritoneal epithelium. An examination of Balfour's specimens led me, however, to conclude that they originated as specialised parts of the body cavity, viz. from the canals in the intermediate cell mass which connect the muscle plate cavities with the general body cavity; and Balfour has now given his adherence to this view ('Comp. Embryology,' vol. 2, p. 570).

These canals having lost their connection with the body cavity of the muscle plates acquire an opening into the segmental duct, and differentiate[1] into the typical Wolffian tubules. The connection with the general body cavity may or may not be retained in the adult. The secondary tubules develop as outgrowths from that part of the primary tubules, which will give rise to a Malpighian capsule. These outgrowths grow forward and eventually acquire an opening into the terminal portion of the tubule of the segment in front. Later they loose their connection with the Malpighian capsules, though a rudiment of this is sometimes retained as a solid cord of cells.

The method of development of the secondary, tertiary, &c., tubules has not been followed.

The primary tubules open into the segmental duct very shortly after the latter has acquired an opening into the cloaca.

The formation of the Malpighian bodies and the outgrowths from them to form secondary tubules occur later.

For a full account of the development of the mesonephros in Elasmobranchs I must refer to the works of Balfour and Semper, to whom we owe the whole of our knowledge.

Development of the Mesonephros in the remainder of the Ichthyopsida.

As a type of this development I will take an Amphibian, Salamandra, in which animal it has been more completely elucidated by Fürbringer than in any other.[2]

Fürbringer describes the formation of the mesonephros as taking place entirely during larval life; no trace of the gland being seen in the newly hatched larva. It arises as a series of ingrowths of the peritoneal epithelium, which soon become separate from the latter. The primary tubules are hollowed out in the cell masses so formed independently both of the body cavity and segmental duct (Wolffian duct), but subsequently they acquire an opening into both.

The secondary tubules arise in a blastema, the origin of which is not clear, but is apparently derived from the just mentioned serial ingrowths. They acquire an opening into the collecting part of the primary tubule and into the body cavity. The remaining dorsal tubules have an equally obscure origin.

---

[1] 'Elasmobranch Fishes,' p. 260 *et. seq.*

[2] Loc. cit.

As the mesonephros becomes more developed the pronephros retrogrades, and is eventually entirely, as far as its function is concerned, replaced by the former.

The development of the mesonephros in Teleostei, Marsipobranchii, Ganoidei, is similary described as taking place in the free young (larva) from strings of cells derived from the peritoneal epithelium. In Marsipobranchii as in Amphibia the young are hatched with a functional pronephros, and no trace of the mesonephros; but the former is, in the further growth of the young animal, gradually replaced functionally by the latter, and more or less retrogrades. In the Teleostei, however, and Ganoidei, it persists with the mesonephros as an important functional organ in the adult. In some Teleostei the pronephros is the only functional adult kidney, the mesonephros not being developed.

I have made some observations on the development of the mesonephros in the Frog (*Rana temporaria*), Salmon and Sturgeon, and my observations lead me very strongly to doubt whether Fürbringer and other observers are right in describing the origin of the cells which give rise to the mesonephros as actual ingrowths from the peritoneal epithelium.

In the case of the Frog this is certainly not the case. In fig. 25 is represented a section through a Tadpole of 11 mm., showing the first trace of the cells (K B) from which the Wolffian tubules arise. At their first appearance they are independent of the peritoneum, and only secondarily become connected with it. Fürbringer figures from the Salamander a section in support of his statement; I have also seen such appearances in the Tadpole, but in this animal these strings are only found in that part of the animal in which, I am confidently able to state, no Wolffian tubules are ever developed. I have examined and compared segment with segment of Tadpoles of various ages, and have never found these strings of cells developing into Wolffian tubules. The cell strings appear to me to arise from a blastema of cells developed *in sitû* becoming connected with the peritoneal epithelium, and they are, no doubt, rudimentary tubules.

Fürbringer in his paper gives no evidence of the origin of these cells from the peritoneal epithelium, except a drawing of a stage in which the blastema is connected with the peritoneal epithelium.[1] I have also seen this stage, as mentioned above,

---

[1] Götte also, in his latest writings on the subject, agrees with Fürbringer as to the origin of the cells which give rise to the mesonephros. But I may draw attention to the fact that Götte has held three views on this point, the last of which did not appear (see Fürbringer, loc. cit.) till 1875, *i.e.* after the publication of Balfour and Semper's works on 'Elasmobranchi.'

in my sections of the Frog, but have completely failed to find the earlier stages of this ingrowth. One would expect to see it preceded by a thickening of the very flat cells lining the body cavity at this point; one would hardly expect the flat cells so specialised to form the lining of the body cavity of the young larva suddenly, and without showing any change to begin to grow inward. Further, if the cell cords described by Fürbringer in the Salamander are really only rudimentary structures belonging to the anterior part of the mesonephros, as is certainly the case in the Frog; and if the process which Fürbringer describes for the posterior part of the mesonephros of the Salamander takes place for all fully-developed parts of the mesonephros, as is the case in the Frog, then part of the difficulty caused by the peculiar secondary development of the peritoneal funnels disappears. In other words, I believe Fürbringer has made a mistake, precisely similar to that which was made about the development of the Avian Wolffian body. He has seen in the anterior part of a young larva the cell cords mentioned above; which were present at a time when there was no trace of the posterior part of the mesonephros. He has also seen in the hinder part of older larvæ the blastema of cells separate from the peritoneal epithelium from which the Wolffian tubules arise. Finally, he has connected these two conditions, which are, as I believe, found in different regions of the trunk, and has concluded that the cell strings of the anterior part have separated from the peritoneal epithelium and given rise to the cell masses of the posterior part which really develop independently of the peritoneal epithelium, and eventually give rise to the Wolffian tubules.

My observations on Teleostei lead me, for similar reasons, to assert an origin, *in sitû,* of a continuous blastema, which later, breaking up, will give rise to the Wolffian tubules.

On the other hand, the older observers, including Vogt and Rosenberg for Teleostei, Rathke, Johan. Müller, Reichert, Vogt, for Amphibia,[2] are quoted by Fürbringer as asserting an origin of the tubules as a series of excavations in a blastema of cells lying just internal to the segmental (Wolffian) duct. And it seems to me that the older observers were,[3] as in their statements concerning the development of the mesonephros in the chick, not far from the truth. In the Sturgeon my observations point to a similar conclusion; in the just-hatched young a few mesoblast cells are seen lying internal to the segmental duct. These, at a later stage, are replaced by a more compact mass of

---

[1] 'Fürbringer,' loc. cit., p. 46.

[2] Ibid., loc. cit., p. 12.

[3] Self, 'Quart. Journ. Mic. Sci.,' April, 1880.

cells, occupying the position of which, in a still older animal, Wolffian tubules are seen.[1]

The point I wish to insist upon is that sufficient proof of an actual ingrowth of cell from the peritoneal epithelium has not been given; but that it is much more probable that the kidney blastema arose *in sitû*, in some cases perhaps in continuity with the peritoneal lining, and in other cases independently of it, but soon becoming united with it to form the nephrostomata.

The development of the mesonephros in the Amniota has been most fully elucidated in the chick.[2]

In a recent paper I have described the development of the posterior Wolffian tubules from a continuous blastema of cells derived from the intermediate cell mass; and in the first part of this paper that of the anterior tubules from the cell cords left connecting the Wolffian duct and intermediate cell mass.

Further, in the chick there is a kind of intermediate method of development of the tubules of the 12th—15th segments (see above).

The question here again recurs which was asked before: Are these tubules of the anterior part of the Avian Wolffian body really tubules of the Wolffian body, or have they something to do with the head-kidney? For a discussion of this question I must refer below to p. 460.

The Metanephros.

In a recent paper[3] I have attempted to show that the metanephros, which is found only in the Amniota, is developed from a blastema of cells which arises continuously with but behind the blastema from which the Wolffian tubules develop.

Although the blastema which will give rise to the greater part of the metanephros arises at a comparatively early stage in development, still it is not till a much later stage that it shifts its position, and begins to show signs of developing into the Wolffian tubules. This late development of the kidney, which in this point to a certain extent resembles the Amphibian mesonephros, is a very remarkable fact. I shall return to it again.

I have thus run over very rapidly the most salient features in the development of the various parts of the Vertebrate excretory system, so far as it is at present known to us. I now turn to

---

[1] Balfour has recently described the existence of solid cords of cells, connected wfth the peritoneal epithelium, in the anterior part of the mesonephros of the sturgeon ('Comp. Embryology,' vol. ii, p. 581). The origin of these cords is not clear, neither is it certain that they undergo full development.

[2] Loc. cit.

[3] Loc. cit.

a consideration of the bearing which these facts have upon any hypothesis as to the phylogenetic connection of these various organs.

But, before so doing, it will be well to consider the nature of the problem which presents itself. It is universally admitted that the Craniata have had a common ancestor. The problem to be solved is contained in these questions: What was the structure and development of the excretory system of that ancestor? How has it been modified to produce the excretory organs which we see in Vertebrates now living?

I am but too well aware how complicated and difficult the problem is, and how insufficient are the data we at present possess to enable us to solve it. Of the two sources (geology and embryology) from which we can hope to obtain these data, palæontology can throw no light whatever upon the primitive Vertebrate or its ancestors, for the Vertebrates have apparently an antiquity greater than that of the oldest fossil-bearing rocks; and even if there are in existence fossiliferous rocks bearing the remains of the ancestor of Vertebrates (excluding Amphioxus), we can hardly hope, when they are found, to obtain any knowledge of the ontogenetic development or structure of soft parts, and the light which palæontology throws upon the later history is at present difficult to use in settling questions of this kind,[1] so that we are thrown almost entirely upon embryology for the facts; but the facts which embryology at present supplies us with are quite inadequate to enable us, even approximately, to solve the problem.

---

[1] In making out the phylogeny of organs which have had an early origin, it seems to me that geology can help us in this way (amongst others). Those forms which are found in the oldest rocks, and which have existed as small isolated groups, very little changed apparently in structure, to the present day, probably retain the same method of development now as then. By examining the embryology of such living forms we might expect to find the development of certain organs different to that in other animals belonging to larger living groups. Turning to the Brachiopoda, a group of great antiquity, we find a development of the body cavity which is shared by but few animals, and which *à priori* we regard as the most primitive method of development of that organ known. Now, of the animals which resemble the Brachiopoda in this respect, Balanoglossus, Amphioxus, and Sagitta are soft bodied, and so not found as fossils; but their very isolation at the present day, with regard to their relations to other groups, suggests that they are survivals of some larger groups, the other members of which have undergone so much evolution that their relationship is unrecognisable. The other group, Echinodermata, which presents this method of development, is found at its greatest development in Palæozoic rocks, and has not undergone any very marked changes since that time. It seems to me that, by following this line, some very important help might be obtained in helping us to decide questions of organ phylogeny.

But still, such as they are, it seems worth while to put them together, and to discuss the conclusions to which they seem to point.

Mr. Balfour[1] has compared the embryonic record to an ancient manuscript in which many leaves are missing, many moved out of their proper order, and many spurious ones interpolated by later hands. It is the duty of an embryologist to try to reconstruct the manuscript and see exactly what it contained when it was first written. In doing this he is aided by the fact that he has access to many copies of the manuscript, which have each been used and altered by very different people. He is thus able, by comparing the different copies, and by studying the characters, &c., of the people by whom they have been possessed, to arrive at a more correct idea as to what the original was like than if he had only one copy.

In studying the various embryonic records we have we can pick out certain features common to all, and which may be assumed to have had their counterpart in the phylogenetic history. But the majority of features have been so altered that it is only possible to arrive at anything like a conclusion by taking into account the complicated conditions in which the animals have lived.

Discussion of the preceding Facts.

While the pronephros is characterised by a very similar structure and development in all the animals in which it occurs, the mesonephros, though possessing in all animals a fairly similar adult structure, presents most remarkable differences in development in the different groups. While the mesonephros is universally (few Teleostei excepted) present, the pronephros is only present in certain forms. Considering first the Ichthyopsida, it is at once seen that the presence or absence of a pronephros is correlated with another peculiarity. When the pronephros is present the egg contains a relatively small amount of food yolk, and the young undergo a considerable part of their development after leaving the egg; while, when the pronephros is absent, the egg contains a very bulky food yolk, and the young undergo far the greater part of their development within the egg (Elasmobranchii).

Further, again considering the Ichthyopsida, we find that one method of development of the mesonephros is found in those animals with a pronephros, while the other method is found in those animals without a pronephros. Of the two methods of development of the mesonephros, while one (that found in

---

[1] 'Comp. Embryology.'

Elasmobranchii) may be considered *as in some respects* primitive, the other must be regarded as very much modified.

Whatever may have been the phylogenetic origin of the Wolffian tubules, the ontogenetic origin, as seen in Amphibia, Teleostei, Ganoids, Marsipobranchii, cannot possibly be regarded as in any way approaching the former. We cannot suppose that a definite serial organ like the mesonephros developed in phylogeny as a series of independent cavities in a mass of mesoblastic cells. At any rate, I think I am justified, in the present state of our knowledge, in making this statement. It is completely opposed to our ideas, and can only be accepted when all other hypothesis as to the origin of the mesonephros in phylogeny, based on the facts of embryology, have been shown to be untenable.

The tubules of the mesonephros in Elasmobranchii, however, in which group they arise from parts of an organ previously developed, present a method of development which is not at all at variance with our *à priori* views as to their phylogenetic origin. From considerations of this kind it seems to me a fair assumption that the development of the tubules in Elasmobranchs from parts of the body cavity more nearly resembles the method by which the organ arose in phylogeny than does that of the Wolffian tubules of the remaining Ichthyopsida.

In Elasmobranchs the Wolffian tubules have a segmental arrangement; one is found in each segment. In all probability this also is a primitive condition.

The arrangement of the tubules in the other vertebrata, although it does not actually afford support to this view, still it does not disprove it. It is a well-known fact that the segmental tubes have very rarely a segmental arrangement in the adult or even in the embryo. But in this connection it must be remembered that the tendency of development always seems to be to render that part of the mesonephros, which is going to function in the adult as an excretory organ, more compact, *i.e.* to bring its constituent parts closer together. I need only refer to the kidneys of the Urodele Amphibia. Here the posterior part of the mesonephros, which is going to function in the adult as kidney, becomes distinguished by its size and the course of its ducts from the anterior part, and in the female by its size only from the anterior part. And Fürbringer has shown, in *Salamandra maculata*, that in correspondence with the increasing size of the posterior region there is found an increased number of primary tubules in a segment, as well as of dorsal secondary tubules.[1]

---

[1] Spengel however asserts, that in the female of those Amphibia he has investigated, the kidney (mesonephros) contains an uniform number of

Spengel has also shown that even in different species of one genus the number of primary tubules in a segment differs, *e.g.* in *Spelerpes variegatus* there is one primary tubule in a segment, in *Spelerpes fuscus* there are two.

Further, Fürbringer states that in the species investigated by him the number of primary tubules in a segment increases with the age of the animal.

"Die Anlagen sind in ihren früheren Entwickelungsstadien leicht zu scheiden; später hingegen lagern sie sich so innig an einander, dass eine Abgrenzung unmöglich wird."[1]

Finally, there seems to be a distinct relation between the closeness of aggregation of the tubules with regard to the body segments and the number of segments found between the mouth and the anus.

In the Anourous Amphibia, where there are very few segments in the adult in this region, we find a very compact and complex kidney.

In the Urodeles, in which the number of segments is greater, the kidney occupies a greater number of segments, and is not nearly so compact, while in Cœcilia, in which the anus is almost terminal, very few segments being placed behind (tail undifferentiated), we find that the kidney is segmental, *i. e.* one primary tubule is found for each segment, and it occupies in the adult as many as sixty segments.[2]

Turning to the Amniota, we find that in Lacertilia[3] the mesonephros has at first a segmental arrangement, one primary tubule for each segment, and although it has not been shown that the fully developed mesonephros of lizards has lost this feature, still there can be little doubt, considering its resemblance to that of Aves, that it has; while in the case of the chick[4] the number of primary tubules in a segment increases with the age of the embryo.

These three facts, viz.—(1) The variability of the number of primary tubules in a segment in closely allied forms, (2) the increased[5] number in a segment as development proceeds, (3) the relation between the compactness of the kidney and the number of segments over which it extends, all point in the same direction. They seem to indicate that the tubules of the Wolffian

---

segmental tubules in each segment over its whole area; while in the male, he finds that they increase in number behind.

[1] Loc. cit., p. 19.

[2] Spengel.

[3] Braun.

[4] Self, 'Quart. Journ. Micr. Sci.,' April, 1880.

[5] There is no evidence that this is effected by intercalation in the chick at any rate.

body are capable of shifting their position according to the wants of the particular species.

We know very well other organs can do this, and I need only mention the anus placed so near the head in frogs, and so far off in Cœcilia, and it seems only probable that an important gland like the kidney should be capable of acquiring a position and arrangement of its constituent parts different from the position of their development, if it is advantageous for the performance of the function of the organ.

The evidence which at the first look appeared so strong against the primitiveness of the Elasmobranch arrangement of one primary tubule to each segment proves on examination to lose a great part of its force.

I now come to a difficulty which apparently at present presents an insuperable obstacle to a successful solution of the question under consideration, viz. What was the structure and development of the excretory system of the ancestral Vertebrate?

Assuming that the development of the Elasmobranch mesonephros presents primitive features in the two details already considered, its development in a third particular can by no means be assumed to be primitive. The fact that the segmental duct develops independently of the tubules cannot, in the present state of our knowledge, be regarded as primitive. Objections of precisely the same kind as those used in arguing against the development of the tubules in Amphibia, &c., being primitive present themselves here.

Any phlyogenetic hypothesis which presents difficulties from a physiological standpoint must be regarded as very provisional indeed. The physiological difficulty present in the conception that in the evolution the mesonephros has arisen by the fusion of two distinct parts, viz. the duct and tubule, is so great that until facts are brought forward to show a different origin we must consent to admit our total ignorance on this point. I think that the observations recorded in the first part of this paper on the development of the Avian Wolffian duct and anterior tubules are of great interest in this relation. Here we have the Wolffian duct and tubules developing in continuity in the anterior part of the excretory system, which has been always admitted to present the most primitive development. But this point I must again keep for later consideration.

So far, then, the following conclusions have been reached—the development of the mesonephros of Elasmobranchii is in part primitive (tubules), and in part very much modified, while the development of the mesonephros of Amphibia, Teleostei, &c., is in all respects modified.

Turning to the development of the segmental duct, we find ourselves obliged, for precisely similar reasons to those already given in the case of the mesonephros, to suppose that that ontogeny is in this respect more primitive in which the duct arises as a continuous groove constricted off from the body cavity than that in which it arises as a solid knob (modified groove) for only a very small part of its course, and undergoing the major part of its early growth quite independently of surrounding structure.

In Elasmobranchii that part which develops as a groove persists as a groove throughout life (abdominal opening of Müllerian duct).

In Amphibia, &c., that part which develops as a groove becomes constricted off first in the middle, and then backwards and forwards, but in front it is constricted in a manner, according to Fürbringer not understood, so as to leave the variable numbers of openings of the pronephros.

However this may be, apparently the openings of the pronephros develop as unclosed portions of the anterior end of the groove from which the duct arose, and they open into a space placed at the root of the mesentery close to the notochord and *close* to the point where in a previous stage the body cavity communicated with the muscle plates.

In the Amphibian, and apparently in the Teleostean, there is no marked structure corresponding to the intermediate cell mass of Elasmobranchii. The muscle-plate cavity is, after its separation from the general body cavity, only separated from the latter by a double layer of cells, forming its ventral wall and the wall of the body cavity; *i. e.* there is no portion of the body cavity at first continuous, but subsequently divided up by the coming together of its walls into a series of canals connecting the general body cavity with the muscle plates.

Now the glomerulus of the pronephros develops in a part of the body cavity anatomically corresponding to the intermediate cell mass of Elasmobranchii, only in Amphibia it does not, in this region, become divided up into chambers corresponding to the segments.

With this part of the body cavity, from the somatic walls of which the original groove arose, the openings of the head-kidney communicate. The number of these openings corresponds with the number of segments occupied by the pronephros in all those animals in which they exceed one, except Myxine; but the development of the pronephros in Myxine is not at all known, and its adult structure is, on the whole, obscure.

Turning again to Elasmobranchs, we find that the anterior knob of the segmental duct arises from the intermediate cell mass, *i. e.* from a part of the body cavity corresponding serially with that with which in the succeeding segments it later unites when the young segmental tubes acquire a communication with the segmental duct.

In Amphibia the segmental duct, when larval life is tolerably advanced, opens into a Wolffian tubule, which arises from a mass of cells, the origin of which is obscure, but which apparently does not appear till after the larva has left the egg. Now the Wolffian tubule of an Amphibian is homologous with that of an Elasmobranch; it is similarly constructed, and opens into the body cavity at a corresponding point. Hence we are driven to the conclusion that the cells from which the Wolffian tubule in an Amphibian arise are homologous with the intermediate cell mass of an Elasmobranch.

But in Amphibia these cells are not developed where, if Elasmobranch development is primitive, they should be; and appear later in a way which gives no clue to their relationship to the intermediate cell mass in Elasmobranchii.

What is the meaning of this extraordinary method of development?

In Elasmobranchs the development of the segmental duct is modified, while the development of the mesonephros is primitive in its segmental arrangement and origin as a specialised part of an organ present at an earlier stage.

In Amphibia the development of the segmental duct is more primitive, but that of the mesonephros very modified, and this very latter fact always goes hand in hand with the presence of a pronephros. Turning to the pronephros, it is found to develop *in continuity* with the segmental duct. It is found to possess, with regard to its openings into the body cavity, a segmented structure. It is also found to possess a structure, the glomerulus, resembling extraordinarily closely the glomerulus of an ordinary Malpighian body of the mesonephros. This glomerulus lies in a special part of the body cavity, just as a glomerulus of a Malpighian body in the mesonephros of an Elasmobranch lies in what from its origin may be called a specialised part of the body cavity; and both these specialised sections in their anatomical position precisely correspond (see above, p. 457).

With all these similarities can the inference be avoided that the head-kidney is descended from the same primitive excretory system as the mesonephros, which has appeared early in development to supply the larva with an excretory organ, and has been able to retain a more primitive development? The larva, having this, has not wanted the hinder part, and in consequence, having all its energy occupied while within the egg in developing those organs which it will really require as a larva, it leaves over the development of the organs not so required until after it is hatched; and in order that it may not be burdened by useless organs, the cells from which the tubules after appear and which should appear, if keeping the phylogenetic order, quite early in embryonic life, in a way already indicated, are reduced so as hitherto to have escaped observation.

It is perfectly true that the pronephros does present peculiarities of structure not presented by the mesonephros, such as the unsegmented nature of the glomerulus, and in the fact that the tube connecting the cavity in which the glomerulus lies with the segmental duct not being coiled. But in the fundamental structure, *i.e.* in the possession of a glomerulus placed close to the main vascular channel (aorta), in the segmental arrangement of the openings of the segmental duct into the cavity (anatomically corresponding in both cases) containing the glomerulus, in the cavity containing the glomerulus being a specialised part of the body cavity; in all these points the pronephros and mesonephros resemble each other.

Assuming for the moment the truth of this suggestion, we find the pronephros to present that method of development which *à priori* we are bound to assume would be if it were not for disturbing causes, the development of the mesonephros, because it represents the most probable method by which the mesonephros and its duct can have arisen in phylogeny.

The question now arises, What are the disturbing causes which in Amphibia have so changed the phylogenetic development? The answer has already been given, but I will repeat it here. It has been brought about by the action of natural selection on the innumerable larvæ produced, so that only those animals reached the adult state which in their prelarval and larval development conformed to the type of development we have before us.

Admitting the possibility of both prelarval as well as larval development varying at any particular stage, the tendency has been to produce a dissimilarity in the early structure of the excretory organs of Elasmobranchii and Amphibia greater than that which exists in the adult state, a result entirely in opposition to what we should expect from the application of that principle which has been laid down as regulating embryonic development, viz. that embryos of different animals, starting as fairly similar, become more and more dissimilar as their development proceeds.

To get any actual proof from embryonic development in favour of the above hypothesis must, from the nature of the case, be very difficult. For the very reason of the existence of the pronephros as an anterior part of the excretory system well marked off from the posterior makes it improbable that anything more than a trace of the hinder part should appear simultaneously in embryonic development with the anterior part. If the rest of the mesonephros developed continuously with the duct and simultaneously with the pronephros, then, on the above hypothesis, we should not be able to distinguish a pronephros from the hinder part; and it is opposed to all our ideas of economy to suppose that a rudiment of the mesonephros should appear at what phylogenetically would be the proper time, remaining over as a rudiment in the larva, *i.e.* as a useless organ forming merely a burden until it was wanted.

It seems to me that we can only expect, at the very utmost, to find a very small trace of the mesonephros in embryonic development at what phylogenetically we should consider, on the above hypothesis, to be the proper moment relative to the pronephros.

I have been examining the development of the segmental duct in an Amphibian, the frog, to see if at the time of closure of the groove of the segmental duct any trace of a discontinuous closure such as we find in the head-kidney existed. If the pronephros is merely the anterior part of a segmental organ of which the mesonephros is the posterior part, and if phylogeny is in any way repeated in the development of the pronephros, we should expect to find that the discontinuous (segmented, see above) closure of the pronephros would be repeated behind, showing some traces at least of the openings of the segmental duct and of the specialised part of the body cavity which later forms the Wolffian tubule and contains the glomerulus. So far it cannot be said that my search has been from my point of view successful. To get any evidence of what I was searching for requires a very complete series of sections in a state of preservation favorable for observation. The difficulties presented by the embryonic Amphibia in their early stages to such a successful result are very great. In the first place they are very brittle, and comparatively very few of the sections, even if thick, can be mounted uninjured. Of these, very few, indeed, can be obtained perfect, and those so obtained are apparently more difficult to see anything in than the thick ones. The cells are full of yolk granules which seem to escape and obliterate the outlines of the cells from the sight.

While my results have not been such as to unable me to speak with any confidence either one way or the other, yet on the whole they have convinced me that a re-examination with a new method of the development of the segmental duct in Amphibia, &c., would repay the trouble.

In the chick, on the other hand, the anterior part of the segmental duct, for the space of five segments, develops exactly in the manner of the segmental duct and head-kidney of the Ichthyopsida. Are the cell cords connecting the duct and peritoneal epithelium in these segments rudimentary Wolffian tubules, or are they rudiments of a head-kidney? In the absence of a continuous glomerulus opposite them they differ from the openings of the pronephros. In their development they resemble the latter. If they are Wolffian tubules they develop quite differently from all other Wolffian tubules. If they are rudimentary pronephric funnels, then the chick possesses a rudiment of a pronephros which resembles exactly the hinder developing Wolffian tubules.

It seems to me that these structures, under the light of the above hypothesis, present no difficulty, and I cannot help thinking that the discovery of their method of development is striking evidence in its favour. They belong, on that hypothesis, to the anterior part of the excretory organ, which has retained the primitive method of development originally characterising the whole organ. They, in some Avian ancestor, have constituted the first developed part of the excretory system, which has been utilised by the larva as its excretory organ. Supposing that Avian ancestor existed now, we should find that its larva possessed an organ which we should call pronephros, having a structure less modified probably from the hinder part of the excretory system than in the case of the Ichthyopsida, *i.e.* an organ the serial homology of which, with the mesonephros, would no more be disputed than is that of the metanephros with the mesonephros.

It may be objected to this view of the anterior part of the Avian excretory system, that it differs in certain marked features from the pronephros of other forms. Of these differences the most important is, perhaps, the fact that there is always found an interval unoccupied by segmental tubes between it and the mesonephros. But in Amphibia Salamandra Fürbringer[1] distinctly states that rudiments, as masses of cells, occupying the same relative position to the segmental duct as do segmental tubes, are found intervening between the two. If these rudimentary tubules underwent full development there would be no such gap as that we now find between the pro- and mesonephros of Amphibia.

But this difficulty is merely part of another difficulty which it seems to me must exist whatever view be taken of the nature of the pronephros, namely, why does this organ, so well developed in the larva and apparently perfectly well performing the functions of an excretory organ, atrophy in the adult? And this difficulty only seems capable of the unsatisfactory explanation,

---

[1] Loc. cit.

that though perfectly well suiting the requirements of the larva, its position is unsuitable for the satisfactory performance of its functions in the adult. Balfour has suggested[1] that the atrophy of the pronephros is due to its position in that part of the body cavity which eventually becomes the pericardium; and has pointed out, as a confirmation of this view, that it only persists in the adult of those animals in which it is completely shut off from the body cavity, *e.g.* Teleostei.

(The enormous size which the pronephros attains in adult Teleostei is peculiar, but, coupled with the remarkably feebly developed mesonephros in the adult, is not astonishing. The pronephros seems capable of carrying on all the excretory work in some adult Teleostei, in which the mesonephros is not present. The absence of the mesonephros in these cases is probably purely secondary, and, no doubt, traces of it would be found if a close examination were made. The survival of a larval character into the adult state is paralleled by the Axolotl's gills.)

A second feature of difference between this anterior part of the Avian excretory system and the Amphibian pronephros, is the absence in the former of a continuous glomerulus. This may be abortion from disuse, and does not really present a serious difficulty.

A third feature of difference is that the Avian pronephros extends over a much greater area than that of the Ichthyopsida, but when I draw attention to the fact that this difference is found amongst the various members of the Ichthyopsida themselves, I think it can hardly be looked upon as a difficulty. In Teleostei the head-kidney is distinguished by one peritoneal opening and a correspondingly short glomerulus. From this we have all stages to the five peritoneal openings of Petromyzon.

Finally, even if the Avian pronephros did differ in certain features from the Ichthyopsidan pronephros, this can hardly be regarded as a serious difficulty.

The pronephros of Teleostei with its Malpighian capsule containing the isolated glomerulus, and with its one peritoneal opening, surely differs considerably from the pronephros of the frog with its three peritoneal openings and its glomerulus lying free in the body cavity.

Again, without laying too much stress upon it, I point to the pronephros of Myxine, which differs still more remarkably from that of other types.

The difficulty presented by the Elasmobranchii, in which the tubules, though retaining certain primitive features of development, do not develop in continuity with the duct, is very great, and in the present state of our knowledge no satisfactory ex-

---

[1] 'Comp. Embryology,' vol. ii.

planation, founded on facts of development, can be given of it. I will suggest a possible, but entirely rough and hypothetical, solution on the lines so far followed.

Before the Elasmobranchii produced eggs with the large food yolk they at present possess, they may have undergone a large part of their development in the surrounding medium as free larvæ. These larvæ must have left the egg at a time when the cavities of the muscle plates were still open to the body cavity, and when the segmental duct had only just commenced to be formed in front, and before the development of the vascular system, and therefore before the glomerulus, the functions of which were probably carried on by the walls of the body cavity. The segmental duct was quickly developed from a groove into a duct, the larvæ thus precociously developing a recently acquired adult structure. With this constitution the larva of the ancestral Elasmobranch quickly developed the rest of its excretory system. In consequence of the larva having been hatched at a very primitive stage, before the muscle plates were separated from the body cavity, certain primitive characters in the development of the segmental tubes were retained. These characters have been more or less transmitted to the present day, this having been rendered possible by the acquisition of food yolk and abolition of the larval state.

However this may be, and it is useless now to make hypotheses of this kind, we can only wait till a more close study of Elasmobranch development has been made to see if any traces can be found of the disturbing cause which has produced the modification in the development of the excretory system assumed on the above hypothesis, and very possibly in the search along the lines which this hypothesis indicates quite a different view as to the phylogeny of the vertebrate excretory system may present itself.

Before concluding I will briefly state what I think to have been the structure of the primitive excretory system in the ancestral Vertebrate.

There was a duct occupying the position of the segmental duct, *i. e.* at the dorsal outer angle of the body cavity, at the point where the latter becomes separated from the cavities of the muscle plates. This duct opened in each segment into the dorsal part of the body cavity. On the inner wall of the latter projected on each side a vascular ridge formed by the aorta. Behind, the segmental duct opened into the cloaca.

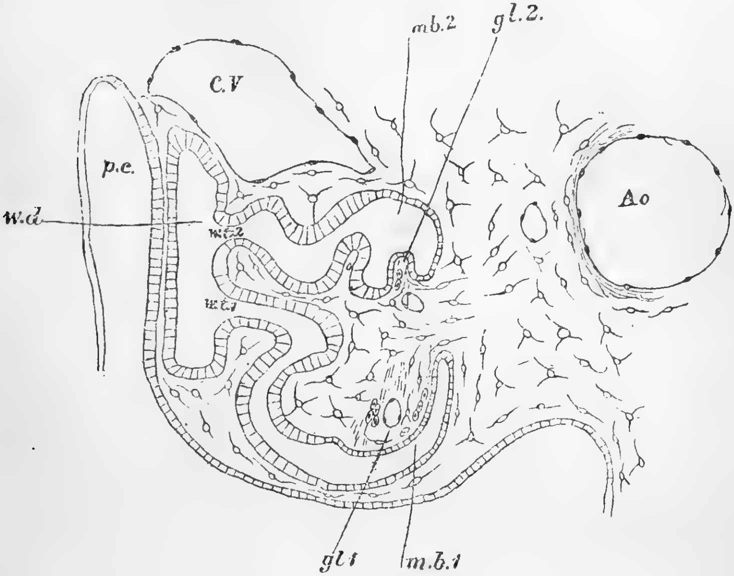
As differentiation proceeded the vascular aortic ridge became more especially developed opposite each opening of the segmental duct, and parts of each of these enlargements became successively enclosed in a special part of the body cavity, giving rise to the commencement of the secondary glomeruli. With this division of the glomerulus segmentally, and of each segment of it into further secondary glomeruli, each lying in a specialised part of the body cavity, the openings of the segmental duct began to fold and divide, incompletely at first, into special openings, one for each secondary glomerulus. Finally, this division was completed, and the segmental duct communicated by a number of openings in each segment with specialised parts of the body cavity containing a portion of the original aortic ridge. The specialised parts containing these glomeruli being still open to the body cavity, and the glomeruli being still all distinctly attached by a common stalk to the walls of the body cavity, and the intermediate parts of the original continuous ridge having completely vanished, now the capsules enclosing the glomeruli became more and more completely marked off from the body cavity. The openings putting them in communication with the segmental duct elongated into tubules which became coiled, and the glomeruli themselves gained a greater independence of each other by a development of intermediate tissue.

A trace of the original state of things has descended to the present time in the pronephros, with its continuous glomerulus opposite the opening of the segmental duct, and placed in a specialised part of the body cavity. Differences in structure from the supposed primitive state of things have of course arisen, in consequence of the specialisation of the pronephros as the larval excretory organ.

In the same way a trace of the division of the primary glomeruli into primary, secondary, &c., glomeruli, is left in the curious development of the external glomeruli of the anterior part of the Avian mesonephros. Only in this case no cause can apparently be given for the retention of this primitive feature of development.

An examination of an early stage in the development of the Avian Wolffian tubules, when the primary and secondary tubules are both fairly well established, but not very complicated in structure, points very distinctly to the fact that the glomeruli of the two tubules are parts of one primitive glomerulus. They appear to be continuous, and while one looks ventrally, *i.e.* the so-called primary glomerulus, the other looks dorsally. A glance at the accompanying woodcut will make this clear.

If this drawing of a section through the Wolffian body of a chick in a part with primary and secondary tubules, be compared with fig. 24, which is from the anterior part of the same chick where there are no secondary tubules, it will be seen that the step between them is not great.[1] It is merely necessary to suppose the division of the glomerulus (in fig. 24) into two parts, and a simultaneous development of certain folds from the Wolffian duct to form the tubules, and the original single tubule would have been transformed into a ventral primary and a dorsal secondary tubule.



Further, as I have pointed out in another paper,[2] the secondary tubule always arises in close proximity, apparently from a blastema continuous with a part of that from which the primary tubule arose.

A modification of development is to be expected, because in those animals in which the mesonephros develops after hatching, it clearly comes gradually into use. The whole is not wanted at once, but with the increasing size of the larva, more tubules are wanted. The first developed (primary) in Salamandra acquire a structure with which they can apparently perform their function when there is hardly a trace of the secondary tubules (Fürbringer, loc. cit., fig. 26).

---

[1] It will be observed that in this figure the tubule connecting the Wolffian duct and capsule is hardly developed. In all probability, this was on the analogy of the pronephros, the primitive state of things, the tubule, being a secondary differention of the duct near each glomerulus.

[2] 'Quart. Journ. Micr. Sci.,' April, 1880.

A cause of abbreviation is so clear in this case that I need not waste time in stating it.

But the whole details of the development of the secondary, &c., dorsal tubules needs reworking, for, with the exception of the observation of Mr. Balfour's for Elasmobranchs, we have no real knowledge of their exact method of development. The result of such an investigation cannot but be exceedingly interesting from a phylogenetic standpoint.

I cannot help thinking, as before stated, that the development of the external glomeruli in the chick may have some interest in this relation.

The modification of the mesonephros of the Amniota is, on the above hypothesis, due to the fact that some Avian ancestor possessed a larva in which the anterior part of the excretory system was early developed, the development of the hinder part being deferred, and consequently modified, just as we see to be the case now in the Ichthyopsida.

The still greater modification und retardation of the development of the metanephros or true kidney of the Amniota, and the great size which the Wolffian body reaches in the embryo, are striking facts which demand consideration in any discussion of the Vertebrate excretory system.

In my paper on the "Development of the Kidney" I have stated my views on the relation of the Amniote kidney to the mesonephros. But one point in that paper is left untouched.

Why does the kidney appear so late? and also why does the Wolffian body become so large and complex—so much larger than the small-sized chicks, in which it is fully developed, can need?

And, further, why should this organ, apparently so well adapted to serve as the excretory organ of the adult chick, atrophy?

It may be said, in answer to the latter question, that only those tubules of the mesonephros which open into the cloaca independently of the Wolffian duct can function in the adult, as those which have not so changed their course would interfere with the function which the Wolffian duct later acquires—the carriage of semen.

It seems to me that the only answer which can be given to the first of these questions is this:

The kidney is thrown back in development for the same reason that the mesonephros of the Amphibia is, viz. because the ancestor of the chick underwent part of its development out of the egg, at which stage of development the testis, not being developed, did not interfere with the excretory functions of the Wolffian tubules, or *vice versâ*. The large size of the mesonephros, then, is to be explained on the supposition that the larva of the chick's ancestor used it for a considerable period of its early life as an excretory organ, so that it may be said that the pronephros holds the same general relation to the mesonephros in the Ichthyopsida as does the mesonephros to the metanephros in the Amniota.

I do not mean to affirm that the above explanation of the lateness of the development of the metanephros is absolutely valid, for I think that a careful consideration of the development of the hind part of the mesonephros in Amphibia and Elasmobranchii might necessitate a slightly different explanation.

But an explanation of that kind must be sought to explain the remarkably late development in the chick of an organ which phylogenetically must be assumed to have had an origin simultaneous with that of the mesonephros.

With regard to the relation which the testes enters into with the mesonephros, it is interesting to notice the modified development which always characterises this connection.

Here it can be definitely affirmed that the lateness and consequent modification of the process is due to the fact that the apparatus has not been required in the larvæ of the Ichthyopsida and of the Amniote ancestors, and consequently has been put off and modified in development. The explanation is exactly similar to that given for the modification in development of the Amphibian mesonephros, except that here we are supposed to be able to assert with greater reason that the putting off and consequent modification is due to the fact that the connection between the testes and mesonephros was not wanted sooner, and so was not developed.

Summary of the Hypothesis and main Arguments used.

The whole of the Vertebrate excretory system, including pronephros, mesonephros, and metanephros, are derived from a primitive organ possessed by the ancestral Vertebrate. This organ had a segmental character, and consisted of a duct, the segmental duct opening in every segment into the body cavity, close to a continuous structure, known now as the glomerulus, which was placed close to the main vascular channels and acted as an excretory organ.

The anterior end of this organ was used by the larva, and developing more or less with regard to other structures at the normal time, retained many primitive features of development originally characterising the whole organ, and is known to us as the pronephros. The posterior part of the organ had its development delayed with regard to other structures, particularly those in connection with which it primitively developed; the development was consequently modified. This part is known to us as the mesonephros.

The same hypothesis was applied to account for the retardation and modification of the development of the metanephros with regard to the mesonephros in the Amniota.

The main facts in favour of the hypothesis are—

1. The development of the segmental tubes in Elasmobranchii and of the pronephros and segmental duct of the Ichthyopsida as parts of the body cavity.
2. The obvious modification in development of the mesonephros, accompanying also the presence of a pronephros in most of the Ichthyopsida.
3. The resemblance in structure between the pronephros and mesonephros, particular stress being laid on the fact that *the glomerulus in both glands is developed in anatomically corresponding,* i. e. *homologous, parts of the body cavity*

I may point out before leaving the subject that other views concerning the nature of the pronephros have been expressed by Gegenbaur, Fürbringer,[1] and Balfour.[2] The two former authors look upon the pronephros as having an antiquity greater than that of Vertebrates, greater even than that of the segmented ancestors of Vertebrates. They regard it as being descended from the primitive excretory system possessed by the unsegmented ancestor, which has been retained in such forms as Turbellaria and Rotifera, the segmented posterior part having been added when the segmented state was reached.

Müllerian Duct.

Balfour's views as to the phylogeny of the Müllerian duct and its homology throughout the Vertebrata are well known. He supposes it is one or, in the chick, more of the head-kidney openings which have become modified for generative purposes.

I still adhere to the view expressed in the paper on the "Rudimentary Head-Kidney of the Chick" as to the meaning of the peculiar structures at the anterior end of the Müllerian duct, and I think that there are grounds, which it is not necessary to enter into here, for supposing that the abdominal opening or openings of the Müllerian duct have been derived from the anterior part of the excretory system after its modification to form the pronephros. But I quite admit that a fuller knowledge of the early development of the Elasmobranch segmental duct may necessitate an alteration in this view.

---

[1] Loc. cit.

[2] Balfour looks upon it as the most primitive part of the excretory system which has been retained by the larva, as so many ancestral organs are, long after they have been lost by the adult. 'Comparative Embryology,' vol. ii.

OBSERVATIONS *on the* CRANIAL NERVES *of* SCYLLIUM.

By A. MILNES MARSHALL, M.A., D.Sc., Professor of Zoology in Owens College; and W. BALDWIN SPENCER, of Owens College. (With Plate XXVII.)

PART I. *The Preauditory Nerves.*

THE investigations recorded here were undertaken in the first instance for the purpose of controlling certain determinations published by one of us in a previous number of this journal[1] concerning the cranial nerves of Elasmobranchs. To this end we have carefully re-examined the specimens upon the investigation of which the former account was based, and have, in addition, made a large number of new preparations, illustrating more especially the later stages of development—stages M to Q of Balfour's nomenclature.[2]

During the course of our work so many altogether new and unexpected points were brought to light, that we soon found it necessary to widen considerably the scope and limits of our investigations, and have finally been led to attempt a complete account of the development of the cranial nerves from stage K to the adult form, and to endeavour in this way to connect together directly the accounts previously given of the early stages[3] with the descriptions of the nerves of adult Elasmobranchs published by Stannius,[4] Gegenbaur,[5] and other anatomists.[6]

Owing to defective supply of materials, our observations on the stages earlier than K are too fragmentary to be reliable; this we greatly regret, inasmuch as many features in the early stages are of extreme importance, and would well repay thorough investigation.

In the present paper we propose to confine ourselves to the consideration of the preauditory nerves, reserving the postauditory, which present many features of peculiar interest, for a future occasion.

---

[1] Marshall, "On the Head Cavities and Associated Nerves of Elasmobranchs," 'Quart. Journ. Micr. Sci.,' Jan., 1881, pp. 71 *seq.* Future references will be to this paper unless otherwise specified.

[3] Elasmobranch fishes, pp. 79 and 80.

[3] Balfour, op. cit. Marshall, loc. cit.

[4] Stannius, 'Das peripherische Nervensystem der Fische,' Rostock, 1849.

[5] Gegenbaur, "Die Kopfnerven von Hexanchus," 'Jenaische Zeitschrift,' Bd. vi.

[6] *Esp.* Jackson and Clarke, "The Cranial Nerves of *Echinorhinus spinosus*," 'Journal of Anatomy,' vol. x.

Our investigations have been conducted almost exclusively by means of sections of hardened embryos of *Scyllium*, and, as on former occasions, we have found a mixture of chromic and osmic acids superior to any other hardening agent. For the specimens from which our best preparations have been made we are indebted to the courtesy of the managers of the Southport Aquarium; our best thanks are also due to Mr. A. J. Moss, of Owens College, for his gift of a fine specimen of *Mustelus*, as well as for valuable assistance in connection with the literature of our subject.

The Third (Oculomotor) Nerve.—We do not propose to deal in the present paper with either the olfactory or optic nerves, inasmuch as the former has been already fully described,[1] while concerning the latter we have nothing new to communicate; we therefore commence with the third or oculomotor nerve.

Concerning the development of this nerve we have very little to add to the account given in the paper already referred to.[2] At stage L it arises from the base of the mid brain, not far from the mid ventral line, by a large posterior ganglionic root and by several smaller anterior ones clearly distinguished from the former by possessing no ganglion cells. The nerve itself runs backwards as a long slender stem to the interval between the first and second head cavities (fig. 11 *o.*, III), where it expands into a ganglionic swelling (fig. 11, *o. c.g.*) wedged in between the tops of the two cavities. From this ganglion the two main branches of the third arise; of these the upper one (fig. 15, III *b*), at a rather later stage, supplies the *rectus superior* and *rectus internus* muscles, whilst the lower one (fig. 15, III *c*) runs down behind the *rectus inferior*, and ends in the *obliquus inferior* muscle (fig. 15, *o. i.*). At stage K, at which our observations commence, the third nerve has the same point of origin and the same relation to the head cavities; it differs from the condition described above chiefly in not possessing anterior non-ganglionic roots, and in not having its terminal branches fully developed. At stages later than N the nerve seems to arise from the base of the mid brain by a single large ganglionic root, no smaller non-ganglionic ones being visible (fig. 14, III), though at stage N itself they are very prominent (fig. 13, III).

Besides the branches of the third nerve, mentioned above, there are two others in direct connection with the ganglion *c. g.*: of these the first, at stage L (fig. 11 *o.*, *N. c.*), is a short nerve, which lies along the top of the second head cavity and serves to

---

[1] Marshall, "Morphology of Vertebrate Olfactory Organ," 'Quart. Journ. Micr. Sci.,' July, 1879, pp. 300 *seq.*

[2] Marshall, loc. cit., pp. 78 *seq.*

connect directly the ganglion *c. g.* of the third nerve with the Gasserian ganglion at the root of the fifth.

The second (figs. 10, 11, and 12, N) passes straight forward from the ganglion *c.g.*, running through the walls of the first head cavity under the *rectus superior* and *rectus internus*, and through the substance of the sclerotic on the inner surface of the eyeball. Passing out from the orbit, immediately above the *obliquus inferior*, it still pursues its course straight forward, but becomes more superficial. In the earlier stages it crosses the root of the olfactory nerve, with which it lies in very close contact: in the later stages (figs. 12 and 15, N) it is, rather more dorsally situated, and then crosses the ophthalmic branches of the fifth and seventh nerves at a considerable angle (fig. 12, N), and ends in the skin at the extreme fore part of the head. After careful and repeated examination we have failed to detect any branch given off from the nerve at any point of its length.

At stage K all the above-mentioned branches of the third nerve are developed, except the upper branch, to the *rectus superior* and *rectus internus*, which we have failed to detect: the ganglion *c.g.* is very conspicuous, and the nerves *N.c.* and *N.* have the same structure and connections as at stage N, the latter of the two stretching forward to the extreme anterior part of the head, in the skin of which it ends.

In the later stages the modifications which the third nerve undergoes are merely ones of detail, all the principal branches of the nerve being already established, and maintaining their relations practically unaltered in the adult. The most important changes concern the ganglion *c.g.*; this, which at stages K and L is a large prominent swelling (fig. 10, *c.g.*), in the later stages becomes far less conspicuous, and the ganglionic cells, instead of being concentrated at one spot, occur in small scattered patches at different parts of the nerve. This change is seen commencing at stage N (fig. 11, *c.g.*), where the ganglion has divided into two main portions, one part retaining its original position, whilst the other becomes connected with the nerve *N.* at some little distance from the third nerve; at stage O-P (figs. 14 and 15) ganglion cells appear to be constantly present at two well-marked points in the course of the third, (1) where the nerve *N.* is given off, and (2) immediately above the *rectus superior*.

At stages K and L (fig. 10) the angle between the nerves *N.c.* and *N.* is very considerable, and this increases in the later stages so much that at stage N (fig. 11) the two nerves are almost at right angles to one another; at stages later than this the nerve *N.* is much more difficult to define, whilst owing to the close proximity of the third to the fifth nerve it is only with extreme difficulty that the nerve *N. c.* can be distinguished at all.

We find, therefore, that the main stem and the branches III *b* and III *c* of stage N become directly the nerves which have the same course and relations in the adult. The ganglion *c.g.* becomes the ciliary ganglion of the adult.[1] The nerves *N.* and *N.c.* become directly continuous with one another, and together form the nerve known as the *Ramus ophthalmicus profundus.* The discussion of the morphological import of these two very remarkable nerves we postpone till after the description of the fifth and seventh nerves.

The Fourth (Pathetic) Nerve.—Concerning the development of the fourth nerve no description has yet appeared, and though our observations do not yet enable us to give a complete account, still, so far as they go, they are of so definite a character that we think it well to record them here rather than wait for the possibility of completing them at some future time.

The condition of the fourth nerve at stage N is well shown in figures 11 and 13, of which the former shows the greater part of its course, and the latter its root of origin. The nerve arises (fig. 13, IV) from the dorsal surface of the extreme hinder border of the mid-brain, so far back indeed that very careful examination is necessary to determine whether its origin is really from mid and not from hind-brain. The roots of the two nerves are in close contact on the dorsal surface of the brain. From its root the nerve runs at first almost directly outwards, following the curvature of the brain, until it comes to lie a very short distance below the surface; it then runs backwards and downwards as a long, straight, and very slender nerve (fig. 11, IV), which very commonly branches early in its course, and terminates in the *obliquus superior* muscle. Just before reaching the muscle (fig. 11) the fourth nerve crosses the ophthalmic branches of the fifth and seventh nerves at right angles, lying at a slightly deeper level than these nerves. It also divides into a number of branches, of which one or more appear to become connected with similar branches from the ophthalmic branch of the fifth, the remainder entering the *obliquus superior,* whilst in the later stages (fig. 16) the main nerve is seen to come into very close contact with the two ophthalmic branches themselves of the fifth and seventh nerves.

It will be seen from the above description that the fourth nerve has by stage N acquired its adult relations and distribution, and at stages O-P (fig. 15) and Q (fig. 16), where it is shown in

---

[1] Marshall, loc. cit., p. 87, and Schwalbe, "Das Ganglion Oculomotorii," 'Jen. Zeit.,' Bd. xiii.

transverse section, it is still the same. In the adult its course, like that of the other nerves, becomes altered owing to the varying rates of growth of the brain, the skull, and the face; the rapid growth of the skull relatively to that of the brain causing the fourth nerve to run some distance forward within the brain case before passing out, when, as before stated, it runs immediately beneath and in very close contact with the ophthalmic branches of the fifth and seventh nerve.

In attempting to trace the fourth nerve in stages earlier than N we have met with considerable difficulties, and have hitherto obtained only a moderate amount of success. At M the relations are the same as at N, the sole difference being that *the nerve is more slender than at the later stage.* At stage L, though we have examined a very considerable number of specimens in excellent histological preservation, we have as yet recognised the fourth nerve in one specimen only, and even in that one not with absolute certainty. In the specimen in question the nerve has the same position and relations as at N, but is very much more slender, so thin, in fact, as to be almost unrecognisable. Earlier than L we have failed, after the most careful search, to find any trace of the nerve.

Though our observations are imperfect they yet seem to point to certain conclusions of importance touching the morphology of this important nerve. In the first place the fourth nerve is the only one in the body which, in the adult, arises from the dorsal surface of the brain; it is, therefore, of great importance to notice that from the very earliest stage at which we have seen it the point of origin is that of the adult nerve.[1] Inasmuch as the majority of the cranial nerves, as well as the dorsal roots of the spinal nerves, arise *at first* from the dorsal surface of the brain or spinal cord, it seems natural to suggest that the fourth nerve differs from all the rest, not in its mode of origin, but in the fact that, whilst all the other nerves shift their attachment to a greater or less extent, it alone preserves the primitive position of its roots of origin. This shifting of the roots is, in part,[2] due to the rapid growth of the dorsal part of the brain forcing the roots of the two sides from each other; and it becomes of interest to notice that the fourth nerve arises from a portion of the brain where this rapid growth of the roof does not occur, and where, consequently, one cause of the change in the other nerves is absent.

The fact that the direction of the fourth nerve is at first at right angles, or nearly so, to the axis of the part of the head

---

[1] Balfour, 'Elasmobranch Fishes,' pp. 156 and 191.

[2] We say "in part," because it will be shown further on in this paper that another process contributes greatly to this shifting.

from which it arises is of importance, as showing that the fourth nerve comes under the category of segmental nerves;[1] and inasmuch as there is no room as regards visceral arches and clefts for a segmental nerve between the third and fifth the fourth would probably be rightly viewed as a separated branch of the third—the only other nerve arising from the mid brain.

On the other hand, certain other facts in connection with the fourth nerve cannot be explained so easily. Thus, segmental nerves not only arise from the neural crest; they also arise early, are from the first large, and have ganglia at or near their roots. Now, so far as our observations go, the fourth nerve, though it may possibly arise from the neural crest, would not appear to arise early, and certainly is not at first a large nerve, being of much greater size at stage O-P (fig. 15) than at stage N (fig. 11); whilst at stage L, in the single specimen in which it has been detected, it is a nerve of extreme slenderness; moreover, at no part of its length have ganglion cells been found—an important point of difference from segmental nerves.

Another curious feature concerning the fourth nerve is that, in our embryos, it appears almost constantly to divide close to its root into two or even more branches, as is well shown in fig. 11, IV. These again subdivide near their terminations (figs. 11 and 15), but *all the branches, whether primary or secondary, are distributed to the superior oblique muscles,* with the possible exception of a few of the smaller ones, which appear to join the ophthalmic branch of the fifth. We would suggest that this branching may possibly be an indication of the fourth nerve having previously had a more extended distribution than its present very limited one.

On the whole, our observations lead us to believe that the fourth nerve is to be regarded as a separated part of that segmental nerve of which the third nerve forms the main portion. A further suggestion concerning the fourth nerve will be made after the seventh nerve has been considered.

The Fifth (Trigeminal) Nerve.—We propose to consider separately the roots and the branches.

A. *The roots of origin.*—The earliest stage in the development of the fifth nerve that we propose to treat of in the present paper is that represented in fig. 1, taken from a transverse section through the hind brain of an embryo at a stage intermediate between I and K, the plane of section passing on the left side through the roots of the trigeminal (V).

As shown in the figure, the roof of the hind brain is very

---

[1] Marshall, "Morphology of Olf. Organ," p. 318, 'Quart. Journ. Micr. Sci.,' July, 1879.

thin, and passes rather abruptly into the much thickened sides. To the top of the thickened portion the fifth nerve is attached by a long slender root (fig. 1, V a). This root passes down alongside the brain, but not in actual connection with it, widening considerably as it does so. Its inner border follows the curvature of the brain until reaching a point about half way down the sides of the neural canal; it then turns suddenly outwards, leaving the brain altogether, and forming, as it does so, a very conspicuous blunt projection (fig. 1, V β), which is in rather closer proximity to the brain than is the part of the nerve immediately above it. The trunk of the nerve then passes outwards and downwards, lying just beneath the superficial epiblast, between it and the outer wall of the second or mandibular head cavity (fig. 1, 2). The whole of the nerve, including its root, consist of closely-packed spherical or polygonal cells, which, like all nerve cells in the early stages, stain very deeply with osmic acid, and differ materially in appearance from the much less closely arranged mesoblast cells.

By stage K the root of the fifth nerve has undergone very remarkable changes; as shown in fig. 4, the dorsal attachment (fig. 1, V a) to the top of the thickened side of the brain has disappeared completely, and the nerve is now attached to the brain at a point about half way down the side (fig. 3, V β), *i. e. at a point exactly corresponding to the conspicuous projection* (fig. 1, V β) *already described at the earlier stage.* Immediately beyond the root of origin the nerve enlarges suddenly, and presents a distinct dorsal projection at the base of the secondary root of attachment. Although hitherto we have not secondary in following all the intermediate stages, and have not yet obtained satisfactory preparations of the stages earlier than that drawn in fig. 1, yet we feel justified in putting forward the following explanation of the appearances we have just described, relying for our justification partly upon the description given by Balfour, and still more on our own observations on the development of the roots of the seventh nerve, which will be described immediately.

Balfour has described and figured the fifth nerve as arising at "stage G, near the anterior end of the hind brain, as an outgrowth from the extreme dorsal summit of the brain, in identically the same way as the dorsal root of a spinal nerve."[1]

He has further described[2] how, by the growth of the roof of the brain, the nerves of the two sides, which at first are in contact dorsally, shift their position and become widely separate. His descriptions and our own somewhat fragmentary

---

[1] Op. cit., p. 191, and Pl. XIV, fig. 3.
[2] Op. cit., p. 196.

observations on these stages, when considered in connection with our much more complete series of observations on the seventh nerve, leave no room for doubt that the root of origin (V α) shown in fig. 1 is the primary root, the one which at stage G was situated at the top of the brain, and which has acquired its present position merely in consequence of the rapid growth of the roof of the brain pushing its two lateral halves apart, and so separating the roots of the nerves.

Concerning the root of attachment (V β) shown at stage K in fig. 3 there is more room for dispute. Balfour appears to hold[1] that this further change in position is due to the same cause as the former one, *i. e.* to growth of the roof of the brain; but this explanation, while it would fully account for the first change, would in no way explain such a shifting of the root down the thickened sides of the brain, as is clearly seen to have occurred on comparing fig. 3 with fig. 1. We believe that what really happens is that about the commencement of stage K the nerve acquires a new and secondary connection with the brain at the point (V β) opposite the projection already noticed, that the primary attachment (V α) is lost, and that the part of the nerve left above the secondary root rapidly diminishes and ultimately disappears altogether, the slight dorsal projection already noticed in fig. 3 being the last rudiment of it. As our arguments in support of this view depend almost entirely on our own observations on the development of the roots of the seventh nerve, we postpone further consideration of the point till a later portion of this paper.

At the commencement of stage K then, the fifth nerve arises about half way up the sides of the hind brain by a single large root in which ganglion cells are abundant, and opposite to which there is a well-marked external bulging of the walls of the brain;[2] beyond this root the nerve expands suddenly into a very large ganglionic swelling, the future Gasserian ganglion.

Before the close of stage K additional roots appear; a long, slender process runs forward from the anterior-superior angle of the Gasserian ganglion, and becomes connected with the brain some distance in front of the main root; in addition to which one, two, or more roots of a similar kind appear in intermediate situations.

These anterior roots of the fifth nerve are well shown at the next stage (L) in fig. 10, V γ, which shows that the fifth nerve at this time arises from the brain by three distinct roots, of which the posterior one is much the largest, and is the ganglionic root (V β) of fig. 3, while the two anterior slender non-

---

[1] Op. cit., p. 196.

[2] Marshall, loc. cit., p. 84.

ganglionic roots are the new ones. We have studied these roots very carefully, but have been unable to determine with certainty whether they are outgrowths from the brain to meet the ganglion, or from the ganglion towards the brain; our observations, however, though inconclusive, tend very strongly towards the latter of the two alternatives. It has also occurred to us that these new roots may possibly be the original primary root of origin (V α, fig. 1), which, after losing its original attachment, has acquired a new one lower down; however, though the dates of the disappearance of V α and appearance of V γ agree fairly well with this hypothesis, we have yet no actual observations in its favour, and do not wish to lay stress upon it.

These anterior roots which, during stage L, may be three or more in number, appear in the later stages to be very constantly reduced to two, one of which is the most anterior of the original roots while the other appears to be formed by the fusion of the intermediate ones. This condition at stage N is well shown in fig. 11, V γ. At a stage between O and P (fig. 14, V γ) they are rather less conspicuous owing to the interval between them and the secondary root (V β) being filled up by dense tissue. They are clearly recognisable in the adult, and form, as will be noticed more fully further on, the first or anterior root of the fifth nerve of zootomists.

B. *The branches of the fifth nerve.*—The Gasserian ganglion is, from its first appearance, wedged in between the dorsal ends of the second and third head cavities in the same manner as is the ciliary ganglion between the first and second (fig. 11). From the Gasserian ganglion, at stage K, two nerves arise; of these, one, which runs straight down between the second and third head cavities, and then along the anterior border of the mandibular arch in front of the ventral portion of the cavity in the latter, is the mandibular branch, and from this, a considerable distance below the ganglion, a small anterior branch—the maxillary nerve—is given off. The second branch from the Gasserian ganglion arises from its anterior inferior angle, runs along the top of the first head cavity and joins the ciliary ganglion; it is the communicating branch between the fifth and third nerves already mentioned, and forms the proximal part of the *ramus ophthalmicus profundus* of zootomists.

At stage L a slender branch arises from the anterior superior angle of the Gasserian ganglion; this is the ophthalmic branch of the fifth (fig. 10, V *a*) which runs forward through the orbit dorsad of all the eye muscles, giving off branches to the neighbouring parts in its course and terminating in the skin of the fore part of the head. One other nerve in connection with the fifth remains to be noticed: this is the connecting branch

(*N.c'.*, fig. 10) between the fifth and seventh nerves: this is present at K, at which stage as well as at L, it forms a very stout, though short nerve, running forward and downwards from the seventh nerve, over the top of the third head cavity, to join the lower part of the Gasserian ganglion.

The branches of the fifth nerve at stage N are well seen in figs. 11 and 12 which have been constructed so that each of them may show the whole course and distribution of certain nerves; the outlines of the figures were drawn, with the camera, from individual sections and the branches of the several nerves carefully filled in, again by the aid of the camera, from other sections of the same series. In this way such a view of the nerve is obtained as might be got from a transparent embryo in which the nerves alone stood out as opaque objects. To prevent confusion, from the overlapping of different nerves, two figures have been given of which the first (fig. 11) shows the roots of the fifth and seventh, the connections of these with one another and with the third nerve, the branches of the latter and of the fifth, the fourth nerve, and some of the branches of the seventh; in the second (fig. 12) the remaining branches of the seventh, with certain of the glossopharyngeal, are shown, and in addition to these, the whole course of the ophthalmics.

The branches of the fifth nerve are seen to be the following:

1. The ophthalmic branch, (V *a*) which arises by a slightly ganglionic root, runs forward over the *obliquus superior* (*o. s.*), crossing, as it does so, the fourth nerve at right angles and giving off branches, some of which appear to be connected with the fourth.

2. The communicating branch (*N.c.*) between the Gasserian and ciliary ganglia, the position and relations of which nerve are sufficiently well shown in the figure. We reserve the discussion of this branch, merely noticing here that, though we describe it with the fifth nerve, it appears to belong to the third quite as much as to the latter.

3. The main stem of the fifth, running down behind the first head cavity (1) and the *rectus externus*, receiving the communicating branch (*N.c.'*) from the seventh, and after passing downwards and forwards for some distance, dividing into two branches, (*a*) an anterior or maxillary nerve (V *b*) which again gives off numerous branches to the skin of the upper jaw, and (*b*) a posterior or mandibular (V *c*) which runs backwards and downwards, lying in close contact with the outer wall of the lower part of the mandibular head cavity (2), and supplying it with branches. The distribution of ganglion cells is sufficiently shown in the figure; there is a small ganglion at the base of the ophthalmic nerve, and the ganglion cells of the main stem

extend some little distance beyond the point of junction with the communicating branch from the seventh.

In figs. 14 and 15 some of these branches (V *a*, V *b*, V *c*) are seen at a stage between O and P: except that the roots of V and VII are much more closely approximated, there is no difference of importance between this stage and the earlier one which we have more fully described. We have traced all these nerves up to what is practically the adult condition, and have identified them with the branches bearing the same names in the adult. Our observations show that in the fifth, as in the third nerve, all the main branches of the adult nerve are fully established by stage L, and that the after changes are comparatively unimportant.

The Seventh, or Facial Nerve.

A. *The roots of origin.*—Fig. 2 represents a transverse section through the roots of origin of the seventh nerve of an embryo between stages I and K, the same, in fact, of which fig. 1 represents the roots of the fifth nerve. The two nerves (VII) are seen to arise from the extreme dorsal summit of the hind brain, the roots of origin of the two nerves (VII *a*) being continuous with one another across the top of the brain. It will also be noticed, as contrasted with figure 1, that the two sides of the hind brain are close together, both above and below, and that the cavity of the hind brain is a mere vertical slit; that, in fact, the growth of the roof of the brain, which we have seen is the first cause of the separation of the roots of the fifth, has not yet commenced in the part of the brain from which the seventh nerves arise. The section further shows that the nerve on either side extends down as a somewhat club-shaped mass of compactly arranged polygonal cells lying between the external epiblast and the neural canal, but distinct from both, its ventral end having a tendency to pass to the outer side of the third head cavity (fig. 2, ^{3}), just as the fifth nerve passed to the outer side of the second cavity (fig. 1, ^{2}).

The next stage is represented in fig. 3, a section through the hind brain and roots of the seventh nerve of an embryo of stage K, of the same age, though not from the same specimen as fig. 4. The figure shows that very important changes have occurred; the roof of the hind brain has grown rapidly and considerably, so as to separate widely the two primary roots of the seventh nerves (VII *a*). On the right side only this dorsal primary root is seen, but on the left side a considerable portion of the nerve is shown, and it is seen *that, in addition to the primary root* (VII *a*), *which is still present, the nerve has acquired a new or secondary root* (VII *β*), *about half way down the sides of the brain.* Both roots of attachment are perfectly clear and unmistakable, while between them the nerve and brain are quite distinct from one another, and separated by an appreciable interval.

If fig. 3, showing the condition of the root of the seventh nerve at stage K, be compared with fig. 1, showing the root of the fifth nerve at a rather earlier stage, it will be seen at once that there is a very close resemblance between the two; the sole point of difference being that in fig. 1, though the nerve still retains its primary attachment, the secondary has not yet been actually acquired. Balfour's figures and description, already referred to, show that at a still earlier stage the fifth nerve has exactly the same appearance and relations which the seventh has in fig. 2; and it is mainly on this fact, coupled with the close similarity between such specimens as those represented in figs. 1 and 3, that we rely in support of the explanation we have given above of the development of the root of the fifth nerve.

Inasmuch as figs. 1 and 2 are taken from the same embryo, it would seem that the fifth nerve appears before the seventh, and is, during the earlier phases of development, just one stage ahead of it in development. At a time (fig. 1) when the primary roots of the fifth have already become widely separated by growth of the brain-roof, and the secondary attachment (V β) is on the point of being acquired, the two seventh nerves (fig. 2) are still in contact with one another across the top of the unexpanded brain-roof; and at stage K the seventh nerve (fig. 3) is in exactly the same condition as the fifth at the end of stage I (fig. 1).

Our observations appear, therefore, to prove conclusively that as concerns the seventh nerve, while the change of position of the dorsal or primary root (VII α) is due *solely* to rapid growth of the roof of the brain, the lower or ventral root (VII β) is a new and purely secondary attachment.

Whilst these results concerning the roots of the seventh are, we believe, new as applied to Elasmobranchs, they are in perfect accordance with the account previously given by one of us of the development of the seventh nerve in the chick, in which the very same series of changes—the separation of the primary roots by growth of the brain-roof, and the acquiring of new or secondary roots—are shown to occur in a precisely similar manner.[1] The close correspondence between these two very different types of vertebrates is of much interest, partly as tending to confirm the correctness of the account, and partly as showing

---

[1] Marshall, "Develop. of Cranial Nerves in Chick," 'Quart. Journ. Micr. Sci.,' Jan., 1878, pp. 34 and 35.

The prediction there made, that the secondary attachment of the nerves in Elasmobranchs would prove on further investigation to be acquired in exactly the same manner as in the chick, is now completely verified.

that this curious shifting of the nerve roots, though clearly a change of a secondary nature, must yet have been acquired very early by Vertebrates.

The later stages of development of the roots of the seventh also present points of great interest. Fig. 6 represents a transverse section through the roots of the seventh nerve of the same embryo of stage N, of which fig. 5 shows the roots of V. The seventh nerve is seen to rise on either side by two roots, one (VII a) from the top of the sides of the brain at the junction of the thickened side with the thin roof, while the other (VII β) arises about half way down the sides of the brain. Between the two roots the nerve is in contact with the brain, but apparently *not connected with it.* We have traced the intermediate steps between figs. 3 and 6, and find that the upper root (VII a) of fig. 6 is the original dorsal or primary root, and the lower one (VII β) the secondary root of fig. 3. In other words, there is an important difference between the fifth and the seventh, *inasmuch as in the former the primary root is lost and the secondary alone retained, whilst in the latter both primary and secondary roots are retained up to stage* N, *and, indeed, as we shall see immediately, throughout life.* The difference between the roots of the fifth and seventh nerves just noticed, does not occur in the case of the chick, in which the primary root of the seventh is lost as completely as is that of the fifth in Elasmobranchs.[1]

This shifting of the roots of origin and acquiring of a secondary connection with the sides of the brain is not confined to the cranial nerves. It has already been shown to occur in the posterior roots of the spinal nerves of the chick,[2] and occurs also in the posterior roots of the spinal nerves of Elasmobranchs. It is a point of much interest to note that the seventh nerve, in the retention of its primary as well as its secondary root, *is not only more primitive than the fifth, but more primitive even than the spinal nerves.*

The condition of the roots of the seventh at stage O is shown in the left hand side of fig. 9, representing half of a transverse section through the hind brain and roots of origin of this nerve. The two roots, the primary (VII a) and the secondary (VII β), are even more distinct than at the earlier stages. The primary root (VII a) arises as before from the top of the thickened side of the hind brain just before its junction with the thin roof; from this origin the root runs downward, alongside of, and closely applied to the brain, but unconnected with it, to join the

---

[1] Marshall, 'Quart. Journ. Micr. Sci.,' Jan., 1878, pp. 24 and 25.

[2] Marshall, "On the Early Stages of Development of the Nerves in Birds," 'Journal of Anatomy,' vol. xi, 1877.

secondary root (VII β). This latter is now situated still nearer to the ventral surface than at its first appearance, the distance between the two roots being considerably greater than at the earlier stages, as is evident from a comparison of fig. 9 with fig. 6. The two roots also differ histologically, the dorsal or primary root consisting almost entirely of elongated fusiform cells, whilst the ventral root (VII β) is mainly composed of spherical ganglion cells.

This ventral root, at stage O, has, as shown in the figure, two distinct attachments to the brain, one just below the other. It is shown in longitudinal and vertical section at stage N in fig. 11, where the brain presents opposite to its point of origin an external bulging precisely similar to that opposite to the secondary root of the fifth (V β). The dorsal or primary root (VII α) is shown at the same stage in fig. 12.

In fig. 14 the two roots of the seventh are seen in longitudinal and vertical section, at an age intermediate between stages O and P. The dorsal root (VII α) arises very far up the sides of the brain, in fact, as in the earlier stages, from the junction of side and roof; it is of considerable length, is widely separated from the secondary root, and still consists mainly of fusiform cells; the secondary or ventral root, which is overlaid and almost concealed by the primary root, is only seen in part, its most anterior portion alone being visible.

The dorsal or primary root is also well shown at the same stage in fig. 15.

B. *Comparison of the embryonic roots of the fifth and seventh nerves with those of the adult.*—It will be convenient here to briefly summarise our results concerning the roots of origin of the fifth and seventh nerves, and to trace their changes up to the adult form.

About the close of stage I the fifth nerve (fig. 1) still retains its primary attachment (V α) to the brain, and is on the point of acquiring its secondary one (V β): owing to the growth of the roof of the brain the two primary roots, which were at first continuous across the top of the brain, are now widely separate. The seventh nerve (fig. 2) arises by its primary root from the dorsal summit of the brain, whose roof at this point has not yet commenced its rapid growth, so that the nerves of the two sides are still directly continuous with one another; there is as yet no trace of the secondary root of the seventh.

At stage K the fifth nerve (fig. 4) has completely lost its primary root and is now attached to the brain by the secondary root alone (V β); a slight trace of the former is still present as a small dorsal projection on the nerve just beyond the root of attachment. Towards the close of stage K, the tertiary or anterior roots have appeared, arising almost certainly as outgrowths of the ganglion towards the brain; but whether these are altogether new developments or merely new attachments of the primary root is uncertain. The seventh nerve (fig. 3) is in the same condition as the fifth at the preceding stage; it is now attached by both primary and secondary roots, the former, owing to the growth of the brain-roof, being widely separate from one another.

At stage N the condition of the roots is much the same as at the end of stage K. The fifth nerve (figs. 5 and 11) is attached by its secondary and tertiary roots, the latter being very constantly two in number, of which the anterior is the larger and attached to the brain some distance in front of the secondary root (V β). The seventh nerve (figs. 6, 11 and 12) is attached by both primary and secondary roots, the nerve between the two being in contact, but not in connection with the brain; the secondary root (fig. 11) is divided into an anterior or facial, and a posterior or auditory division.

At stage O (figs. 8 and 9) the chief differences are—first, that, owing to increased growth of the brain, the distance between the primary and secondary roots of the seventh nerve is much greater than before; secondly, that the roots of the fifth and seventh nerves, which, from the first, have been quite independent of one another, are now situated much closer together than they were at the earlier stages.

At stage O-P (fig. 14) the two roots of the seventh (VII a and VII β) are still further apart from one another, but are now very close to those of the fifth. The connection between the two nerves which we have already seen is fully established at stage K, and which is shown at stage L in fig. 10 (*N.c′.*) and at stage N in fig. 11 (*N.c′.*), is, by stage O-P, very much more extensive and intimate than previously. The roots of the nerves are still quite distinct from one another (fig. 14), but immediately beyond these roots the two nerves become so closely and extensively united together that it is impossible to draw a line of separation between them. The connection is rendered still more intimate by the crossing of one of the branches of the seventh, as will be described fully later on, over the main stem of the fifth, so as to lie in front of the branches of this nerve.

The condition of the roots, as of the branches, of the fifth and seventh nerves at stage O-P differs but little from that of the adult, the sole change of importance as concerns the roots being that the ventral roots (V β and VII β) approach still closer together, and come into actual contact.

The primitive distinctness, gradual approximation, and ultimate more or less complete fusion of the roots of the fifth and seventh is of great interest, as proving that the fusion of these two nerves, so characteristic of Pisces and Amphibia, is a purely secondary feature, and that the two are at first as independent of one another in these forms as they are throughout life in the higher Vertebrates.

In adult Elasmobranchs the combined roots of the fifth and seventh nerves are usually described together, and the descriptions of different observers, though not quite in harmony with one another in certain details, yet agree fairly well on the main points. Stannius, whose descriptions are the most elaborate, describes the combined fifth and seventh nerves as arising in Plagiostomes by three roots,[1] of which one is seen on closer examination to be double, giving four roots in all; of these the first, or most anterior one, arises from the ventral surface of the medulla by two short non-ganglionic roots, which unite together shortly after leaving the brain. This root is in *Raja*, according to Stannius, mainly motor, supplying the muscles by which the respiratory movements of the anterior wall of the spiracle are effected, and also certain others in connection with the jaws. The second root of Stannius is large, lies posterior to the first, and is in close proximity behind with the auditory nerve; it may be distinguished into an anterior part which belongs to the trigeminal, and a posterior, more ventrally situated portion, which belongs to the facial. The third root is very large and much more dorsally situated than the other; it is connected by its deeper fibres with the second root, whilst, from its superficial fibres are derived, according to Stannius, the *ramus ophthalmicus superficialis* of the fifth, and also, in part, the maxillary and buccal nerves.

Gegenbaur,[2] in his account of the cranial nerves of *Hexanchus*, distinguishes between the roots of the fifth and the seventh. He describes the fifth as arising by the union of two trunks of about equal size, an anterior and a posterior; of these the anterior (*a*) arises from the ventral surface of the medulla by two roots situated very close together; the posterior (*b*) has also two distinct roots, a dorsal one (α) arising from the side of the medulla by a large swelling projecting into the fourth ventricle, and a ventral one (β) situated immediately above the root of the facial, and in front of, and above that of the auditory.

The seventh nerve in *Hexanchus* is described as arising by two

---

[1] Stannius, 'Das peripherische Nervensystem der Fische.' Rostock, 1849, pp. 29 and 30.

[2] "Ueber die Kopfnerven von Hexanchus," 'Jenaische Zeitschrift,' Bd. vi, 1871, pp. 501, 502, and 513, 514.

roots, a larger one immediately in front of the auditory, and a smaller one passing to it from the fifth.

Jackson and Clarke[1] describe the combined fifth and seventh nerves in *Echinorhinus* as arising by three roots; an anterior inferior root (v α), itself with two well-marked rootlets, a second root (v β) arising by a well-marked superior rootlet from the lobus trigeminus and by a smaller inferior one, and a third root (v γ and VII) closely connected with the second one.

Balfour[2] describes the fifth nerve in *Scyllium stellare* as arising by three roots:—(1) an anterior more or less ventral root; (2) a root rather behind this arising by two strands, a dorsal and a ventral, and closely connected behind with the root of the seventh; and (3) a quite distinct dorsal and posterior root situated slightly behind the dorsal strand of the second root. The seventh nerve is described as arising by a single root close to, and behind, the second root of the fifth.

On comparing these descriptions of adult Elasmobranchs with our own observations on embryos and adults we are led to the following conclusions:

The fifth nerve in the adult arises by two roots:

a. An anterior one arising from the ventral surface of the medulla by two non-ganglionic rootlets, whose distinctness varies much in different adult Elasmobranchs. These rootlets are the tertiary or anterior roots of our embryos (figs. 10, 11, and 14, v γ). This root corresponds to the first root of Stannius, the anterior root (*a*) of Gegenbaur, the anterior inferior root (v α) of Jackson and Clarke, and the anterior root (1) of Balfour.

b. A posterior larger ganglionic root, the ventral or secondary root of our embryos (figs. 10, 11, and 14, v β). This is at first quite distinct from the root of the seventh, but during the later stages of development gradually approaches this latter, and in the adult cannot be clearly distinguished from it.

This root is the anterior part of the second root of Stannius; the ventral division (β) of the posterior root (*b*) of the fifth of Gegenbaur; apparently the inferior rootlet of the second root (v β), and possibly part of the third root (v γ and VII) as well, of Jackson and Clarke; and the second root (2) of the fifth of Balfour.

The seventh nerve in the adult arises by two roots:

a. A dorsal root arising far up the side of the medulla, at the junction of the thickened sides and thin roof of the fourth ventricle. This root is the primary or dorsal root of the seventh nerve of our embryos (figs. 2, 3, 6, 9, 12, 14, and 15, VII *a*).

---

[1] "The Brain and Cranial Nerves of *Echinorhinus spinosus*." 'Journal of Anat. and Phys.,' vol. x, p. 81.

[2] Op. cit., pp. 194 and 195.

It has by previous observers been almost invariably described as a root of the fifth, and never as a true root of the seventh; *our description and figures here given leave no room for doubt that it belongs to the seventh.* As already noticed, the retention of this root marks the seventh as being more primitive than the spinal, and possibly more so than any of the other cranial nerves, all the other nerves apparently retaining their secondary roots only.

This root is the third or dorsal root of Stannius; the dorsal rootlet (*a*) of the posterior trunk (*b*) of the fifth of Gegenbaur; the superior rootlet of the second root (V β) of Jackson and Clarke, and the dorsal and posterior root (3) of the fifth of Balfour.

b. A ventral root arising from the side of the medulla at a rather lower level than the posterior root of the fifth. This is the secondary or ventral root of the seventh of our embryos (figs. 3, 6, 9, 10, 11, and 15, VII β). The auditory nerve is at first derived from this root, but in the adult appears to be more distinct from the facial than is the case in the embryo. This root is, at first, some little distance behind the secondary one (V β) of the fifth nerve (figs. 10 and 11), from which it is perfectly distinct; in the later stages the two roots gradually approach one another (fig. 14), and in the adult are usually in close contact.

This root is the posterior part of the second root of Stannius, the root of the seventh of Gegenbaur; part, or possibly the whole of the third root (V γ and VII) of Jackson and Clarke; and the single root of the seventh of Balfour.

It would appear, therefore, that the fifth nerve loses its primary root, retains its secondary, and acquires tertiary roots, while the seventh retains both primary and secondary. Concerning the fourth nerve we would suggest the possibility that it may prove to be the primary root of a nerve of which the third nerve is the secondary root, which has, in this case, acquired complete independence.

C. *The branches of the seventh nerve.*—At stage K the seventh nerve, which, as we have already seen, has acquired its secondary as well as its primary roots of origin (fig. 3), expands below the secondary root into a large ganglionic swelling lying immediately behind the third head cavity. From this enlargement three branches arise: (1) from the anterior and superior angle of the ganglion a large nerve with a ganglionic base arises, and runs forward along the dorsal surface of the head, lying just beneath the superficial epiblast; this is the ophthalmic branch of the seventh, and is referred to in our figures as VII *a*.

(2) The second branch, which is also large, and has a

ganglionic base, arises from the front part of the ganglion immediately below the root of the ophthalmic; its deeper portion runs forwards and slightly downwards over the top of the third head cavity, and becomes connected with the main stem of the fifth; it is referred to in the figures as *N. c′*. The more superficial portion passes on further forward in the same direction, crosses the mandibular arch, and enters the maxillary process, lying immediately superficial to the maxillary nerve, and just beneath the external epiblast; it is referred to in the figures as VII *d*.

(3) The third branch is the direct continuation of the main stem of the facial nerve; it runs downwards and backwards along the anterior border of the hyoidean arch, and is the rudiment of the posterior or hyoidean branch of the seventh in the adult; it is referred to in the figures as VII *c*.

Of these branches the first, or ophthalmic, is from its earliest appearance connected with the dorsal or primary root of the seventh rather than with the ventral root. The second branch is the most remarkable of the three; its deeper portion forms, as we have seen, a direct connection between the fifth and seventh nerves, a communication which appears to be very early established, inasmuch as by stage K the connecting branch is already a nerve of considerable size; the superficial portion of this branch (VII *d*) is noteworthy, mainly on account of its very close relations with the maxillary division of the fifth nerve.

At stage L the only changes of importance are, (1) that the several branches have increased in size, and, excepting the branch VII *d*, which has a very straight course, and ends abruptly in the skin, have divided into secondary branches near their terminations; and (2), that a small anterior branch has arisen from the hyoidean nerve (VII *c*), some distance from the brain, which runs forward over the top of the spiracular or hyomandibular cleft, and then down in the anterior wall of the spiracle, *i. e.* in the posterior portion of the mandibular arch; this branch will be referred to as VII *b*.

The several branches of the seventh nerve at stage N are well shown in the diagrammatic figures 11 and 12. The ophthalmic branch (VII *a*) is seen in fig. 12 arising from the base of the primary or dorsal root (VII *a*) as a stout nerve, which expands very shortly after its origin into a large somewhat fusiform ganglion, beyond which the nerve runs forward as a stout trunk to the extreme anterior part of the head. Throughout its course it lies just beneath the external epiblast, and immediately dorsad of the ophthalmic branch of the fifth (V *a*), with which it is in very close relation; like this latter nerve it gives off branches along its whole course to the integument of the neighbouring parts, the branches being few in number at the proximal end, and much more numerous distally. A short distance before its termination this nerve, like the ophthalmic branch of the fifth, is crossed at a considerable angle by the nerve *N* (fig. 12).

The connecting branch (*N. c′.*) between the seventh and fifth nerves is well seen in figs. 11 and 12; it is now shorter and wider than at stage L (fig. 10), and contains very numerous ganglion cells along its whole length.

The superficial portion of this nerve (VII *d*) is not shown in fig. 11, but is represented along its whole length in fig. 12; it is a stout nerve with a remarkably straight course; it gives off no branches at all along the greater part of its length, but near its distal termination divides rather suddenly into a number of branches, which end in the integument of the maxillary process, the most anterior of the branches extending forwards almost as far as the hinder border of the olfactory pit. The relations of this nerve to the maxillary branch of the fifth are very curious; the two nerves are very close together, the branch of the seventh lying, as at the earlier stage, immediately superficial to that of the fifth. These relations are well seen in the transverse section drawn in fig. 6. This shows, as already noticed, the primary and secondary roots of the seventh, and also the proximal portion of the nerve VII *d*. This nerve is seen to be a direct continuation of the primary root; its inner, or deeper, portion is seen on both sides to become continuous with the maxillary branch of the fifth, (V *b*), the junction of the two forming the connection between the fifth and seventh nerves already noticed. Beyond this point of union the nerve VII *d* is continued downwards, lying immediately superficial to the maxillary nerve (V *b*). The two nerves preserve this relation up to their terminal distribution, two of the ultimate branches being represented in figure 5 (V *b* and VII *d*). This very remarkable branch of the seventh puzzled us greatly for a long time, and it was only after working out the whole history of its development up to stage Q that we succeeded in determining its import. The nerve has already been described and figured by one of us,[1] and named tentatively the palatine. This determination now proves to be erroneous; the palatine is a deep-lying nerve, whereas the nerve VII *d* retains its superficial position in the adult.

This nerve (VII *d*) we have now identified as the buccal nerve, the proof of this determination, consisting in our having traced the nerve directly up to the adult. The buccal nerve has always hitherto been regarded as a branch of the fifth, and is described

---

[1] Marshall, loc. cit., pp. 86, 87, and Pls. V, fig. 15; and VI, figs. 28 and 29.

as such by Stannius,[1] Gegenbaur,[2] Jackson and Clarke,[3] Balfour,[4] and others. Stannius[5] points out that the buccal nerve in fishes is very variable; that it may either arise from the main stem of the fifth, from the maxillary or the mandibular, or from both these nerves, or, finally, may be absent altogether.

Up to stage N, as shown in figures 6, 11, and 12, the buccal nerve is clearly a branch of the seventh, and could not possibly be taken for a branch of the fifth. After stage N, however, the connection between the roots of the fifth and seventh nerves becomes, as we have seen, very much more intimate; and at the stage between O and P, represented in fig. 14, the buccal nerve (VII *d*), which is now situated completely in front of the maxillary (V *b*), might very easily be taken for a branch of the fifth rather than of the seventh; careful examination shows, however, that the buccal, which is, as before, the most superficial of all the ventral branches, can be traced up to the dorsal root of the seventh from which it arises, as in the earlier stages.

This origin of the buccal nerve from the root VII *a* has already been noticed by Stannius,[6] who, however, as we have seen, did not refer the root in question to the seventh. Stannius' figure of the nerve in *Chimæra*[7] shows clearly the very superficial position of the buccal nerve and its independence of both maxillary and mandibular nerves.

Of the remaining branches of the seventh the anterior one (VII *b*) is shown in fig. 11 at its origin arising from a large ganglionic swelling on the main or hyoidean branch of the seventh, and running forward in close contact with the top of the spiracle (*sp.*), in front of which it divides almost at once into two branches, the distribution of which is shown in fig. 12; of these the anterior one (VII *pa*) runs downwards, forwards, and inwards, giving off numerous branches to the roof of the mouth. In fig. 12 the anterior branches of this nerve appear to cross the posterior branches of the buccal, but it must be borne in mind that at this point the two nerves are at very different levels, the buccal being very superficial and the nerve (VII *pa*) lying very deep. This latter is seen in transverse section in fig. 7, which shows, on the right side, its origin from the ganglion, and, on the left, its distribution to the mucous membrane of the mouth. By comparing this figure with fig. 6, the

---

[1] 'Handbuch der Zootomie,' p. 158.
[2] Loc. cit., p. 509.
[3] Loc. cit., p. 86.
[4] Op. cit., p. 195.
[5] 'Das Peripherische Nervensystem,' pp. 41 and 42.
[6] Loc. cit., p. 30.
[7] Loc. cit., Taf. 1, fig. 1.

difference of levels between the two nerves will be at once apparent. This anterior branch (VII *pa*) is the palatine nerve; it has already acquired by stage N its characteristic distribution, and undergoes no further change of importance from this period up to the adult stage.

The second or posterior division (fig. 12, VII *sp*) of the nerve (VII *b*) runs downwards and slightly backwards along the anterior border of the spiracular cleft; it gives off branches along the whole of its length, the great majority of which run backwards to the mucous membrane of the border of the cleft and to the spiracular branchia. This nerve, which at this stage is of about equal size with the palatine, is the spiracular or præspiracular nerve of zootomists.

The only branch of the seventh still left for description is the main trunk or hyoidean branch (fig. 11, VII *c*), which forms the direct continuation of the main stem of the nerve. This, as is seen from fig. 11, arises from the ventral or secondary root of the seventh, and is at its origin closely connected with the auditory nerve (VIII). Immediately after the auditory nerve leaves it, the facial forms a ganglionic swelling from which the communicating branch (*N. c′.*) to the fifth nerve is given off; beyond this point it is continued for a short distance as a stout nerve with comparatively few ganglion cells; this very speedily dilates into the large ganglionic swelling on the top of the spiracular cleft, from which the anterior branch (VII *b*) is given off. The main stem of the seventh (VII *c*) continues its course downwards, running along the anterior border of the hyoid arch and very close to the posterior border of the spiracular cleft; during this part of its course it contains few or no ganglion cells, it gives off a number of branches, of which the first is the largest, from its posterior border which supply the muscles derived from the wall of the third head cavity (3). A short distance below the lower edge of the spiracular cleft the nerve divides into two branches, of which the anterior (VII c^1.) runs forward along the lower border of the mandibular arch, sending numerous branches to the integument of this part and extending forward so as to come into very close relation with the posterior branches of the maxillary division of the fifth (V *b*). The posterior of the two branches (VII *c*, 2) into which the seventh divides continues the direction of the main stem, and runs down in the hyoid arch just in front of the third head cavity, in the terminal dilatation of which it ends. Of these two terminal branches of the seventh, the anterior, sensory, and superficial one is the *ramus mandibularis externus* of Stannius[1] and Gegenbaur,[2] while the

---

[1] Loc. cit., p. 65.

[2] Loc. cit., p. 514.

posterior, muscular, and deep branch is the *ramus mandibularis internus* v. *profundus* of the same authors. Both these branches have already acquired, by stage N, not only the characteristic distribution of the adult nerves, but nearly all the minor branches as well.

To recapitulate; we find that by stage N the seventh nerve has acquired all the important branches of the adult nerve, the main trunks and many of the branches being fully developed at a much earlier period—stage L. The seventh nerve at stage N has two roots, a dorsal or primary, and a ventral or secondary. From the dorsal root (VII *a*) arise two branches: (1) the ophthalmic (VII *a*) and (2) the buccal (VII *d*), both of which appear to be purely sensory nerves. The connecting branch (*N. c'.*) to the fifth nerve, though it appears in longitudinal section (fig. 11) to be a distinct branch, in transverse sections (fig. 6) seems to be only the deeper portion of the buccal nerve. From the ventral root arises the main or hyoidean branch (VII *c*), from which the branch VII *b* runs forward over the top of the spiracle, dividing, almost immediately, into the palatine (*pa*) and spiracular (*sp*) nerves, whilst the hyoidean itself divides distally into the sensory *ramus mandibularis externus* (V *c*, 1) and the motor *ramus mandibularis internus* (V *c*, 2).

The Sixth (Abducens) Nerve.—Concerning the development of the sixth nerve our observations simply confirm the account already given by one of us.[1]

The whole length of the nerve is shown in longitudinal and vertical section in fig. 13 (VI), where it is seen arising from the base of the brain by a number of small non-ganglionic roots which unite to form a slender nerve; this nerve runs forwards a short distance, then turns downwards, pierces the investing mass (*i. v.*), and ends in the posterior extremity of the *rectus externus* muscle (*r. e.*).

Fig. 7 shows the sixth nerve in transverse section at the same stage (N): on the left side of the figure the termination of the nerve in the *rectus externus* is seen; while on the right side, which is taken from a more posterior section, one of the roots of origin is seen. This figure shows that the roots of the sixth are considerably nearer the mid ventral line than are the secondary roots of the seventh nerves, and also that the sixth and seventh nerves are quite independent of one another. At stage N the sixth nerve appears to be altogether behind the seventh, but in stage O it is situated rather further forwards, so that the same section may pass through the roots of both nerves.

---

[1] Marshall, loc. cit., pp. 89—93.

We have not yet detected the sixth nerve in embryos younger than stage L: concerning the morphological value of this nerve we adhere to the opinion already expressed that it is to be viewed as bearing the same relation to the seventh that the anterior root of a spinal nerve does to its posterior root.

The Eighth (Auditory) Nerve.—This nerve also we can dispose of briefly: at stage K it appears as a large ganglionic posterior branch of the seventh nerve, given off immediately beyond the root of origin. It is from the first connected with the ventral or secondary root (VII β). The condition at stage L is shown in fig. 10 (VIII). At stage N (fig. 11) its root, though still intimately connected with that of the facial, shows a very evident line of separation from it; the ganglionic character of the auditory nerve placing it in marked contrast with the non-ganglionic root of the facial. This distinction between the two roots becomes more marked in the later stages.

General Considerations.—Several questions of a more general character arise out of the facts we have recorded above, and we propose to conclude the present paper with a brief notice of the more important of them. The problems in connection with the roots of origin of the nerves have been already sufficiently discussed, so that we turn at once to the consideration of their branches, concerning which the most important points are the determinations of the equivalence of the branches of the different nerves to one another.

We commence with the ophthalmic branches of the fifth and seventh nerves, the branches named V *a* and VII *a* in our figures. These two nerves, whose courses and relations are well shown in figs. 11 and 12, accompany one another very closely along their whole length; they appear to be both sensory nerves, their branches being distributed exclusively to the skin of the top and front of the head, and more especially to the mucous canals of these parts. Of the two nerves the branch of the fifth nerve (V *a*) is the smaller and the more ventrally placed of the two: though the smaller, its branches are, especially in the earlier part of its course, more numerous than those of the seventh. The two branches in their course through the orbit *lie dorsad of all the other contents of the orbit.* They are at first quite distinct from one another (figs. 11 and 12) and lie close beneath the external epiblast (fig. 5, VII *a*); the branch of the seventh being the more superficial of the two. In the later stages of development, as in the adult, the two nerves lie in very close contact with one another (fig. 16, V *a* and VII *a*), the branch of the seventh lying immediately dorsad of the branch of the fifth;

they also, as shown in fig. 16, lie at a deeper level than at the earlier stages.

The fourth nerve bears, as already noticed, a very close relation to these branches. As shown in figs. 11 (IV) and 16 (IV) it crosses the ophthalmic branches at right angles, lying at a slightly deeper level but turning outwards immediately beneath them, to end in the superior oblique muscle (*o. s.*). At the point of crossing the branches of the two nerves are in very close relation with one another, and we are inclined to believe that a communication exists between the fourth nerve and the ophthalmic branch of the fifth at this point, though we have failed to determine this with certainty.

In determining the morphological value of these ophthalmic branches of the fifth and seventh nerve, very valuable evidence, by which we have been much influenced, is afforded by the condition of the glossopharyngeal nerve. This nerve, at stage L, gives off, just beyond its root of origin, a slender dorsal branch (fig. 10, IX *a*), which, at first passing upwards and backwards, soon curves round the hinder end of the auditory vesicle (*aud.*), and reaching the dorsal surface of the head, runs forward a short distance, lying immediately beneath the superficial epiblast. It gives off branches along its whole course, which are distributed, as shown in the figure, to the integument of the top of the head. At stage N this nerve (fig. 12, IX *a*) has the same course and appearance, the only differences being that it extends rather further forwards than at the earlier stage, so as to reach some distance in front of the middle of the auditory vesicle; and, secondly, that its branches are now seen to be in connection with the commencing mucous canals of this region. This branch of the ninth nerve is clearly the *ramus dorsalis*, and an examination of the figures 11 and 12 will, we think, leave no doubt that the nerves VII *a* and V *a*, which have a similarly superficial course and a like distribution to mucous canals, must be viewed as the equivalent branches of the seventh and fifth nerves. We are, therefore, led to adopt the view put forward by Balfour, that the ophthalmic branches V *a* and VII *a of the fifth and seventh nerves are the rami dorsales of these nerves.*[1] Stannius and

---

[1] In my paper on the head cavities of Elasmobranchs I abandoned the view previously put forward (this Journal, Jan., 1878, p. 30), that the ophthalmics were persistent remains of the commissure connecting together the roots of the nerves at their first appearance, but did not expressly adopt the view that they were *rami dorsales.* I have been led to adopt this view mainly because it now appears that, instead of being perfectly exceptional in their course, as I had previously supposed them to be, the ophthalmics merely express an exaggerated condition of a state of things shown in a less extreme form by the *ramus dorsalis* of the glossopharyngeal.—A. M. M.

Gegenbaur speak of the ophthalmics as *rami dorsales,* but refer them entirely to the fifth.

What the causes are which have led to the very marked extension forwards of the *rami dorsales* of these nerves is not very evident; we would suggest that it is due mainly to an extension forwards and accumulation at the anterior end of the head of the special tegumentary sense organs—the mucous canals—this extension forwards involving a corresponding extension of the nerves supplying these organs; in connection with this suggestion it is of interest to note that no one of the nerves in front of the fifth sends any branches to these organs. Whether there is any trace of a *ramus dorsalis* to the third is very doubtful; at any rate the fourth nerve cannot be the *ramus dorsalis* of the third, as its course is, at first, at right angles (fig. 11) to the *rami dorsales* of the fifth and seventh nerves; and, secondly, it is a motor and not a sensory nerve.

We now come to a far more intricate problem, viz. the import of the connecting branches between the third, fifth, and seventh nerves, with which it will be convenient to consider the nerve *N.* (figs. 10, 11, 12, and 15).

These three nerves, *N. c.*, *N. c.′* and *N.* all appear very early; we have failed to determine the date of their first origin, but by stage K they are fully established. The posterior one (*N. c′.*), connecting the fifth and seventh nerves together, is the most difficult to investigate, owing to its appearing from the first as merely the deeper portion of the buccal nerve (fig. 6); in longitudinal sections, however, it appears very distinct (*vide* fig. 10). It is from the first much shorter than either of the other two nerves we are considering, and in the later stages (fig. 14) and the adult condition, owing to the close approximation of the fifth and seventh nerves, ceases to be visible as a distinct trunk.

The second of the three nerves (*N. c.*, figs. 10 and 11) forms, as already noticed, a direct connection between the Gasserian ganglion of the fifth and the ciliary ganglion (*c. g.*) of the third nerve, and is much more slender than *N. c′*. Concerning the nerve in question, it is of the utmost importance to notice that not only is it fully established at the stage at which our observations commence, but *that it is from the very first a connecting nerve, and that there is no reason whatever in the early stages for considering it as belonging to the fifth rather than to the third nerve.* We have, therefore, in this paper given it a perfectly neutral name.

The last of these nerves, *N.*, is still more remarkable; like the others it is present at K. *Starting at this stage from the ciliary ganglion it runs an almost perfectly straight course to the anterior* *end of the head, ending abruptly in the external epiblast, and giving off no branches whatever.* At stage L it is in very close relation to the olfactory nerve, and in some specimens seems to be connected with it, though we cannot speak with certainty on this point.

As soon as the eye muscles are established they have very definite relations to this nerve; the *rectus superior* and *internus*, and the *obliquus superior* lying above it, and the remaining three muscles below it (fig. 11). It also passes through the substance of the sclerotic, as noticed in a former portion of this paper.

Concerning the morphological significance of these nerves, bearing in mind their very early appearance and the total absence of evidence for regarding them as branches of either of the nerves they serve to connect, we are disposed to view the nerves *N. c.* and *N. c.′* as persistent portions of the neural ridge between the outgrowths to form the third, fifth, and seventh nerves, and as being, therefore, homologous with the primitive commissure connecting the posterior roots of the spinal nerves together.[1] As to the nerve *N.* we are much more in doubt; its apparent connection with the olfactory nerve at L, if confirmed, would tell in favour of its being regarded as a similar commissure between the third and olfactory nerves, and would greatly support views previously advanced by one of us concerning the morphological value of the olfactory nerve.[2] On the other hand, the extension forwards of the nerve *N.* beyond the olfactory nerve to the extreme anterior end of the head must, for the present, be regarded as almost conclusive against its commissural nature. *In this case it can only be a branch of the third nerve,* for the only other nerve with which it is in direct, or indirect, connection is the connecting nerve (*N. c.*) between the third and fifth, which, if it does not belong to the third, there is at any rate no reason for referring to the fifth.

In the adult *Scyllium* this nerve retains the relation to other nerves which it has clearly acquired by stage N; it is described in the adult by Schwalbe[3] as "dieser scheinbare Zweig des Oculomotorius." In *Mustelus* Schwalbe describes it as a branch of the fifth. We much regret that we have had no opportunity of studying the development of this nerve in *Mustelus*; should it prove to arise as in *Scyllium,* then it must definitely be regarded as a branch ofthe third.

As we have already pointed out, the nerves *N. c.* and *N.* toge-

---

[1] For these commissures in Elasmobranchs, *vide* Balfour, op. cit., pp. 158—160, and Pl. XI, fig. 18, and Pl. XIV, fig. 15 *b*. In the chick, Marshall, 'Quart. Journ. Micr. Sci.,' Jan., 1878, Pl. III, figs. 27 and 28.

[2] Marshall, 'Quart. Journ. Micr. Sci,' vol. xix, pp. 300 *seq.*

[3] Schwalbe, 'Das Ganglion Oculomotorius,' p. 16.

ther make up the *ramus ophthalmicus profundus* of zootomists, a nerve which seems to have escaped Balfour's notice both in the adult and in the embryo. Balfour does, indeed, in his description of the nerves of the adult *Scyllium*, speak of a *ramus ophthalmicus profundus*, but inasmuch as he says concerning it that " this latter nerve arises from the anterior root of the fifth, separately pierces the wall of the orbit, and takes a course slightly ventral to the superior ophthalmic nerve, *but does not (as is usual in Elasmobranchs) run below the superior rectus and superior oblique muscle of the eye*,"[1] itis clear that *he is describing the ophthalmic branch of the fifth and not the true profundus*, whose existence he has overlooked. There appears to be considerable confusion in the use of the terms *ramus ophthalmicus superficialis* and *ramus ophthalmicus profundus* by different writers, a confusion which our observations on *Scyllium* may help to remove. We find, as already stated, three perfectly distinct nerves to which the term *ophthalmic nerve* can be, and is, applied; of these the two dorsal ones (V *a* and VII *a* of our figures) are the *rami dorsales* of the fifth and seventh nerves, and may be spoken of as the ophthalmic branches of the fifth and seventh nerves respectively. Both these nerves are very superficial along their whole course, and *both lie dorsad of all the eye muscles and other contents of the orbit*. The two nerves are at first perfectly distinct, but in the adult unite more or less closely together, the extent of their union varying much in different Elasmobranchs; the two together constitute the *ramus ophthalmicus superficialis*.

The third of the ophthalmic nerves, the *ramus ophthalmicus profundus*, has a very different course, and is of a totally different nature; it is formed in *Scyllium* by the union of the connecting branch between the fifth and third nerve (*N. c.*) with the branch N of the third nerve. It is very definitely characterised by its course ventrad of the *superior rectus, superior oblique*, and *internal rectus muscles*, by its close relation with the inner wall of the eyeball, by the fact that the ciliary ganglion is either in its trunk or is connected with it directly, by its having at first no branches and by its close connection with the olfactory nerve.

We believe that the *ophthalmicus superficialis* and *ophthalmicus profundus* always maintain these relations; that the *profundus*, which is clearly the nasal nerve of *Mammalia*, is a primitive and very constant nerve, and that it never shifts its position so as to lie dorsad of all the eye muscles, as supposed by Balfour.

The two divisions of the *ophthalmicus superficialis*, on the other hand, appear to be very variable indeed in different Verte-

---

[1] Op. cit., p. 194: the italics are our own.

brates; they attain their maximum development in the Elasmobranchs, probably on account of the great development and importance of the organs they supply—the mucous canals. In Mammalia the ophthalmic branch of the fifth is represented by the frontal and lachrymal nerves, while the seventh nerve has no ophthalmic branch.

It would hardly be profitable to discuss the various descriptions of these nerves by different writers; we will merely point out here that Schwalbe[1] clearly distinguishes the three ophthalmic nerves. He calls, as we have done, the ophthalmic branches of the seventh and fifth nerves together the *ramus ophthalmicus superficialis*, distinguishing the component parts as *portio major* (VII *a*) and *portio minor* (V *a*) respectively. He also employs the term *ramus ophthalmicus profundus* in the same sense as we have done. Balfour, who was the first to clearly recognise the double nature of the *ophthalmicus superficialis*, is in error in calling the lower portion of it (V *a*) the *ophthalmicus profundus*.

Concerning the other branches of the nerves in question, there can be little doubt that the hyoidean branch (VII *c*) of the seventh and the mandibular branch (V *c*) of the fifth are homologous nerves, supplying respectively the anterior walls of the hyoidean and mandibular arches; and there appear to be good reasons for viewing the nerve (III *c*) as the corresponding branch of the third.[2] All the three nerves in question are either mainly or exclusively motor in function.

The seventh, like the hinder cranial nerves, forks over a visceral cleft—the spiracle. As shown in figs. 11 and 12, there are two branches of the seventh which run down in front of the spiracular cleft, viz. the buccal (VII *d*) and the mandibular (VII *b*), which latter divides almost at once into the palatine and spiracular nerves. Of these two a history of their development and a comparison of the branches of the seventh with those of the glossopharyngeal (IX, fig. 12), leave no possible room for doubt that the mandibular branch (VII *b*) is the homologue of the anterior branch (IX *b*) of the glossopharyngeal. This latter nerve (IX *b*, fig. 12) extends very far forwards in the hyoidean arch, being in this respect very closely imitated by the palatine nerve (VII, *pa*), so that we are disposed to regard the whole of the mandibular division (VII *b*) of the seventh, *i. e.* both palatine and spiracular nerves, as together equivalent to the anterior or hyoidean branch (IX *b*) of the glossopharyngeal.

---

[1] "Das Ganglion Oculomotorius," 'Jenaische Zeitschrift,' Bd. xiii, pp. 11 *seq.*

[2] Marshall, loc. cit., p. 88.

Balfour[1] describes the mandibular branch of the seventh as being large in the embryo, so large in fact that he feels difficulty about identifying it with the adult spiracular nerve. His figures,[1] however, show perfectly clearly that what he describes as the mandibular branch of the seventh is really the nerve we have shown to be the buccal.[2]

The maxillary nerve (v *b*) is, from its time and mode of development, almost certainly to be regarded as the true anterior branch of the fifth corresponding to the mandibular branch of the seventh, although in the absence of a visceral cleft in this region this determination cannot be considered absolutely proved. Whether there is any equivalent branch of the third nerve is very doubtful; at any rate no such branch can be pointed out with certainty.

There now remains for consideration the buccal nerve, the determination of which, as a branch of the seventh, is one of the most striking points we have brought to light. Whether this remarkable nerve has any homologue among the branches of the fifth is a point our investigations have not yet enabled us to determine. It is perhaps worth while pointing out that there are many points of resemblance between this nerve and the *ramus ophthalmicus profundus*, points of sufficient importance to render a comparison between the two nerves at any rate a possible and suggestive one. In each case the proximal portion of the nerves in question connects together directly the ganglion of one segmental nerve with that of the nerve next in front, while the distal portion passes forward into the segment anterior to that in which the main branches of the nerve are contained. The early origin, the curiously straight course, and the absence of branches until close to their termination, are features common to the two nerves, and ones in which they stand in marked contrast to most other branches. The deep course of the *profundus* as contrasted with the very superficial one of the buccal nerve may perhaps be attributed to the great development of the eye: in front of the orbit the *profundus* is a superficial nerve, and like the buccal, is purely sensory in its distribution.

On the other hand, it must be noticed that, as already pointed out, the evidence is distinctly in favour of the distal portion of the *profundus* (beyond the ciliary ganglion) being a branch of the third rather than of the fifth nerve. Another point of distinction between them lies in the fact, that the former (the *profundus*) is distributed to what is, morphologically, the dorsal surface, the buccal to the ventral.

In the present paper we have purposely refrained from

---

[1] Op. cit., p. 202.

[2] Op. cit., Pl. XIV, fig. 2 and fig. 15 *a*.

attempting to determine the homologies between the nerves of *Scyllium* and those of other vertebrates, preferring to wait until by the study of the development of other types we are enabled to bring forward positive evidence in support of our determinations.

In conclusion, we would express the hope that, by working out the development of the roots and branches of the cranial nerves in a very typical vertebrate, and following these roots and branches through their subsequent changes up to their adult condition, we have rendered more practicable than has hitherto been the case comparisons between the descriptions of embryos and of the corresponding adult animals, and have contributed something towards the establishment upon a firm basis of comparative neurology.

On the OCCURRENCE *of* CORPUSCLES *in the* RED VASCULAR FLUID *of* CHÆTOPODS. By J. E. BLOMFIELD, B.A., and A. G. BOURNE.

IT used to be (or we may even say that it is) an accepted commonplace of zoological science, that the red vascular fluid of the Chætopoda is devoid of corpuscles. Certain exceptions have been admitted, but they have been regarded as exceptions. In reality they appear to be no exceptions but the rule. Professor E. Ray Lankester ('Quart. Journ. Mic. Soc.,' vol. xviii, 1878, p. 68) has demonstrated the existence of colourless corpuscles in the red vascular fluid of the earth-worm, and has described them as "small, oblong, flattened, fusiform bodies, with clear, sharp outline, beyond which occasionally appears a small quantity of ragged protoplasm," and considers them to be merely nuclei of the cells forming the walls of the vessels which have become "free."

In the above-mentioned paper there is a complete account of what was hitherto known through the researches of M. Ed. Claparède and M. De Quatrefages, as to the existence of similar corpuscles in the red vascular fluids of other Chætopods.

Since the publication of this paper, Dr. Franz Vejdovsky ("Beiträge für Vergleichenden Morphologie der Anneliden. 1. Monographie der Enchytræiden." Prag, 1879) has described similar corpuscles in Criodrilus, and mentions them as occurring in Tubifex, about which latter genus Dr. Vejdovsky promises to say more on a future occasion. These corpuscles have then been hitherto observed in the following genera:—Lumbricus, Criodrilus, and Tubifex, among the Oligochæta; Ophelia, Cirrhatulus, Terebella, Staurocephalus, and Syllidea, among the Polychæta.

We are enabled to add to this list Eunice and Nereis, in both of which genera we find similar corpuscles. These corpuscles are rendered evident by treating a portion of part of the tissue which is well supplied by these vessels, *e.g.* a muscular septum or parapodium, or even better, merely a portion of one of the larger vessels removed to a slide with two pairs of forceps, the blood being kept in it, with osmic acid in 1-per cent. solution, followed by picrocarmin, the excess of the latter being removed by blotting-paper, and the tissue washed first with water and afterwards with glycerin. To guard against error it is important to see the corpuscles while actually within the walls of the vessels, and to move them up and down by gentle pressure on the coverslip to distinguish them from the nuclei of the cells of the wall of the vessel itself. This method is the one applied by Professor Lankester to the investigation of the red vascular fluid of the earth-worm, and it seems exceedingly probable that in most Chætopoda when carefully applied, it will yield the same result as it did in that case, viz. evidence of the existence of corpuscles in the red vascular fluid.

The corpuscles occur either singly or in small masses. In Eunice they are either round and average $\frac{1}{4000}$th of an inch in diameter, or are oblong with a long diameter of $\frac{1}{2600}$th of an inch. In Nereis the corpuscles are mostly round and rather smaller, varying from $\frac{1}{4000}$th to $\frac{1}{4500}$th of an inch in diameter.

PACINIAN CORPUSCLES *in the* PANCREAS *and* MESENTERIC GLANDS *of the* CAT.

By VINCENT HARRIS, M.D., Lond., Demonstrator of Practical Physiology at St. Bartholomew's Hospital.

a. In the Pancreas.—In examining some specimens of the pancreas of a cat, from a part which was closely adherent to the duodenum, I observed a large number of Pacinian corpuscles. In one small section no less than three were seen grouped together, with here and there a solitary one; they were cut in various ways by a transverse section of the tissue, and so evidently lay with their long axes placed in various directions. The largest number of the corpuscles was seen near the outside of the sections, in the connective-tissue capsule of the organ, but in one or two instances a solitary corpuscle appeared in the midst of the gland amongst the scanty interlobular connective tissue. The difference in the size of the bodies was very marked, some being twice as large as others, but not any so large as those generally observed in the mesentery proper.

As regards structure, they showed extremely well the hyaline ground membranes lined with endothelium, but here and there an appearance as though they were separated from one another by an albuminous material in addition. The average number of the ground membranes in a corpuscle was about twenty-two.

β. In Lymphatic Glands.—In the mesenteric glands of a cat examined in sections, I also noticed a large number of Pacinian corpuscles. These again were found to be situated to the outside of the sections, and were evidently contained less in the gland itself than in the loose connective tissue surrounding it. They presented the structure of the ground membranes and of the central mass perfectly, and the termination of the axis cylinder, closely invested with large nuclei, was in most cases contained in a sheath filled with a brownish material, which would not stain well with logwood. This material was probably similar to that which was observed between the capsules of the corpuscles in some specimens of the pancreas.

The appearance of Pacinian corpuscles in the localities mentioned above is most likely to be explained by the fact that the tissues in which they were found are closely connected with the mesentery, which in the cat is well known to be abundantly supplied with such nerve endings. In support of this supposition I may add that, in examining some mesenteric lymphatic glands with the naked eye, I observed that in the peritoneum surrounding at least two, there was a large number of very small Pacinian corpuscles, and that some might be traced almost to the gland capsule itself. I cannot say definitely whether the mesentery proper was well supplied with Pacinian corpuscles in the animals from which the mesenteric gland and pancreas first mentioned were respectively obtained, but in the last instance this was the case.

LIMULUS *an* ARACHNID. By E. RAY LANKESTER, M.A., F.R.S., Jodrell Professor of Zoology in University College, London. (With Plates XXVIII and XXIX).

A. INTRODUCTION AND BIBLIOGRAPHY.

THE prevailing opinion among zoologists at the present day, with regard to the affinities of the King Crab, is that it must be regarded as one of the Crustacea. Even when this view is not fully accepted the King Crab is placed in a special position of isolation and its relationship with Crustacea strongly insisted upon, whilst more remote affinity with the Arachnida is grudgingly admitted.

My friend Edouard Van Beneden, of Liège, is the only zoologist who has definitely taken a divergent line, and has frankly endorsed the instinctive perception of Straus Durkheim in declaring that Limulus is no Crustacean, but simply and unreservedly an Arachnid. Ed. Van Beneden bases his opinion upon embryological data. I have elsewhere expressed my full concurrence in that opinion, but the grounds upon which my conclusion rests are not solely embryological —they have reference to the structure of the adult Limulus and Scorpion. In the following pages I hope to show that Limulus is best understood as an aquatic scorpion, and the Scorpion and its allies as terrestrial modifications of the King Crab.

My views on this subject were formed some eight years ago, and I have to acknowledge the kindness of Mr. Carrington, F.L.S., of the Royal Westminster Aquarium, by which I have been enabled to dissect and make histological study of perfectly fresh specimens of Limulus sent to me in the living state.

It is not desirable at the outset to follow the history of the discussion relative to the zoological position of Limulus. Those who desire to become acquainted with the most important contributions to the subject should consult the memoirs of Anton Dohrn and A. S. Packard, who have given very ample references to the literature of the subject.

I shall here give in alphabetical order a list of the chief works referred to in the following pages, the number attached to an author's name when cited, having reference to the number in the present list. After I have put forward the facts and inferences with reference to the structure and affinities of Limulus which appear to me to be well-established, I shall briefly review the various opinions which have been advanced by recent writers of authority.

Special Memoirs.

1. DOHRN, Anton. "Bau und Entwickelung der Arthropoden," 'Jenaische Zeitschrift,' Bd. vi, 1871.
2. GEGENBAUR, Carl. "Anatomische Untersuchung eines Limulus," 'Abhandlungen der naturforschenden Gesellschaft in Halle,' 1858.
3. GRENACHER. "Unters. uber das Sehorgan der Arthropoden," 1879.
4. LANKESTER, E. Ray. "Mobility of the Spermatozoa of Limulus," 'Quart. Journ. of Micr. Science,' 1878.
5. MILNE-EDWARDS, Alphonse. "Recherches sur l'Anatomie des Limules," 'Annales des Sciences Naturelles,' 5th series, Zoologie, vol. xvii, 1873.
6. NEWPORT, George. "Nervous and Circulatory Systems in Myriapoda and Macrourous Arachnida," 'Philos. Transactions of the Royal Society,' part ii, 1843.
7. OWEN, Richard. "Anatomy of the King Crab," 'Transactions of the Linnæan Society of London,' 1873.
8. PACKARD, A. S., Junr. "The Development of Limulus polyphemus," 'Memoirs of the Boston Society of Natural History,' 1872.
9. PACKARD, A. S, Junr. "The Anatomy, Histology, and Embryology of Limulus polyphemus," 'Anniversary Memoirs of the Boston Society of Natural History, 1880.
10. VAN BENEDEN, Edouard. "De la place qui les Limules doivent occuper dans la classification des Arthropodes," Société Entomologique de Belgique, October, 1871 (translated in the 'Annals and Mag. of Natural History,' 1872).
11. VAN DER HOEVEN. 'Recherches sur l'Histoire Naturelle et l'Anatomie des Limules,' Leyden, 1838.
12. WALCOT, C. D. "The Trilobite," 'Bulletin of the Museum of Comparative Zoology at Harvard College,' vol. viii, April, 1881.
13. WOODWARD, Henry. "A Monograph of the British Fossil Merostomata," 'Palæontographical Society of London,' 1866—1878.

Text-books of Zoology.

14. CLAUS, Carl. 'Grundzüge der Zoologie,' fourth edition, first volume, third part, p. 638, 1880.

15. Gegenbaur, Carl. 'Elements of Comparative Anatomy,' English translation, 1878, p. 230.
16. Huxley, Thos. H. 'The Anatomy of Invertebrated Animals,' 1877, p. 260 and p. 374.
17. Haeckel, Ernst. 'Generelle Morphologie,' vol. ii, p. xc.
18. Owen, Richard, 'Lectures on Invertebrate Animals,' 1843, p. 181.
19. Straus Durkheim in Appendix to the sixth volume of the French translation of Meckel's 'Comparative Anatomy,' 1829.

Embryology of Arachnida.

20. Balfour, F. M. "Notes on the Development of the Araneina," 'Quart. Journ. of Micr. Science,' vol. xx, 1880.
21. Metschnikoff, Elias. "Embryologie des Scorpions," 'Zeitschrift für Wiss. Zoologie,' Bd. xxi, 1870.

As I am about to endorse the conclusion arrived at by an eminent naturalist of the first half of this century, viz. Straus Durkheim, it will be well to give here at once the grounds upon which he based that conclusion.

Straus Durkheim maintained that Limulus should be classified with the Arachnida, but the publication of his views on the subject appears never to have taken a very definite or satisfactory form. In fact the only record of Straus Durkheim's teaching on this subject which I can find is in the French translation of Meckel's 'General Treatise on Comparative Anatomy.' MM. Riester and Alph. Sanson carried out this translation, and added many notes in the form of appendices to each volume. At the end (p. 497) of the sixth volume, which bears the date 1829-1830, there is a note headed, "Sur l'appareil locomoteur passif des Arachnides," which appears to be an abstract of a memoir 'On the Comparative Anatomy of the Arachnida,' read to the Academy of Sciences, June 1st, 1829, but never, I believe, published. M. Straus Durkheim communicated its contents to MM. Riester and Sanson. From this note I submit a few extracts. The authors commence, "La classe des Arachnides, dans laquelle M. Straus comprend le genre Limule, formant à lui-seul un ordre designé sous le nom de Gnathopodes, et dont il isole les Pycnogonides qu'il renvoie aux Crustacés, offre dans la disposition de son squelette et des muscles qui en meuvent les diverses pièces, des particularités tellement tranchées qu'on ne peut y méconnaître un type different. C'est de ce squelette que sont tirés les traits principaux propres à characteriser la classe des arachnides en general, et qui consiste dans la *disposition des pattes rayonnant sur un sternum commun, dans la présence d'un sternum cartilagineux intérieur, dans l'absence d'antennes.*"

The Arachnida are then divided into three orders, "les pulmonaires, les branchifères, et les trachéens," but it is not explained whether the term "gnathopodes" is to be regarded as simply a synonym of the order "branchifères."

With regard to the internal sternum, the citation of the views of M. Straus runs as follows:—"Dans l'interieur du thorax de tous les *arachnides*, à l'exception peut-être des *acarides* dont la plupart des espèces sont trop petites pour qu'on puisse les disséquer et connaître leur organisation, on trouve une pièce cartilagineuse diversement configurée suivant les familles, et placée dans le thorax au-dessus du *sternum*. Cette pièce, à laquelle convient le nom de sternum interieur est maintenue librement par le moyen de plusieurs muscles qui se soudent de différents points de sa surface sur le bouclier, ou sur le sternum extérieur auquel ils se fixent. Elle sert en outre de point d'insertion à un certain nombre de muscles des pattes."

Since the time when Straus Durkheim put forward these views a mass of knowledge has accumulated which has tended to throw light on the affinities of Limulus. Of most importance has been the discovery of the complete form of the body of the palæozoic scorpion-like Arthropods known as the Eurypterina, and the quite recent (1873) thorough investigation of the nervous system of Limulus, by Alphonse Milne-Edwards, and further, the investigation of the developmental history of Scorpio, by Metschnikoff, and of Limulus, by Dohrn and by Packard. The gradual growth of the recognition of the Arachnidan affinities of Limulus during the last twenty-five years is obvious enough, and yet all systematic writers, and all who have especially discussed the question, continue to classify Limulus among the Crustacea whilst speculating as to the possible derivation of the Arachnida from that form, or else place Limulus in a distinct group, neither Crustacean nor Arachnidan.

I shall endeavour to show in the following pages that there is a much closer agreement of parts between Limulus and the Arachnida (especially Scorpio) than has been hitherto admitted by any one writer, even by Straus himself. It appears to me that the full extent of the agreement between Limulus and the Arachnida has never yet been stated, for whilst this or that observer has recognised *one* set of facts he has overlooked or misinterpreted another, and thus undervalued the indications of affinity between the two forms which he had admitted to exist. That the King Crab is as closely related to the Scorpion as is the Spider has for years been an open secret, which has escaped notice by something like fatality.

B. Comparison of Limulus and Scorpio.

The Arachnid which comes nearest in structure to Limulus is the Scorpion. In some few points the Spiders and, yet again, the Phrynidæ are more closely similar to Limulus than is that animal. I shall proceed, systematically, through a comparison of the skeletal and chief internal organs of Limulus with those of Scorpio, pointing out where other genera of living Arachnida come into closer agreement with the former than does the Scorpion.

§ *a.* **NERVOUS SYSTEM.**—As the view which may be adopted in regard to the agreement or distinctness of apparently corresponding parts in Limulus and Scorpio depends, to a considerable extent, on the indications afforded by the nervous system, it will be as well to proceed at once to state what is now known with regard to that system in both Limulus and Scorpio.

For a long time our knowledge of the nervous system of Limulus was very defective, owing to the fact that only badly preserved spirit-specimens had been dissected. Hence it has been held by Van der Hoeven (11) and by Owen (7) that the nerves which supply the first two pairs of appendages take their origin from a nervous mass in front of the œsophagus. Dohrn (1) and Huxley (16), on the other hand, have stated that only the nerves to the first pair of appendages are præ-œsophageal in origin. It was reserved for M. Alphonse Milne-Edwards (5) to demonstrate by the dissection of perfectly fresh specimens of Limulus the true arrangement of these parts. I am able, from my own dissection of a fresh specimen of the same animal, to confirm M. Milne-Edward's description, though I must say that such confirmation is a mere formality, since the beautiful memoir in which that author has published his results bears throughout unmistakable evidence of care and accuracy.

With regard to the nervous system of Scorpio, we are not in the same favourable position. No zoologist, so far as I am aware, has studied the nervous system, or, indeed, any of the viscera of Scorpio by the aid of fresh specimens, and I cannot but expect that some very important modifications, in accepted conclusions, may result from a renewed investigation of the anatomy of that animal carried out upon freshly killed individuals. Nor has the nervous system of the adult Scorpion been studied by the aid of the microscope, in regard to which deficiency we are in the same difficulty so far as

Limulus is concerned in spite of Packard's recent work in that direction (9); in fact, the comparative anatomy of the nervous system of Arthropoda generally has yet to be placed on a firm histological basis, and until this is done we must not attach a very great importance to the results of simple dissection. With regard to the naked-eye appearance of the nervous system of Scorpion, we have, however, the exceedingly careful work of George Newport (6), which is worthy of all confidence, and what is of more importance we have certain embryological data furnished by the investigations of Metschnikoff (21) and of Balfour (20). The observations of the latter zoologist relate to the Araneina, but may fairly be considered as confirmatory of those of Metschnikoff.

The central nervous system of Limulus consists, according to M. Alph. Milne-Edwards, (A) of a distinctly emarginated brain or cerebral mass which I have elsewhere proposed to call the ARCHI-CEREBRUM,[1] and of two strands of nervous tissue, which embrace the œsophagus and unite behind it, so as to form (B) an oval ŒSOPHAGEAL COLLAR, being continued backward from their point of union along the ventral surface of the animal as (C) the ABDOMINAL CORD to a point some distance in front of the anus. The limbs of the collar are united by from three to eight transverse commissures in front of their point of union with one another and behind the œsophagus. From the archi-cerebrum are given off five nerves only, namely, those to the ocelli, to the compound eyes, and to the frontal integument. From the œsophageal collar a great number of nerves radiate, including those to the first as well as to all the other pediform appendages, and also the nerves to the chilaria (or metathoracic sternites) and to the genital operculum. We find a distinct nerve to each appendage, and a number of large tegumentary nerves also given off from the œsophageal collar. It is important to note that the pair of nerves to the genital operculum is derived from this region and not from the cord-like prolongation of the united strands of the collar. It is also important to observe that at present we have no knowledge of the existence of distinct ganglia or enlarged masses of nerve-cells in the œsophageal collar, so that it is not possible to infer from any such fact of structure how many ganglia corresponding to an equal number of segments are represented by the œsophageal collar. M. Alphonse Milne-Edwards, who holds the "chilaria" to be the equivalents of the Scorpion's "pec-

---

[1] This Journal, April, 1881. 'On the Appendages and on the Nervous System of *Apus cancriformis*.'

tiniform organs," considers that eight pairs of ganglia are thus represented, a pair for each of the walking legs, a pair for the chilaria, and a pair for the genital operculum. The "chilaria" appear to me (as explained below) to be simply "sternites," and not related to the Scorpion's "combs;" and and I should therefore consider only seven pairs of segmental ganglia to be represented in the œsophageal collar. The history of development is not yet quite definitely ascertained, but it should decide this point, and should show, supposing the views which I am about to advocate are correct, that there is no ganglionic enlargement of the cord corresponding to the "chilaria," whilst the ganglonic enlargement from which the genital operculum is innervated should at first be more distinctly abdominal in position, and at a later period become fused with the six ganglion-pairs corresponding to the pediform appendages.

The third portion of the central nervous system of Limulus distinguished as the ABDOMINAL CORD, stretches from the œsophageal collar into the abdominal region, and gives off no nerves over a space equalling half its total length; it then enlarges and gives origin to a series of five groups of nerves, of which the first four correspond to and supply the four first pairs of branchial feet, whilst the fifth supplies not only the fifth pair of branchial feet, but also the præanal and perianal regions and the postanal spine. As to the disposition of nerve-cells in this abdominal cord we have no information, that is to say, as to whether it is possible anatomically to define separate ganglia in connection with the five groups of nerves in its hinder part, or in any region in front of them.

A very important relation between the arteries of Limulus and the main nerve trunks was first indicated by Owen (18), but more fully elucidated by Alphonse Milne-Edwards. This consists in the *ensheathing* of the œsophageal collar and of the abdominal cord in an actual arterial trunk; not only this but many of the larger nerves (those to the limbs) are ensheathed also by branches of the same arterial trunk. M. Milne-Edwards has pointed out that this arrangement is most nearly approached in Scorpio, and has recognised the remarkable agreements between the arterial system of the two animals—to which reference will be made further on—though he nevertheless is led by other considerations which are, I think, erroneous, to refuse to Limulus a position among the Arachnida.

When we compare the nervous system of Scorpio, as far as it has been made known by Newport and Metschnikoff, with that of Limulus we find portions precisely corresponding to the three main regions above distinguished in the latter animal. Anteriorly we have (A) a cerebral mass supplying the central and marginal eyes with nerves, (B) a large œsophageal collar, from which radiate the nerves to the appendages and some other parts, and (C) an abdominal cord which terminates in the fourth of the narrow præanal segments of the body.

When we look into details a little more closely we find some very obvious differences between these regions as presented in the Scorpion on the one hand and in Limulus on the other. But it must be remembered, in regard to these differences, that we have no account of the Scorpion's nerve-centres derived from the dissection of fresh specimens, nor of the actual arrangement of nerve-cells and nerve-fibres as revealed by microscopic examination.

In the first place the brain and the œsophageal collar of Scorpio are more intimately fused with one another than are the corresponding parts of Limulus. Moreover, the œsophageal collar is relatively more massive, and exhibits but a small perforation for the passage of the very narrow œsophagus. Instead of being bridged over behind the œsophagus by transverse commissures, as in Limulus, the two halves of the collar appear to be flattened out here and fused with one another. It is possible that a more accurate knowledge of this region in Scorpio might show structure representing the transverse commissures of Limulus.

A long tract of the most anterior portion of the abdominal cord in Scorpio, as in Limulus, gives off no nerves. But in accordance with the elongated form and well-marked segmentation of the hinder region of the body, we find that after this first tract there are, in Scorpio, seven well-marked ganglia placed at intervals on the cord, the most anterior of them sending off nerves to the *third* pair of lung-sacs, but to nothing in front of this.

With regard to the actual origin of nerves, it has always been stated that the first pair of appendages of Scorpio receive each a nerve from the præ-œsophageal ganglion. If this were absolutely the case it would mark a considerable difference between Scorpio and Limulus. But as a matter of fact mere inspection of Newport's drawing is sufficient to show that the nerves to the chelicerae of the Scorpion have a lateral position embracing the true "archi-cerebrum," which supplies the lateral and central eyes between them, and whatever may be the result to be obtained in the future by microscopic sections or study of fresh specimens, we have the important embryological fact due to Metschnikoff (and confirmed for other Arachnida by Balfour) that the nerve-ganglion mass from which the nerve to the chelicera on each side takes its origin is *quite independent of the archi-cerebrum,* and in the embryo is placed behind the latter, and to the side of the œsophagus right and left. This seems to me sufficient to justify a complete assimilation of the two regions in Scorpio and Limulus, the difference being merely that post-embryonic fusion of the archi-cerebrum and lateral ganglia has proceeded a little further in Scorpio than in Limulus.

From the collar, then, in Scorpio, as in Limulus, the nerves to all six of the pediform appendages take their origin. But the agreement extends even further than this, for the nerves to that region of the Scorpion's body which corresponds with the genital operculum of Limulus also proceed from the œsophageal collar. The *attraction* (if I may use the term) of nerve origins to the œsophageal collar appears to have proceeded further in the Scorpion than in Limulus, for, whereas, in Limulus, the first and remaining four pairs of branchial feet are supplied from the abdominal cord, in Scorpio those parts, which for reasons to be given below, I consider to represent the first, second, and third of the branchial feet of Limulus, all appear to receive their nerves from the œsophageal collar, so that it is not until we come to the representatives of the fourth pair of branchial feet of Limulus (viz. the third pair of lung-books, see below) that we find in the Scorpion a nerve supply from the abdominal cord. This phenomenon of the travelling forward and concentration of nerve origins and their connected ganglia is one sufficiently familiar in various groups of animals. The fact of the dislocation in this way of the nerve supply of the genital operculum of Limulus above remarked on, receives illustration by the still further carrying out of the same process in Scorpio.

The difference in the disposition of the nerve orgins (such as it is) in regard to the *hinder* part of the abdominal cord in the two animals receives its explanation from the difference of general form and segmentation of the hinder region of the body which they respectively exhibit.

It appears, then, that there is when the most recent results of anatomical and of embryological observation are taken into consideration, no important difference between the central nervous system of Limulus and of Scorpio, and more especially it is to be noted for the purpose which we have next in view, viz. that of comparing the skeleton and appendages of the two animals, that there is not a difference of origin in the large nerves supplying the appendages, or the genital or the respiratory region, which can forbid us from unreservedly accepting as exactly representing one another, parts, which on the ground of numerical sequence, appear to reciprocally correspond.

§ *b*. **SKELETON.**—I. **Tergites, or Dorsal Sclerites.**—It is difficult to separate the description of one part of the skeleton

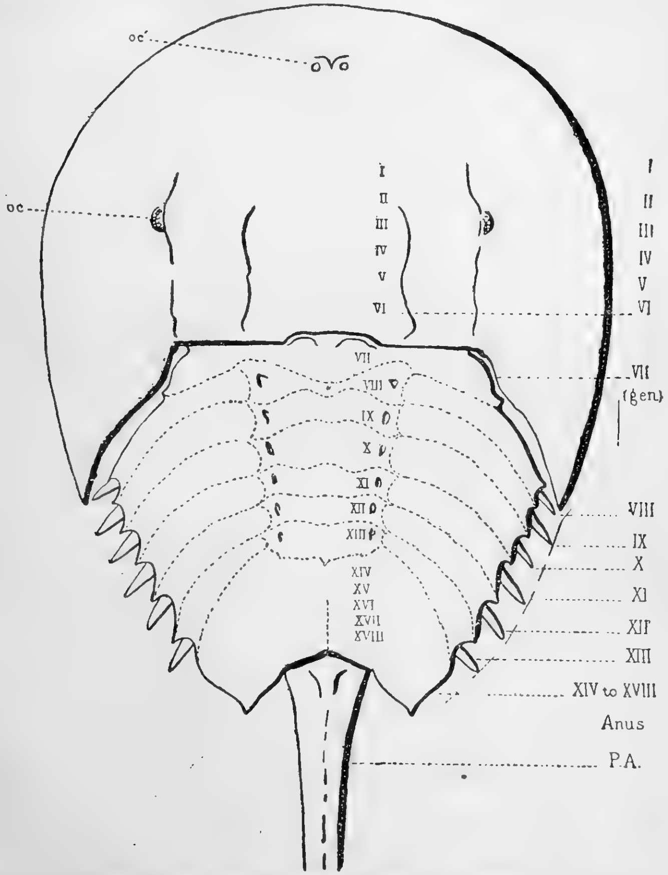


FIG. 1.—Outline of the tergal surface of *Limulus polyphemus* (drawn from the object). The dotted lines correspond to the markings on the abdominal carapace, which in the adult indicate what were separate segments in the embryo. *oc'*. Simple eyes (mesial). *oc*. Compound, or grouped eyes (lateral). *P.A.* Post-anal spine.

of Limulus and Scorpio from that of another, and in commencing with the tergal elements, we must necessarily refer simultaneously to the general disposition of the appendages.

Cephalothoracic tergites.—In Limulus (woodcut, fig. 1),[1] as in Scorpio (woodcut, fig. 2), the anterior region of the body is covered in by a large sclerite, which is known as the cephalothoracic plate or carapace.

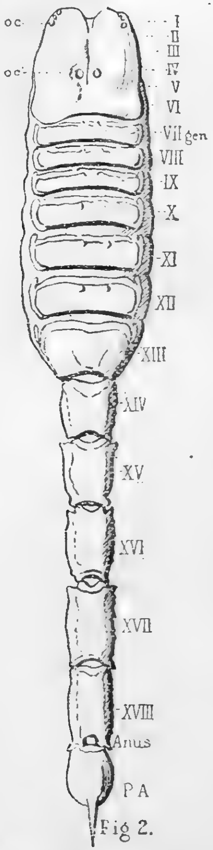


Fig 2.

Fig. 2.—Outline of the tergal surface of a scorpion, *Buthus Kochii* (drawn from the object). *oc'*. Simple eyes (mesial). *oc.* Grouped eyes (lateral). *P.A.* Post-anal spine. The anus is on the sternal surface.

In Limulus its margins are produced and its posterior angles extended, so as to produce a form which differs from

---

[1] It is necessary to state once for all that where not otherwise expressed I always allude by the term Scorpio, or Scorpion, to the species *Buthus Kochii*, of India, which happens to have been that studied by me. Other species differ in trifling details from this.

that seen in the Scorpion, but in essential points there is remarkable agreement. In both the carapace carries two paired groups of eyes. Nearer the middle line is a single pair of simple eyes (*oc′*), which in Scorpio have an almost central position; more laterally placed (quite laterally in Scorpio) is a group, on either side, of simple eyes (*oc*), which in Limulus *are so closely aggregated* as to form what is often called "a compound eye." The compound eyes of Limulus have, however, been shown by Grenacher (3) to differ very much in structure from the compound eyes of either Crustacea or Insects, to which they have usually been asssimilated. They are more correctly interpreted—as the comparison with Scorpio would suggest—as an aggregation of simple eyes. Such an aggregation (varying, according to the genus, in number from two to five) we find in a less compact form than in Limulus on the right and left side of the Scorpion's cephalothoracic tergite.

In both Limulus and Scorpio the cephalothoracic tergite covers in an area corresponding to the six leg-like appendages which are present in both animals, and may therefore be considered as representing six coalesced tergites (I to VI). In Limulus the genital operculum which follows upon the legs, and also the metathoracic sternites or chilaria which lie between it and the bases of the last pair of legs, have been by some morphologists regarded as also indicating segments which should be reckoned to the cephalothorax, and accordingly eight coalesced tergites have been supposed to constitute the carapace of the King Crab, whilst only six can be reckoned for the Scorpion. In reality, however, the chilaria are not appendages at all, as is proved by their late appearance in development (Packard, 8) and their form; they are simply sternites corresponding to the pentagonal sternite placed between the bases of the last pair of legs in Scorpio (woodcut, fig. 5). As to the genital operculum of Limulus, though in the adult it is in some measure adherent to the region of the cephalothorax, yet it has a tergal area corresponding to it in the *abdominal* carapace, and in the embryonic Limulus is clearly seen to belong to that region, and not to the cephalothorax. The innervation of the genital operculum from the œsophageal nerve-collar has, as already pointed out, no weight as an argument in favour of the association of that coalesced pair of appendages with the cephalothorax, for on the very same grounds it would be necessary to associate a large part of the middle region of the Scorpion's body (as far as and inclusive of the second pair of pulmonary sacs) with the cephalothorax.

Abdominal tergites.—Following upon the cephalothoracic plate we have in the Scorpion seven wide band-like sternites, to which succeed five narrow cylinders, the dorsal part of each of which is tergite, and solidly fused with the ventral half or sternite. In the last of these twelve segments is placed the anus (in fig. 2 its position is marked, though it is not seen on account of its ventral position), and beyond the anus is the postanal spine or sting.

In Limulus (fig. 1), in place of the seven band-like and five half-cylindrical tergites, we find one large chitinous plate, which is known as the "abdominal carapace." In its posterior region is placed the anus, and to it succeeds a postanal spine, sometimes, but erroneously, compared to the cylindrical segments of the Scorpion's body. Clearly enough the postanal spines in the two cases correspond to one another.

If there is correspondence between Limulus and Scorpio of segment for segment and piece for piece throughout (as it is the purpose of this essay to demonstrate), then in the abdominal carapace of Limulus we must find the representatives of the twelve segments, which in the Scorpion exist between cephalothorax and anus. The embryonic Limulus, as has been shown by Dohrn and Packard, exhibits in this region of the body a series of *separated* segments, which fuse together as growth advances, and constitute the one immovable abdominal carapace. In the adult the indications of the former existence of these separate segments is more obvious than has been supposed. In fig. 1 I have indicated by dotted lines the series of ridges, which can be made out in the abdominal carapace of an adult *Limulus polyphemus*, and which clearly mark off a number of the original segments.

With regard to the general form of this region as compared with the body of the Scorpion, it may be pointed out that here, just as in the region of the cephalothorax, there is an excessive development and exaggeration of the margin of the dorsal integument, so that the central area marked out in the figure is the real "body" of the *Limulus*, and the wide spreading lateral areas are only enormous excrescences of a relatively superficial character. It is not difficult to find numerous parallels to these pleural developments in all groups of Arthropoda.

Returning to the examination of the actual number of segments indicated in the abdominal carapace of the adult Limulus, we find areas corresponding to the seven wide tergites of the Scorpion marked in the drawing of *Limulus* by the numbers VII to XIII. Corresponding to these areas are a series of marginal processes, the first corresponding to the first area, is a mere angular process of the integument, but the six which follow are in the form of movable spines.[1]

Corresponding also to the six segments which bear the six spines (that is, to the six hinder segments of the seven in question) are a series of pits in the axial region of the tergum, a pair in each segment.

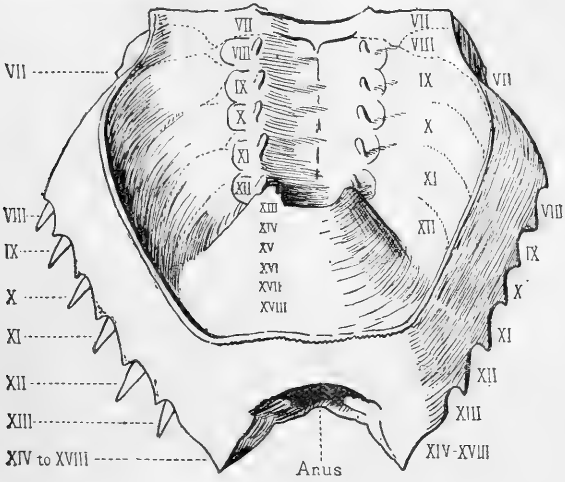


Fig 3.

Fig. 3.—View of the abdominal carapace of *Limulus polyphemus* from below, the soft sternal region and appendages of the anterior six segments and the viscera having been removed.

The figures VII to XII upon the drawing (drawn from the object) are placed by the sides of the tergal entapophyses. The continuation of the same series (XIII to XVIII) is placed upon the chitinized sternal surface of the unsegmented region, which in Limulus represents the seventh abdominal and the five cylindrical præanal segments of the Scorpion.

These are deep invaginations of the integument forming hollow processes, pushed as it were into the body cavity and clothed internally with cartilage, the structure of which has been described by Gegenbaur (2); they give attachment to muscles and are well termed "entapophyses" by Owen (7).

When we look in the abdominal carapace of Limulus for representatives of the five cylindrical præanal segments of the Scorpion, we find nothing but a broad smooth area extending from the marking which indicates the hind

---

[1] These spines I have seen slowly moving, independently of one another, in the living King Crab, indicating a separate musculature for each spine.

border of the thirteenth segment (seventh of the abdominal series) to the soft membrane which forms the hinge of the postanal spine.

In the embryo Limulus, however, this area is further segmented. We do not find the five segments of the Scorpion, but we find two of which (as segments) no indication is left in the adult, and the foremost of these carries a movable spine on each side like those in front of it.

The anterior margin of the segment or tract of the body which carries the anus appears to be uniformly in Arthropoda, and in some other segmented animals, the part from which new segments grow and become individualised, and it is to this tract of the body including its præ- and postanal regions that the name "telson" is applicable as, for example, in the Lobster. It not unfrequently happens that this segment-producing region does not produce the full number of segments in given examples of an Arthropodous class, which is characteristic of the majority or of the more fully segmented members of the class. Thus, both in Crustacea and Arachnida we find numerous forms with a reduced number of abdominal segments.[1] Usually, however, as in the spiders, the embryo exhibits at some time of its development the full complement of segments, the hindermost of which subsequently become obliterated by fusion or atrophy. Limulus so far conforms to this plan as to show the segmental potentiality of its præanal area, but fails to exhibit to the observer the full complement of segments even as a temporary arrangement of its living substance.

Accordingly the whole area posterior to the ridge marking the posterior border of the thirteenth segment may be regarded in Limulus as belonging to the "telson," or area of potential segmentation, a certain reservation being observed in respect to the one or two minute segments which appeared and disappeared in this region in the embryo.

We may, when comparing this condition of things with that exhibited by the Scorpion, *either* consider the telsonic area and spine of Limulus as representing the five cylindrical segments and the sting of the Scorpion in an unsegmented state, *or* we may insist rather upon the actuality than the protentiality, and identify the telson or fifth of the cylindrical segments of the Scorpion (viz. that carrying the anus), and the postanal spine with the telsonic area and spine of Limulus, whilst regarding the four anterior cylin-

---

[1] Note also the evanescent character of the three last segments of Thelyphonus (fig. 12).

drical segments of the Scorpion as something over and above and not developed in Limulus at all.

It seems, however, probable from the evidence of extinct forms, as well as from the abortive segmentation of the embryo, that Limulus is *not* derived from an ancestor in which the telsonic area was as limited in its production of segments as it is in Limulus itself, but on the contrary, that the ancestor of Limulus had the full complement of segments (and possibly more) which is seen in Scorpio and the Eurypterina. In that case the præanal area and spine of Limulus would not merely be an area representing the five cylindrical segments and sting of Scorpio in *potentiality*, but would be the *actual* representative of those segments gradually reduced and fused in the course of an historic process of change.

II. **Appendages.**—At each stage of the comparison between Limulus and Scorpio, the proofs of the intimate affinity of the two animals become more convincing, since we find that the view which it is necessary to adopt in order to make one set of structures agree closely in the two animals, is precisely the view which it is necessary to adopt, when a second set are considered, in order to make agreement possible.

We have just dealt with the tergites and have found an exact correspondence of piece for piece, with the exception that four præanal segments are suppressed or five fused in Limulus which are discretely present in Scorpio. In order to admit such an agreement of piece for piece as to tergites, we have to reject the view that the chilaria and the genital operculum represent segments belonging to the cephalo thoracic tergite, for in that case the cephalothorax of Scorpio would be a fusion of six, whilst that of Limulus would be a fusion of eight pieces.

When we come to examine the sternites, we shall find that the exclusion of the chilaria from the series of appendages is exactly what is required in order to identify the sternites of Limulus with those of Scorpio, and the removal of the genital operculum of Limulus from the cephalothorax makes its identity with the genital operculum of Scorpio even more obvious than it would otherwise be.

The six pairs of appendages of the cephalothorax of Limulus may be compared one by one with the six pairs of Scorpio.

Cephalothoracic appendage, No. I.—We have already disposed of the obstacle which has been always raised hitherto when the cheliceræ of the Scorpion have been

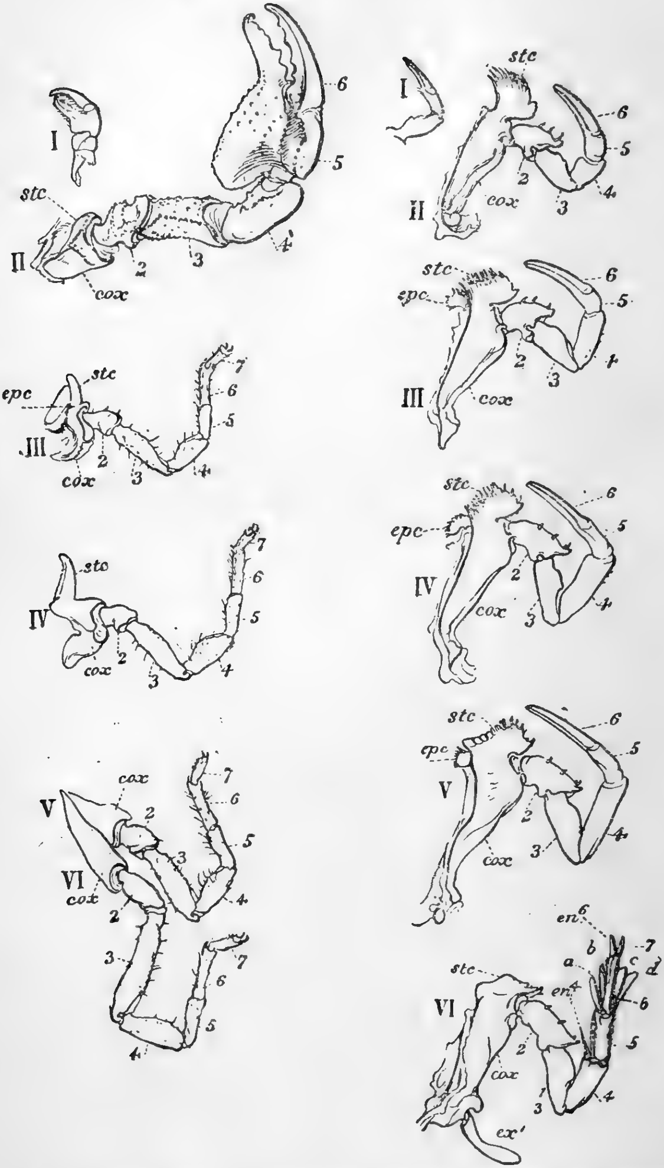


FIG. 4.—Cephalothoracic appendages of Scorpio (left), and of Limulus (right), drawn from the object. *cox*, coxa. *stc*. Sternocoxal process of the coxa. *epc*. Epicoxite. *ex'*. Exite of the coxa of the sixth appendage of Limulus. *en'*. Endite of the fourth segment of the same limb. *a, b, c, d*. Endites and exites of the fifth segment of same. *en*^{6}. Endite of the sixth segment of the same.

assimilated to the cheliceræ or first pair of limbs of Limulus. Instead of there being a difference as to innervation we have seen that there is a real identity.

In Limulus, each of the first pair of appendages is a short pair of nippers (woodcut, fig. 4, I, right) composed of three sclerites; at the base of the two appendages and between them and the mouth is placed an ovate sternite, the camerostome or upper lip. (Plate XXVIII, fig. 4).

In Scorpio (woodcut, fig. 4, I left) a similarly small pair of appendages is found similarly composed of three sclerites, and similarly overhanging an oval "camerostome."

Ceph. thor. app., No. II.—In this and the following leg-like appendages of Limulus six chief sclerites are developed, the basal one or "coxa" being much enlarged, and its interior border produced into a well-marked process provided with tooth-like hairs. The arrangement of the limbs around the mouth and the central sternite which follows it (*pmst* in Pl. XXVIII, fig. 4), is such that the processes of the coxæ of all ten limbs act together as manducatory organs. The process of the coxa may be called "the sterno-coxal process" (*stc.* in the woodcut, fig. 4). The second cephalo-thoracic appendage in the female *Limulus polyphemus* is like the third, fourth, and fifth, a chela—that is to say, the penultimate sclerite is produced so as to form with the last sclerite a pair of nippers. In the male this is not the case, the second pair of appendages being thicker and heavier than in the female, and the penultimate joint not prolonged. The form of appendage seen in the male *L. polyphemus* in this position is similar to appendages seen in other Arachnida than Scorpio, viz. Thelyphonus (woodcut, fig. 12).

The second pair of appendages in the Scorpion is like that of the female Limulus, but relatively larger. It consists of six sclerites as in Limulus, and has a sterno-coxal process on its coxa, which acts with its fellow of the opposite side as a jaw (woodcut, fig 4, II).

Cephalo-thoracic appendage, No. III.—In *Limulus polyphemus* this has, in both sexes, the same form as has the second appendage in the female. It is similarly composed of six sclerites, but in addition to these we find a distinct movable sclerite developed on the median border of the coxa. This sclerite may be termed the "epicoxite" (woodcut, fig. 4, III, *epc.*, right). The epicoxite is a remarkable feature, and is not easily paralleled among Arthropoda. The basal "endite" of the limbs of the Crustacean Apus is similar to it, and perhaps derived from a common ancestral origin.

In Scorpio the third cephalothoracic appendage is in the form of a walking leg, and as such has seven sclerites. It is a remarkable fact that in Limulus the sixth cephalothoracic appendage, which is non-chelate, also presents seven axial sclerites (woodcut, fig. 4, VI, right), so that the Scorpion's ambulatory limbs do not depart from the possibilities of Limulus in developing axial sclerites beyond the number six. It is also important to notice in this connection that the Arachnida exhibit a great variability in the number of joints present in their legs. Thelyphonus develops a four-jointed "tarsus" at the end of the five proximal segments of its ambulatory limbs (woodcut, fig. 12), whilst Galeodes presents a curious increase in the number of segments in the proximal region of its hinder limbs (woodcut, fig. 10).

The most important feature in which the third and subsequent cephalothoracic limbs of Scorpio resemble those of Limulus is in the great development of the coxæ. The sterno-coxal process is present on the third and fourth cephalothoracic appendages, and is even larger relatively than in Limulus. In the third and fourth limbs it is free, overlying a very soft minute sternal region below the mouth, and playing with its fellow of the opposite side the part of an ingestive organ for the mouth. The narrow cleft between the opposed sterno-coxal processes probably acts by capillary attraction in the taking up of such food as the blood of other animals.

The coxæ of the fifth and sixth appendages of Scorpio have, on the other hand, no free sterno-coxal process.

The great enlargement of the coxæ of these four pairs of appendages, and their encroachment upon the median area, is accompanied by, and related to, the suppression of any representative of the sternal sclerite (*pmst.*, fig. 4, Pl. XXVIII) which is present in Limulus. The coxæ of the third pair and of the fourth pair meet one another in the middle ventral line, but are separated by soft membrane. The coxæ of the fifth and sixth pairs do not meet their fellows in the middle line, but are kept apart by the wedge-shaped extremity of a sternite (*met.* in woodcut, fig. 8). They differ from the coxæ of the third and fourth pairs in that the fifth is adherent to the sixth (woodcut, fig. 4, V, VI, left.)

The base of the third appendage in Scorpio exhibits a development internal to the sterno-coxal process, which corresponds to, and probably represents, the "epicoxite" of Limulus. This is in the form of a movable plate (woodcut, fig. 4, III, *epc.*, left), which presents parallel ridges on its surface.

Cephalothoracic appendage, No. IV.—Appendage No. IV in Limulus closely resembles No. III. As in No. III, an epicoxite is present.

The corresponding appendage of Scorpio has been already mentioned. It has seven joints and a large sterno-coxal process, but no epicoxite, such as occurs in the limb next in front of it.

Cephalothoracic appendage, No. V.—In Limulus this resembles Nos. III and IV, like them having an epicoxite.

In Scorpio, No. V, is a seven-jointed ambulatory limb, with large coxa fused to the coxa of the next following appendage, but devoid of sterno-coxal process.

Cephalothoracic appendage, No. VI.—In Limulus this is the characteristic digging limb, unlike in the special modification of its parts and their remarkable function (for which see the citations of Lockwood and of Lloyd in 'Owen's Memoir,' No. 7) any other arthropod appendage.

In structure it is remarkable for exhibiting the feature of secondary movable arthrites diverging from the axis of the limb, unusual in Arthropoda other than the Crustacea. Seven axial sclerites or segments can be distinguished, the coxa being large, as in the other limbs, but devoid of an epicoxite. On the other hand, whilst the "endite" is thus absent, an "exite" is developed in the form of a flattened elongated piece articulated to the external border of the coxa (woodcut fig. 4, VI *ex′* right).

The second and third segments of the axis are devoid of apophyses, but the fourth bears a large spine-like articulated endite. The fifth joint of the axis carries four flattened apophyses (endites and exites), which are articulated and capable of active movement. The sixth joint bears one articulated endite, and, further, the short terminal seventh or ultimate segment of the axis, which is relatively much longer in newly hatched individuals than in the adult.

The sixth cephalothoracic appendage in Scorpio is quite similar to the three preceding walking legs. Its large coxa is fused to that of the fifth appendage of the same side. The spinous outgrowths on the sixth and seventh segments of this and the other legs are in character somewhat similar to the more highly developed apophyses of the digging limb of Limulus.

The seventh pair of appendages or genital operculum.—In Limulus lying between the bases of the sixth pair of cephalothoracic appendages is a pair of sclerites, the chilaria of Owen, actually the metathoracic division of the sternum (woodcut fig. 5, *st.* right), which belongs to the segment carrying the sixth pair of appendages. Precisely similar in position in Scorpio is a pentagonal[1] sclerite divided into a right and a left half by a median groove (woodcut fig. 5, *st* left upper figure). This is, in like manner, the metathoracic sternite, of which more will be said below.

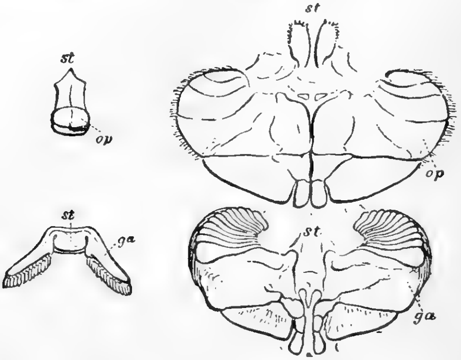


FIG. 5.—The seventh (*op*) and eighth (*ga*) pairs of appendages of Scorpio (left) and Limulus (right), together with the thoracic metasternites (*st* of the upper figures), and sternites of the eighth segment (*st* of the lower figures). The anterior face of the appendages is shown Drawn from the object.

Following in Limulus as in Scorpio upon the metathoracic sternite, is a lid-like plate, the hinge of which is transverse to the long axis of the body, and on the inner face of which are placed, both in Scorpio and in Limulus, the genital apertures, male or female, as the case may be (woodcut fig. 6, VII, right Limulus, left Scorpio).

The history of development in Limulus shows that this genital operculum starts as two independent processes of the body, which are to be regarded as the appendages of the seventh segment. The operculum retains throughout life evidence of its double origin, and closely resembles in form the five succeeding pairs of appendages which carry the respiratory lamellæ.

In Scorpio, on the other hand, the genital operculum is relatively of very small size, as seen in figs. 5 and 8 *go*; in fig. 6, it and the following appendages are drawn on an enlarged scale for the purpose of comparison with the corresponding parts in Limulus. Very little trace of having been formed by the union of two lateral appendages is exhibited by the genital operculum of Scorpio. At the same time its

---

[1] Pentagonal in the subgenus Buthus, from which my drawings and notes are taken, but more triangular and reduced in size in the subgenus Androctonus.

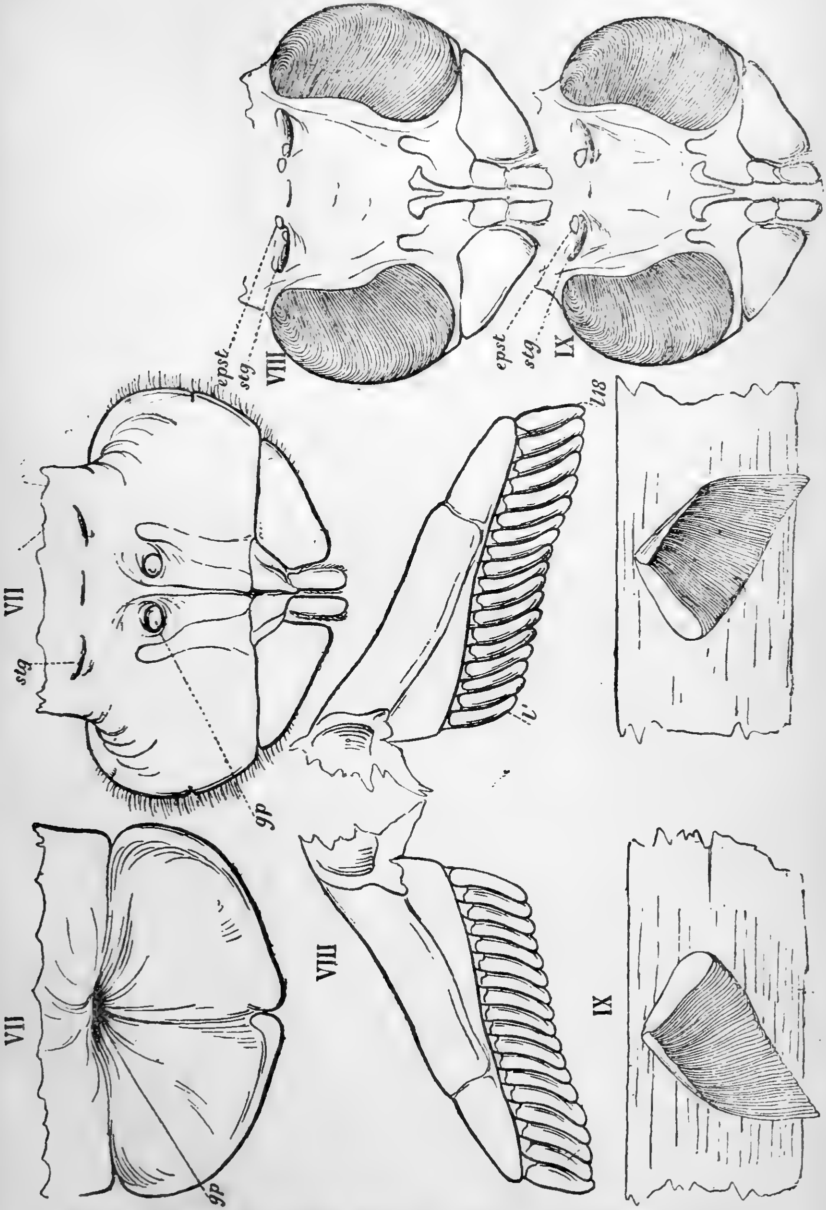


FIG. 6.—The seventh, eighth, and ninth pairs of appendages of Scorpio (left) and Limulus (right). The posterior face of the appendages is shown. *gp*. Genital pore. *stg*. Parabranchial muscular stigmata (tendons of the thoraco-branchial muscles) of Limulus. *epst*. Epistigmatic sclerites of same. Drawn from specimens.

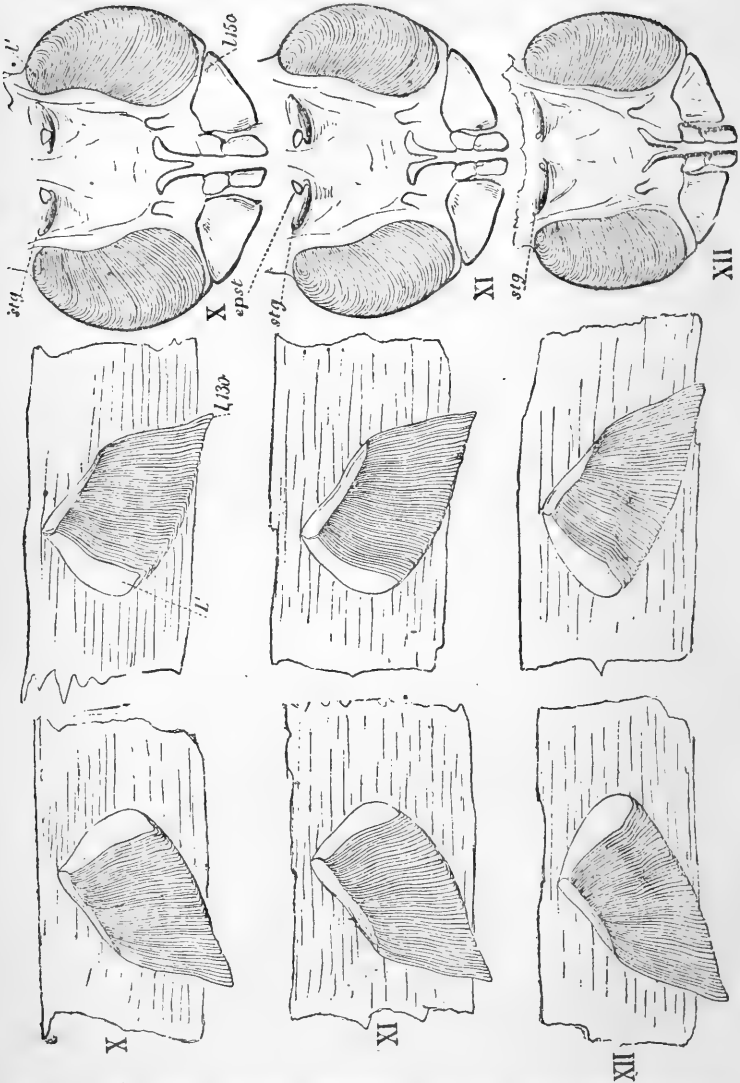


FIG. 7.—The tenth, eleventh, and twelfth pairs of appendages of Scorpio (left), and of Limulus (right). The posterior face of the appendages is shown. *stg*. Parabranchial stigmata of Limulus. *epst*. Epistigmatic sclerites. *l'*. Mediad, or first lamella of the lamelligerous appendages. *l*130. External, or one hundred and thirtieth lamella of the same in Scorpio. *l*150. External, or one hundred and fiftieth lamella of the same in Limulus. Draw from specimens. It is important to note that in these and other figures the lung-books of the Scorpion are represented as *entirely freed from the delicate pulmonary sac which invests them.*

bifid margin speaks of such an origin, and, as a matter of fact, such appears to be its embryological history.

I shall here quote a passage from 'Balfour's Embryology,' recounting Metschnikoff's observations upon the existence of rudiments of appendages in the segments of the Scorpion's body following upon the cephalothorax with its six pairs of limbs. The observations have great importance, not only in reference to the genital operculum but also in regard to the pulmonary sacs and their "branchial books" which are found in succeeding segments.

Balfour says, "Rudimentary appendages appear on the six segments behind the ambulatory legs. . . . They persist only on the second segment, where they appear to form the comb-like organs or pectines. The last abdominal segment, *i. e.* that next the tail, is without provisional appendages. The embryonic tail is divided into six segments, including the telson. The lungs are formed by paired invaginations, the walls of which subsequently become plicated, on the four last segments, which bear rudimentary limbs, and simultaneously with the disappearance of the rudimentary limbs" ('Comp. Embryology,' vol. i, p. 359).

Hence it appears that, in Scorpio, in front of the comb-like organs, that is to say, in the position subsequently occupied by the genital operculum, there is in the embryo, as in that of Limulus, a pair of rudimentary appendages. We know that in Limulus these grow together to form the genital operculum. It is in the very highest degree probable that the same history obtains for the similarly related genital operculum of Scorpio.

In discussing the tergites, it has already been pointed out that the genital operculum corresponds to a separate band-like tergite in Scorpio (VII, in woodcut, fig. 2), and to an emarginated area on the anterior border of the abdominal carapace of Limulus (VII, in woodcut, fig. 1), which is more distinctly marked in the embryo.

The eighth pair of appendages.—In Scorpio we find, on the ventral surface corresponding with the eighth tergite (six tergites being reckoned to the cephalothorax) a pair of appendages carrying fine lamellæ set like the teeth of a comb along the inferior margin (woodcuts fig. 5 *ga*, left, and fig. 6 VIII, left; see also Plate XXVIII). They are developed from the second pair of rudimentary abdominal appendages of the embryo.

In Limulus, in the corresponding position, we find a pair of appendages, the first of a series of five pairs (woodcuts fig. 5 *ga*, right, and fig. 6 VIII, right). The appendages of the two sides, as in the case of the genital operculum, do not diverge from one another but are directed towards one another and united across the middle line by a soft plate-like fold of the sternal integument; the result being that a plate-like body is formed from two originally distinct right and left appendages. On the under surface of each of the combined appendages a series of very delicate lamellæ is found *corresponding to the lamelliform teeth of the Scorpion's comb-like organs.*

Ninth, tenth, eleventh, and twelfth appendages.—In Limulus, corresponding to the tergal areas marked IX, X, XI, XII, we find a series of pairs of appendages precisely similar to that belonging to the eighth segment.

In Scorpio it will be remembered that in the embryo rudimentary appendages appear corresponding to the first six abdominal segments, or the seventh, eighth, ninth, tenth, eleventh, and twelfth of the whole body. Of these the first pair we have seen, become in all probability the genital operculum; the second pair are known to become the "pectines;" the pairs on the ninth, tenth, eleventh, and twelfth segments *disappear*, as the lung sacs on those segments develop by a process of invagination.

They disappear, but only from view. It has not been shown by actual observation, but it cannot well be doubted, that these rudimentary appendages sink within the lung-invaginations, and become the lamelligerous appendages which are found in them in the adult Scorpion.

The four pairs of stigmata on the ventral surface of the ninth, tenth, eleventh, and twelfth segments of the Scorpion's body (woodcut, fig. 8) lead into sacs, each of whch contains, concealed within it, an appendage consisting of an axis bearing a series of delicate lamellæ (woodcuts, figs. 6 and 7, IX, X, XI, XII, left).

Each of these concealed appendages is strictly comparable in structure to one of the comb-like organs of the eighth segment, the axis corresponding to the axis, and the delicate lamellæ to the teeth of the comb.

Thus, then, we find five pairs of lamelligerous appendages on the five segments of the Scorpion's body numbered 8, 9, 10, 11, 12, of which the first pair is external, and accordingly modified, whilst the next four are sunk below the surface, and accordingly modified. In Limulus, on the exactly corresponding segments, namely, those numbered 8, 9, 10, 11, 12, we find five pairs of lamelligerous appendages, but these are all external, and all alike modified for the purposes of aquatic respiration.[1]

---

[1] Latreille, though holding the Limuli to be Crustacea, and not Arachnida, was the first to insist on the branchia-like character of the Scorpion's lung-books

Furthermore, it is important to notice that in Scorpio neither in the embryo nor at any other time does the seventh abdominal segment (thirteenth of the whole series) carry a pair of appendages, nor do any of the subsequent cylindrical segments. Similarly in Limulus no appendages or rudiments of appendages are to be detected after the last pair of lamelligerous organs—the twelfth of the whole series.

The segmented region, devoid of appendages in the Scorpion, is represented by an unsegmented region devoid of appendages in the King Crab.

Before entering into a more minute comparison of the lamelligerous appendages of the Scorpion with those of Limulus, with the object of establishing the identity of origin of the two series by the detection of agreement between them in details of structure, it will be most convenient to examine another series of skeletal elements, namely, the sternites.

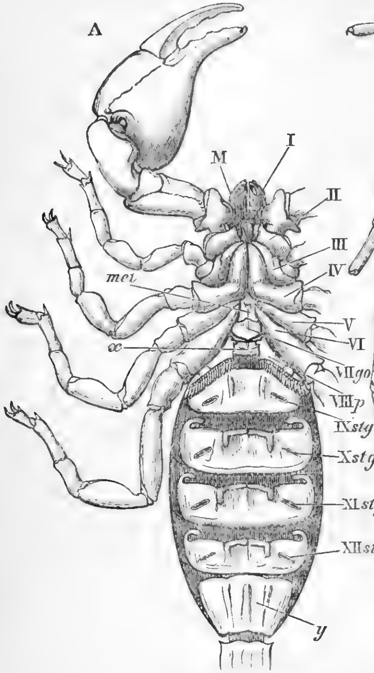
III. **Sternites.**—In Limulus, in the cephalo-thoracic region, we find that the integument of the sternal area, though to a large extent soft and devoid of hard chitinous plates, yet presents here and there well-marked sclerites. On the sub-frontal area, a small discoidal piece, the sub-frontal sclerite is found (Pl. XXVIII, fig. 4, *sf*). Between the mouth and the bases of the first pair of appendages a much more important sclerite occurs, to which the term used by Latreille for the similarly placed sclerite in Arachnida, viz. (camerostome), may be used.

In the Scorpion (fig. 8, in front of the mouth to which the line M points) a similar tubercular sclerite is found. There is advantage in not merely designating this piece "labrum," since there is but little ground for holding it to be equivalent either to the labrum of Insecta or to that of Crustacea.

In the Spider Mygale (fig. 9) and in Galeodes (figs. 10 and 11, *cam*), this same piece is observed, attaining a remarkable development in the latter.

When we come to the region behind the mouth, we find in Limulus a large median sclerite extending from the pharynx backward. It lies between the bases of the third, fourth, fifth, and sixth pairs of cephalothoracic appendages. On account of its position, it may be termed the thoracic promeso-sternite (Pl. XXVIII, fig. 4, *pmst*), since it appears to represent elements which, in other Arachnida, are marked off as distinct prosternite and mesosternite.

In Scorpio we find nothing corresponding to this piece. By the enlargement and mesiad production of the coxæ of the four hinder cephalothoracic appendages it has been as it were



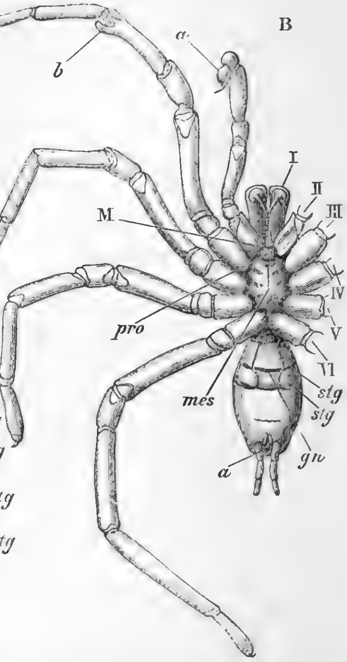


Fig. 8 (a).—Ventral aspect of a scorpion (*Buthus Kochii*), with the terminal segments omitted. Drawn from the object. i to vi. The cephalothoracic appendages. ii. Points to the sterno-coxal process of the great chelæ. iii. To the sterno-coxal process of the first walking leg. iv. To the sterno-coxal process of the second walking leg. *met.* Thoracic metasternite. vii *go.* The genital operculum. viii *p.* The pectines, or eighth pair of appendages. *x.* Sternite of the eighth segment. ix *stg,* x *stg,* xi *stg,* xii *stg.* Stigmata leading into the pulmonary sacs, containing the appendages of the ninth, tenth, eleventh, and twelfth segments. *y.* Sternite of the thirteenth segment devoid of appendages. m. Mouth, in front of which is seen the camerostome.

Fig. 9 (b).—Ventral aspect of a bird's nest spider (Mygale sp), the hairs removed. Drawn from the object. i to vi. Cephalothoracic appendages. m. Mouth, in front of which is seen the camerostome. *pro.* Thoracic prosternite. *mes.* Thoracic mesosternite. *stg.* The apertures of the two pulmonary sacs of the left side. *gn.* Genital aperture. *an.* Anus.

obliterated. A similar obliteration has taken place in Galeodes (fig. 10), but in Thelyphonus (fig. 12), a triangular sternite (*st′*) is found (though erroneously omitted in the figure given in the last edition of Cuvier's 'Regne Animal') in front of the coxæ of the fourth pair of cephalothoracic appendages.

The Arachnids, which come nearest to Limulus in the

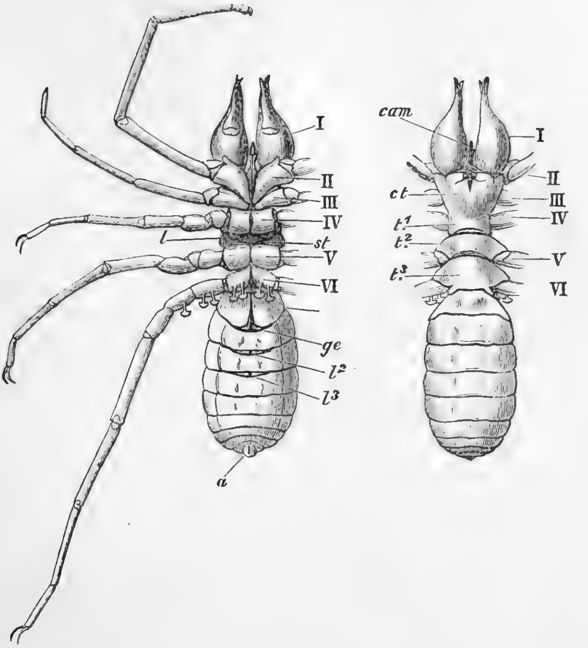


FIG. 10 and 11.—Ventral and dorsal aspect of Galeodes sp. (from the object). I to VI. The cephalothoracic appendages. *l*. Thoracic right tracheal aperture. l^2, l^3. Abdominal tracheal apertures. *ge*. Genital aperture. *st*. Sternal surface. *a*. Anus. *cam*. Camerostome. *ct*. Cephalothoracic tergite. t'. Prothoracic portion of the cephalothoracic tergite. t^2. Separate mesothoracic tergite. t^3. Separate metathoracic tergite.

character of this portion of the sternal area, are the Spiders. In Mygale (*M. avicularia*) the coxæ of the five hinder cephalothoracic pairs of appendages are arranged around a large oval sternite (fig. 9), which is divided into two portions, an anterior small prosternite (*pro*) and a larger mesosternite (*mes*). This double piece appears to correspond to the sternite of Limulus, marked *pmst* in fig. 4, Pl. XXVIII.

It is not a little remarkable that, in a structural feature observed in Limulus and *not* repeated in Scorpio nor in any Crustacean or Insect, the closest parallel should be found in another Arachnid; it is remarkable because it tends still further to determine the association of Limulus with the Arachnida in classification rather than with any other group.

Behind the thoracic promesosternite of Limulus, separated from it by soft integument and posterior to the coxæ of the

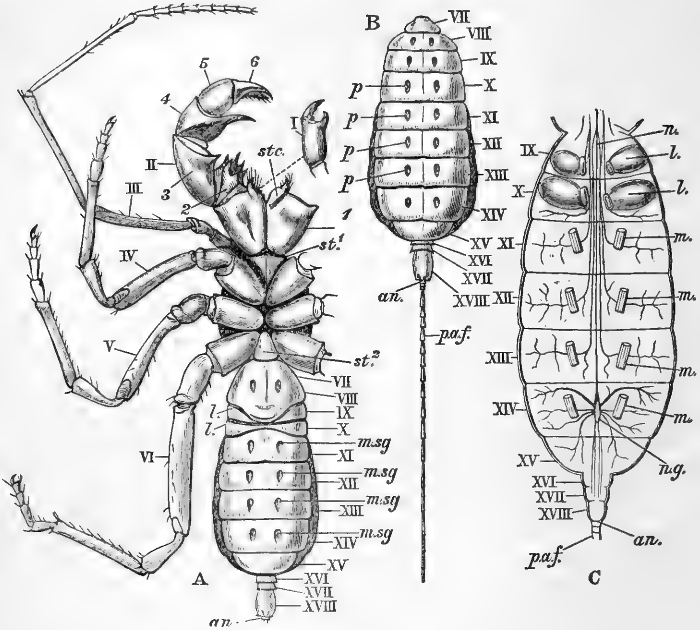


FIG. 12 (A).—Ventral aspect of Thelyphonus (from the object). I to VI. Cephalothoracic appendages; the first, which is concealed by the coxa of the second, is represented as removed from its attachment. *stc.* Sterno-coxal process of the coxa of the left second appendage. *st*^{1}. Thoracic prosternite. *st*^{2}. Thoracic metasternite. VII to XVIII. Segments of the abdomen. *l, l.* Apertures of the right lung sacs in the ninth and tenth segments. *msg.* Muscular stigmata on the sternites of the tenth, eleventh, twelfth, thirteenth, and fourteenth segments. *an.* Anus.

FIG. 13 (B).—Dorsal aspect of the abdominal segments of the same. *p.* Muscular pits corresponding to the entapophyses of Limulus. *paf.* The jointed postanal filament.

FIG. 14 (C).—Abdominal segments of the same, with the terga and viscera dissected away (after Blanchard). *n.* Nerve cord. *ng.* Abdominal nerve ganglion. *l, l.* Pulmonary sacs in the ninth and tenth segments. *m, m, m, m.* Muscles attached to muscular stigmata of the four following segments. *an.* Anus. *paf.* Postanal filament.

sixth pair of cephalothoracic limbs, we find a pair of closely opposed upstanding sclerites, the chilaria of Owen (*metst* Pl. XXVIII, fig. 4, and woodcut, fig. 4). The late development of these pieces, as determined by Packard, as well as their position, leaves no doubt that they are not to be regarded, as is supposed by some, as rudimentary appendages. They are a paired development of the metathoracic sternal area and may be designated metasternites.

They have no representative in Mygale (fig. 9), but here Scorpio returns to its allegiance and exhibits a well-developed sclerite exactly corresponding to them. The pentagonal sclerite wedged between the coxæ of the last pair of cephalothoracic limbs in Scorpio (woodcut, fig. 8, *met*) clearly enough agrees in position precisely with the chilaria of Limulus (see also woodcut, fig. 5). It is true that the form of the pentagonal metasternite of Scorpio differs from that of the two little tubercles of Limulus, but the exclusion from the functions of the mouth of the former sufficiently accounts for the difference.

In Thelyphonus (woodcut, fig. 12, *st.*[2]) a triangular metasternite corresponding in position to that of Scorpio is found.

It is exceedingly astonishing that so careful an observer as M. Alphonse Milne-Edwards should have suggested, as he has done, that the "chilaria" of Limulus correspond to the "pectines" of the Scorpion, since the former are in front of and the latter are behind the genital operculum. When the possibility of such homologies is entertained, it is but a natural consequence that the complete series of agreements of segment for segment and appendage for appendage which obtains between Limulus and Scorpio, should be entirely overlooked.

When we pass to the abdominal segments we find a very considerable difference between Limulus and Scorpio in the development of sternites.

The sternal integument of the region at the base of the genital operculum and the gill-bearing appendages, is almost entirely soft and free from sclerites in Limulus. In Scorpio, on the other hand, whilst the sternal region around the genital operculum is soft, a well-developed sternite (woodcut, fig. 8 *x*) is found supporting the pectiniform appendages; and for each of the five following segments a broad bandlike sternal sclerite is developed. The four anterior of these are perforated, each by a pair of slit-like apertures leading into four pairs of recesses, in each of which a lamelligerous appendage is sunk. The fifth is imperforate, and bears no appendage. The segments of the so-called "tail" which follow present a complete chitinisation of the integument, so that the sternites of each segment is confluent with the tergite.

When we examine the sternal area of the segments of Limulus which carry lamelligerous appendages, we find that although the integument is mostly soft and flexible, yet there are small sclerites present, *and, in fact, stigmata or apertures leading into pits corresponding to the stigmata of the pulmonary sacs of Scorpio.*

These parabranchial stigmata of Limulus have hitherto escaped observation.[1] They are found on the posterior face of the median sternal elevation or lobe which unites the two lateral elements or appendages which go to form one of the double lamelligerous organs of that animal (Plate XXVIII, fig. 10 *stg*, and woodcuts, figs. 6 and 7 *stg*). The lips of the stigma are chitinised, and the opening leads into a funnel-like cavity with chitinised walls. The sternal integument further shows one or two small sclerites, the "epistigmatic sclerites" (*epst*), by the side of the stigma. These stigmata occur in the position mentioned, not only at the bases of the appendages of the four segments corresponding to those which carry the pulmonary stigmata in the Scorpion, namely, the ninth, tenth, eleventh, and twelfth, but also at the base of the appendages of the eighth segment, which represent the pectines of the Scorpion, and at the base of the genital operculum. They are connected with the attachment of a series of powerful muscles, the thoraco-branchials, which, taking their origin in the thorax, are inserted into the integument right and left at the base of each of the six pairs of abdominal appendages. The function of these muscles is clearly enough to agitate this series of plate-like organs, either for the purpose of respiration or for that of locomotion, probably for both simultaneously.

The fact that the insertion of a muscle into the integument of Limulus is connected with a "cupping" of the area of attachment is remarkable but not without parallel. The series of dorsal entapophyses have a precisely similar significance, and in other Arachnida, *e.g.* Thelyphonus (fig. 12 *msg* fig. 13 *p*, and fig. 14 *m*), we find an identical arrangement on both ventral and dorsal surface, the stigmata being, however, much shallower than in Limulus.

I am not aware of the occurrence of such "muscular stigmata" in any other Arthropoda than the Arachnida, at any rate, of stigmata comparable to those of Limulus. Usually the tendons of muscles are in Arthropoda formed by solid fibrous extensions of the subepidermic layers of the integument.

The tendons or processes connected with the parabranchial stigmata, and with the dorsal entapophyses of Limulus, are by no means entirely formed by the invaginated epidermis and its chitinous product. The tissue below the epidermis is developed in a very special manner, and forms part of an endoskeleton which in the thoracic region gives rise to a very remarkable internal sternum or entosternite. The struc-

---

[1] I communicated an account of their occurrence and probable significance to the Royal Society on May 26th, 1881.

ture of this deep skeletal tissue has been investigated by Gegenbaur, who has shown that it may have the form either of a fibrous or of a more distinctly cartilaginous modification of the connective tissue into which it gradually passes, and from which, on the other hand, is developed in other regions a series of vascular channels constituting the capillaries, veins, and arteries. On the present occasion I do not propose to enter into histological details with regard to Limulus, but I may just mention that whilst the hollow entapophyses are invested on their visceral surface by a richly developed cartilaginous modification of the connective substance, with a well developed capsular arrangement of the intercellular substance, the funnel-like invaginations connected with the parabranchial stigmata are clothed and continued by a fibrous tissue not unlike the tendon of Vertebrata. The same tendon-like tissue also forms the entosternite.

In Plate XXVIII, fig. 11, the internal connection of the pair of parabranchial stigmata of a lamelligerous appendage-pair of Limulus is drawn. The integument has been dissected away from the whole of the anterior face of the appendages and their uniting sternal bridge, so as to show the inner aspect of the integument of the posterior face. The pouch-like character of the invaginations into which the stigmata lead and the attachment of the thoraco-branchial muscle is thus exhibited. In fig. 13, Pl. XXVIII, one of the funnel-like tendons, consisting internally of chitin borne on epidermis, and externally of fibrous tissue, is shown in an isolated condition. It is possible to introduce a probe into the funnel to the depth of an inch, the axial cavity of invagination extending to that distance. The funnel-like pouch of Limulus thus constituted, I consider to be the homologue (that is, the genetic representative or homogen) of the pulmonary sac of Scorpion.

It will now be convenient to give, in a tabular form, a summary of the view which has been set forth in the preceding pages. Having thus exposed what I conceive to be the legitimate conception of the morphological relations of Limulus and Scorpio, I shall endeavour to justify, by a closer examination, the identification (which forms an essential part of it) of the pectines of the Scorpion and its four pairs of book-like organs sunk in recesses of the integument with the five pairs of lamelligerous appendages of Limulus.

(The tabular statement is given on the next page.)

Segments.	LIMULUS.			
	Tergites.		*Sternites.*	*Appendages.*
1	Cephalothoracic carapace with central and peripheral eyes.		Camerostome (small tubercular sclerite) in front of the mouth.	Small chelæ.
2			The fused pro- and mesothoracic sternites (a narrow elongate sclerite stretching from the mouth to the chilaria).	Chelæ.
3				Chelæ.
4				Chelæ.
5				Chelæ.
6			The chilaria or paired metastoma, or metathoracic sternites.	Digging legs.
7	Narrow emarginate area at the anterior border of the abdominal carapace. No dorsal pits.	Abdominal carapace.	Soft integument and stigmatic pits (muscular), posterior to base of operculum.	Genital operculum.
8	1st pair of lateral spines. 1st pair of dorsal pits and entapophyses.		Epistigmatic pair of sclerites and stigmatic pits.	1st gill-book pair projecting.
9	2nd pair of lateral spines. 2nd pair of dorsal pits and entapophyses.		Epistigmatic pair of sclerites and stigmatic pits.	2nd gill-book pair projecting.

Segments.	SCORPIO.		
	Appendages.	*Sternites.*	*Tergites.*
1	Cheliceræ.	Camerostome (of Latreille), or upper lip.	Cephalothoracic carapace with central and peripheral eyes.
2	Chelæ.	Obliterated by the mesiad extension of the coxæ of the four walking legs; the two anterior movable, the two posterior fixed. (In Mygale a distinctly marked small prothoracic sternite is followed by a large oval mesothoracic sternite.)	
3	Walking legs.		
4	Walking legs.		
5	Walking legs.		
6	Walking legs.	Pentagonal elongate sclerite or metathoracic sternite.	
7	Genital operculum.	Soft integument.	A separate narrow band-like sclerite.
8	Pectinæ, or pair of comb-like organs; modified gill-book projecting.	Separate small rectangular sclerite.	A separate narrow band-like sclerite.
9	1st gill-book pair sunk in pulmonary sacs.	A separate broad transverse sclerite with stigmata leading to pulmonary sacs.	A separate band-like sclerite.

Segments.	LIMULUS.			
	Tergites.		*Sternites.*	*Appendages.*
10	3rd pair of lateral spines. 3rd pair of dorsal pits and entapophyses.	Abdominal carapace.	Epistigmatic pair of sclerites and stigmatic pits.	3rd gill-book pair projecting.
11	4th pair of lateral spines. 4th pair of dorsal pits and entapophyses.		Epistigmatic pair of sclerites and stigmatic pits.	4th gill-book pair projecting.
12	5th pair of lateral spines. 5th pair of dorsal pits and entapophyses.		Epistigmatic pair of sclerites and stigmatic pits.	5th gill-book pair projecting.
13	6th pair of lateral spines. 6th pair of dorsal pits and entapophyses.		Large and solid sclerite forming the sternum of the "Telson," *i.e.* of the præ-anal region of potential segmentation, which includes a soft invaginate area on which opens the ANUS.	None.
14	Only in the embryo this segment is separate, and has a 7th pair of lateral spines.			None.
15	Only in the embryo this segment is indicated.			None.
16	These three segments are never expressed and are represented by the præanal region of the telson.			None.
17				None.
18				None.
	Post-anal spine.			

Segments.	SCORPIO.		
	Appendages.	*Sternites.*	*Tergites.*
10	2nd gill-book pair sunk in pulmonary sacs.	A separate broad transverse sclerite with stigmata leading to pulmonary sacs.	A separate band-like sclerite.
11	3rd gill-book pair sunk in pulmonary sacs.	A separate broad transverse sclerite with stigmata leading to pulmonary sacs.	A separate band-like sclerite.
12	4th gill-book pair sunk in pulmonary sacs.	A separate broad transverse sclerite with stigmata leading to pulmonary sacs.	A separate band-like sclerite.
13	None.	A separate broad transverse sclerite devoid of stigmata.	A separate broad band-like sclerite.
14	None.	Ventral half of a distinct cylindrical sclerite.	Dorsal half of a distinct cylindrical sclerite.
15	None.	Ventral half of a distinct cylindrical sclerite.	Dorsal half of a distinct cylindrical sclerite.
16	None.	Ventral half of a distinct cylindrical sclerite.	Dorsal half of a distinct cylindrical sclerite.
17	None.	Ventral half of a distinct cylindrical sclerite.	Dorsal half of a distinct cylindrical sclerite.
18	None.	Ventral half of a distinct cylindrical sclerite, in which is placed the ANUS.	Dorsal half of a distinct cylindrical sclerite.
			Post-anal spine or sting (a jointed filament in Thelyphonus).

IV. **The common characters of the lamelligerous appendages of Scorpio and Limulus.**—When we have once, on the ground of a certain general agreement in structure and of a definite identity in relation to other parts which correspond one to another, started the hypothesis that the lamelligerous appendages of the Scorpion agree each to each in their order with the lamelligerous appendages of the King Crab, two further proceedings are naturally the consequence. We inquire first of all whether there are any less obvious agreements in the structure of the organs compared which may be brought out and made to give their testimony in favour of our hypothesis, and, secondly, we inquire how can we form a plausible conception of the origin of the two sets of structures from one set of organs present in a common ancestor of Limulus and Scorpio? this last inquiry having especial value, in that it may lead us to give due value to structures present either in Scorpio or Limulus which had appeared previously to have no special significance in the matter.

A close comparison[1] of the lamelligerous appendages of Scorpio and Limulus—including under this head the pectines and the pulmonary books of the former, and the branchial books of the latter—reveals the important fact that they agree closely with one another in the mode in which the lamellæ are set upon the supporting axis.

In all, we find an axis springing from the body wall, transverse to which, on its posterior face, are set a series of lamellæ. In order to compare one of these appendages with another, it is necessary that all should be placed in one and the same position. We must be careful not to compare the anterior aspect of one with the posterior aspect of the other. In the woodcuts, figs. 6 and 7, the posterior face of the appendage as it hangs from its sternal attachment has been represented.

There is no difficulty about determining this face for the pectines of the Scorpion or for the branchial appendages of Limulus, but the pulmonary books of the Scorpion require some consideration. Supposing them to have once been external, we must suppose that, with the gradual invagina-

---

[1] The account which I give in the text of the lung-books of Scorpio differs a good deal from that which is current, due to Joh. Müller as long ago as 1828. I have not had specimens sufficiently well preserved to enable me to determine the relation and possible adhesions of the proper wall of the pulmonary sac (the invaginated sternal surface) to the lamellæ, but have freed the appendage from the investing membrane. I hope to be able by the examination of fresh specimens to give on a future occasion a more thorough account of the pulmonary sacs and lamelligerous appendages of the Scorpion.

tion of their surface of attachment, they have become more and more deflected into the cavity of invagination, moving on their fixed base at first backwards, then upwards, and finally forwards. As we now find them (in a spirit specimen!), on viewing the inner surface of the ventral sclerites by removing the terga and viscera, they can be rotated on their hinge line so that they may be made to lie prone forwards, exposing the stigma or opening of the pulmonary recess posteriorly, as in Pl. XXVIII, fig. 1 *a*, or they may be made to lie prone in the reverse direction, hiding from view the stigma, as shown in Pl. XXVIII, fig. 2 *a*, and in the woodcuts, figs. 6 and 7. The position which corresponds with that of the external appendages the pectines and the branchial organs of Limulus, when viewed from the posterior face, so as to show (in the case of Limulus) the lamellæ, is that in which the lung-book is directed backward so as to hide the stigmatic aperture and is looked at from within the Scorpion's body, that is, by dissecting off the terga, viscera, and muscles.

When the pectines, lung-books, and branchial books are thus placed we find that the lamellæ are not set precisely at right angles upon the axes, but obliquely, so that there is an imbrication of the successive lamellæ. In all three it is the posterior lamella which is uppermost (see Pl. XVIII, fig. 2 and [illegible] fig. 10 *l′*). *The imbrication is identical in all.*

As to *number* of lamellæ, we find in the pecten of *Buthus Kochii* eighteen (in other scorpions there are more or less); in the lung-books of some scorpions[1] as many as 130, and in the Limulus gill-book as many as 150. These numbers vary slightly, increasing with growth in all probability.

As to *structure* of lamellæ, those of the pecten are more solid and strongly chitinised than those of the other two organs, but are, nevertheless, true lamellæ flattened transversely.

Those of the lung-books are exceedingly delicate plates composed of two closely approximated membranes, between which the blood circulates; they are, in fact, flattened bags. They carry on their free margins a few chitinous spinules (Pl. XXVIII, fig. 8). The lamellæ of the gill-books of Limulus are similarly delicate flattened bags with a setose free border. I am not able to institute any comparison of the histological structure of the lamellæ of the Scorpion's lung-book with that of the King Crab's gill-book, for although I have been able to work out that of the latter on

---

[1] I believe the form in which I counted these to be a species of Androctonus.

fresh material, no such opportunity has yet presented itself of investigating the Scorpion.

As to the *shape* of the lamellæ, those of the pecten are narrower and relatively thicker than those of the lung-books or gill-books; the whole eighteen are also more nearly equal to one another in size and shape. In the lung-books the shape differs at the two extremities of the series a little, and in size the proximal laminæ are much larger than the distal. The average shape may be described as that of a broad scythe-blade (Pl. XXVIII, fig. 8) with a narrow base support (*a b*). The lamellæ of the gill-books of Limulus, on the other hand, are approximately semicircular in shape, with a wide base of origin (*a b* in fig. 9, Pl. XXVIII). Moreover an important difference, which is explained by the convergence in place of divergence of the axis of the limb relatively to the mid line of the body, is seen when the lamellæ of the gill-book and of the lung-book are compared, in the fact that in the gill-book the proximal laminæ are the smallest (Pl. XXVIII, fig. 10 *l′*), whilst in the lung-book they are the largest.

Further comparison of the grouping and form of the lamellæ is facilitated by the figures on Pl. XXVIII, where fig. 1 and fig. 1 *a*, fig. 2 and figs. 2 *a* and 2 *b*, fig. 3 and figs. 3 *a* and 3 *b*, give representations of the three varieties of lamelligerous appendages in a series of identical positions. Fig. 1 should, for comparison with fig. 1 *a*, be looked at by inverting the plate.

The axes which support the lamellæ in the three varieties of lamelligerous limb differ much from one another, but in a manner directly corresponding with obvious functions.

The pecten has a large free axis firmly chitinised, imperfectly divided into two joints. It is flattened by antero-posterior pressure. The function of the pecten is not actually known, but it appears to be tactile. It is not respiratory, and the Scorpion is of terrestrial habit; hence its comparatively solid character and protective development of chitin.

The gill-book of Limulus is supported on an axis, which is flattened by dorso-ventral pressure, protection being thus afforded to the otherwise naked and very delicate lamellæ. It is not free except at its extremity, where it exhibits a jointing of separate chitinous plates. Its base is very wide, and is attached, not to a flat sternal surface, but to an outstanding sternal lobe, which extends between the bases of fellow-appendages, and gives rise to a teat-like soft process in the median line (Pl. XXVIII, *m d*, fig. 10). The character of this axis is obviously an adaptation to the branchial function of the lamellæ combined with a locomotor function.

The lung-book of Scorpio has no locomotor function, and it is protected by the recess of the sternum, in which it lies. It is not tactile, nor is it exposed to desiccation and rough usage, as are the pectines. It is specialised for respiratory purposes. The axis is exceedingly small and simple, for the greater part of its length adherent to the invaginated sternal wall, leaving, however, a small free distal portion (see Pl. XXVIII, fig. 2 *a*). Its walls are quite free from chitinisation, and of great delicacy. It is little else than a horizontal vascular tube supporting the lamelliform bags into which its cavity leads (Pl. XXVIII, 2 *a*, *b*).

Though the axis is here reduced to its simplest expression, it is not possible to overlook in it the representative of the vertically compressed chitinised axis of the pecten, and of the horizontally compressed chitinised axis of the gill-book.

7. Hypothesis as to the mode of origin of the three varieties of lamelligerous appendages in Scorpio and Limulus.—The view which I have advanced in this memoir as to the practical identity of the gill-books of Limulus and the lung-books of Scorpio implicitly contains the affirmation that either the structures of Limulus have been derived from those of Scorpio, or those of Scorpio from those of Limulus, or that a third (now extinct) form has given rise to both Limulus and Scorpio. Further, it is to be observed that such extinct form might be more like to Limulus than to Scorpio, or *vice versâ,* in respect of any particular element of structure.

To make a long story as short as possible I may say that, without prejudicing the recognition of the (as I think) well-established morphological identities above pointed out, we may best explain their existence by assuming that an aquatic form breathing the dissolved oxygen of the water inhabited by it, by means of book-like gills, was the common ancestor of Limulus and of Scorpio. From the book-like gills of this ancestral form the broad series of Limulus and the narrower lung-books, *as well as the pectines or combs* of the Scorpion, have been derived. The form of the book-like gills of this Arachnidan ancestor was probably something intermediate between the three existent modifications of it, and best conceived of, perhaps, by imagining the teeth of the Scorpion's "pectinate organs" to become soft and flattened and increased in number (see Pl. XXIX, fig. 1).

To obtain from these the Limulus gill we have but to suppose certain definite changes of dimension, the imbrication and character of the lamellæ, and their external position remaining unaltered (Pl. XXIX, figs. 2 *a* and 3 *a*).

To arrive at the book-lungs of the Scorpion, we have to imagine the ventral surface on each side in close proximity to the short appendages carrying the gill-books—to have become deeply cupped or depressed, so that two series of cup-like pits should be formed, a right and a left, a pair being placed in each segment, corresponding to each pair of gill-books. Each cup must have become so large in area and so deep as to embrace within its limits the relatively small adjacent gill-book (XXIX, fig. 2 *b*). Further, when once the gill-book had been involved in this cup-like depression, the walls of the cup must have tended to grow together so as to form a pulmonary chamber with only a narrow slit-like opening to the exterior (Pl. XXIX, fig. 3 *b*), and *pari passu* with this closing in of the cupped area, and the protection of the respiratory lamellæ, the Arachnid must have acquired the power of leaving the water and of breathing the atmospheric oxygen admitted to the damp chamber formed by the cave-like areas of depression.

Whilst framing such a hypothetical account of the way in which the transition from naked "gill-book" to insunken "lung-book" could have taken place, one naturally asks—"Is it not somewhat gratuitous to assume that cupped areæ should form conveniently alongside of the gill-books of the aquatic ancestor, so as to be ready to increase in size, and ultimately draw into themselves, as it were, the gill-books?" "Is there," we are led on further to ask, "any known instance in Arachnida of the formation of cupped areæ on the chitinous surface of the body? If so, can we show in what mechanical relation they are formed? And, lastly, can it be demonstrated that such mechanical relation probably existed in connection with the gill-books of the assumed common ancestor of Limulus and Scorpio?" If all these questions can be affirmatively answered, then our hypothesis as to the transition of the aquatic Arachnid to the pulmonate condition acquires great plausibility.

The answer to these questions appears to me to have more than ordinary interest, since the formation of cupped areæ on the chitinous surface of the body and the mechanical relations connected with their formation have, as pointed out a few pages back, come to light as demanded by the hypothesis. They exist in Limulus itself and in Thelyphonus. In Limulus there are two great muscles, a right and a left, inserted into the soft ventral integument near the base of each double gill-plate. These muscles serve (together with others that enter the appendage itself) by their contractions to move the gill-plates in the water and so aid in aquatic respiration. The position of the insertion of each muscular mass is marked by a deep funnel-like depression of the integument. From the external surface this depression appears as a "stigma," which we have already described as the parabranchial stigma. The funnel-like depression has a narrow mouth which is often as much as half an inch in length. Internally the invaginated cuticle stands up as a flexible tendon clothed with fibrous tissue and giving attachment to the muscle already mentioned.

In Limulus we find a pair of these "muscle-stigmata," right and left behind the genital operculum, and a pair (right and left) behind each of the lamelliform fused appendages which carry the gill-books.

We have only to suppose the appendages carrying the gill-books *not* to have fused as yet in the middle line, and the muscular stigmata to have become greatly developed (perhaps by increased development of the muscle aiding in aquatic respiration when the appendage itself grew small and therefore less efficient) and we have at once the gill-book sinking within the area of the stigmatic pit, Pl. XXIX, fig. *2 b*.

A very important feature in the supposed further development is the correspondence of the atrophy of the muscle (which atrophy is required to fit in with our hypothesis, and to convert the muscle-pit into a pulmonary sac) with the changes in the structures which would necessarily result were the physiological conditions gradually to become such as to favour aërial in place of aquatic respiration. The violent agitation of the gills by the muscle attached to the stigmatic pit would become useless, supposing an exposure of the gill-lamellæ to the atmosphere became by degrees habitual with the ancestral Arachnidan. In proportion as these hypothetical creatures acquired the habit of aërial repiration—the deepening and arching in of the stigmatic pit would be favoured, and the atrophy and final disappearance of the muscle which was attached to its inner surface, and mechanically brought it into existence, would also be directly promoted.

A further confirmation of the view now advanced is found in the remarkable East Indian Arachnid Thelyphonus. This Arachnid has not four pairs of lung-sacs like Scorpio, but only two pairs, corresponding to the two foremost lungs of

Scorpio, and to the second and third gill-book-pairs of Limulus (woodcut, figs. 12, 13, 14). Nevertheless, as we have seen in a previous section of this Essay, the *four* segments of the abdomen posterior to these are *each marked by a pair of shallow stigmata placed in line with the orifices of the pulmonarg sacs of the two anterior segments, msg.* When the internal structure corresponding to these parts is examined, it is found that a large muscle (similar to the similarly placed muscle of Limulus) is inserted into each of the four right and four left stigmata in the segments posterior to the pulmonary sacs (woodcut fig. 14, *m.*) The two segments into which the two pairs of pulmonary sacs are sunk, have *no such muscles.* The pulmonary sacs are, therefore, to all appearance, enlarged muscular stigmata, from which their former muscles have disappeared by disuse and atrophy.

VI. **The Entosternite.**—Leaving now the comparison of segments and appendages, which is undoubtedly the most important element in determining our judgment as to the affinity of Limulus with Scorpio, we come to the consideration of a number of other structures, which we shall find some more and some less favorable to the hypothesis of a close relationship between the two animals.

Connected with the exoskeleton and locomotor system is that remarkable development of an endoskeleton in the Arachnida, which Straus Durkheim put forward in the passage cited at the commencement of this article, as one of the leading characteristics of the class and one of the obvious features in which Limulus shows itself to be a true Arachnid.

As already remarked, in speaking of the entapophyses and parabranchial stigmata, Limulus shows a marked tendency to the development of cartilage and fibro-cartilage by the modification of its connective substance at certain points and in certain areas.

The most striking result of this tendency, is the formation of a large fibro-cartilaginous plate which lies in the cephalothoracic region between the alimentary canal above and the nerve collar below, and unconnected by hard parts with any portion of the exoskeleton. It is represented of the natural size as seen from the ventral (sternal) aspect in fig. 7, Pl. XXVIII. It has been previously figured by Van der Hoeven (12), whose figure is not very accurate, and is copied by Owen (7).

This body is the base of origin of a large number of muscles, and may be regarded as an enlargement and interlacing of their respective tendons. In the figure, nothing but the skeletal structure, cleaned of its muscles, is represented.

In Scorpio, a similarly shaped loose skeletal piece is ound, which gives attachment to muscles in the same way and has a similar relation to the ventral nerve-mass and artery, by which in Scorpio it is perforated (Pl. XXVIII, figs. 6 and 6 *a*). The corresponding body in Mygale is (Pl. XXVIII, figs. 5, 5 *a*) more closely similar in form to that of Limulus than is that of Scorpio.

In order to make a close comparison of these Entosternites, it will be necessary to determine exactly the insertions of the muscles to which they give origin; and further, to ascertain how far the histological structure of those of Scorpio and Mygale agrees with that of Limulus. The results of this investigation I hope to make the subject of a future publication. In the meantime the close correspondence in general character of the three Entosternites figured on Pl. XXVIII cannot escape notice, and fully justifies the importance which Straus Durkheim attached to them. The two pairs of tendinous outgrowths right and left of the central plate in Limulus correspond with the three pairs seen in Mygale, whilst the deep anterior notch in the latter corresponds with the shallower excavation in Limulus, in which the number 7 is placed in the drawing, and in which in the animal itself the bend of the alimentary canal is placed, the mouth being actually below the central region of the plate, so that the alimentary canal passes first forwards beneath the plate and is then reflected so as to pass backwards whilst resting on the upper surface of the plate.

Whilst of this as of so many other structures of the Arachnida (such as the lung-books, &c.) which have been compared in the present memoir with structures in Limulus, a renewed and critical examination is absolutely needed, yet we have sufficient ground, even in our present incomplete knowledge, for concluding that the agreement as to them presented by the two animals is a very close one.

In no Crustacean is a free entosternite at all similar to the organ under discussion known. The apodemes of the sternal surface of Decapodous Crustacea do not resemble it in form though of a similar function. The nearest approach to it is seen in the rod-like skeletal organ found in the abdomen of Lepidoptera, and described by Leydig ('Bau des Thierischen Körpers,' Atlas, pl. vi, fig. 1). Its shape and position are very different, however, from the entosternites of Limulus and other Arachnida. It agrees with these only so far as that it is a free internal skeletal piece.

In the abdominal region of Limulus small loose fibro-cartilages, similar in nature to the Entosternite, are found, connected with the tendons of muscles. I have isolated four such pieces. They are mentioned by Straus Durkheim.

(*To be continued.*)

MEMOIRS.

The ORGAN *of* JACOBSON *in the* RABBIT. By E. KLEIN, M.D., F.R.S., Lecturer on Histology and Embryology in the Medical School of St. Bartholomew's Hospital. (With Plates XXX and XXXI.)

IN continuation of the research of the organ of Jacobson in mammals, that of the guinea-pig having been described in the January and April numbers, 1881, of this Journal, I now propose to give the results of the minute examination of this organ in the rabbit. The differences in the microscopic anatomy of Jacobson's organ in these two rodents are very great indeed, much greater than could be anticipated. That of the rabbit in many respects comes very close to the ideal type which Gratiolet[1] draws and describes, and is also similar to the organ in the sheep as described by Balogh.[2] The points of resemblance between that of the rabbit and the sheep and the points of dissemblance between that of the rabbit and the guinea-pig will be fully discussed below.

In describing the organ of the rabbit, we shall take note, as we did in the guinea-pig, of the surrounding parts, in the region of the organ of Jacobson, since all the parts constituting the organ of Jacobson bear an intimate relation to the tissues around them.

In the rabbit, the facial part of the head being longer than in the guinea-pig, it naturally follows that the nasal septum, and consequently also the organ of Jacobson, are of greater length in the former than in the latter animal. When fully exposing the front part of the septum nasale, from the dorsum of the nose to the lower nasal furrow, by removing the lateral wall of the nose as well as the lower concha or lower turbinated bone—which by its very beautiful plication contrasts in a conspicuous manner with the comparatively smooth lower concha of the guinea-pig—the

---

[1] 'Recherches sur l'organe de Jacobson,' Paris, 1845, p. 19, and *passim*.

[2] 'Das Jacobson'sche Organ des Schafes, Sitzungsb. d. K. Akad. d. Wiss.,' xlii, p. 449.

organ of Jacobson is easily perceived as a bilateral tubular bulging, extending in the septum along the lower nasal furrow.

The outer wall of each organ or tube, *i.e.* the one actually seen—the other, or median wall, being hidden in the septum—is marked by its rich supply of large blood-vessels, constituting, as will be mentioned below, the cavernous layer of the tubes.

The length of the tubes in a medium-sized rabbit is from 1·5 to 2 centimètres, and already with the unaided eye it can be ascertained that each tube rather sharply tapers towards its anterior and posterior extremity.

Making transverse sections through the front part of the nasal organ, the following cartilages are met with :—

1. The cartilage of the septum. This is hyaline cartilage, staining deeply in logwood, except the part next to the dorsum of the nose, this part being triangular in section, and not staining well in logwood. Between the two divisions there is this great difference : that in the former the cartilage cells are very much closer in position than in the latter, they being here arranged more distinctly in groups of two, four, six, or eight cells, separated by relatively large masses of hyaline ground substance. In logwood-stained specimens the hyaline matrix, as well as the cells, are very slightly stained in the upper part, whereas they assume a deep colour in the lower part of the septum. In both divisions, but especially in the upper one, the cartilage cells contain in their substance several small oil globules or one large oil drop. Next to the perichondrium, the cartilage cells are small, flattened parallel to the surface, whereas in the centre of the cartilage the cells, or rather their groups, are arranged more or less transversely ; a similar distinction, as is well known, F. E. Schultze,[1] has noticed in the bronchial cartilages.

The close position of the cells in the lower part and the deep staining of this latter, appear to indicate that a more active growth is taking place in it than in the upper portion of the septal cartilage.

This cartilage increases in height and thickness, especially its lower margin becomes conspicuous by its enlargement, as we pass backwards in the region of the organ of Jacobson.

2. The cartilage of the lateral wall. A transverse section made in the region of the incisors, shows us on each side in the lateral wall of the nasal cavity, rising from the lower nasal furrow, and forming the support of that wall, a cartilage plate, extending with a somewhat broad projection into the front or smooth part of the lower concha. In connection with it is a thin cartilage plate, extending laterally and upwards in a curved manner. In fig. 1 these cartilages are well shown. The first, *i.e.* the one

---

[1] 'Stricker's Manual of Histology,' article "Bronchi and Lung."

forming the support of the lateral wall of the nasal furrow, will be spoken of as the lower limb; the second, *i.e.* the one forming the support of the front part of the lower concha, as the upper limb; and the third, *i.e.* the one extending outwards and upwards, as the lateral limb. In a section anterior to fig. 1, the lower limb, shown in fig. 1, is not present yet, but outside it is a small cartilage plate coming off from the lateral limb and extending a short way downwards. Where the other limbs join the lower limb, this latter has its thickest diameter. The upper and lateral limbs increase also in thickness towards their upper margin.

As regards the structure of these different parts, the same distinction can be drawn as in the septal cartilage, viz. the upper portions of both the upper and lateral limbs stain much less in logwood, and the cartilage cells are relatively fewer than in the lower parts.

In a preparation in front of the one of which fig. 1, is a representation, the three limbs of the above cartilage form one continuous whole; but in the preparation shown in fig. 1 there is already a discontinuity noticed, at any rate on one side, between the upper and lower limb, as well as between the latter and the lateral limb. Going a little further back, *e.g.* fig. 1, we notice not only a permanent discontinuity between the two last-named limbs, but we perceive that the upper limb of the cartilage becomes greatly reduced in height, being now represented only by its upper less stained portion, soon to disappear altogether, and to be replaced by the rudiments of spongy bone, forming now the support of the plicated lower concha. While this happens with the upper limb, also the lower limb undergoes considerable changes, viz. it increases in height and thickness, and its lower margin, while greatly expanding in breadth, curves downwards and inwards, in the manner of a trough or hook, but so that the nasal furrow is now forming the lining of its (*i.e.* the trough's) concavity. In fig. 2 this so changed lower limb is accurately delineated.

It is here seen that the trough consists of an inner or median labium, terminating close to the lower margin of the septal cartilage with a pointed extremity and an outer or lateral labium continuous with the lower limb of the cartilage above mentioned. From the convex surface of the trough a short cartilage extends downwards into the upper maxillary bone. This cartilage is not well seen in fig. 2, but is observed well in sections taken between the parts of figs. 1 and 2. In a part of which fig. 2 is a representation it is just disappearing.

As will be shown soon below, this trough-shaped cartilage is the front end of the cartilage that forms the support of the organ or tube of Jacobson, and it is known as Jacobson's cartilage.

Going a little further back than fig. 2, we find that Jacobson's cartilage changes in two ways: first, the curve itself is not any more the lowest point, but has become turned slightly inwards, *i. e.* in a median direction, and, secondly, there is an indication of its severance from the rest of the lower limb of the cartilage of the lateral wall of the nasal furrow.

In fig. 3 both these changes are well shown. Still further back the severance is complete, and Jacobson's cartilage forms now an independent and well-defined organ, of the shape and position shown very accurately in fig. 3. It is here noticed that the inner or median labium is considerably different in shape from that in fig. 2; it still terminates with an upper sharp margin next the septal cartilage, but is possessed of a short triangular projection.

As is shown in fig. 3, the lower nasal furrow becomes invaginated into the concavity of Jacobson's cartilage as the mouth of the organ of Jacobson, in exactly the same way as was pointed out in my last paper (' Quarterly Journal of Micr. Science, April, 1881) of Jacobson's organ of the guinea-pig. We must for the present omit to enter into a detailed description of this opening, since we shall return to it further below; at present we will only concern ourselves with Jacobson's cartilage, and trace this backwards all along the organ of Jacobson, of which it forms the chief support.

The next shape of Jacobson's cartilage after the one shown in fig. 3 is one illustrated in fig. 4, that is, the extremities of the two labia have joined, and Jacobson's cartilage forms now a complete capsule around the organ of Jacobson, which is a complete tube (13, fig. 4). In this shape it resembles Jacobson's cartilage in the guinea-pig in about the anterior half of the organ of Jacobson, as illustrated in fig. 1 of my first paper on this subject (' Quarterly Journal of Micr. Science,' January, 1881). In some sections through this region I notice that the median walls of the capsule of Jacobson's cartilage of the two sides are more or less continuous with one another to a very small extent in the lower part. But in this shape Jacobson's cartilage does not extend for any considerable distance, any more than was the case in the guinea-pig, for in the rabbit it soon changes in this manner, that the capsule is discontinuous in the upper wall, but slightly directed outwards, so that we again distinguish an outer or lateral from an inner or median labium. Owing to this discontinuity being established, not in a straight upward, but slightly oblique and outward direction, it follows that the median labium is longer than the lateral one.

The next change is this: the extremity of the outer labium becomes much thickened, while the median labium is prolonged in an upward direction. The thickening of the extremity of the lateral labium is soon again lost, but the median labium, to the hind end of the organ of Jacobson, goes on steadily increasing in height, the lateral labium in its height remaining tolerably stationary. The greater part of the organ of Jacobson is surrounded by the cartilage of this nature, *i. e.* a trough- or hook-shaped plate, of which the median labium is much higher than the lateral one, the former resting with its upper extremity alone against the thickened lower margin of the septal cartilage. The cartilage becomes, at the same time, thinner as it is traced backwards. Figures 5 and 6 show these points very clearly. All the figures having been drawn with the camera lucida the relations of size, shape, and position are perfectly exact.

From the relative length of the median labium of Jacobson's cartilage alone it is easily possible to decide which of two sections is more anterior, viz. the one whose median labium is shorter.

As the hind extremity of the organ of Jacobson is approached this important change takes place, viz. the lateral labium turns inwards with its upper extremity, as if to close against the median labium, and the part of this latter that extends *above this line* sooner or later becomes discontinuous from the rest (see figs. 8 and 9), and, gradually becoming shorter, altogether disappears, so that at the very extremity of Jacobson's organ, viz. when the organ of Jacobson has dwindled down to an exceedingly fine tube, the cartilage of Jacobson appears in a transverse section of an annular shape, open inwards and outwards.

The cartilage of Jacobson extends a very short distance beyond the tube of Jacobson, and it is then only with the median wall, which, however, soon altogether disappears.

Comparing, then, Jacobson's cartilage in the rabbit with that of the guinea-pig, as described in my former papers, we see this remarkable difference, that in the rabbit the cartilage extends as far as the organ of Jacobson, and even beyond it, while in the guinea-pig a considerable posterior portion of the organ of Jacobson is without any cartilage, but is surrounded entirely by the bone of the crista nasalis of the superior maxilla (see Plate XVII, fig. 7, of this Journal, April, 1881).

The anterior extremity of Jacobson's cartilage is in both animals very different, as is noticed on a comparison of figs. 3, 4, 5, 6, of my paper in this year's April number of this Journal with the figures of the present memoir.

In the guinea-pig Jacobson's cartilage having formed a complete capsule, going backwards becomes again incomplete, the deficiency affecting the lower and outer wall, the plough-shaped upper wall (see fig. 2, Pl. VII, in this year's January number of this Journal) being the last part of Jacobson's cartilage to disappear. In the rabbit, on the other hand, it is the outer and upper part of the capsule which becomes wanting, so that Jacobson's cartilage represents a hook-shaped or trough-shaped organ, the opening being in the upper part of the wall.

As regards the presence of an inner and outer labium, and as regards the elongation of the former as we pass backwards, there is nothing of the kind in the guinea-pig. The cartilage of Jacobson agrees in its general shape, *i.e.* being a trough-shaped plate with its opening directed upwards, more with the ideal cartilage of Jacobson described by Gratiolet[1] of the mammal, and to some limited extent also with that of the sheep, mentioned by Balogh,[2] and figured by him in figs. 15, 16, and 17 of his plate iv, being here represented in some places as a trough-shaped capsule with an upper opening.

Another point of dissemblance between the cartilage of Jacobson in the rabbit and guinea-pig is its relation to the upper maxillary bone.

As I have shown in figs. 4, 5, and 6 of Plates XVI and XVII in the April number, 1881, of this Journal, the cartilage of Jacobson *in the guinea-pig* is supported already in the most anterior part of the organ of Jacobson, and even at the mouth of this latter, by a lamina of osseous substance extending on each side from the superior maxilla on the inner or median surface of Jacobson's cartilage. This bone is in reality the front part of the crista nasalis of the superior maxilla. When Jacobson's cartilage has assumed the shape of a more or less perfect capsule, the bone forms an almost complete capsule around Jacobson's cartilage, as is shown in figs. 1 and 2 of Plate VII in this year's January number of this Journal. In the posterior portion of the organ of Jacobson the cartilage of Jacobson, as mentioned previously, disappears altogether, and now the organ of Jacobson is altogether surrounded by the bone of the crista nasalis of the superior maxilla. Thus it is in the guinea-pig; in the rabbit the relations are altogether of a different nature, as is shown in figs 4 to 8.

In the rabbit Jacobson's cartilage is supported on its lower wall by the intermaxillary bones separated in the median line by their respective inner periosteum. This relation is noticed already before any trace of the organ of Jacobson is reached, and it remains the same past the region in which Jacobson's cartilage has assumed the shape of a complete capsule (see fig. 4). Soon after this

---

[1] L. c., p. 21.

[2] L. c., p. 451.

place, when Jacobson's cartilage becomes changed into a trough-shaped capsule with an upper opening, there is seen along its outer or lateral labium, and gradually elongating so as to reach up to the upper extremity of this latter, a lamina of bone extending from the lateral portion of the intermaxillary bone; this condition is accurately illustrated in figures 5—9. It is also noticed that this osseous lamina supporting the outer labium of Jacobson's cartilage increases slightly in thickness towards its upper extremity. In the posterior extremity of the organ of Jacobson also the inner or median labium of Jacobson's cartilage receives a bony support from the median portion of each intermaxillary bone, in the shape of an osseous lamina extending for a relatively short distance in the median line separating the Jacobson's cartilage of the two sides (see fig. 8). But in no place does the organ of Jacobson, or rather Jacobson's cartilage, receive a support from the intermaxillary bones to such an extent as is the case in the guinea-pig.

In addition to the cartilages described hitherto there exists a cartilage in the rabbit which, as far as I can see, is not represented in the guinea-pig, viz. a curved or trough-shaped plate of hyaline cartilage, the concavity of which coincides with the lower nasal furrow; the mucous membrane of this latter forms indeed the lining of that trough-shaped cartilage plate. The most anterior point where this cartilage is met with is the one depicted in fig. 4; it will be seen that Jacobson's cartilage is a closed capsule and that the mucous membrane of the lower nasal furrow is supported by a trough-shaped cartilage plate; on one side the lower nasal furrow appears closed as if to form a tube. The fact is, that we have here on one side of the section the upper extremity of the Stenson's or Stenonian duct, or the naso-palatine canal, while on the other side the communication of this duct with the lower nasal furrow is seen widely open.

Just as in the guinea-pig so also in the rabbit, the Stenonian canals open into the nasal furrow, and are not in any way in a direct communication with the organ of Jacobson.

The trough-shaped cartilage plate just named as supporting the mucous membrane of the lower nasal furrow is in reality a continuation of Stenson's cartilage, *i.e.* the cartilage forming the support of Stenson's duct, and for this reason the former may be called the Stenson's or Stenonian cartilage. It extends as far as the organ of Jacobson does (see fig. 9) and terminates with Jacobson's cartilage. It is uninterrupted in its whole extension and does not alter in shape, size, or thickness, except at its posterior extremity, where it suddenly becomes shorter and thinner.

I now come to the essential part of this paper, viz. the description of the structure of the tissues forming the wall of the organs or tubes of Jacobson.

In the guinea-pig, as has been pointed out in my paper of this year's April number of this Journal, the mucous membrane lining the depth of the nasal furrow is in the front part of the nasal organ covered with stratified pavement epithelium, and the mucosa itself contains, like the mucosa of the neighbouring parts, a plexus of veins longitudinally arranged and is infiltrated with numerous lymph-corpuscles. The same epithelium and structure of the mucous membrane of the nasal furrow, except that the infiltration with lymph-corpuscles is here much less marked than in the guinea-pig, is also met with in the front part of the nasal organ of the rabbit, and I can therefore pass this over without any further details, and will refer the reader to figs. 1, 2 and 3, of the present memoir.

As in the guinea-pig, so also in the rabbit, there exists an open communication of the tubes or organs of Jacobson with the lower nasal furrow by means of a narrow mouth, such as is shown in fig. 3.[1] In front of this opening we notice in the mucous membrane of the nasal furrow a conspicuous plexus of large veins, the tissue between which contains bundles of muscular tissue, that is to say, we have here already to deal with a cavernous tissue similar to that occurring in the lateral wall of the organ of Jacobson, at and about the mouth, such as is represented in fig. 3. The cavernous tissue is a conspicuous part of the mucous membrane and occupies the part of this latter which corresponds to the lower wall of Jacobson's cartilage. The part of the mucous membrane corresponding to the median labium of Jacobson's cartilage is more or less occupied by glands extending downwards and upwards, so as to form a continuity with those contained in the mucous membrane of the nasal furrow and septum respectively.

All these glands, be it said once for all, whether in the wall of Jacobson's organ, or in the mucous membrane of the nasal septum, or the concha inferior, or the walls of the nasal furrow, are *always serous glands* of exactly the same nature as those described in my former papers; the ducts are lined with a single layer of columnar cells, whose outer portion is conspicuously fibrillated, just as in the salivary tubes of Pflüger.

The mouth of Jacobson's organ is lined with stratified pavement epithelium; the subepithelial tissue contains numerous lymph-corpuscles. Immediately past the mouth the epithelium is stratified columnar all round the lumen of the now closed tube

---

[1] My friend Dr. Reuben Harvey, of Dublin, informs me that he found the same condition also in the rat and cat.

or organ of Jacobson. The lumen here is circular in transverse section. The superficial layer of the epithelium is made up of conical or cylindrical, the deepest of inverted conical cells, and between the two are pushed in more or less numerous spindle-shaped cells. The glands occupy all parts of the cavity of Jacobson's cartilage except the part corresponding to its lower walls, which is occupied by *the cavernous tissue.* Compare fig. 3.

Immediately after this point, *i.e.* the closed tube with circular lumen—but the Jacobson's cartilage is not yet a closed capsule—the shape of the organ and the disposition of its parts change in this manner: the lumen is now *oval* in transverse section, the long diameter being placed in an upward and downward direction. The walls of the organ of Jacobson may consequently now be considered as the *median* and *lateral* wall, and the sulci where the two meet will be considered, as was the case in the guinea-pig's organ (see my former paper), as the *upper* and *lower sulcus.* The epithelium lining the lumen is stratified columnar as described above, except on the median wall, where it is *sensory epithelium,* the structure of which will be considered minutely below. The sensory epithelium is not directly continuous with the epithelium lining the upper and lower sulcus of the organ. In connection with the epithelium of the median wall is a lymph follicle of considerable size. This follicle occurring in several successive transverse sections it follows that we have in reality to do with a patch of lymph-follicles extending in a longitudinal direction.

The lymph-follicle, together with the numerous bundles of the olfactory nerve branches (see below), occupy a great part of the median wall; the upper part of this wall, and the whole region above the upper sulcus, is filled with *serous glands whose ducts open into the upper sulcus.* The lower part of the median wall is occupied by a plexus of large veins which, different from the cavernous tissue, do not contain any non-striped muscle in the interstitial tissue. The part of the wall immediately below the lower sulcus is occupied by *serous glands which send their ducts into the lower sulcus;* they are far less in number than those of the upper sulcus.

The remainder of the wall of the organ of Jacobson, i. e. the lateral wall, *is occupied by the cavernous tissue*—a plexus of venous vessels or sinuses separated by, and embedded in, a meshwork of bundles of what appears to be non-striped muscular tissue. The venous vessels extend in a longitudinal direction, while the bundles of muscle cells extend prevalently in a radiating direction from the cartilage towards the lumen of the organ.

At this point which we are now describing the epithelium of the lateral wall rests on a thin layer of mucous membrane densely infiltrated with lymph-corpucles; this *subepithelial layer* is limited by a thin layer of elastic fibres running chiefly in a longitudinal direction, and it is *this elastic layer*, which, while forming the inner boundary of the cavernous tissue represents at the same time the inner insertion of the bundles of non-striped muscular cells mentioned above.

The next point we have to consider is the constitution of the walls of Jacobson's organ at a point a little further behind the one just described, *i. e.* when the cartilage of Jacobson has become closed so as to form a complete capsule, such as is represented in fig. 4. The distribution of the different tissues as just described is very much the same, except that the epithelium of the lateral wall is separated from the elastic layer by a measurable stratum densely infiltrated with lymph-corpuscles. The elastic layer is very conspicuous.

The cavernous layer, the distribution of the glands at the upper and lower sulcus, the sensory epithelium and the occurrence of lymph-follicles, the presence of olfactory nerve trunks and plexuses of fine bundles of these in the median wall, is the same as in the parts anterior to this place.

We have mentioned on a former page that past the region of the closed Jacobson's cartilage capsule we again find this cartilage open in the upper part, assuming the shape of a trough, so that its cavity is in a free communication with the tissue at the side of the septal cartilage (see fig, 5), and we have also stated above that, beginning with this region, and through the greatest part of the organ of Jacobson, the relations remain of this nature, with this difference, that as we pass farther backwards the median or inner labium of the trough gradually increases in length.

Now, with the change of the Jacobson's cartilage from a closed capsule into a trough-shaped plate, there occurs a very interesting change in the wall of the organ of Jacobson; the median wall, it is true, remains the same, and so does the disposition of the glands of the upper and lower sulcus as well as that of the cavernous tissue and of the elastic layer in the lateral wall, but the subepithelial layer of the lateral wall alters so that this whole wall assumes a different aspect. Previously, we saw the epithelium of the lateral walls separated from the elastic layer by a thin layer of connective tissue densely infiltrated with lymph-corpuscles; now, however, this subepithelial layer becomes greatly thickened, owing to the presence of serous glands, which occupy the middle of the layer, so that this middle part of the lateral wall becomes changed into *a fold forming a conspicuous projection into the cavity of the organ of Jacobson.* This fold may be therefore called the *glandfold* of the lateral wall. These glands send their ducts through the epithelium of the lateral wall vertically and straight through the middle of the lateral wall. These glands do not extend through the whole subepthelial layer from the upper to the lower sulcus, but are limited chiefly, as stated above, to the middle part of the lateral wall, hence the fold; above and below the gland the subepithelial layer contains numerous lymph-corpuscles as before.

This glandfold remains now through the whole length of the organ of Jacobson to near its posterior extremity.

The presence of this glandfold in the lateral wall necessitates a change in the shape of the cavity of the organ of Jacobson; whereas the cavity in transverse section through the anterior regions appears more or less oval, it now for obvious reasons possesses the shape of a bean or kidney (compare figs. 10 and 11).

The cartilage of Jacobson, being trough-shaped with an upper opening, it follows that the glands in the upper part of the wall of the organ of Jacobson, viz. those, the ducts of which open in the upper sulcus, form an unbroken continuity through that opening with the serous glands of the mucous membrane covering the septal cartilage. These points, *i.e.* the presence of a glandfold in the lateral wall, the continuity of the glands of the organ of Jacobson with those of the mucosa covering the septal cartilage, together with the above-mentioned trough-shaped nature of Jacobson's cartilage, form the chief differential characters by which to distinguish at once the sections placed through the most anterior portions of the organ of Jacobson from those made of the rest, except the posterior extremity, which will be considered presently.

In some places the median wall of the organ of Jacobson contains a few alveoli of serous glands; these are evidently outrunners from the glands of the lower wall, that is, those opening into the lower sulcus.

In these respects, then, the organ of Jacobson differs considerably from that of the guinea-pig, as described in my former papers, while it approaches to a certain limited extent that of the typical organ of the mammal, as described by Gratiolet, and that of the sheep, as described by Balogh. Gratiolet[1] speaks of the upper wall as possessed of a "bourrelet saillant," and containing numerous glands, but it is quite clear from his description that this "bourrelet saillant" is not the same thing as our "glandfold," since, besides its different position, he ascribes it not to a separate group of glands, but to the glands in general. He also speaks[2] "d'un grand sinus veineux qui règne dans toute l'étendue du bourrelet."

---

[1] L. c., p. 20.

[2] L. c., p. 21.

Balogh[1] says that in the sheep's organ there exists a "gland-projection" (Drüsenwulst) in the mucous membrane of the organ of Jacobson, which extends from the upper and outer parts of the mucous membrane into the lumen of the organ of Jacobson. The bourrelet of Gratiolet and the Drüsenwulst of Balogh are evidently the same thing, but they differ from the glandfold of the middle of the lateral wall as described by me of the rabbit. In the guinea-pig there is nothing of the kind, as I have pointed out in my former paper.

In the posterior position of the organ of Jacobson we find the following disposition of the several layers:—The median wall in its sensory epithelium, and its numerous plexuses of olfactory nerve bundles remains unaltered, so does the epithelium of the lateral wall, the subepithelial layer, the glandfold, and the elastic layer. The cavernous layer, however, becomes greatly increased in thickness and extent, encroaching considerably on the lower and upper wall; hence the glands opening into the upper sulcus appear very greatly diminished in numbers. The same is the case with the glands opening into the lower sulcus; these sooner or later cease altogether.

At the very extremity of the organ of Jacobson we find the lumen reduced to a minute opening; in the median wall we still find a trace of the sensory epithelium, but soon this also disappears, the lumen becomes circular in transverse section, and the epithelium is altogether made up of columnar cells. In the posterior extremity of Jacobson's organ the lumen and the lining epithelium in so far alter their position as they are now close to the median labium of Jacobson's cartilage, and they shift also a little more in a downward direction (see figs. 8 and 9).

A plexus of thin olfactory bundles is still to be recognised in the upper part of the median wall, but this latter is greatly reduced in thickness. The glands opening into the upper sulcus reach lower down than before.

Of a glandfold in the lateral wall, or of a subepithelial layer, nothing is left; the lateral and lower wall are occupied by the cavernous tissue, which possesses a considerable thickness, and in which the bundles of non-striped muscular cells, running in all directions, still form a conspicuous feature.

We see, then, that in all repects the organ of Jacobson of the rabbit differs materially from that of the guinea-pig; the shape and size of Jacobson's cartilage, the disposition of the several structures in the median and lateral wall, are quite different in the two cases.

Before describing the minute structure of the different parts

---

[1] L. c., p. 457.

constituting the median and lateral wall of the organ of Jacobson, I will give here the results of measurements carried out in a transverse section through the organ of Jacobson, such as is illustrated in fig. 4, viz. at a point where the cartilage of Jacobson forms a complete capsule.

Thickness of Jacobson's cartilage at the upper angle	0·56	mm.
„ „ „ at the lower angle	0·225	„
„ „ „ in the middle of the lateral wall	0·225	„
„ „ „ in the middle of the median wall	0·18	„
Short transverse diameter, *i.e.* across the middle of Jacobson's cartilage capsule, from side to side, inclusive of the thickness of the cartilage of both the lateral and median wall	1·295	„
Long transverse diameter, *i.e.* from the upper to the lower wall, inclusive of the thickness of the cartilage of both the upper and lower angle	3·145	„
Thickness of the lower wall of the organ of Jacobson, exclusive of the epithelium lining the lumen	0·225	„
„ „ epithelium at the lower sulcus	0·20	„
„ „ lateral wall in about the middle, exclusive of the epithelium lining the lumen	0·315	„
„ „ epithelium of the lateral wall	0·045	„
„ „ gland layer at the lower sulcus	0·225	„
„ „ sensory epithelium in about the middle of the median wall, at a place where there is in it a lymph-follicle	0·388	„
„ „ remainder of the mucosa of the median wall	0·1125	„
Long transverse diameter of the lumen of the organ of Jacobson	0·45	„
Short „ „ „ „ „	0·135	„

The measurements taken from a section such as is illustrated in fig. 11 are:

Thickness of Jacobson's cartilage in the middle of the lateral wall	0·148	mm.
„ „ „ in the middle of the median wall	0·135	„
„ „ „ in the middle of the lower wall	0·18	„
„ „ „ at the extremity of the upper labium	0·18	„
Diameter from the extremity of the median labium to the lower wall of the cartilage, inclusive of the thickness of this latter	4·235	„
In a section further back, *e.g.* one shown in fig. 7, this diameter increases to	5·12	„
Diameter from the extremity of the lateral labium to the middle of the lower wall of the cartilage, inclusive of the thickness of this latter	2·2	„
Transverse diameter of the opening between the extremity of the lateral labium, right across to the middle of the median labium of Jacobson's cartilage	0·45	„

Thickness of osseous lamina outside the lateral labium of Jacobson's cartilage	0·05	mm.
Long transverse diameter of the lumen of Jacobson's organ	0·6875	„
Short „ „ „ „ „	0·135	„
Short transverse diameter of the whole organ of Jacobson, exclusive of the cartilage, measured across the middle	1·125	„
Thickness of the epithelium of the lateral wall	0·043	„
Greatest thickness of the glandfold, inclusive of epithelium	0·1656	„
Greatest height diameter of this glandfold, *i.e.* in an upward and downward direction	0·252	„
Thickness of the elastic layer	0·008 to 0·016	„
„ „ cavernous layer in the middle of the lateral wall	0·338	„
„ „ mucous membrane of the median wall, exclusive of the sensory epithelium	0·338	„
„ „ sensory epithelium	0·136 to 0·225	„
Transverse diameter of a large olfactory nerve trunk in the median wall	0·144 by 0·216	„
Diameter of a lymph-follicle within the sensory epithelium	0·194 by 0·252	„

In addition to the description given in the preceding pages of the structure of the different parts constituting the wall of the organ or tubes of Jacobson, we have now to state various points of detail not yet mentioned.

1. As regards the lateral wall.

(*a*) As mentioned above, the epithelium lining this is stratified columnar, being composed of three layers of cells; a superficial layer of conical or cylindrical cells, each with an oval nucleus; a deep layer of inverted conical cells, *i.e.* cells whose base is fixed on the subepithelial layer and whose pointed process is directed towards the free or inner surface, each of these cells possess a more or less spherical nucleus. Between these two layers of cells, especially between the superficial cells, there is a middle layer of cells composed of spindle-shaped cells, each with an oval nucleus. The nuclei of the superficial cells are the largest. In specimens hardened with spirit, the nuclei of the superficial conical cells and of the spindle-shaped cells appear as if situated in the same layer, and it then appears as if the epithelium were made up of two layers only.

On the inner or free surface of the superficial cells is a distinct and sharp boundary line, but there are no cilia projecting beyond this boundary line. In the guinea-pig the superficial cells of this epithelium possess cilia, as I have pointed out in my first paper, and I have then doubted Loewe's[1] assertion as to the non-existence of cilia on this epithelium in the rabbit's organ. But I now find that in all my specimens of the rabbit's organ, be they prepared with osmic acid, Müller's fluid, chromic acid, or spirit,

---

[1] 'Beitr. zur Anatom. d. Nase und Mundhohle,' Berlin, 1878.

there are never any cilia to be met with on the surface of the epithelium, and in this respect there does really exist a remarkable difference between the rabbit's and guinea-pig's Jacobson's organ.

The epithelium of the lateral wall now under consideration is always infiltrated with lymph-corpuscles, each with two or three small nuclei; these cells evidently migrate from the subepithelial layer into the epithelium; they are found in all parts of the epithelium.

(*b*) The subepithelial layer contains always a great number of lymph-corpuscles, both in the glandfold and beyond it; the lymph-corpuscles are relatively large cells, each of them possessed of a well-formed cell body. Where they are crowded together they appear pressed against one other, and therefore more or less polyhedral in outline.

(*c*) The elastic layer is composed chiefly of networks of fine elastic fibrils extending in a direction parallel to the long axis of the organ. This layer, increases in thickness (to almost double) as we pass backwards; it is always very conspicuous in transverse sections stained with dyes, since its bright fibres do not stain, and therefore contrast well both with the subepithelial layer and with the cavernous layer outside. The elastic layer is evidently the layer in which the muscular fibres of the cavernous layer insert themselves.

(*d*) The cavernous layer. This is the most conspicuous part of the lateral wall. As mentioned above, it increases both in thickness and breadth as we pass backwards (compare figs. 4 to 9). Its venous sinuses take up a plexus of small vessels, chiefly veins, situated close to the elastic layer. The sinuses extend in a longitudinal direction and are separated from one another by, or rather are embedded in a tissue, which consists pre-eminently of muscular substance. This is arranged in bundles of various sizes, directed chiefly in a radiating manner from the periphery of the organ, *i.e.* from the outer wall of Jacobson's cartilage towards the lumen of the organ, and connected into plexuses. Between these bundles we meet always a few bundles running in an oblique or even longitudinal direction. In preparations prepared with spirit the muscular tissue does not differ from non-striped muscular tissue, but in osmic acid specimens, hardened afterwards in chromic acid, the elements of this muscular tissue appear materially to differ from ordinary non-striped muscular tissue. We find, namely, that the individual elements are twice and three times as thick as ordinary non-striped muscle cells; that they are composed of coarse fibrillæ, and that they appear much longer and each to possess a number of nuclei; further, that they appear as if branched and connected into a network, so that they resemble the muscular fibres of the heart much closer than ordinary non-striped muscular cells. When a bundle of the muscular fibres of the cavernous layer is viewed in cross section the individual small elements appear in their size and structure very much like the muscular fibres of the heart.

In the matrix of the cavernous tissue may be met with small and large bundles of nerve fibres chiefly following a longitudinal course; in most cases the nerve fibres are medullated fibres, but there are here and there bundles to be seen, the greater majority of whose nerve fibres are non-medullated.

The outer boundary of the cavernous layer and the outer insertion of its muscular bundles is formed by fibrous tissue intimately connected with the perichondrium of Jacobson's cartilage.

(*e*) As regards the glands occupying the upper wall, *i.e.* those opening in the upper sulcus, as well as the glands in the lower wall, *i.e.* those opening into the lower sulcus, their structure, position, and change in amount has been mentioned above, and is easily understood from an inspection of the figures. The epithelium of the ducts of both groups at their mouth forms one continuity with the epithelium of the lateral wall, but is quite distinct from the sensory epithelium; the same relation has been pointed out in connection with the guinea-pig's organ, where it was shown that these ducts form in reality the boundary between the epithelium of the lateral wall and the sensory epithelium, lining the median surface of the lumen. So it is also in the rabbit; the two epithelial structures, *i.e.* the epithelium of the lateral wall and the sensory epithelium being well marked of from one another, and the mouth of the ducts of the serous glands, both at the upper and lower sculcus form the boundary between them.

2. The median wall.

The sensory epithelium consists, like that of the guinea-pig, of two distinct strata—a superficial one composed of thin conical spindle-shaped or cylindrical *epithelial cells,* each with an oval nucleus.

The cells forming this stratum vary from one another in this respect, that the nucleus does not lie in all cells at the same level, but is placed at different depths, so that, taking this stratum as a whole, it appears to contain several layers of nuclei; generally there are between three and five such layers. The nuclei are oval, some more elliptical than others; they stain always readily in dyes, and hence they are very conspicuous in specimens so stained.

The part of the epithelial cell between the nucleus and the inner or free surface varies between 0·012 and 0·016 mm., and it appears clear and longitudinally striated. On the free surface it appears limited by a membranous structure, similar to the limiting membrane of v. Brun in the olfactory organ.

The thickness of the layer containing the oval nuclei of these epithelial cells is between 0·027 and 0·028 mm.

The size of these nuclei is 0·0054 by 0·0072, or 0·0036 by 0·009 mm.

Underneath the epithelial cells, or rather underneath the nuclei indicative of them, follows the deep stratum or the stratum of the *sensory cells*. These cells are spindle-shaped or multipolar, possessed of a cell body of clear, granular-looking substance, and including a spherical nucleus, the size of which is about 0·0072 mm.; being spherical these nuclei are therefore larger than the oval nuclei of the "epithelial cells." Besides, the nuclei of the sensory cells are more transparent and less stained in preparations stained with hæmatoxylin, and they include a beautiful uniform network, or in some cases a convolution of fibrils.

In a preparation hardened with spirit and stained with logwood the contrast between the two strata, *i. e.* the stratum of the epithelial cells with deeply-stained homogeneous nuclei, and the stratum of the sensory cells, with the large clear spherical nuclei, is very great indeed, and no one looking at such a specimen can for a moment fail to see it. The number of sensory cells, or their nuclei indicative of them, differs in different parts; it is greatest in about the middle of the median wall, and decreases towards the upper and lower sulcus. The greatest thickness of the stratum of sensory cells is about 0·18 mm., and the greatest number of nuclei, from the superficial stratum to the subepithelial layer, in a vertical direction, is about eight or ten.

The boundary of the sensory epithelium towards the subepithelial mucosa is not well defined, the sensory cells *being prolonged singly or in small groups into the mucosa.* This fact does not come out so well in spirit preparations, but is distinct in specimens prepared in Müller's fluid. In these latter the deepest sensory cells, and especially those that extend into the mucosa, appear larger in the amount of the cell protoplasm than the more superficially situated sensory cells.

Everywhere the cell body is very distinct, owing to its relatively large size, and when isolated appears, as in the guinea-pig's organ, spindle-shaped or multipolar. The processes, like those of the sensory cells in the olfactory nasal membrane, may be distinguished as an *outer process,* extending between the "epithelial cells" to the surface, and *one or more inner* ones passing downwards, *i. e.* into the depth towards the mucosa. I have described this in my former paper in connection with the organ of Jacobson of the guinea-pig, and I have there also referred to the assertions and observations on these points by Balogh[1] in the sheep's organ, so that it is needless to enter into this subject here again.

In the rabbit's organ I have been able to follow what at first I omitted to do in the guinea-pig's organ, but what I have now done also in this last named animal, viz. the distribution of the olfactory nerve bundles and nerve fibres in the median wall, and their relation to the sensory cells.

All along the inner surface of the median labium of Jacobson's cartilage we find olfactory nerve bundles following a longitudinal direction; spreading from behind toward the anterior regions of the organ of Jacobson,[2] it is natural that we should find the number of nerve bundles greater in the posterior than in the anterior regions of the organ.

The transverse diameter of a large nerve bundle in the anterior portion of the organ is about 0·144 by 0·216 mm. From these nerve bundles, which, as mentioned just now, run in a longitudinal direction in the outer parts of the mucosa of the median wall, *i.e.* near Jacobson's cartilage, numerous minute bundles branch off, which run in an oblique direction towards the sensory epithelium; they are very numerous, and by branching and re-uniting form a plexus of small bundles of olfactory fibres occupying the inner part of the mucosa of the median wall. The nearer the sensory epithelium the smaller the branches and the closer the plexus. This plexus, *subepithelial plexus*, extends pre-eminently in a direction parallel with the short axes of the organ of Jacobson. The ground substance of the mucosa in which this plexus is embedded is made up of fibrous connective tissue. The relation of this plexus to the sensory cells is this: those sensory cells which, as mentioned above, extend into the mucosa, are situated in the meshes of the plexus, but are very closely applied to the branches of it, and from what I have been able to make out in thin sections appear to become continuous by their deep process with the nerve fibres in such a way that the process constitutes a primitive fibril of an axis-cylinder of the nerve branch.

In the sensory epithelium the sensory cells appear all to be contained in the meshes of a plexus of fibrillar bands, which appear directly continuous with the branches of the subepithelial nerve plexus. As is the case with the sensory cells extending into the mucosa, so also with the other sensory cells, it can be ascertained that with their deep process or processes they join as primitive

---

[1] L. c., pp. 465 and 466.

[2] See Gratiolet and Balogh on the general distribution of the olfactory nerve branch.

fibrils the nerve plexus. I have given an illustration of these appearances in fig. 12, and from this it will be seen that in this respect the relations appear very similar to those described by Max Schultze and others of the olfactory nasal mucous membrane, but different from what is maintained by the latest investigator of this subject, viz. Cisoff. Both in teased and non-teased specimens of vertical and horizontal sections I have convinced myself of the continuation of the subepithelial plexus of nerve fibres *as plexus* into the sensory epithelium, and of the intimate apposition of the deep process or processes of the sensory cells to the plexus, from whose fibrils they could not be distinguished either in aspect or position.

The naso-lachrymal duct in the rabbit, in the whole extent of of the organ of Jacobson, is considerably larger than that of the guinea-pig. For some distance anterior to the commencement of the organ of Jacobson the naso-lachrymal duct is seen on each side in the lateral wall of the nasal furrow, *inside the cartilage,* forming the support of this latter between it and the surface epithelium. In a section a little way further behind, like that shown in fig. 1, the naso-lachrymal duct is seen *outside the cartilage plate,* mentioned in the introduction as the lower limb of the cartilage, forming the support of the superior concha.

This changed relation between the naso-lachrymal duct and the cartilage is not due to any change in position of the naso-lachrymal duct, but to the fact that, as mentioned above, there is a small cartilage plate present in the most anterior parts which is not continued as far as the section represented in fig. 1; and, on the other hand, the lower cartilage limb shown in this figure is not present yet in sections anterior to the one shown in this same figure.

Behind the section represented in fig. 1, until the disappearance of the cartilage in the lower concha (fig. 3) the naso-lachrymal duct retains the same position as before, viz. outside the cartilage. It is situated just in the angle formed by the lower limb and lateral limb of the cartilage forming the support of the lateral wall of the nasal furrow. Compare figures 2 and 3.

Behind the mouth of the organ of Jacobson, the naso-lachrymal ducts are seen at the roots of the plicated part of the inferior concha between its osseous support and the alveolar process of the upper maxilla (see fig. 4).

In the region of the posterior part of the organ of Jacobson the naso-lachrymal duct changes slightly its position, inasmuch as it descends a little lower down, *i.e.* nearer to the lower nasal furrow.

As regards the shape of the naso-lachrymal duct, its cross section appears in all preparations more or less oval, but is more flattened from side to side anterior to the organ of Jacobson. As regards the size, it appears greatest in about the region of the mouth of the organ of Jacobson; anteriorly and posteriorly of this it appears to decrease in size.

The following are the measurements of its lumen only, anterior to a point shown in fig. 1:

The short transverse diameter	0·135 mm.
The long „ „	0·9 „

At a point represented in fig. 1:

The short transverse diameter	0·135 mm.
The long „ „	0·7875 „

A little behind this point, but before the mouth of Jacobson's organ is reached:

The short transverse diameter varies between	0·56 and 0·68 mm.
The long transverse diameter between	1·24 „ 1·58 „

In the region of the middle of the organ of Jacobson the naso-lachrymal duct becomes again smaller, as is shown by the following numbers:

The short transverse diameter varies between	0·38 and 0·6 mm.
The long transverse diameter between	1·01 „ 1·24 „

As regards the structure of the naso-lachrymal duct it is everywhere the same:

1. The stratified columnar epithelium lining the lumen is of the same nature as that described of the guinea-pig; the thickness of the epithelium varies between 0·048 and 0·068 mm.

2. This epithelium is placed, just as was the case in the guinea-pig, on a thin subepithelial layer infiltrated with lymph-corpuscles. In many places there are large lymph-follicles present in the wall of the naso-lachrymal duct; these lymph-follicles have their seat really in the subepithelial layer, and extend from here both into the epithelium and in the outer parts of the wall of the duct. Between more or less well-defined lymph-follicles and diffuse adenoid tissue infiltrating the subepithelial layer are all gradations. The diameter of a medium-sized lymph-follicle, such as is shown in fig. 14, amounts to about 0·45 mm. The adenoid tissue of these lymph-follicles penetrates into the surface epithelium to the extent of the disappearance of this latter at these places, just as is the case in other places where lymph-follicles reach to the surface, *e.g.* Peyer's patches of the intestine, tonsils, and pharynx, &c.

But independent of the lymph-follicles, the adenoid tissue, at any rate the lymph-corpuscles and capillary blood-vessels of the subepithelial layer, extend into the epithelium in many places in a more or less uniform manner. This is evidently analagous to the fact described of the naso-lachrymal duct of the guinea-pig, viz. an extension of capillary blood-vessels and nucleated cells from the subepithelial layer into more or less well defined cavities in the epithelium. In the rabbit the intraepithelial spaces containing the capillary blood-vessels and lymph-corpuscles do not appear to be of this same well-defined nature.

3. Outside the subepithelial layer is a plexus of smaller and larger veins, running chiefly in a longitudinal direction, and a few arterial branches also extending in a longitudinal direction. The tissue in which these vessels are embedded is a very loose connective tissue infiltrated with numerous lymph-corpuscles.

As was pointed out in my former paper, Henle[1] already described the occurrence of lymphatic tissue in the wall of the naso-lachrymal duct of man.

The last point that I wish to describe here are the structure of the mucous membrane lining the lower nasal furrow, and covering the inferior turbinated bone, and the nasal septum in the region of the organ of Jacobson.

In the most anterior portion of the nasal cavity, anterior to the part illustrated in section, fig. 1, the epithelium covering the free surface of all the above regions, viz. the nasal furrow, turbinated bone, and nasal septum, is stratified pavement epithelium; the subepithelial connective tissue is infiltrated in many places with lymph-corpuscles; rudiments of papillæ are present in the region of the lateral wall of the nasal furrow.

The mucosa contains serous glands only over the inferior concha. But there is everywhere an indication of venous plexuses, situated in the superficial part of the mucosa, and extending more or less parallel to the long axis of the nasal organ; these plexuses are well developed in the upper part of the septum and in the inferior concha.

A little further behind, *e. g.* in fig. 1, the epithelium lining the nasal furrow and the nasal septum is still stratified pavement epithelium, but the epithelium covering the surface of the inferior concha is already stratified columnar, the superficial cells being ciliated. Numerous serous glands in the deeper layer, and very rich plexuses of venous vessels in the superficial layer, are contained in the mucosa of the inferior concha, and in a limited degree also in the mucous membrane of the nasal septum.

In a place, of which fig. 2 is a representation, the lower nasal furrow alone is lined with stratified pavement epithelium, all the

---

[1] 'Eingeweidelehre,' ii, p. 713.

other parts with stratified columnar cells, of which the superficial layer is made up of ciliated cells, with the usual goblet cells amongst them. The subepithelial layer contains numerous lymph-corpuscles and a rich plexus of veins; the deeper layer of the mucosa includes serous glands, forming a continuous layer in the mucous membrane of the lower concha and in that of the upper part of the nasal septum; but they are scarce in the lower part of the latter, and are altogether absent in the mucous membrane of the lower nasal furrow.

The same relations obtain past the mouth of Jacobson's organ until the part is reached which is illustrated in fig. 4, *i.e.* showing Jacobson's cartilage a closed capsule, except that the mucous membrane covering the lower part of the nasal septum contains very numerous serous glands; the epithelium lining the lower nasal furrow is still stratified pavement epithelium.

A little further back, viz. where Jacobson's cartilage has assumed the shape of a trough (see figs. 5 and 6) with a long median labium, also the epithelium lining the lower nasal furrow, *i.e.* the concave surface of Stenson's cartilage, is stratified columnar, the superficial cells being ciliated, with the usual goblet cells amongst them.

The thickness of this ciliated columnar epithelium is the same as in other parts of the nasal cavity, and amounts to about 0·086 mm., exclusive of the cilia, which are about 0·0072 mm. long.

The serous glands in the mucosa of the lower part of the nasal septum have now greatly increased, and, as has been pointed out before, form a continuity with the glands of the organ of Jacobson.

Of interest is the occurrence of diffuse adenoid tissue, and of smaller and larger lymph follicles, isolated and in continuous patches in the mucous membrane lining the concave side of Stenson's cartilage, as is shown in figs. 6 and 7.

The diameter of a well-defined lymph-follicle of the larger kind varies between 0·216 by 0·25 mm., and 0·3 by 0·45 mm.

The extension of the adenoid tissue of these lymph-follicles into the epithelium of the surface is the same as described above of the lymph-follicles of the naso-lachrymal ducts.

On the FURTHER DEVELOPMENT *of* WELWITSCHIA MIRABILIS.

By F. O. BOWER, M.A., Camb., Assistant to the Professor of Botany in University College, London. With Plates XXXII and XXXIII.

SINCE the publication of my paper "On the Germination and Histology of the Seedling of *Welwitschia mirabilis*" ('Quart. Journ. Micr. Sci.,' Jan. 1881), I have been put in a position to study the structure of older plants, some specimens of these having been supplied to me from the Kew collections, others being kindly presented by Chev. D. J. de Nauet Monteiro. It is the object of the present paper to describe the structure of these older plants, and to show how it corresponds with that of the young seedling as already described.

Before proceeding to this I must put on record information received from Chev. Monteiro by letter. In plants of Welwitschia, which he has cultivated for three to four years, he tells me that in one case "the first pair of leaves (or cotyledons) have dropped off, one plant has one still on, and the remainder have them still." Further, he states that while they remain the cotyledons do not change or grow; that as the stem enlarges they become "jagged at the base, and on dropping they leave a tumid scar, observable in the dried specimens as a circle under the true leaves. In the older specimens it is so torn up that you might take it for part of the cortical integument, unless you were aware of the circumstances." These observations of Chev. Monteiro supply us with the direct proof that the cotyledons wither, and since there has never been observed a further development of leaves of the main axis after the first plumular pair, we may conclude that the latter are the leaves which remain persistent throughout the life of the Welwitschia plant (cf. 'Quart. Journ. Micr. Sci.,' Jan. 1881, p. 29.)

Root and Hypocotyledonary Stem.—External Characters.

The primary root of the seedling has already been described as attaining a length of four to five inches without branching (l. c., p. 19). In a letter Chev. Monteiro describes older plants as developing a tap root "about eighteen or twenty inches in length;" after attaining this length the root branches repeatedly. This was the case in the plants four months old which he supplied to me. In external appearance the young roots present no peculiarity. The roots of plants of medium age are, however, covered with a thick fluffy layer of tissue (effete corky tissue) of a light yellow colour. This gives place in still older plants to a darker and even black covering of greater strength and hardness. These remarks apply also to the lower part of the hypocotyledonary stem. The fluffy layer, being very friable, could not easily be cut into sections by the razor; it will therefore not be represented in the figures of sections accompanying this paper.

Internal Structure.—Root.

It was shown in my former paper (p. 25) that the cortical tissue of the root, being cut off from physiological connection with the central cylinder, ceases to grow, and is thrown off, so that the older root consists of tissues derived only from the central cylinder. We will now trace the further development of that cylinder. Its periphery (as seen in fig. 22, l. c.) is occupied by a formative tissue derived from the pericambium. This gives rise to a constantly and successively renewed cork tissue, which is always to be found covering the root externally. Beyond its presence it is not of any special interest; we may, therefore, pass on to the consideration of the internal tissues.

In the specimens described in my former paper the fusion of the diarch xylem system at the centre of the root was not observed (l. c., p. 25, fig. 22). The two xylem masses were in all cases separated by a parenchymatous tissue. It is true Bertrand ('Ann. d. Sci. Nat.,' série v, tom. xx, p. 9) has described the central fusion of the xylem masses of the lateral root of Welwitschia, but the question as to the main root still remained open. On comparison of older plants, however, it appears that in the main root, for a short distance below the transition from stem to true root, such a fusion does not occur, the centre of the transverse section being permanently occupied by a mass of parenchyma. As this tissue bears an important relation to the further development of the part where it occurs, it will be again referred to later (cf. fig. 22 of first paper, which was taken at a point close below the feeder). In the lower portion of the root a complete coalescence of the two originally separate xylem masses takes place, so that a single plate is formed; the junction is followed by a further development of xylem at the central part of each side of the plate, produced by an active cambium layer, which lies between the primary phloem and the xylem plate. The secondary xylem thus produced appears as a narrow band on each side of the xylem plate, the two bands being separated laterally from one another by very broad parenchymatous rays. The whole xylem system thus produced attains a stellate form (fig. 1.). Thus far the structure of the root of Welwitschia corresponds to that of Ephedra (cf. Van Tieghem, La Racine, p. 211).

Meanwhile a further development has been going on in the parenchyma which surrounds the vascular tissues: this results in the formation (*a*) of a number of irregularly arranged spicular cells (*sp. c.*), whose axes are parallel to the longitudinal axis of the root; (*b*) of long sclerenchyma fibres with no crystals in their walls (*scl. f.*, cf. Hooker, l. c., p. 15). The spaces between these are filled with thin-walled parenchymatous tissue, whose cells retain their cell nature, and play an active part in the further development (cf. below). At the periphery of the section lies a layer of cork derived from a phellogen layer, as already described. Since the same is the case in sections from older roots, the fact will not be again mentioned. It may be noticed that the cork in fig. 1 has at certain points attained a considerable thickness; this corresponds to the fluffy layer already mentioned.

No secondary free vascular bundles are to be seen in fig. 1, which represents a section cut far down the root of the youngest plant in the Kew collections. Thus far the structure presents no great peculiarities. The same root was, however, cut about one inch further from the apex, and by comparison of a series of sections, the first secondary bundles were observed to originate in the following way:—One of the cambian bands becomes laterally extended (fig. II., 1), then a plate of parenchymatous tissue is intercalated between the extension and the main mass (fig. II, ii.). A separate bundle is thus formed by branching of the main bundle; it is, therefore, a direct derivative of it, and does not end "blind" (cf. De Bary, 'Vergl. Anat.,' p. 303, &c.). As the new bundle pursues its course up the root it leaves its original position relative to the main bundle system and takes a spiral course till it is placed opposite to one of the protoxylem masses (fig. II, iii, b_2). A similar development goes on at the opposite side of the root, and a symmetrical arrangement is thus attained.

It must, however, be remarked that the central bundle system of the roots of Welwitschia is not always arranged as regularly as here described, though this seems to be the typical arrangement.[1]

This arrangement of the central vascular bundles of the root is well seen in the section represented in fig. IV. This was taken at a point far down the root of the plant (fig. III). This root

---

[1] The description which follows is mainly derived from the study of one plant which was handed over for dissection from the collections at Kew. It is represented in outline in fig. III, i, ii. From a comparison of it with other rather younger plants, it is seen that the swelling of the stock starts near the top, and proceeds downwards, the lower limit of the swelling being pretty strongly marked, as seen in fig. III, i.

being much older than those before examined, the bundles of the central system are further developed; it will be seen, however, that they correspond exactly in their arrangement to the description given above. The protoxylem masses can still be recognised though the protophloem masses cannot be traced. Around the former are grouped symmetrically four large vascular wedges, two of which are connected centrally with one another; these are derived directly from the activity of that cambium layer which appears between the protophloem and the primary xylem plate, those which alternate with them having been derived from them by branching, as above described. It is true there are frequent deviations from the type, due to the sinuous course pursued by the bundles of the central group, and by their frequent anastomosis both with one another and with the members of the later developed peripheral system; but the tendency is to return after these irregularities to the typical arrangement. Though in older roots the bundle system becomes complicated by the formation of large numbers of peripheral bundles, still as a rule the centre of the root is occupied by the original fourfold group. Since these bundles, and in fact all bundles of the root of Welwitschia, remain "open" for a long time, they attain in old roots a very considerable size. The discrepancy of this description with the figures and account given by Hooker depends upon the fact that the xylem is there omitted, while the phloem is represented as the whole bundle (cf. also De Bary, 'Vergl. Anat.' p. 633).

On regarding the arrangement of the bundles of the central system comparatively, we see that there is here in the root of Welwitschia a case similar to that in the stems of many Dicotyledons, in which it is found that the broad medullary rays are traversed by intercalary bundles (Zwischenstränge, cf. De Bary, 'Vergl. Anat.,' p. 408, &c.); these keep up a vascular connection between the primary bundles on either side of the ray. We have as the counterpart of these intercalary bundles those branches (fig. IV, b_2), which pursue a sinuous course along the very broad medullary rays of the root of Welwitschia, and which from time to time fuse laterally with the primary bundles.

As the central group develops, there appear in the peripheral part of the root a number of fresh bundles, which, though not disposed regularly in rings, still show a tendency to that arrangement. Such bundles are to be seen in fig. IV, *per. bun.* As to their ending I cannot speak definitely, but this much is certain, that whereas I have often been able to observe anastomosis between members of this peripheral series, as well as between them and the central group, I have never obtained evidence of their ending "blind." Having the analogy of the central group before us, we may therefore consider it as probable that the peripheral series is connected terminally with the central group; that is, that the bundles of the root form a single system having a common origin.

To the question of the communication between the bundles of the central group and those of the peripheral system, I have paid special attention, since Bertrand ('An. d. Sci. Nat.' série v, vol. xx, p. 10) asserts that the latter series are always separated from the central system by fundamental tissue. I have, however, been able to satisfy myself that the contrary is the fact, and by comparison of long series of transverse sections, I have succeeded in tracing the passage of branch bundles from the central group to the peripheral series. As to the lateral communication of different members of the same series, it occurs so frequently that it can be observed in any series of transverse sections; moreover tangential sections demonstrate that the bundles of either series form a cylindrical network. It is owing to the irregularity of the frequent anastomosis that the central series, as well as the peripheral, show those varieties of arrangement to which they have already been described as being subject.

As the root increases in age its bulk also increases. This is due partly to growth of the old bundles and formation of new ones, partly to growth of the tissues in which the bundles are embedded.

It has already been stated that the bundles of the central group remain "open" and increase in size, while fresh series of open peripheral bundles are formed outside them. The disposal of the members of the later-formed series is more regular than that of those earlier developed, so that in old roots the appearance is presented of a number of definite rings of bundles surrounding others less regularly arranged. The new bundles are formed by active division at certain points in the parenchymatous "ground tissue." Fig. v represents the points of origin of three new bundles of a peripheral series. Though at first sight this figure gives the idea of a definite cambium layer in which the new bundles appear at certain points (as in the monocotyledonous stems with secondary thickening), it will be seen, on observing it more closely, that at the limits of the figure the tissues bear traces of less active division, the fact being that the activity is specially localised around the point of origin of new bundles (cf. *infra*).

The arrangement of the other tissues of the older root now demands further attention. The spicular cells seem here, as in other parts of the plant, to be pretty uniformly distributed; they are more numerous towards the periphery of the root. Fresh spicular cells may be formed from single cells of the parenchyma at any point outside the vascular bundles; occasionally they are even to be found embedded in the secondary phloem, in which case they must have originated from cells of the bast parenchyma, or directly from the cambium. The longitudinal axis of the spicular cells is usually, but not always, nearly parallel to the axis of the root. Sclerenchyma fibres are also to be found in considerable numbers in the old root: they lie scattered singly in the parenchyma, chiefly around the periphery of the vascular bundles; they take a sinuous, longitudinal course. Gum passages are also to be found in the roots: they appear irregular in their distribution (fig. IV).

The parenchyma in which all these several tissues are embedded deserves special attention, since many of the abnormalities of structure of the root and also of the stem of Welwitschia are the outcome of its peculiar properties. The most remarkable of these is its constant capability of cell division (Halb-meristemetisch, De Bary, 'Vergl. Anat.,' p. 634). The parenchyma seems in no living part of the plants which I have examined to have passed over to the permanent condition; it seems normally to be subject during its whole life to occasional division. It is true that the activity of this division is not uniform, but that it is specially localised at certain points, such as (*a*), the base and sides of the leaf groove (fig. X); (*b*) the point of origin of new vascular bundles (fig. V); (*c*) the phellogen layer; but that does not alter the fact that cell division may and usually does occur (at least in moderately young plants) more or less actively wherever there is in stem or root a parenchymatous ground tissue. We shall now be able better to realise what occurs when new peripheral bundles are formed, as in fig. V. An active division takes place at the point where the new bundle is to be formed; the surrounding tissue keeps pace with the radial extension thus produced, but the activity is merely local; there is no tangentially continuous ring of activity dividing tissue, such as that in the monocotyledonous stems with secondary thickening.

Though this activity of division is well marked in the parenchyma of the root, it is in the stem hereafter to be described that it is most striking; and, indeed, the swelling of the stock, which starts from the apex and proceeds downwards is mainly due to this activity.

A further peculiarity of the parenchyma is this—that apparently any cell may develop into a spicular cell or sclerenchyma fibre, and that this development is not restricted to any particular zone, but seems to be another proof of the general activity of the parenchyma. We find spicular cells in very different stages of development, occurring side by side, both in root and stem (fig. XX). (For the development of the spicular cells, see below, p. 590).

The lateral roots appear externally to the protoxylem masses, and are produced by the activity of the pericambium only, the cells of the bundle sheath apparently taking no part in the process.

Transition from Root to Hypocotyledonary Stem.

We have found no difficulty in recognising the relation of the structure of the older root to that of the young root, as described in my former paper (cf. l. c., fig. 22). As we now proceed to trace the transition from true root to hypocotyledonary stem, we must bear in mind the structure of the corresponding part of the young seedling as there described. It has already been shown how in the lower portions of the main root the two originally separate protoxylem masses unite centrally to form a single xylem plate, but that for some distance below the point of transition from root to stem this coalescence does not take place, the centre of the root being occupied by a mass of parenchyma. Here, as in other parts of the plant, the parenchyma retains its activity, and is capable of increase. This may be observed in plants at a comparatively early stage. In those sent me by Chev. Monteiro (four months old), divisions in this medullary tissue were already observable.

The course of the vascular bundles at the point of transition from root to stem was traced carefully in the plant represented in fig. III, and the following description is based chiefly upon results obtained from it:—The main root retains throughout its length the same arrangement of its vascular system (fig. IV), the only change being that higher up (*i.e.* nearer the stem); the peripheral system is more complicated. In the series of figures (VI, 1—6) which illustrate the transition from root to stem, the younger peripheral bundles, as well as the spicular cells and sclerenchyma fibres, are omitted. In all cases the xylem is shaded, while the phloem is left white. The numbered dotted lines in fig. III show the points at which the successive members of the series fig. VI were cut.

If we compare fig. IV with fig. VI, i, we see at once a difference in the arrangement of the central vascular group. In the latter figure it is divided into two parts by a plate of parenchymatous tissue. The protoxylem masses can easily be distinguished (*pr. xy.*). They have the same relative position as in fig. IV. Probably the fig. 22 of my former paper represents a section cut at a corresponding point in a younger plant: since a central fusion of the vascular tissues did not take place at this point in the young root, the secondary products, as seen in the older roots, also remain permanently separated by a plate of parenchymatous tissue, which divides the central vascular group into two parts. As we pass further upwards, the variation from the true root type extends. In fig. VI, 2, we have a condition in which the protoxylem masses, though they are still only two in number, are separated further from one another. This separation is due to an active increase in bulk of the parenchymatous tissue originally lying between them. It will be noticed that the components of each group tend to arrange themselves radially around the protoxylem masses as centres.

As we again pass further upwards, at a short distance above Section 2, each protoxylem mass is seen to divide into two (fig. VII, 3), and at the same time each of the two groups of bundles is divided by a plate of parenchyma into two, so that the central system of bundles now appears arranged in four groups. Each of these includes one protoxylem mass, around which the other bundles of the group tend to arrange themselves radially. It will be noticed that the distance of the two pairs of protoxylem masses in 3 from one another is greater than that of the single mass in 2, while the whole section is elliptical in form. This is doubtless the result mainly of the secondary activity of the parenchyma so often alluded to. The number of peripheral bundles is increased. Referring to fig. III, it will be seen that between the points 3 and 4 there occurs a sudden swelling of the stock, hence the difference in area between 3 and 4 of fig. VI. As they pursue their course upwards from 3, the four protoxylem masses, with the groups of bundles surrounding them, separate from one another till all four are at equal distances apart. Meanwhile the bundles of each of the groups arrange themselves nearly symmetrically round the four centres. The peripheral bundles undergo frequent anastomosis at the point of swelling, both with one another and with members of the four central groups. A comparison of the sections represented in figs. VI, 3 and 4, shows that though the plant increases very largely in thickness between the planes of these two sections, still the vascular system does not become much stronger, but that the parenchyma increases very much in bulk. (N.B.—The bundles in fig. VI, 4, are drawn rather too thick.) Here, then, we have to deal with a more prominent instance of the effect of the activity of the parenchyma. A comparison of fig. VI, 1—4, with fig. 15 G H and fig. 22 of my former paper will throw some light on the process of development. If allowance be made for the difference of scale of the two series, it will be seen that a great increase of bulk has taken place, and that in proportion to that increase in bulk the original protoxylem masses have become separated further from one another, *i. e.* that the increase is due not to a process of external apposition of tissues, as would be the case if the increase were due to a peripheral cambium layer, but to an intercalary growth throughout the section.

From the figures of Hooker illustrating the structure of older plants, and from observations of dissections of older specimens with the naked eye, I conclude that after a certain time this intercalary activity is diminished at the centre of the plant, and becomes localised in the peripheral portions.

Up to fig. VI, 4, we have been able to trace the relationship of the primary bundles of the seedling to the vascular system of the older plant. We must, however, leave the study of the upward course of the bundles at this point; before we are able properly to estimate the relations of the vascular tissues of the upper part of the stem, we must become better acquainted with the external conformation of the apex of the plant.

Apex of the Plant.—External Characters.

From plants of three to four months growth I was able to obtain early stages in the development of those two structures, which lie between the cotyledons; these were mentioned in my former paper (p. 19, note), but I was not then able to make any definite statement concerning them.

If a thick horizontal section be cut through the apex of a plant of about four months growth, it will be found to include (1) the bases of the cotyledons, the two being distinguished only by two indentations of the margin of the section; (2) the bases of the plumular leaves (*p. l.* fig. VIII); (3) the apical cone of the stem (*ap.*), which appears hardly any further advanced than in the younger seedlings described in my former paper (fig. 15 A); and (4) the two structures in question, which lie between the plumular leaves on either side of the apical cone (*l. c.*, fig. VII). In this figure they appear of unequal size; this appearance is, however, due to the fact that the plane of section was not exactly horizontal in the case figured. In reality they are as a rule nearly equal in size. (Note.—Fig. 10 of former paper is an exception to this.) I have observed no sign of fresh lateral appendages either on them or on the apical cone, with the exception of the fertile branches, the origin of which is clearly adventitious, and will be dealt with later. As the plant increases in size these lateral cones grow more strongly than the apical cone, and overtop it, so that in the older plants they are the only prominent structures to be found between the plumular leaves (fig. III, and fig. 10 of former paper). These two cones widen as the plant increases in age and form the crown of the plant. In plants with a crown of three inches in diameter it is possible still to trace a division of the crown into two areas corresponding in position to the original lobes; but in plants above this size this character is usually lost, since the comparatively small fissure which marks off the two areas from one another is masked by the much more deep fissure of the crown, which, as described by Hooker (l.c., p. 8), runs in a direction parallel to the bases of the plumular leaves of old plants. In all plants, however, of suitable age which have passed through my hands, the same conformation appears between the plumular leaves. We may then conclude that the apical cone of Welwitschia ceases to grow either apically or laterally, and that the crown is derived directly and solely from two lateral cones arising on either side of the apical cone between the plumular leaves; further, that these maintain a constant increase in width throughout the life of the plant.[1]

The question naturally arises, what is the morphological value of the two lateral cones? I conclude, on the following grounds, that they are morphologically axillary buds in the axils of the cotyledons.

(*a*) Their position supports this view, if allowance be made for lateral compression by the plumular leaves, to the development of which every part of the plant seems to be subordinate. The lateral cones do not appear to be genetically connected with the apical cone of the plant.

(*b*) Such axillary buds are to be found in the axils of the cotyledons of Ephedra.

(*c*) Their structure, when older, has very little resemblance to a leaf (the alternative view being that they are a second pair of plumular leaves).

(*d*) The development of axillary buds is the rule in the fertile branches of Welwitschia, and this occurs in the axils of the *first pair* of leaves of the male branch.

The position of the leaves and their insertion at the base of the leaf grooves are facts already so well known that they need hardly be again described. Reference need only be made to figs. III and VIII, in which the well-known arrangement will be easily recognised.

Hypocotyledonary Stem and Apical Region.—Internal Structure.

We shall now be in a position to continue the study of the course pursued by the vascular bundles as they proceed up the hypocotyledonary stem. We have seen the central bundles arrange themselves round four centres (fig. VI, 4), while in the peripheral part of the section appear a number of small bundles, which anastomose freely both with one another and with the

---

[1] As a parallel case to this is cited by Mr. W. T. Thiselton Dyer *Mora excelsa* of British Guiana.

members of the central groups. Successive sections higher up the stem show a gradual deviation from this arrangement. The four central groups split up into smaller central bundles (fig. VII, 5); these, however still retain a position obviously related to four centres. Above fig. VII, 4, it was not possible to identify the primary bundles. As they proceed again further up the stem, the bundles nearer the centre of the transverse section begin to rotate separately on their axes, so that each assumes a position with its xylem directed towards the centre and its phloem towards the periphery of the section (cf. the rotation of bundles in the hypocotyledonary stem of the seedling; former paper, p. 22). At the same time the courses of the bundles diverge, so that the whole bundle system becomes separated into (*a*) a group of bundles which arrange themselves into two wavy nearly parallel lines, and which are clearly the bundles which come in from the plumular leaves (blatt-spurschicht); this is seen to be the case in fig. VII, 6, where the section includes the margins of the plumular leaves; (*b*) a central group of small bundles, which will be afterwards seen to run up into the lobes of the crown; and (*c*) a peripheral series, which are regularly arranged with their xylem towards the centre and their phloem towards the periphery. Between these various series are to be seen bundles which pursue a horizontal course, and keep up connection between them.

For the further elucidation of the bundle system of the apex we must refer to longitudinal sections. Since the lobes of the crown have a bundle system of their own, it is to be expected that the arrangement of bundles will appear different according as sections are made in a median plane (fig. III, B), in which case the central lobes would be only slightly touched, or in a tangential plane, which would pass through the lobe as in fig. III, A. Such sections are represented in fig. VIII, A, B. In both of these may be seen the two deep leaf grooves, with the bases of the plumular leaves still adherent. Between the two grooves rise the lobes of the crown. In the one case (A) one of these lobes is cut through longitudinally, in the other (B) it presents its central edge. The dark shaded peripheral portions of these sections represent the masses of brown corky tissue, which are specially large at the upper part of the stock, and are produced by the degradations of portions of tissue originally active. The process of degradation extends as the plant becomes older, and even vascular bundles are often to be found included in the effete mass, especially in older plants. A similar degradation is also to be found at the apex of the lobes of the crown. The limit of the degraded tissue at the upper part of the plant is not marked by any very definite cork layer specially developed; it appears rather that a process of change goes on in the cell walls of the tissues already formed, and extends gradually inwards, accompanied by loss of the cell contents.

From the bases of the leaves bundles are seen to enter the stem ("blatt-spurschicht," De Bary, 'Vergl. Anat.,' p. 632. "Vascular stratum," Hooker). These pursue for a certain distance a nearly parallel course; later they break up into irregular branches. This is best seen in fig. VIII, B. This fact is easily reconciled with what we have already seen in transverse sections. In comparing the several figures it must be remembered that the dotted line in fig. VIII, B, indicates the point to which the transverse section, fig. VI, 6, corresponds: while the section, fig. VI, 5, is taken at a point some distance below the branching of the bundles of the leaf trace (Blatt Spurbündel). From these bundles of the leaf trace branches are given off in different directions; for clearness sake these, and others which intertwine with them, may be ranged into several categories according to the direction of their course; these categories merge, however, into one another.

(*a*) Branches which run horizontally. These take a course along the inner margin of the bundles of the leaf trace (fig. VI, 6) and send out branches between these bundles towards the periphery; or they take a direct course between the bundles of the leaf trace, and run out into the peripheral part of the stock; this is most common at the centre of the stock, fig. VIII, B.

(*b*) Branches which *ascend* either (i) into the central lobes, or (ii) into the upper peripheral parts of the stock (fig. VIII, A).

Lastly (*c*), there is a third series of bundles which are apparently not in direct connection with the bundles of the leaf trace: these run up the stem, and passing between the bundles of the leaf trace (apparently without any anastomosis) continue their course into the lobes of the crown. We have thus a direct vascular connection kept up between the following several parts of the plant (1), between the leaf and the peripheral part of the stock (2), between the leaf and the lobes of the crown, and (3) between the lower part of the stock and the crown. It will be seen later that these connections are similarly kept up in the older plant.

The mutual vascular connection of different parts of the periphery of the stock is also very complete. In fig. VIII it is shown that amastomosis of bundles in this part of the plant is frequent. This is more evident in tangential sections at the periphery, and more especially in old plants, where the bundles are seen to form a most complicated and irregular network. In the special plant under consideration (fig. III) these ramifying branches collect as they pass from the summit of the stock, so that they appear in transverse section (fig. VI, 6) as a regular peripheral series. It may be seen, in fig. VIII, how this happens. I have no evidence of "blind" endings of bundles in this part of the plant, though, owing to the great irregularity of course of the bundles, the proof of such endings would be very difficult. It cannot, therefore, be asserted that they do not exist (cf. "Cycas," De Bary, 'Vergl. Anat.,' 630).

In horizontal section the lobes of the crown present an appearance as in fig. IX, A, B, the former being a section near the base, and the latter near the apex, of one of the lobes. Externally the lobe is covered with epidermis; beneath this is a tissue capable of division, in fact, this is the chief formative tissue of the lobe, by means of which it increases in bulk (cf. *infra*). Vascular bundles are scattered somewhat irregularly through the section, but they are evidently arranged with relation to the centre, the xylem being usually central. The peripheral bundles pursue a less regular course than those nearer the centre; they anastomose, and run horizontally, &c. These irregularities are again more marked in the later developed bundles, which are formed as the lobe increases in size. In large plants the course of the peripheral bundles of the crown is very sinuous, and difficult to follow (cf. *infra*). As before stated, the tissues at the apex of the lobe suffer a corky degradation, which starts at the apex and spreads backwards, including, as in the stock, not only parenchymatous tissue and spicular cells, but also vascular bundles.

We must now turn our attention to the region of greatest vegetative activity, viz. the base of the leaf groove. If a longitudinal section be made through this, the whole mass of tissue is found to be in a state of active increase. The direction of the divisions is shown in fig. X, which represents under a higher power the area marked off (x) in fig. VIII, A, and includes part of the outer and inner lips of the leaf groove, together with the basal part of the plumular leaf. The divisions in the tissues, beneath the epidermis, which covers both lips of the groove, are most frequent in a direction parallel to the surface, so that the chief increase of bulk of tissue is in a direction perpendicular to the surface. The divisions in the tissues of the stock below the base of the leaf groove are mainly transverse (as regards the axis of the stock). A growth in length of the stock is thus effected. The divisions at the base of the leaf itself are mostly in a similar direction, that is, at right angles to the surfaces of the leaf; an increase in length of the leaf structure is thus produced. The divisions in these several parts being constantly repeated in the same direction, the cells assume an arrangement in rows at right angles to the direction of division. Such rows may be traced distinctly throughout the greater part of the upper portion of plants of the stage of development of fig. III, and this shows that the greater part of their tissues have been or are concerned in a similar division. Of course the rows of cells are not so clearly marked in the older parts as at the base of the groove, where the increase is most active: still they are easily traceable. I have accordingly tried to represent, by means of lines in fig. VIII, A, the direction of these rows of parenchymatous cells (cf. Sachs, 'Anordnung der Zellen in jüngsten Pflanzen-theilen,' fig. 5). Young spicular cells may often be found pushing their growing ends between the young tissues (for their development, cf. *infra*). Fresh vascular bundles also appear below the surface of the lips of the groove, but their course is, as a rule, so tortuous that it can only be followed with difficulty. In the leaf, however, their course being rectilinear, it can easily be traced (fig. X).

Cell division continues throughout the life of the plant in the directions described, relatively to the leaf groove. The fresh cells thus produced assume an approximately cubical form. Bearing these facts in mind, we shall be able to understand how the further development of the plant proceeds. Since the divisions in the tissue immediately below the epidermis of both lips of the groove are mostly parallel to the surface of the groove, an increase in bulk takes place, both in the crown and in the upper part of the stock, mainly in a direction perpendicular to the surface, *i.e.* both the crown and the upper part of the stock become wider, at the same time the depth of the leaf groove does not materially increase. We have seen that below the base of the leaf groove the divisions are mainly in a direction perpendicular to the axis of the plant; hence there results an increase in length of the whole plant. But as the width of the crown increases the activity of the central portion falls off, till finally growth in length ceases in that part. The centre is, therefore, overtopped by the more actively growing peripheral part. Hence originates the cup-like form of the apex of old plants (cf. Hooker's figures). We have already an indication of this in fig. XI. Finally, the divisions in the base of the leaf itself are mainly transverse, and thus the growth of the leaf is strongest longitudinally. For the verification of these several conclusions the figs. X and XII of this paper should be compared with the figures of older plants given in Sir J. Hooker's memoir. It will then be easily understood how, by the increase in width of the lobes of the crown, the bundles of the leaf trace (vascular strata of Sir J. Hooker) of the plumular leaves, which are in the young plant parallel to one another (fig. VIII), become wedged apart. As the plant grows the angle between them constantly increases till, in very old plants, the bases of the leaves almost lie in the same plane.

The section fig. XI serves as an intermediate stage between the plant represented in fig. III and the older plants described and figured by Sir J. Hooker. On comparison it will be seen that, though the relative positions of the several parts are slightly altered by growth, the arrangement of the vascular bundles corresponds to that already described in the younger plant. We are able here, as before, to trace a direct vascular connection (1) between the leaf and the peripheral part of the stock, (2) between the leaf and the crown, and (3) between the stock and the crown. It may further be observed how in this older plant the central bundles of the stock collect at some distance below the entry of the leaf bundles into groups, which are identical with the central vascular groups described in the lower part of the stock of the younger plant (cf. fig. VI, 4, 5). On the other hand, the arrangement of the vascular bundles in fig. XI obviously corresponds to that described by Sir J. Hooker in older plants. We have here (using his nomenclature) the "vascular stratum," consisting of the bundles which enter from the leaf, the "ascending bundles," which rise into the crown, and the "descending" system, which passes into the stock.

The scheme of vascular arrangement proposed by Bertrand ('Ann. d. Sci. Nat.,' série V, vol. XX, pl. 12, fig. 14) may be here noticed. A glance at the figures illustrating this paper will be enough to show that his scheme does not accord with my observations.

In order to confirm our results we may turn to the appearance presented by sections taken from an old plant in the plane of the bundles of the leaf trace, so as to follow their course into the stem (fig. XII). From these it is learnt that the leaf bundles do not all run the same distance into the stem; that they vary in this respect, some pursuing a direct course almost to the centre before they pass out of the plane of section, others successively less distances; also, that there is a certain amount of regularity in their arrangement, those which continue their course for a less distance in the plane of section alternating with those which pursue a direct course for a greater distance. Further, that before leaving the plane of section the bundles usually divide, and the branches often anastomose with bundles pursuing a course perpendicular to them; or they may simply curve out of the plane of section without anastomosis, and take a longitudinal course upwards or downwards. Lastly, of the bundles, which appear cut transversely in fig. XII, while some are connected by anastomosis with the bundles of the leaf trace, others appear to be quite separate from them; these are the bundles already mentioned which pass between the bundles of the leaf trace, and maintain a direct vascular connection between stock and crown. It will be seen in fig. XII that fresh bundles running perpendicular to the section are formed at the peripheral part of the stem. *i. e.* nearer the shaded portion of the figure, which represents the region of greatest vegetative activity at the base of the leaf.

On comparing the description now given for the older plant with that before given for the younger plant, we must conclude that the vascular arrangement corresponds in the two cases, and that no fundamental change appears in the mutual vascular connections as the development of the plant proceeds. The chief difference lies in the fact that the bundles of the leaf trace do not all proceed for an equal distance into the stem in the older plant; and this has an important bearing (1) upon the mode of increase in number of the bundles in the leaf as its development proceeds, and (2) on the arrangement of the bundles in the stock in the older plant.

It is an obvious fact, which may be observed on comparing plants of different ages, that the plumular leaves increase in width as they grow older, and that this is accompanied by an increase in the number of their vascular bundles. The question therefore arises—How and where the fresh bundles are formed?

The course of the first two bundles of the plumular leaf was described in my former paper (cf. fig. 15, A). Comparing this with our fig. VIII, we see that at first, at all events, the number of bundles is increased by the successive development of fresh bundles near to the margins of the leaves. In the case in question we have two pairs of such secondary bundles, but this mode of increase in number of the bundles is not continued in the later stages of development; this is proved by such an appearance as that presented in fig. VI, 6, 7. On carefully examining such sections as the former of these no trace of young marginal bundles in course of development is to be found. On the other hand, there appear between the older bundles of the leaf trace younger bundles, as shown in fig. VI, 7, which are evidently freshly intercalated between the pre-existing bundles of the leaf trace. In fig. XII we have further evidence of this intercalation of fresh bundles, which seems to be more common in older than in younger plants. These younger intercalated bundles run parallel to the older series, but do not extend so far into the stem; in fact, those bundles which are youngest follow the plane of the leaf for the shortest distance into the stem. We have, then, an explanation afforded us of the difference of the bundles in this respect, and we may presume that the same rule applies to the bundles of the leaf trace throughout, that is, that the oldest bundles are those which extend furthest into the stem in the plane of the leaf, while those which extend successively a less distance are younger. And this leads us to the second point, viz. the bearing of these conclusions upon the arrangement of the bundles in the lower part of the stock. It has been described by Hooker (p. 14) how in the stock and root of old plants the peripheral bundles are arranged in rings. The outermost of these rings is the youngest. This is the natural result of a process of continued intercalation of fresh bundles of the leaf trace as above described, followed in each case by a curvature of the newly-formed bundle out of the plane of the leaf trace into the peripheral part of the stem: and since before this curvature each new bundle proceeds a shorter distance towards the centre of the stem than the next older bundles, it will appear in transverse section of the stem nearer the circumference of the section than they. A similar explanation of the arrangement of bundles may be given for the crown; here, however, the bundles are less regularly arranged.

Immediately below the surface of the lips of the leaf groove is to be seen a very complicated network of anastomosing bundles, usually of small size. These are represented in fig. XII, which includes the lower lip of the groove. I did not observe any blind endings of these bundles, though such endings may exist. Still to prove this is very difficult, since the bundles pursue a most tortuous course. A similar system of bundles is found at the periphery of the crown. These bundles are accompanied by tracheids with reticulated walls (v· Mohl's "transfusions Gewebe;" cf. Strasburger, 'Coniferen,' p. 99). In old plants these bundles attain a considerable size, so that they can easily be recognised with the naked eye. Further from the lip, most of these bundles join some few main trunks, which pursue a more regular course, and finally join the descending bundles of the leaf trace. But all of them do not end thus. As before mentioned the corky degradation of tissue at the apex of the plant often includes vascular bundles, and this is especially common in older plants. It is certain of these ramifying bundles which are thus included in the degraded tissue.

We have seen in my former paper that in the earlier stages of development the vascular system of Welwitschia does not differ greatly from that of other allied plants. The primary structure of the root corresponds closely to that of Ephedra, while the arrangement of the bundles of the hypocotyledonary stem presents no very remarkable peculiarities. It is only when the plumular leaves begin to develop that the vascular system assumes an arrangement peculiar to this plant. Still it is interesting to trace, even in plants of considerable age, how close is the correspondence in arrangement of bundles to that described in my former paper on the seedling of the plant.

In the root the secondary thickening proceeds at first in the normal manner, and though, as the age increases, fresh peripheral bundles are formed, still even in very old roots the primary xylem masses and the first products of secondary thickening may be observed with certainty.

Passing upwards from the root to the hypocotyledonary stem, we have seen that in a plant of considerable age (fig. III) the course of the primary xylem groups could be accurately followed; that as we pass from the root upwards the two primary xylem groups separate; that each divides into distinct parts; that these four parts could be traced into the hypocotyledonary stem, in a transverse section of which there appears round each of the four primary xylem groups a number of secondary bundles arranged in a radiate manner. Comparing these facts with the observations recorded in my former paper on the seedling, it will be seen that the lower part of the plant retains for a considerable time an arrangement of the vascular tissues which is very closely related to that of the seedling. This grouping of the secondary vascular bundles round four centres corresponding to the original vascular bundles of the seedling may also be observed in plants of much greater age than that represented in fig. III. Sir Joseph Hooker observed and figured it, though, as the structure of the seedling was then unknown, the explanation of the fact was not obvious.

As we approach the plumular leaves the arrangement of the secondary vascular bundles corresponds less closely to that of the primary bundles of the seedling, and seems to be determined rather by the form and position of the plumular leaves than by relation to the pre-existing bundles. The vascular arrangement at the upper part of the plant having been fully described above need not be here recapitulated; we must, however, for a moment notice the system of small frequently anastomosing bundles which is found immediately below the surfaces of the leaf groove. Those are, as far as I know, unique. They do not extend far, and appear as a rule to be thrown off with the masses of effete tissue which cover the apical part of the stock and crown. They appear, therefore, to be in the main a temporary system. It is with them that the vascular system of the fertile branches is connected, as will be stated below.

Comparisons have been drawn between the bundle system of Welwitschia and that of other plants. For instance, Strasburger ('Conif. ü. Gnet.,' p. 376) compares the stem with that of the Piperaceæ, Amaranthaceæ, and Nyctagineæ, while De Bary ('Vergl. Anat.,' p. 634) cites, in connection with it, the Chenopodiaceæ, Amarantaceæ, and Mirabilis. There are undoubtedly points in common between these and Welwitschia. It still remains, however, to investigate the structure of various plants cited by Hooker and Strasburger as having an external conformation more or less like that of Welwitschia, and it is perhaps in these plants that we may hope to find the closest similarity of vascular arrangement to that in Welwitschia, which appears at present unique, and more especially so at the upper part of the plant.

Development of Fertile Branches.

The origin of the fertile branches is a point hitherto unobserved. I have been supplied from the Kew collections with material for the study of the development of the male branches. Since the position, appearance, and structure of the female branches is similar to these in the mature state, we may, for the present, assume that they resemble them also in their mode of development.

The development begins at some distance from the base of the leaf groove, and usually on the inner lip of the groove, though exceptions to this rule are cited by Hooker (p. 20). The point at which the development of a fertile branch is about to begin may be recognised externally as a dark dot, the change of colour of the tissues at that point being due to the increase in the quantity of starch and protoplasm which they contain. First there appears a ring-like depression of the surface (fig. XIII, 1); this depression deepens, while the central part enclosed by it grows on (2). As the development proceeds, that part of the ring furthest from the base of the leaf groove is more depressed than the part nearer to it. The result of this is that the central cone, which assumes the functions of the apical cone of the young branch, is turned upward (3, 4). The apex of the cone does not rise above the level of the surface of the lip, and it is thus protected during its early stages from pressure of the plumular leaf. The tissues surrounding the depression grow meanwhile more rapidly, so that the apex of the young branch is gradually arched over by flaps of irregular shape, which give to the branch, when seen from above (fig. XIII, 5), an appearance as though it had been developed endogenously, and were breaking through the external tissues. This appearance remains after the branch has finally developed, the base of it being surrounded by an irregular margin (fig. XIV). Leaves are produced laterally on the apical cone of the young branch; these appear in successive decussating pairs, the first pair being anterior and posterior. The process of extension I have not been able to trace, but there is no doubt that it begins below the lowest pair of leaves, since (1) no traces of leaves are to be found at the base of the mature fertile branch, (2) the lowest pair of leaves of the mature branch are in the same position relatively to the plant as the first pair developed on the young fertile branch, *i.e.* anterior and posterior. As to the further external characters of the fertile branches I need only refer to writings of Hooker, Strasburger, and MacNab. I have nothing to add to the detailed account given by Strasburger (' Coniferen,' pp. 141, &c.) of the structure of the fertile branches. As he describes, the bundles run nearly parallel down them. As they enter the main stem of the plant they anastomose with one another, and passing inwards, are almost immediately lost in the anastomising network of bundles already described beneath the surface of the lip. With these bundles they appear to fuse (fig. XV).

We thus see that the origin of the male branches is *exogenous,* and since they first appear on the lip of the groove at some distance above its base they are *adventitious.*

Development of Spicular Cells.

As before stated, it appears that any cell of the active parenchyma may develop into a spicular cell. It is, however, at the lower part of the leaf that the first stages of this process are most easily followed, since there the tissues are in a state of rapid differentiation from a mass of uniform tissue, such as that seen in fig. X. In the mesophyll, which is still actively dividing, certain cells cease to divide, increase in size, and put out protrusions in two or more directions. These grow apparently at their apex, and push their way between the other tissues at the angles where the cells meet (fig. XVI). These cells remain singly nucleated, and I have never observed a spicular cell with more than one nucleus. In spicular cells developing in the stock or crown, the growing ends seek out and follow the intercellular spaces, where growth is naturally easiest (fig. XVII). As the cell increases in size the cell wall becomes differentiated into an outer cellulose wall and an inner lignified wall. The well-known crystals of calcium oxalate assume, at a very early stage, their final development, as may be seen in figs. XVII—XX. They are so placed as to remain with one side in contact with the cellulose wall, while the other sides are contiguous with the inner lignified wall (fig. XIX). That the outer cellulose wall is not merely composed of the cellulose walls of the surrounding cells (as might be concluded from such a case as that in fig. XX) is shown by the existence of it in those parts of the wall of spicular cells which abut on intercellular spaces (fig. XVII). The cellulosew all remains permanent. As the cell develops the lignified inner wall increases in thickness, till in the fully-formed spicular cell the cavity is almost obliterated (fig. XX). This inner lignified wall is not uniform. In both longitudinal and transverse sections pits are to be seen scattered irregularly between the crystals; these are represented in fig. XVIII as dark dots. They extend from the outer cellulose wall to the cell cavity, and are filled with plugs of protoplasm (fig. XIX), which are continuous as far as the outer cellulose wall. The protoplasmic contents become more and more reduced as the development of the lignified walls proceeds, till they appear to be finally lost.

The mode of development of the spicular cells may be compared with that of the internal hairs in the intercellular spaces of the Nymphæaceæ, and in the tissues of certain Aroideæ (cf. De Bary, 'Vergl. Anat.,' p. 230, &c., where the literature is cited). The spicular cells are an interesting intermediate between these and sclerenchyma fibres, which they resemble in many respects.

Sieve Tubes.

The sieve tubes of Welwitschia have already been described by Strasburger ('Coniferen,' p. 380), and figured by Bertrand ("Cellules grillagées," 'Ann. d. Sci. Nat.,' série v, vol. XX). But in longitudinal sections from the root I have obtained different results from the latter writer. I find the sieve tubes to resemble those of the other Gymnosperms in their general characters, but the sieve plates are collected almost exclusively on their sloping ends (fig. XXI). Occasionally one is met with on the lateral walls. The sieve plates are often coloured a sherry-brown with Schultz solution, while the rest of the walls is blue. The contents are transparent and almost free from granules. Between the sieve tubes appear cells of the bast parenchyma.

Results.

1. The cotyledons wither and fall off, and the pair of leaves of the mature plant are the first pair of plumular leaves.

2. The crown is derived entirely from the continued growth of two lobes (axillary buds), which appear in the axils of the cotyledons. The apical cone of the plant remains rudimentary.

3. The parenchymatous "ground tissue" throughout the plant remains, for a long period at least, capable of active growth and division (halbmeristematisch). The results of this are (*a*) increase in bulk of the tissue itself; (*b*) production of fresh spicular cells, and sclerenchyma fibres by the growth of single cells of it; (*c*) formation of fresh vascular bundles by means of repeated divisions at certain points.

N.B.—It should here be observed that the active tissue has a different origin in different parts of the plant; in the root it is derived from the "tissu conjunctif" of Van Tieghem, in the stem from the fundamental tissue generally.

4. The directions of cell division at the base of the leaf groove are such as to bring about—

(*a*) Increase in length of the leaf.

(*b*) Increase in length of the whole plant.

(*c*) Increase of the tissues in a direction perpendicular to the surface of the groove, and hence increase in bulk of the crown on the one hand, and of the stock on the other.

5. The two originally separate primary xylem masses of the root unite centrally at a short distance below the feeder, so as to form a single plate. Above the point of union a parenchymatous pith remains permanently between them.

6. Further development proceeds in a direction at right angles to this plate, by means of a cambium layer. The result is the formation of two masses of secondary vascular tissue separated laterally from one another by two broad medullary rays. Thus far the structure corresponds to that described for Ephedra (cf. Van Tieghem, La Racine, p. 211).

7. Smaller vascular bundles separate laterally from these masses of secondary vascular tissue, and pursue a sinuous course along the medullary rays, fusing from time to time (*a*) laterally with the system from which they were derived, and (*b*) with the later developed system of peripheral bundles. These bundles may be compared with the intercalary bundles of many dicotyledonous stems (Zwischenstränge, De Bary, 'Vergl. Anat.,' p. 468).

8. Peripheral bundles appear later; their terminations I have not been able to trace. They are formed by the specially localised activity of division in the parenchymatous "ground tissue," and are arranged in more or less regular rings.

9. All bundles of the root remain for a considerable time open bundles, and by the activity of their cambium may attain a large size. This is especially the case in the central group.

10. In the arrangement of the vascular bundles of the older plant at the point of transition from root to stem, there may be traced a very close relationship to the arrangement at the same point in the seedling. The differences are due to (1) increase in size and number of vascular bundles, the later formed bundles being, however, mainly grouped around the original bundles; (2) to increase in bulk of the "ground tissue," and consequent increase in absolute distance of the original bundles one from another.

11. As they pass up the stock the bundles retain for a time an arrangement obviously related to that in the seedling, but as the apex is approached this is gradually lost sight of by the breaking up of the groups of secondary bundles which lower down surround the primary bundles.

12. The vascular system at the apex of the plant is so arranged as to keep up the following direct vascular connections:

(*a*) Between the leaf, and both peripheral and central parts of the stem.

(*b*) Between leaf and crown.

(*c*) Between stock and crown (cauline bundles).

No fundamental change takes place in these relations as the plant develops.

13. Increase in number of bundles at the base of the leaf is effected at first while the leaves are young by the development of fresh bundles at the margin of the leaves, but later by intercalation of new young bundles between the older ones, and the successive peripheral rings of bundles in the stock correspond to the successive series of bundles thus intercalated.

14. The development of the fertile branches is adventitious and exogenous. Their vascular system is directly connected with the network of bundles which ramify below the surface of the leaf groove. There is no direct connection between the successive series of fertile branches and the successive rings of peripheral bundles of the stock, though both probably owe their serial development to the same causes, *i.e.* alternating periods of activity and dormancy of the plant.

15. Spicular cells are developed from single cells of the parenchyma. These undergo apical growth, pushing their way between the surrounding tissues. They often follow the intercellular spaces. Their walls are differentiated into an outer cellulose layer and an inner later developed lignified layer. The crystals of calcium oxalate lie between the two. The lignified wall is pitted. The cells remain uninuclear.

16. The sieve tubes have their sieve plates collected on their sloping ends; here and there a sieve plate is found on their sides.

Postscript.—Since the above paper was written there has appeared in 'The Gardener's Chronicle,' August 13th, 1881, an account by M. Naudin of fresh observations on young plants of *Welwitschia mirabilis,* which seem to show that the type of development, which is *constant* in the specimens which I have had the opportunity of observing, is not the only one for this remarkable plant. In all my specimens the series of members, as above described, has been—(1) two *cotyledons* present in the mature embryo, (2) two *plumular leaves* decussating with these, and capable apparently of indefinite growth, (3) two structures which appear between these, and which, for reasons given above, I regard as *buds in the axils of the cotyledons,* (4) *apical cone* of the whole plant, which does not develop further. I gather from M. Naudin's description that there may be another and different type of development, which, moreover, he seems to find constant. In this case the following succession of members is found by him—(1) two *cotyledons,* (2) two *small plumular leaves* decussating with the above, (3) a *stem* about two lines long, bearing (*a*) an almost imperceptible *bracteole,* (*b*) a *true leaf,* (*c*) *two further leaves,* "alternate but very close together, so that they appear opposite, and which seem to terminate the tigellum."

I do not doubt that this description points to a further development of the plumule than any which has occurred in the plants at Kew. In these the axis remained in all cases very short, and certainly did not attain a length at all approaching two lines, which is the length stated by M. Naudin for his plant.

I conclude, then, that the observations of M. Naudin do not affect the above interpretation of my observations on the plants at Kew, but rather point to the conclusion that the type of development which I have described is not universal.

Further, I think it is unlikely that the Kew plants will enter on the further stage of plumular development as described by M. Naudin. The oldest seedlings now living at Kew were sown in August, 1880 (*i.e.* five or six months before those of M. Naudin). These, when last I saw them (July, 1881), showed no outward signs of any deviation from the type which I have described as normal. Their two plumular leaves were of healthy appearance, and had attained a size almost equal to that of the cotyledons. This condition may be contrasted with that described by M. Naudin. He expressly mentions that the pair of leaves succeeding the cotyledons are "*very small,*" and speaks of their "growth being apparently arrested." May we not have to deal with two alternative types of development—(1) one in which the first pair of plumular leaves are large, while further development of the main axis is arrested; (2) one where the first pair of plumular leaves are small, while the main axis grows in length and forms further appendicular members?

Lastly, M. Naudin suggests the possibility of the two members, which have been hitherto regarded as leaves, being really flattened branches. In connection with this I can only say that if (as the specimens I have examined lead me to believe is the case) the two leaf-like members of the mature plant are *normally* derived from the first pair of plumular structures, as described in my first paper, there can be little doubt of their foliar nature. The only alternative would be the assumption that they are extra-axillary, *i.e.* adventitious axes, developed nearer to the apex than the youngest leaves, a supposition which is warranted neither by their structure, appearance, or origin.

August 29th, 1881.

On the STRUCTURE *and* SIGNIFICANCE *of some* ABERRANT FORMS *of* LAMELLIBRANCHIATE GILLS. By K. MITSUKURI, Ph. B., of Tokio, Japan, late Fellow of the Johns Hopkins University, Baltimore. (With Plate XXXIV.)

THE following contribution to the morphology of the Molluscan branchiæ is part of an investigation on which I have for some time past been engaged, under the direction of Dr. W. K. Brooks, in Professor Martin's laboratory at the Johns Hopkins University. The gills, of which the description is here given, are those of *Nucula proxima* and *Yoldia limatula*. They are extremely interesting because of their simple structure, and this account of their minute structure is published with the hope that it may throw some additional light on the nature of Lamellibranchiate gills. I wish to express here my sincere thanks to Dr. Brooks for his constant advice and assistance. I am also deeply indebted for specimens used in the investigation to Professors A. E. Verrill and S. J. Smith, of Yale College, and to Mr. Richard Rathbone, of the United States Fish Commission.

Nucula proxima, SAY.

This Lamellibranch shows many departures from the structure which is generally regarded as characteristic of the class. Fig. 1 gives a fair idea of what is seen when the left valve of the shell has been taken away, and the mantle of the same side removed along the lower border of the visceral mass near the line *x y*. *a. a.* is the anterior abductor muscle made up of several fasciculi; *p. a.* is the posterior abductor. It will be noticed that *Nucula* possesses one of the few shells in which the umbo is turned toward the posterior end. In the specimen figured, the visceral mass (*v. m.*) shows convolutions on the surface, which, under the microscope, proved to be the male reproductive organ, probably enormously developed for the breeding season, and this character enables one to distinguish the sex of a specimen without difficulty. All the males have these convolutions, and, when preserved in alcohol, are of a greyish colour. The females show hardly any convolutions, and are much more darkly coloured. The foot (*f*) is folded longitudinally at its end, and can accordingly be spread out into a flat circular disc. The labial palpi (*l*) are unusually developed, and might at first sight be taken for gills. The inside of the outer and the outside of the inner palpus are raised into numerous parallel ridges, which, as shown in the figure, can be seen from the outside, and do not extend to the lower margin. At their posterior end there are two remarkable structures. One of them is a hood-like structure (*l. b.*, figs. 1 and 2), which is the posterior prolongation of the united upper edges of the inner and outer palpi. The other (*l. a.*, figs. 1 and 2), lying immediately below the first, is a long tentacular appendage. It is a hollow tube, open, however, along a line on its posterior aspect, and having its cavity continuous with the space between the two palpi. As it has been seen protruded, with the foot outside of the shell (Woodward's 'Manual of Mollusca,' p. 426), and since, in alcoholic specimens, a great deal of dirt and sand is found along its length and between the palpi from its base to the mouth, it is no doubt a food-procuring organ, probably sending a constant stream of nutritive matters to the mouth by means of its cilia. It is interesting to notice in connection with this appendage that in *Nucula*, the gills, unlike those of ordinary Lamellibranchs, must be practically useless for obtaining food, as will be evident from the following descripion of them.

The gill (*g.*, figs. 1 and 3) is comparatively small. It is situated quite posteriorly, and is suspended by a membrane (*m.*, figs. 1 and 3), which is attached to the body along the broken line $x\,y\,z\,w$. It is united to the visceral mass (*v. m.*) from x to y, and to the upper part of the foot (*f*, fig. 3) from y to z (see figs. 1 and 3). At the last point, having come to the median line of the body, it joins with its fellow of the opposite side, and they continue in this way as far as w. Here they separate again, each proceeding to the posterior tip (p) of the gill of its own side. It should be remarked that, as the point x is further from the median line of the body than the point y (fig. 3), there is a considerable free space beneath the suspending membrane of the gill.

When we turn to the gill itself, we find an altogether unusual structure. Fig. 4 shows it dissected out and seen from below and slightly from one side. In general appearance it resembles a boat which is suspended by its keel. $x\,c\,p$, fig. 4 (seen in cross section at $i\,j$, fig. 5), is the line of attachment and corresponds to the keel; $x\,d\,p$, fig. 4 (seen in cross-section at d, fig. 5), represents the bottom line of the hollow of the boat. The latter is bounded by the two surfaces $x\,a\,p\,d$ and $x\,b\,p\,d$ (fig. 4; seen in cross-section at $b\,d$ and $a\,d$, fig. 5). The anterior end (x, fig. 4) is rather blunt, while the posterior end (p, figs. 1, 3, and 4) is quite pointed. The resemblance of the gill to a boat is, however, only very superficial, as the gill is not one solid mass, but is made up of a series of paired plates of a peculiar shape, placed one after another from the anterior to the posterior end. A little dissection under a lens will show that the part above the line *x d p* (fig. 4) and below the line of suspension (*x c p*), is continuous along the entire length of the gill, and that, with this part for the *stem*, the plates are given off, one after another, in pairs to the two sides (see fig. 5). The plates constitute the proper respiratory parts of the organ. They are largest in the middle, and diminish in size toward the two extremities.

It is evident from this description that the gill in *Nucula* is of quite an exceptional nature. It does not, as in most Lamellibranchs, extend along the whole length of the side of the body, constituting the most conspicuous object of the mantle cavity, but is comparatively insignificant, being pushed back and freely suspended in the mantle cavity. It cannot, therefore, divide the latter into the suprabranchial and infrabranchial chambers, and is, of course, utterly devoid of any structure like the ciliated water-passages in the ordinary gill, for driving water from the lower to the upper. It cannot, also, as has been said, serve as an effective food-procuring organ. The gill in *Nucula* must for these reasons be of vastly less functional importance to the animal than it is in common Lamellibranchs, and, so far as I am able to see, serves only as the organ of respiration. It seems to me, however, that the division of the mantle cavity into the upper and lower chambers is begun in the posterior part. It has been seen that ventral to the membrane suspending the gill (*m*, figs. 1 and 3) there is a large space continuons with the general branchial cavity, and there certainly is a space dorsal to this membrane. These spaces seem to be the rudiments of the supra- and infrabranchial chambers. Moreover, the arrangement of the different parts at the posterior end, as seen in fig. 3, recalls that of the corresponding parts in many of those genera in which the mantle cavity is divided into two parts. It is not difficult to conceive how the same division might be brought about in the case of *Nucula*, by proper development of the gill and the membrane.

Fig. 5 shows a pair of opposed plates considerably enlarged. The solid part (*i d j*) which I have called the *stem*, and which is continuous throughout the whole length of the gill, together with the suspending membrane (*k i j l*) is seen in cross section in the middle, and from this middle portion the paired plates (*e. e.*) are seen to proceed. The coloured part at the bottom represents the complex chitinous framework. The membrane (*k i j l*) is made up of fibrous tissue, the bundles of which this is composed crossing each other in many directions. Its free surfaces are covered with columnar epithelium. The stem consists mostly of a solid mass of large irregular cells with rather large nuclei. There are, I am almost certain, *two* blood-channels excavated through it; a lower larger (*n*), and an upper smaller (*o*). The latter seems to be in connection with a free space (*q.*) found often in sections of the suspending membrane. The large channel (*n*) sends a branch (*r*) into each plate. The fibrous tissue found in the upper membrane dips down into this part at regular intervals, viz. between every branch (*r*) of the lower blood channel (*n*). How these fibres end below, when they reach the chitinous framework, I have not been able to make out. A few fibres (*u*) are sent down into the plate a little above the blood-channel (*r*), and gradually approach and finally touch the latter near its lower end. A few more fibres (*t*) are seen along the upper edge of the plate. Exactly what this fibrous tissue is I am unable to make out, but it seems to be some sort of tough connective tissue, with perhaps muscular fibres more or less intermixed. That it is very tough and serves as a support to the whole structure is seen by the fact that the fibres often stick out beyond the broken edge of the soft tissues. The trough of the chitinous framework is seen at *s*, in cross-section. It extends along the whole length of the gill and sends out two branches into each plate. I have obtained the appearances, in some sections, of a bundle of fibrous tissue running in it and filling it. The framework will be described more fully further on. The plates (*e*), the proper respiratory organs, are comparatively speaking very broad and quite thin, and hang down from the solid part of the gill. The epithelium of the plates which is represented in the figure as ending abruptly at the edges *i d* and *j d*, turns at a right angle at these lines to cover the *stem*, and is soon reflected outwards again to form the epithelium of the next plate in the series. This is evident from an inspection of fig. 8. Each plate may be said to be simply an enormously widened blood-channel (fig. 6), and as the blood is necessarily spread out in a thin layer over a large area, the purposes of aëration must be admirably served. The columnar epithelial cells seen at *a d*, fig. 5, are very characteristic of the plates under a microscope, and are the cells (*d a*, fig. 6) around the chitinous bars (*h*, figs. 5 and 6) seen in optical section. The surface of the irregularly rectangular cells placed just inside these columnar cells in fig. 5, ought therefore to be continuous with the outer edge of the columnar cells, but in order to avoid confusion is not so represented in the figure. This is also the case with the cubical cells along the upper edge. The chitinous support (*h*, figs. 5 and 6) of the plate runs near the lower edge (fig. 5) to its tip (*a* or *b*, fig. 5), and is made up of two entirely separate parts (seen in cross-section in fig. 6) applied closely together. Owing to the shape of these parts there is, however, a narrow oval space between them. This space, as will be shown further on, is continuous with the space in the trough (*s*, fig. 5) of the *stem*. The cells along the lower edge of the plate are columnar, and surround the chitinous support in a characteristic manner shown in fig. 6. Their surface outlines are irregularly rectangular, contrasting with the irregularly polygonal cells covering the rest of the plate. The branch (*r*, figs. 5 and 6) of the lower blood-channel (*n*) in the *stem*, is seen to be circular in cross-section and to bulge out the surface of the plate. These points are not, however, constant, as the vessel is sometimes constricted into more or less separate channels, while the amount of bulging seems to depend on the quantity of blood present. The remaining part of the plate (*e*, figs. 5 and 6) is flat and quite thin, enclosing a broad blood-channel between its two epithelial surfaces. It is here no doubt that the aëration of blood is accomplished. The cells of this part are cubical, as seen in fig. 6. Some of them send processes inward to join others from the opposite side. This gives a labyrinthine appearance to this part of the plate. The course of the blood is evidently from one blood-channel in the *stem* to the other, through the space in the plate. For instance, the blood may start from the upper channel (*o*) in the stem, go to the broad flat part (*e*, fig. 5) of the plate where it gets aërated, then enter the branch (*r*), along its upper edge, and run up this to reach the lower blood-channel (*v e*) in the stem. This is, however, a purely hypothetical course. I have had no means of determining whether the blood goes from the upper to the lower channel or *vice versâ*.

The framework which supports the gill can be separated out by heating it in dilute caustic potash, as it is insoluble in weak acids and alkalies. It is stained by carmine and other colouring reagents. Whether it is really formed of chitin I do not know, but as previous writers have described the substance as of that nature it will be convenient to use the term "chitinous support" for the present. The framework consists of a trough (seen in cross-section at *s*, fig. 5; longitudinally from below in fig. 8; diagramatically represented in fig. 7), which runs along the whole length of the gill, and from which a pair of closely-applied parallel branches (*h*, figs. 5, 6, 7, and 8) is given off into each plate. The trough is divided into two unequal parts: an upper larger and a lower smaller, by a cross piece (*c. p.*, figs. 5 and 7), which stretches from one side of it to the other, a little below the middle. This cross piece is not, however, continuous, but is pierced through by oral openings (*o v*, figs. 7 and 8) whenever branches are given off laterally to the plates. The space enclosed between each pair of closely-applied branches (see *h*, figs. 6, 7, and 8) is connected with the lower compartment of the trough by means of somewhat circular openings (*o p*, and *o′ p′*, figs. 7 and 8) found near the bottom. In fig. 8 the letters *a, a, a,* are placed opposite each pair of the branches that go into a plate. It will be seen how one half of the chitinous support of one plate, after forming an arch at the trough, turns round to enter the next plate in succession, and to constitute there one half of the support of that plate. The framework treated with potash, and sometimes without any treatment, shows marked longitudinal striation (fig. 8), and some of its fibres sticking out at the broken edge beyond the others resemble in appearance the fibres found in the suspending membrane, at *t* and *u*, fig. 5, and give reasons for thinking that the whole chitinous framework is nothing but the fibrous tissue found in other parts cemented closely together and forming one cohering mass.

Although, owing to the state of the specimens, I have obtained only here and there evidences of cilia, it seems reasonable to suppose that the whole gill is covered with cilia. On two rows of cells (*l. f.*, fig. 6; *d. a.*, fig. 5) on the lower edge of the plate I believe there are larger cilia than on the rest, as I have now and then seen their remains, and as, without any question, cells in the corresponding positions in Yoldia have long and conspicuous cilia.

Yoldia limatula, SAY.

Yoldia resembles *Nucula* in several structural peculiarities—in its well-developed labial palpi, with their peculiar food-procuring appendage, in its feather-like gills, in the posterior position and comparatively small size of the gills, and the consequent absence of the division of the mantle cavity into the supra and infrabranchial chambers. It differs from

Nucula in having a siphon, and further shows its departure from the ordinary lamellibranchiate structure in having a highly specialised tactile organ in the siphon.[1]

The gill, although different in details from that of *Nucula*, is essentially of the same structure as the latter. It is suspended by a membrane, as in *Nucula*. Fig. 9 shows it dissected out by itself. The line of suspension is *x c p*; *x d p* is the ventral median line, and corresponds to *x d p* in fig. 4. As in *Nucula*, the gill is made up of a series of paired plates, placed one after another, and attached to the central solid *stem* continuous throughout the whole length of the gill. The plates do not, however, project downward, as we have seen in the case of *Nucula*, but here turn upward (see fig. 11). The plates are largest in the middle, and gradually become smaller toward the extremities. At the front end (*x*, fig. 9) there is a rather interesting arrangement. Fig. 10 shows diagrammatically the relations of the various parts at the anterior termination of the gill. It will be seen that the plates of the gill gradually become smaller and finally die out toward the front, and the gill is continued simply as a flat membranous structure (*x*, fig. 10), which goes into the visceral mass (*v. m.*, figs. 9 and 10). A cross-section of this part shows that at its lower portion, at least, there is a blood-channel, probably continuous with one of the channels in the *stem* of the gill. In some specimens this membrane-like portion of the branchia is longer than in others, and goes some distance around the visceral mass.

Owing to the rather poor state of preservation of the alcoholic specimens, I have not been able to make out the histology of the *Yoldia* gill as fully as I should like, but the following description I believe to be correct in essential points :—Fig. 11 represent an opposed pair of plates, and corresponds to fig. 5 of the *Nucula* branchia. The suspending membrane (*k i j l*) consists of fibres crossing each other in several directions, and is covered on its two surfaces by columnar epithelium. The solid *stem* (*i d j*) of the gill has two blood-channels, an upper (*n*) and a lower (*o*). The latter seems to be in communication with a comparatively free space (*q*) in the middle of the suspending membrane. Directly below the upper blood-channel (*o*) there is a bundle of tissue, which appears to be fibrous, running the length of the gill (seen in cross-section at *f*, fig. 11). It serves no doubt for support. The floor of the lower blood-channel (*r*) is covered by a **V**-shaped bundle of longitudinal fibres (*s*).

---

[1] W. K. Brooks, 'Proc. Amer. Ass. Adv. Sci.,' 1874 (end of note).

This would seem to be homologous with the trough-shaped chitinous structure in *Nucula*, but seems to be formed of the same fibres already referred to several times, which are found in the suspending membrane and other parts of the *Nucula* and *Yoldia* gills, and I cannot establish any connection between this bundle and the chitinous bars (*h*, fig. 11) in each plate. The latter, when they reach the longitudinal bundle (*s*), make a bend and turn out again to enter the next plate in the series. In some sections I have obtained indications of a very thin layer of chitin beneath the fibrous bundle (*s*), which may, therefore, correspond to the fibres found *in* the trough of the framework in the *Nucula* gill (see above). If, however, this V-shaped structure is really homologous with the *trough* of the *Nucula* gill, it goes far in support of the view advanced above, that the chitinous framework is really made up of the fibrous tissue which is found in other parts, here cemented into one compact mass. In such a case fusion has gone further in *Nucula* than in *Yoldia*, and we see in the first genus the trough well united with the branches (*h*) in each plate. The plates (*e*, fig. 11) in *Yoldia* spread themselves upward instead of downward, as in *Nucula*. The chitinous bars (*h*), of which there are two in each plate, follow the curve of the plate and end rather bluntly about half way up, at the point *a*. That the part from *d* to *a* corresponds to the lower inner edge of the *Nucula* plate (*d a*, fig. 5) is shown by the characteristic rows of columnar cells having longer cilia than those found in other parts of the gill. There is another system of chitinous strnctures (*ch*, fig. 11). Many fine chitinous filaments come down together in a bundle on each side from the suspending membrane, and as soon as each bundle reaches the plate of its own side filaments spread themselves out like the frame of a fan over the whole plate. Several fibres sometimes proceed together, and then separating give the appearance of branching. They are found directly beneath the epithelial cells that cover the plate. The effect of this framework must be to keep the plate well spread out for the purpose of aëration. I have not succeeded in obtaining any single section which shows the structure of the plate well, but from the comparison of a good many sections which I have made, I feel tolerably sure that the whole space between the epithelial surfaces is pervaded by what Peck[1] calls "lacunar tissue" (fig. 12). It is

---

[1] R. Holman Peck, "The Minute Structure of the Gills of Lamellibranch Mollusca," 'Quart. Journ. Micros. Sci.,' 1877.

a loose trabecular tissue with many nuclei and within whose network blood can flow. The space between the chitinous bars (*h*, fig. 11), which is quite large in *Yoldia*, seems to be tolerably free from this lacunar tissue. Fig. 11 *a* gives the outline of the plate seen from one side.

Theoretical Considerations.

The gills, here described, of *Nucula* and *Yoldia* are, I think, the most rudimentary of any that have been studied so far. In fact, at first sight, the resemblance to the ordinary Lamellibranch gill is not apparent, and they suggest more the Cephalopod gill. But I believe, the homology of their various parts with those of more complex gills in *Unio, Mytilus, Arca*, &c., is not difficult to make out. After consulting the articles by Peck (loc. cit.), Posner,[1] Lacaze-Duthiers,[2] Bonnet,[3] and others, and also after examining the sections I myself have obtained of *Unio, Modiola, Scapharca*, &c., I have no doubt whatever that the *plates* in *Nucula* and *Yoldia* represent the descending or attached limb of the filaments in the *outer* and *inner* gill-plates in forms like *Mytilus, Modiola*, and *Arca*, and accordingly are homologous with the folds on the *inner* lamella of the *outer* gill-plate, and on the *outer* lamella of the *inner* gill-plate in *Unio, Anodon*, and *Dreissena*. If a comparison is made of my fig. 6 with any of the cross-sections of gill-filaments given by Peck, it will be seen at once how similarly the paired chitinous bars are placed, how almost identically the epithelial cells are arranged around them, how two rows of those cells (*l. f.*, fig. 6)—called by Peck latero-frontal epithelial—have longer cilia than the rest. In fact, Peck's fig. 12 (a transverse section of a filament of the Anodon gill) agrees with my fig. 6 in all essential points. The left hand-figure in his fig. 5 (the superficial view of the edge of a gill-filament of *Mytilus* showing the latero-frontal and other epithelial cells) and the upper part of his fig. 20 (the same view of a gill-filament of Anodon) would pass very well for the corresponding part in *Nucula*. So far as I can make out from rather poor specimens, the latero-frontal cells in *Nucula* are strikingly like those represented in Peck's

---

[1] Carl Posner, "Ueber den Bau der Najadenkieme," 'Archiv. für mikros. Anat.,' 1875.

[2] Henri de Lacaze-Duthiers, "Mémoire sur le Developpement des Branchies des Mollusques Acephales Lamellibranches," 'Ann. d. Sci. Nat.,' Ser. iv, tome v, 1856.

[3] Robert Bonnet, "Der Bau u. die Circulations-verhältnisse der Acephalenkieme," 'Morphologisches,' Jahrbuch iii, 1879.

fig. 20. If, then, the plates in the gills of *Nucula* and *Yoldia* represent the gill-filaments in other genera, it follows from the embryological observations of Lacaze-Duthiers' (loc. cit.), and from the position of the chitinous bars in the plates, that they are homologous with the *descending* limb of the gill-filaments in ordinary Lamellibranchs. Professor Huxley seems to have no doubt whatever of the homology stated here, as will appear from the quotation given further on. Admitting, then, that this supposition is correct, and that the gills in *Nucula* and *Yoldia* are in an unusually rudimentary condition, what light, if any, do they throw on the organogeny of the Lamellibranchiate gill? But, before proceeding to the discussion of this point, let us review briefly what theories have been advanced as to what is the most primitive type of the branchiæ of this group. Setting aside older authors like Williams and Hancock, I consider the articles, already alluded to, by Peck, Posner, and Lacaze-Duthiers as having the most important bearing on the subject. Posner, after a careful histological examination of the gills of *Anodon, Unio, Cardium, Mya, Mytilus, Ostrea, Pecten, Pholas, Pinna, Scrobicularia, Solen, Solecurtus,* and *Venus,* puts forward, although with hesitation, the theory that the pouch-like gills of the Unionidæ are the most primitive type of the Lamellibranchiate gill. Stepanoff,[1] so far as I can gather, inclines to this view. Peck, on the other hand, after an investigation of *Arca, Mytilus, Anodon,* and *Dreissena,* comes to the conclusion that "the gill-plates of the Unionidæ are a highly modified form derived from a simple condition in which the gills consist *not* of plates but of a series of juxtaposed independent *filaments,* such as we see in a less modified state in Arca and Mytilus." This view is the more generally accepted of the two. The only complete history of the development of the Lamellibranchiate gill by Lacaze-Duthiers (loc. cit.) and all the fragmentary embryological observations on the organ show that the gills are at first of a tentacular or filamentary character. Those who read carefully Mr. Peck's paper, will, I think, feel convinced by the arguments he brings forward. So high an authority as Professor Huxley is entirely of this view. He says:—"In its simplest form, the branchia of a Lamellibranch consists of a stem fringed by a double series of filaments (*e.g.* *Nucula*). The next degree of complication arises from these filaments becoming, as it were, doubled

---

[1] Paul Stepanoff, "Ueber die Geschlectsorgane und die Entwicklung von Cyclas," 'Archiv f. Naturgesch.,' 1865.

upon themselves at the free ends, the reflected portions lying on the outer side of the outer, and on the inner side of the inner, series of filaments . . . (Mytilus Pecten). In most Lamellibranchs, the gills are four elongated plates, each of which is in fact a long narrow pouch, with its open end turned toward the hæmal face of the body" ('Invertebrates,' pp. 408-9, Am. Ed.). My own observations lead me to the same conclusion. In fact, it is difficult to see how the pouch-like gills of *Unio* can give rise to such forms of branchiæ as are found in *Nucula* and *Yoldia*. By a very circuitous route they may have degenerated into their present rudimentary state, it is true, but all recent observations tend to show that while other organs in the Lamellibranchiata have been steadily degenerating, the gills, on the contrary, have become highly developed and perform functions which the probable change of the animal from the motile to the sedentary habits of life has forced on these gills. If, then, there has been no considerable degeneration, and if the homologies of different parts of these branchiæ are, as I have stated above, the filamentary character of the primitive Lamellibranchiate gill is placed beyond doubt.

I believe further light is thrown on the subject by the gills of *Nucula* and *Yoldia*. Peck shows that the gills primarily consisted of a series of filaments, but does not attempt to account for the fact that these filaments have come out in long rows on the side of the body. I venture to suggest an explanation. If we reflect for a moment, I think we shall see that the gills of *Nucula* and *Yoldia* may be considered as a stem which, being folded on either side to increase the surface of contact with the water, gives rise to the flat plates which I have homologized with the descending limb of the gill-filament of *Mytilus* and other like forms. The plates are, strictly speaking, nothing but the epithelial covering of the stem raised into folds and enclosing between the two sides of the folds a blood-channel. In the case of *Yoldia* mesoblastic lacunar tissue is carried out into the folds. According to this theory, the gill of the Lamellibranchiata was originally a longitudinal ridge on the side of the body. Probably in this a blood-vessel ran, and must have served as the organ of respiration. In course of time, however, this ridge became folded for the increase of the surface of contact with the water and thus produced papilla on its two sides—rudiments of the future gill filaments. The gills of *Nucula* and *Yoldia* have gone but little beyond this stage. I think there is much to support this view. Stepanoff (loc. cit.) observed in *Cyclas* that the gills arise first as a ridge on each side of the body. Leydig[1] makes the same statement. M. Lovén's[2] observations have a still more important bearing on the point. He says:—"Nous avons, si je ne me trompe, vu la première formation des branchies; nous en savons assez pour être sûr qu'elles se montrent sous la forme d'un cordon fin, renflé à certains intervalles; que ces renflements se contournent plus tard en anses, qui s'allongent de plus en plus, et sur lesquelles se développent les cils vibratiles regulièrement disposés et d'un forme particulière."[3] "Un cordon fin renflé à certains intervalles" is, it seems to me, nothing but a ridge with slight swellings or papillæ. Lovén's figures are not exactly clear to me, but what he designates as the gills are certainly in favour of my view. In all the fragmentary embryological observations, the gills are generally seen as papillæ, or nothing but the folds of a blood-channel. I have already called attention to the anterior part of the *Yoldia* gill where the plates die out and the gill is continued simply as a ridge containing a blood-channel. Whether this is a remnant of the primitive ridge or not it is difficult to determine, but the fact that there *can be* on the side of the body a thin-walled ridge which, containing a blood-channel, must serve more or less for respiration, goes far in support of the view here advanced.

To review the whole matter, the Lamellibranch gill was perhaps originally a simple ridge on the side of the body, but to increase the surface of contact with the water folds may have arisen on two sides of this ridge. If such was the case, *Nucula* and *Yoldia* are still in a stage only very little advanced from this primitive condition. In course of time, however, as some of the Lamellibranchiata, either owing to degeneration or some other cause, become incapable of extensive locomotion, these buds or folds were perhaps prolonged to form tentacular filaments, which, going on in their development, finally produced such complex gill structure as we see in *Mytilus*, *Unio*, *Ostrea*, and other forms, taking on at the same time functions totally foreign to their

---

[1] Franz Leydig, "Ueber *Cyclas cornea*," 'Müller's Archiv,' 1855. He says:—"Die letzte Hauptmändering im äusseren Habitus esfährt der Embryo durch die Bildung der Kiemen. Auch sie wachsen als *Leisten* von hinten nach vorne und zwar geben sie ursprünglich von Mantel aus" (p. 62).

[2] "Bidrag till Kämedornen om utvecklingen af mollusca acephala Lamellibranchiata," 'Memoirs of the Academy of Stockholm,' 1848, lately reprinted in an abridged form in German.

[3] Translated by M. Young, and quoted by Lacaze-Duthiers in the article already referred to.

original one. Between the simple gills of *Nucula* and most complex ones known, there are a great many intermediate stages, some going more in one direction, others in another. For instance, *Lucina* and *Corbis* are said to have only one gill-plate on each side ('Owen's Inverteb.'). According to Sars, *Pecchiola* is in the same condition ('Remarkable Forms of Animal Life,' G. O. Sars). *Chamostrea* and *Myochama* are described by Hancock ('Ann. and Mag. of Nat. Hist.,' 1852-3) as having the inner gill-plate complete, but the outer plate lacking the outer lamella. In these tentacular filaments seem to be fused with each other. On the other hand, although *Arca, Mytilus, Modiola,* have all the lamellæ present, the filaments composing them have not fused with one another. It is interesting to notice that *Nucula* and *Yoldia,* in which the gills have remained rudimentary, have, as Dr. Brooks first pointed out to me, an unusual power of locomotion, while forms wholly or almost wholly unable to move, as *Ostrea, Pholas,* &c., possess highly-developed gills.

For some reason the inner gill-plate seems to develop further than the outer. For instance, in many genera, the inner is much larger than the outer. In *Chamostrea* and *Myochama,* already referred to, it is the inner gill-plate that is complete, and the outer gill-plate that lacks a lamella. It will also be seen a little further on that in *Anodon* the inner gill-plate has gone further than the outer in its development. In the embryological study of the branchiæ of *Mytilus* Lacaze-Duthiers observed that the filaments of the inner gill budded out first.

It is very instructive to see the process of secondary folding going on in higher varieties of the gill. The two lamellæ of a gill-plate are, in such a case, no longer parallel, but wavy, and the surface of a lamella is thus considerably increased. In *Anodon* this process is perhaps going on, for Peck shows that in that genus the cross-section of the outer gill-plate has parallel and straight edges, but that the outer lamella of the inner has a wavy margin. Posner shows successive stages of secondary folding in the gills of *Pholas dactylus, Venus* (sp), *Mya arenaria, Ostrea edulis, Solen vagina, Cardium edule, Pinna nobilis.*

Diametrically opposite, as the views advocated by Posner and Peck may seem, it is not difficult to reconcile the two.

If we look over the list of the genera examined by Posner, we shall find all of them, except *Mytilus* and perhaps *Pecten,* to possess more complex gills than *Unio,* and starting, as he did, from the last genus, it is no wonder that he considered it to possess the primitive gill. On the other hand, Peck investigated forms simpler than *Unio,* and arrived at the probably true conclusion. Posner simply began where Peck ended. The two investigators, therefore, supplement each other, and now, with the addition of the extremely simple gills of *Nucula* and *Yoldia,* the series is fairly complete, and it seems to me that the filamentary character of the primitive Lamellibranch gill is made tolerably certain.

LIMULUS *an* ARACHIND. By E. RAY LANKESTER, M.A., F.R.S., Jodrell Professor of Zoology in University College, London.

(*Continued from p.* 548.)

Further remarks on the pulmonary sacs and lung books of Scorpio.—In the comparison of the lung books of Scorpio and the gill books of Limulus, given in a preceding portion of this memoir, and in the attempt to derive the two modifications of lamelligerous appendage from a common ancestral form, I have carried the supposed history only so far as to reach a hypothetical Scorpion-like form in which the lamelligerous appendage is supposed to be filled with blood, the "pulmonary sac," or "investing sac," or "cave of invagination" (the homologue of the funnel-like cavity of the tendon of the thoraco-branchials of Limulus), being still filled with air and communicating persistently with the atmosphere by means of a stigma, which in this case is the original orifice of invagination of the investing sac.

Such *was* probably the condition of an ancestral Scorpion. *In living Scorpions a further development has taken place.* The original stigma has become entirely closed up; the investing sac—that which I have spoken of as pulmonary sac—contains no longer air but blood. A *new* opening (the stigmatic slit) has formed within the area formed by the closure of the *primitive* opening of the cave of invagination in the form of a slit-like fissure in the delicate membranous wall of the axis of the in-sunken pulmonary appendage (see woodcut, fig. 15). By this aperture air now enters where, in Limulus and the

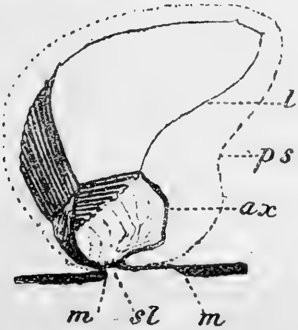


Fig.15

FIG. 15.—Diagram of a Scorpion's lung-book, enclosed in the pulmonary sac and divided by a cut at right angles to the lamelligerous axis. *ax*, axis; *l*, lamella; *ps*, pulmonary sac or cave of invagination; *m*, raised margin of the stigma; *sl*, slit leading from the exterior into the axis of the lamelligerous appendage.

early Scorpion ancestors, there was blood. A blood space has become converted into an air space just as an air space (that of the investing sac) has become converted into a blood space.

That a blood space should have become converted into an air space is not exceptional. All tracheæ in Arthropoda are potential blood-vessels, and their blood-vessels are potential tracheæ. The air-carrying spaces of Arthropoda have been in fact in many cases probably produced by a direct conversion of blood-vessels.

The changed contents of the Scorpion's as compared with the King Crab's respiratory appendage does not affect the morphological significance of its parts nor the importance to be attached to the evidences of its having once projected on a free surface, although now sunk within a sac formed by a recess of the body surface.

The minute embryological history of the Scorpion's lung book is the evidence which we now want in order to actually demonstrate that the *primitive* stigma is the orifice of invagination of the investing sac into which the lamelligerous appendage sinks, and that the opening into the axis of the appendage from the surface is a secondary formation, produced after the primitive stigma had been occluded and completely closed by the adhesion to the lips of that orifice of the axis of the in-sunken lamelligerous appendage.

So much as is already known of the embryological history of the Scorpion's lamelligerous lung sacs is not opposed to the view here advocated. Rudimentary appendages, which in the embryo project from the surface of the segments in which the pulmonary organs are subsequently found, disappear from view at the same time as certain pits are formed in their immediate vicinity. These pits and their orifices of invagination are, according to my view, not the air-containing chamber and its permanent external opening, but the investing sacs (the homologues of the hollow parabranchial tendons of Limulus) in which the lamelligerous appendage is enclosed, and which cease after their formation to communicate with the exterior.

§ *c*. **ALIMENTARY CANAL.**—Though there are very considerable differences between the alimentary canal and its offgrowths in Limulus and in the Scorpion, yet there are some remarkable agreements of a fundamental character. The differences, such as they are, can be viewed as the results of special adaptation. There is the same difficulty with regard to the facts relative to the Scorpion's alimentary system as in regard to all its viscera. I shall rely upon Newport, but I

Fig.16.

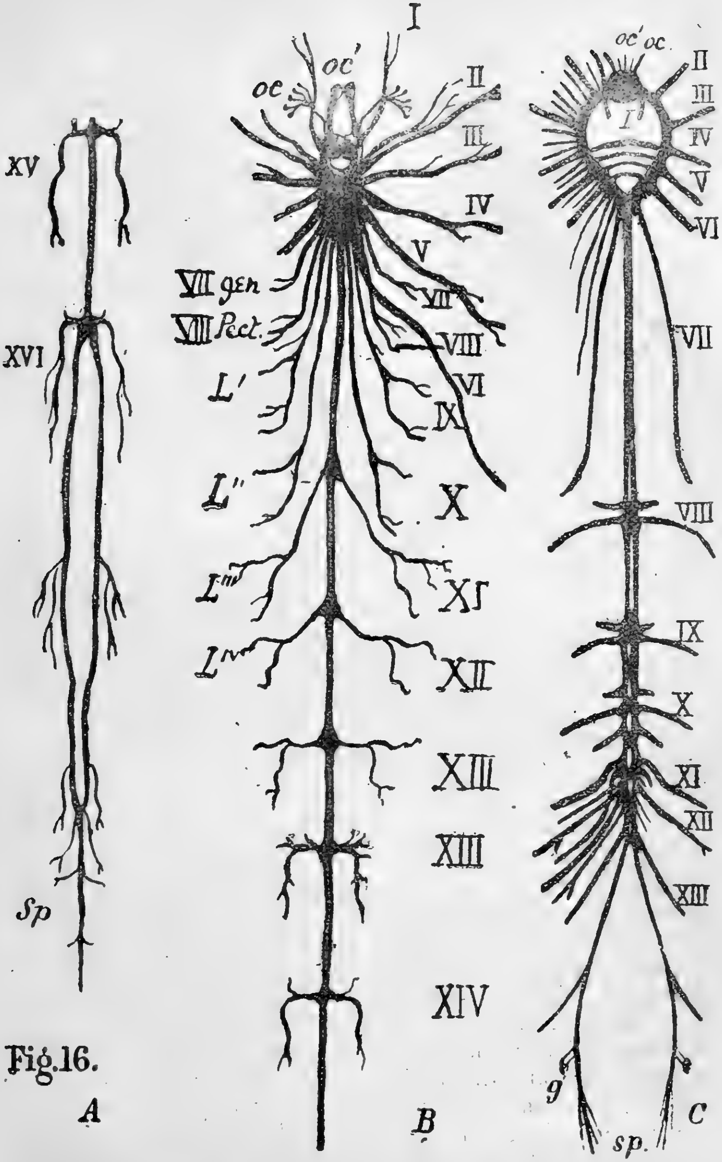


Fig. 16.—Diagrams of the nervous system (see page 508); *A B*, of Buthus (Scorpion), after Newport. *C*, of Limulus, constructed from Milne-Edwards. I to VI, nerves to the cephalo-thoracic limbs; VII, nerve to the genital operculum; VIII to XII, nerves to the lamelligerous appendages, of which all arise from the ventral cord in Limulus; whilst VIII, IX, and X are, as it were, drawn up to the œsophageal collar in Buthus. *oc*, nerve to the compound or grouped eyes; *oc'*, nerve to the simple eyes; L^{i} to L^{iv}, position of the four respiratory appendages of the left side of the Scorpion; *g*, rectal ganglia of Limulus; *sp*, nerves to the post-anal spine.

may mention that Dufour ('Mémoires de l'Institut Sciences Math. et Phys.,' vol. xiv, 1856,) has given an account which is based upon the examination of freshly killed specimens of *Scorpio occitanus*. In regard to the alimentary canal and its appendages, Dufour is probably correct where he differs from Newport, the divergence being due to the fact that Newport studied the large oriental species of Buthus, whilst Dufour made use of the species above-named. Differences in the account given by Dufour of the nervous and circulatory systems are not thus to be explained, and without definitely concluding that Dufour is entirely wrong in his statements, I have felt justified in accepting Newport's account as accurate. Newport, unfortunately, did not complete his figures of the alimentary system nor give any proper account of them. Hence Dufour's contribution to this part of the subject is of increased value.

In both Scorpio and Limulus the alimentary canal consists of an axial tube which takes a median course without lateral convolution from mouth to anus. It presents a suctorial or ingestive portion in front, and a widened "proctodæum," or hind-gut posteriorly. The mouth in Limulus is not placed so far forward as in Scorpio, but has a considerable area of the cephalothorax in front of it. Resulting from this position we find that the suctorial or pharyngeal portion of the tract is bent sharply upon itself, passing from the mouth forwards to the front of the head, and then turning upwards and backwards to pursue a median course to the anus. This bent pharynx of Limulus is a powerful suctorial organ, and is lined within by chitinous ridges. The food of Limulus consists of soft-bodied worms, which are sucked into this organ and crushed by it.

Corresponding to the bent pharynx of Limulus is the pharyngeal sac of Scorpio, the mechanism of which was described by Professor Huxley in this Journal (1860). The Scorpion's pharynx is adapted to the sucking up of the juices of other animals which it kills, but never draws within the boundaries of its exceedingly minute oral aperture.

Following upon the pharynx of Limulus, and separated from it internally by a valvular arrangement, is the digestive portion of the alimentary tract. It is remarkable for presenting two pairs of tubular outgrowths, which are the stalks of the huge saffron-coloured gland which fills up the space offered by the horseshoe-shaped carapace, and even extends into the region of the abdomen. The digestive section of the alimentary tract runs through the whole series of appendage-bearing segments, and in the telsonic region joins the proctodæum or anal invagination, from which it is very sharply marked off by a groove and by the expansion and plication of the walls of the proctodæum.

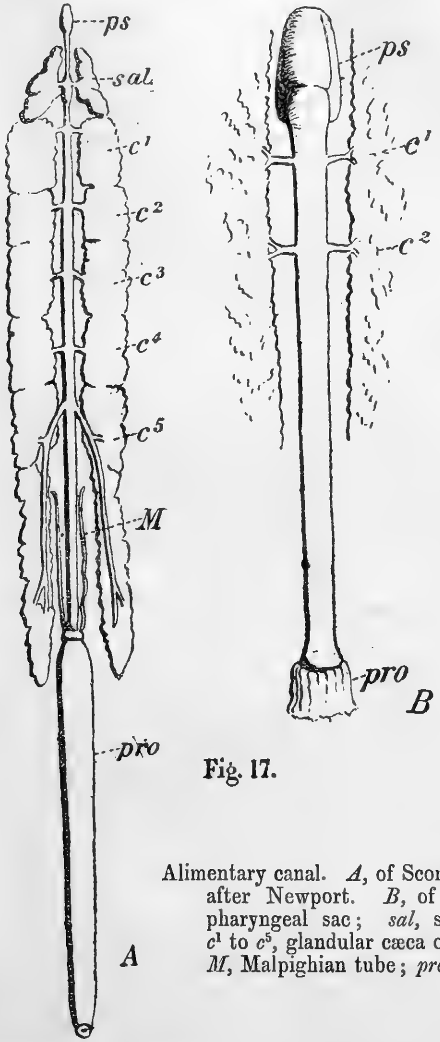


Fig. 17.

Alimentary canal. *A*, of Scorpion (Buthus); after Newport. *B*, of Limulus. *ps*, pharyngeal sac; *sal*, salivary gland; c^1 to c^5, glandular cæca of the mid-gut, *M*, Malpighian tube; *pro*, proctodæum.

The corresponding section of the Scorpion's alimentary tract *also* carries *more than one pair* of glandular cæca, and ends in a voluminous proctodæum, which commences, precisely as in Limulus, in that region of the body which succeeds the thirteenth segment, as indicated by the superficial sclerites.

Very marked differences, however, exist between the two animals in regard to the number of the glandular cæca. Whilst Limulus has but two pairs of ducts opening at an interval into the mid-gut (mesenteron), the Scorpion has (according to Newport) six. The first pair are connected with a peculiar pair of glands recognised by Newport, Dufour and Huxley (loc. cit.) as salivary glands. The opening of these ducts is placed at a point where the alimentary canal is slightly dilated. The following five pairs of ducts are the conduits of a huge glandular mass, which corresponds undeniably to the great saffron-coloured gland of Limulus. But in Limulus the relative enlargement of the cephalothorax results in the packing of the gland into that region, whereas in Scorpio the relative enlargement of the anterior part of the abdomen (segments VII to XIII inclusive) results in the packing of the gland more posteriorly. The same difference of external proportions in the two animals results in a similar contrast in the position occupied by their generative glands; in Limulus they ramify anteriorly to the genital orifice, in Scorpio posteriorly to it.

According to Newport, the glandular mass (which I shall call the hepatic gland, in accordance with custom, and not as implying that it is the morphological equivalent of the vertebrate liver rather than of any other outgrowth of the mesenteron) is divided into lobes or lappets, corresponding to the separate ducts. Dufour also admits this to be the case for the large oriental species of Buthus, but describes the organ as continuous, and with only four pairs of ducts (in place of five) in *S. occitanus*. The minute structure of this gland has not been investigated in any Scorpion nor yet in Limulus. By earlier writers it was spoken of in Scorpion as "the fat body."

The Scorpions appear, then, to vary in the number of pairs of ducts possessed by the hepatic gland, and the fact that Limulus has but two pairs is, accordingly, not an important point of divergence. The absence of salivary glands is a more serious departure from the arrangements prevailing in the Scorpions. It is, however, to be remarked that on comparing allied aquatic and terrestial forms of animals, salivary glands are not unfrequently found to be present in the latter whilst absent from the former.

When we come to compare the proctodæum of the two animals we find, perhaps, the most important difference which can be pointed to as obtaining between

Limulus and Scorpio. The exceeding shortness of the proctodæum of Limulus is only a part of that general reduction of its hinder segments which is paralleled in many other groups of Arthropoda. But in Scorpio there are given off from the anterior border of the proctodæum two delicate tubes. According to Dufour, in *Sc. occitanus* there are four of these tubes, of which one pair is branched. Newport, however, figures only one pair in Buthus. These delicate tubes are the Malpighian glands, found alike in Myriapoda, Hexapoda, and Arachnida, but never in Crustacea. They have been shown in Spiders by Mr. Balfour (20) to develop from the proctodæum, or anal invagination of the epiblast; they have a renal function, and *possibly* represent morphologically 'nephridia,' such as those of Gephyræa and Rotifera.

Their absence from Limulus is a difficulty in the way of associating Limulus and Scorpio, but it is also a difficulty in the way of associating the Crustacea with the other Arthropoda. Leydig has pointed out, in the proctodæum of Copepoda, structural evidence of the existence of a region which may functionally represent the Malpighian tubes of the tracheate Arthropoda, and careful histological study may give similar evidence with regard to Limulus. As to the development of actual cæcal tubes in this region, two views are admissible: either the common ancestor of the Arthropoda possessed these tubes and they have been lost by Crustacea and by Limulus (and some others) among the Arachnida, but retained by the various tracheate classes, *or* the common ancestor possessed only the functional "renal region" of the proctodæum, which has remained undifferentiated in form in Crustacea and in Limulus, but has taken on the form of cæcal tubes in the air-breathing forms, perhaps independently, in the course of the evolution of different groups. If we are to hold that Malpighian tubes can only once have originated, and that all forms possessing them have a common ancestor, we must suppose either that Limulus has lost them or that all Tracheata are descended from the Arachnida. Amongst these possibilities we have no decisive indications. The whole question of the genealogy of the various classes of Arthropoda is involved in the issue.

§ *d.* **VASCULAR SYSTEM.**—The close agreement between the vascular system of Limulus and Scorpio has been ably insisted upon by M. Alphonse Milne-Edwards, who, eight years since, gave the results in his beautiful memoir, already cited, of a series of injections carried out upon perfectly fresh specimens of Limulus. It is not possible to say, in the absence of any adequate investigation of fresh specimens of Scorpio, how far the resemblance may go; but, depending upon the careful dissectious by Newport of spirit specimens (and discarding those of Dufour which are very incomplete), we are able to point to very close agreements.

In Limulus a more complete vascular system has been demonstrated than in any other Arthropod, and Scorpio comes nearest to it in this respect of all members of the group. The arterial channels do not end in wide spaces bounded by the connective (vasifactive) tissue which clothes muscles and viscera, but the connective tissue here, as in other animals in which fine vessels are developed for the passage of the blood, forms in most regions of the body a series of canals, which constitute a capillary system and lead into definitely constituted veins.

It is worthy of remark by the way that canalisation of the connective tissue is the same phenomenon and due to the same processes of growth in all Arthropoda, whether the canals so formed are connected with the atmospheric air by stigmata or are filled by the blood fluid of the primitive cœlomic cavity.

It does not fall within the scope of this memoir to give a detailed account of the vascular system of Limulus; for that the reader is referred to the memoir of M. Milne-Edwards. I shall content myself with drawing attention to the agreement between Scorpio and Limulus in respect of—(1) the existence of capillaries and veins; (2) of the well-developed vessels conveying blood to the limbs and viscera, and more especially in respect of the great spinal artery and its mode of origin; (3) of the intimate association of the arteries and nerves; (4) of the details of the structure of the heart.

The memoir by George Newport, in the 'Philosophical Transactions' for 1843, and that by M. Alphonse Milne-Edwards, in the 'Annales des Sciences Naturelles' of 1873, contain the exposition of the facts in detail relatively respectively to the Scorpion and the King Crab. Of the latter animal, M. Milne-Edwards says: "The venous blood, in place of being distributed in interorganic lacunæ, as in the Crustacea, is in a considerable portion of its course enclosed in special vessels whose walls are perfectly distinct from the adjacent organs; they often take their origin in ramifications of a remarkable delicacy and lead into reservoirs which are for the most part definitely circumscribed. The nutrient liquid passes from these reservoirs into the branchiæ, and after having traversed these respiratory organs, passes by a system of branchio-cardiac canals into a pericardial chamber, and then penetrates the heart. From the heart, the dimensions of which are considerable, it is forced into the tubular arteries with resisting walls, the distribution of which is exceedingly complex, with frequent anastomoses, whilst their terminal ramifications, which are of marvellous tenuity and abundance, can be followed into the substance of the most delicate membranes." These capillaries are figured by M. Milne-Edwards, but we have not of them, any more than of the tissues of Scorpio, a satisfactory histological account. Gegenbaur (2), whose observations were made on spirit specimens, did not observe these finer ramifications of the vessels, but supposed the arteries to lead into intercommunicating lacunæ without definite walls.

As to Scorpio, it may be justly said that it was the main purport of Newport's memoir to make known just such an extended vascular system in the Myriapoda and Arachnida as above indicated for Limulus, though M. Alphonse Milne-Edwards does not cite Newport's work, but unjustly appeals to the second-hand authority of M. Blanchard, for the few facts which he mentions relative to the Scorpion. And further, the general description of the circulation above given as to Limulus is strictly applicable as a summary of Newport's observations upon the course of the blood and distribution of the vessels in the Scorpion.

Newport's description and figures of the heart and its main arteries in Scorpio show a close agreement with these parts in Limulus, as described by Milne-Edwards. A revision of these structures in the Scorpion, in the light of what is now known as to Limulus, would probably show a still closer agreement in some details, especially were injection practised upon freshly killed specimens.

The diagrams here given will enable the reader to judge of the general features of the arterial system in the two animals.

The heart of both Limulus and Scorpion is an elongated organ, constricted so as to form eight successive chambers, which are imperfectly marked in the Limulus, but more obvious in the Scorpion, since in that animal imperfect transverse septa occur within it, less complete, according to Newport, than in other Arthropod hearts. In front of the eight chambers the heart is continued in both animals as a truncus arteriosus towards the head. Posteriorly it is continued as a posterior aorta in Scorpio into the cylindrical tail; but in Limulus, in accordance with the reduction of that region of the body, it ends blindly. The eight chambers of the Scorpion's heart appear to be the exact equivalents of the less strongly marked divisions of the King Crab's heart, *being originally placed in corresponding segments of the body.* At the

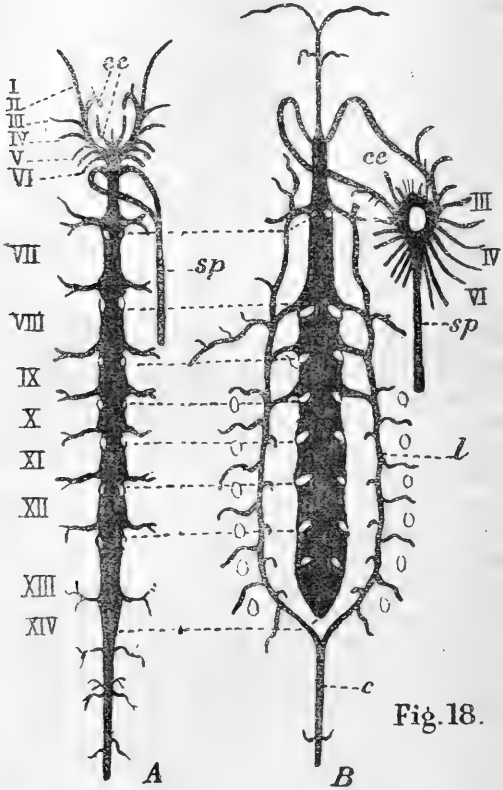


Fig.18.

FIG. 18.—Heart and origin of the supra-spinal artery. *A*, of the Scorpion (Buthus), after Newport; *B*, of Limulus, after Milne-Edwards. I to VI, arteries to the six pediform limbs; VII to XIV, the eight chambers of the heart; *sp*, supra-spinal artery; *ce*, cerebral arteries; *c*, caudal artery; *l*, lateral anastomatic artery of Limulus.

anterior margin of each division there is a pair of valvular apertures, and there are accordingly eight pairs in each heart. At the hinder margin of each division in the Scorpion a pair of lateral arteries is given off (eight pairs in all); such lateral arteries exist only in connection with the first *three* divisions of the King Crab's heart, their place being taken by secondary longitudinal trunks (woodcut, fig. 19, *l*). From the base of the truncus arteriosus, that is, just in front of the most anterior pair of valvular apertures, a pair of lateral arteries is also given off in both hearts.

The eight chambers of the Scorpion's heart are placed in the seven anterior abdominal segments, the first corresponding to the segment which bears the genital operculum, the last two being placed in one segment (thirteenth of the whole series), the broad triangular segment which precedes the first cylindrical caudal segment.

The eight chambers of the King Crab's heart have a similar relation, though not so obvious. The anterior portion of the heart is somewhat drawn forward, so that the segments indicated by the valvular apertures are (like the corresponding nerve ganglion of the genital operculum) a good deal shifted to the front of the appendicular portions of the skeleton to which they are segmentally related.

In place of the five hinder pairs of lateral arteries present in Scorpio, we find in Limulus large lateral arteries (fig. 18 *l*), which take origin by an anastomosis from the three pairs of anterior lateral arteries of the heart, and from the pair of lateral arteries of the base of the truncus arteriosus.

The truncus arteriosus (or anterior portion of the heart, as M. Milne-Edwards prefers to call it) presents a remarkable agreement in the two cases in regard to the distribution and character of the vessels given off from it, although upon the basis of a fundamental agreement very wide differences in detail are to be noted. At the base of the truncus, just in front of the most anterior pair of valvular apertures of the heart, we have the pair of lateral arteries similar to those given off from the heart. Then the trunk is continued forwards (through the cephalothoracic region in Scorpio), and gives off two branches, which form a small vascular collar around the œsophagus in the Scorpion, but a wide pair of arterial commissures in the King Crab, which meet upon the postœsophageal portion of the nerve collar. *In front* of the vascular collar in Scorpio the trunk divides into a median and two lateral stems, and from these, arteries are given to the cephalothoracic appendages, to the brain and to the eyes, as shown in the woodcut. Its main continuation, however, is in the vascular collar, the arches of which form a large vessel which, as the *supra-spinal artery*, takes a course backwards along the upper surface of the ventral nerve-cord (see woodcut, *s p.*). The association of this part of the arterial system with the nerve-cord and its branches is very intimate, so as to have excited special remark on the part of Newport.

A parallel but more intimate association of the corresponding part of the vascular system in Limulus, with its nerve-ganglion collar, cord, and main nerves, was first observed by Owen ('Lectures,' 1855), but has been fully demonstrated and described in detail by Milne-Edwards. The supra-spinal artery of Scorpio is represented by a complete arterial investment of the nerve-ganglion collar, including the brain, and of the chief nerves, as well as of the ventral nerve-cord arising from it, so that the nerves actually lie *inside* arteries and the brain, nerve-collar and nerve-cord are placed in the interior of a great arterial trunk corresponding to the supra-spinal artery of the Scorpion.

The agreement of these parts in Limulus and the Scorpion has been insisted upon by M. Milne-Edwards at page 19 of his memoir (5).

No Crustacean presents so complete a vascular system as Limulus, nor can we find anywhere but in Scorpio an artery originating by arterial arches embracing the œsophagus and passing through the body in close association with the nerve-cord as a main channel for the distribution of the blood.

The chief difference (by no means a small one) between this part of the arterial system in Limulus and Scorpio is that the arteries to the cephalothoracic limbs and brain are in the former given off from the œsophageal vascular collar, or from its united factors, whilst in Scorpio they originate from a distinct trifurcate anterior continuation of the dorsally placed truncus arteriosus (see woodcut, fig. 19).

§ *e*. **GENERATIVE GLANDS.**—The position of the external openings of the generative organs has already been shown to correspond exactly in Limulus and Scorpio, being placed in both in the segment next following that to which the sixth pair of leg-like appendages are attached, and being covered in by an opercular plate with a bifid margin, the plate being formed by the coalescence of the two appendages proper to this segment.

Limulus and Scorpio agree in having the sexes distinct. They also agree in the general form and character of the ovaries and testes respectively, and in the fact that the ovary and the testis are in fundamental form like to one another.

Though it might be possible to find an ovary or a testis similar in form to those of Limulus and Scorpio among Crustacea (I do not know of one), yet it is an important fact, as part of our cumulative evidence of affinity between the two, that in both these animals the ovaries and the testes present the same characteristic form, and that that form is an unusual one. The tubular genital gland is not disposed as a simple central body with two ducts, nor as right and left lobes united by a central isthmus, nor as a single or double bunch of simple or arborescent cæca, but it is distinctly *retiform*. There are two genital ducts, which pass from the two genital pores right and left, and are continued into a widely diffused meshwork. The meshwork may be regarded as a continuation of the two genital ducts which give rise to branches, which anastomose and also join their fellows of the opposite side ; it has a tubular structure, and its walls present follicles in which the generative cells are produced. In Scorpio the ovarian follicles are less numerous and more highly developed individually than in Limulus, and also in the former animal the meshwork formed by the gland is more symmetrical and its meshes larger than in the latter, but the reticular arrangement of the genital gland is the same in both.

The main differences in the genital glands of Limulus, as compared with those of Scorpion, are related to two modifying causes : firstly, the greater relative size of the cephalothorax in Limulus ; and, secondly, the terrestrial mode of life of Scorpion which replaces the aquatic mode of life of Limulus.

Owing to the first of these causes we find that, whereas in Limulus the retiform generative gland extends *both* in front of and behind the genital pore, that is to say, into the cephalothorax (segments 1 to 6) and into the abdominal segments (segments 7 to 13), in Scorpio we find its meshworks spread entirely in the region posterior to the genital pore, that is, in the wide and thick abdominal segments (7 to 13).

The second cause has brought about a very important difference in the secondary arrangements of the generative system. Limulus does not copulate, but the male discharges the spermatozoa into the water on to the surface of the eggs which have just been laid by the female. Such a method of fertilisation is impossible in any animal of strictly terrestrial habits. Copulation is a necessity in such animals. It is only those terrestrial animals which pass into the water during the breeding season which can dispense with intromission. Accordingly we find the efferent ducts, both in male and female Scorpions, modified to subserve copulation. The ejaculatory apparatus in the male is complicated ; the distal portion of each of the efferent ducts is modified so as to form an intrommittent organ, and accessory glands are developed from its sides. The two oviducts in the female are enlarged to form vaginæ. There are thus two penes and two vaginæ in the male and female Scorpion respectively. In copulation the female appears to lie upon her back and, it has been suggested, with much plausibility, that the pectens (the lamelliferous appendages of the eighth segment) serve as tactile organs, guiding and stimulating the movements which result in the coitus.

The female Scorpion is even further specialised in reference to its genitalia as compared with Limulus. Whilst it retains the reticulate gland and the *two* ducts, each with its external aperture as in Limulus, it develops no special spermatheca or receptacle for the spermatozoa received in copulation, but the semen passes along the tubular oviduct and into its net-like branches. Here the semen fertilises the ova, which are placed in follicles set upon the sides of the mesh-forming ovarian tube. The development of the egg proceeds actually within the follicle and the Scorpion produces her young in the living condition.

Connected with this viviparous character is the specialisation of the egg-bearing follicles carried by the ovarian meshwork. In Limulus more numerous eggs are produced, and there is no specialisation of follicles, but from all parts of the ovarian reticulum egg-cells appear to develop and to become free in the lumen of the tubular structure of which the reticulum consists.

The best account extant of the generative organs of the Scorpions appears to be that of Dufour (loc. cit.), who studied fresh specimens, but his account leaves everything to be done in respect of the histology, and one may even hesitate to feel confidence in his description of large features.

There is, also, no complete account of the generative glands of Limulus. We may hope that the American naturalists, who have abundant Limuli on the sea-shore, will soon give us a precise account of the form of the fully developed ovary and testis, as well as an account of their histology. At present our knowledge is confined to the figure given by Owen of a *portion* only of the ovary, and to his description, which is very definite as far as it goes, and sufficient for the purpose of a general comparison with Scorpio. The testis was immature in a male specimen recently dissected by me, and in an earlier dissection I was unable to clear out this organ fully on account of the special objects which I had in view.

I was, however, able on that occasion to determine an important point of agreement between Limulus and Scorpio, namely, in regard to the character of the spermatozoa. It is a familiar fact that the spermatozoa of the Arthropoda exhibit the greatest diversity of form, and also great want of uniformity, as to the presence or absence of a motile flagelliform tail.

In Crustacea generally they are immobile and of very various shapes; but in Cirrhipedia, and possibly some others, they are filamentous, with a motile tail. In chilopod Myriapods they have a vibratile tail. In Chilognaths they are motionless. In hexapod Insects they have a vibratile tail. In some Arachnida (*e.g.* Spiders) they are devoid of such a process. We owe to Kölliker the observation that in the *Scorpio europæus* the spermatozoa are filamentous in form, with a vibratile tail. Accordingly, it is compatible with Arachnidan affinities for the spermatozoa to be either motile or immobile; at the same time, as an element in the cumulative evidence of affinity between the King Crab and Scorpion, which it has been my object in this essay to bring together, the presence of vibratile spermatozoa in Limulus is a fact of value. The spermatozoa of Limulus are, as I observed four years ago (4), provided with a long vibratile tail; they agree, therefore, with those of the Scorpion.

C. The Eurypterina as a Connecting Link between Limulus and Scorpio.

The intimate affinity of the extinct Eurypterina with Limulus is no longer doubted. The researches of Hall, Huxley, and Woodward, have thoroughly established the fact that Pterygotus, Eurypterus, Slimonia, and Stylonurus, are to be regarded as Limuli, in which one pair of leg-like organs (probably the most anterior) has been suppressed, and in which the telsonic region, instead of exhibiting but an imperfect development of segments posterior to the twelfth, and that only in the embryo, gives rise to a series of segments forming a large tail-like region of the body. The result of this development of segments between the anus and the last appendage-bearing segment (the twelfth of Limulus) is that the so-called "macrourous" form of body is produced, and consequently a general similarity in appearance is observed between the Eurypterina and Scorpio.

The two woodcuts (figs. 19 and 20) sufficiently exhibit this general resemblance. In other respects, allowing for the suppression of an anterior pair of appendages in the Eurypterina, we find obvious agreements with Limulus. The actual fifth pair of limbs—theoretically the sixth—present constantly in all the genera that enlarged form and specialisation of their terminal joints which are noticed in the corresponding limbs of the King Crab. The coxæ of these and of the three pairs of limbs in front are brought up to the mouth, and denticulated so as to serve as jaw-organs.

A genital operculum of the same proportions as that of Limulus is present, and traces of appendages (sternal plates), corresponding to the five pairs of branchial plates of that animal, have been detected on the following segments. The cephalothoracic tergum is, in some Eurypterina, horseshoe shaped as in Limulus, though relatively smaller in size, and the eyes appear to have been similar to those of Limulus in character and position, though the compound eyes are close to the margin of the carapace instead of at some distance from it. Though in many Eurypterina the cephalothoracic appendages are simple tactile or ambulatory organs, yet in others we find (as in Pterygotus) the chelate form appearing, as with the majority of these limbs in Limulus.

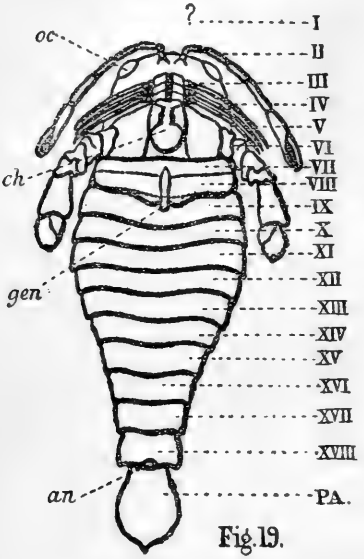


Fig. 19.

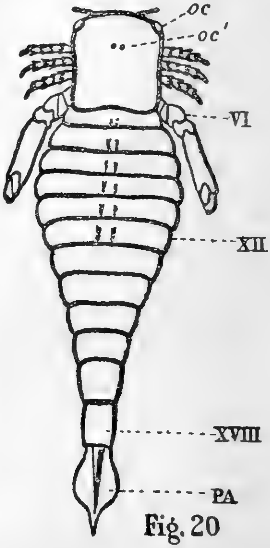


Fig. 20

Fig. 19.—*Pterygotus Angticus.* The segments are numbered to show their agreement with those of the Scorpion (see Fig. 2). *oc*, compound eye; *ch*, chilarium; *gen*, genital operculum; *an*, anus; PA, post-anal spine or plate.

Fig. 20.—*Slimonia acuminata.* *oc*, compound eye, lateral; *oc'*, simple eye, central; PA, post-anal spine.

I am anxious here to point out that there is not only a general resemblance of the Eurypterine body to that of the Scorpion, but that in many of the most important points in which they differ from those of Limulus the Eurypterine body and appendages agree precisely with those of the Scorpion, and not in a merely general way. The Eurypterina in fact serve in a most important manner to directly confirm the assimilation of segments and appendages in the two animals which I have already insisted upon.

In the first place, it is to be admitted once for all that Limulus and Scorpio agree with one another, and differ from the Eurypterina in possessing six pairs of cephalothoracic appendages. An anterior pair has disappeared in the Eurypterina, and this reduction is the distinctive character of the order. That such a loss of an anterior pair of limbs has occurred is rendered probable by the fact that there is evidence of a tendency for this abortion of anterior appendages to go on further still. The actual anterior pair corresponding to the second pair of Limulus and Scorpio is very small in some Eurypterina (see fig. 20), and suggests the existence of causes tending to the suppression of appendages in the anterior region. Such a suppression of anterior appendages is not without parallel among the Arthropoda (*e.g.* certain Crustacea), and for the Arachnida it has always been regarded as characteristic whenever the attempt has been made to compare the appendages of those forms with those of either the hexapod Insects or of the Crustacea. It is not, therefore, assuming too much when we admit that just as possibly (though I do not at the moment assert the fact) one pair of appendages is suppressed in all Arachnida as compared with other Arthropoda, so a second pair has been suppressed in the Eurypterine order of Arachnida.

Counting the segments of the Eurypterina upon this assumption, we find that they exactly agree with those of the Scorpion. The segments succeeding the cephalothorax and anterior to the anus are twelve in number, gradually towards the anus, though not *suddenly*, diminishing in size after the seventh, as in Scorpio. Posteriorly to the anus is the postanal spine, broad and flat in most Eurypterina for swimming, and neither rod-like, as in Limulus, nor globose, as in Scorpio. Any difficulty which the unsegmented telsonic region of Limulus may have presented in the comparison with Scorpio is removed by the simple inspection of the abdomen of the fossil Limuloid (woodcut, fig. 20).

Secondly, a difference between Scorpio and Limulus of some importance is seen when the form of the cephalothoracic limbs is compared, since in Scorpio certain of those which are chelate, in Limulus are simple ambulatory organs. Here, too, the admittedly Limuloid Eurypterina remove all difficulty; for among them all the cephalothoracic appendages are in some genera non-chelate (fig. 20), and exhibit a considerable range of character, being (as in other Arachnida) either ambulatory or tactile organs. The chelate limbs are thus seen to be a special feature of Limulus, and not essentially characteristic of the Limuloid Arachnida. Accordingly there is no difficulty in deriving the Scorpion's ambulatory limbs from those of such Limuloids.

Thirdly, certain features are presented by the cephalothorax of the Eurypterina, in which they agree very closely with the Scorpions, and in which Limulus differs from them.

A great difference between Limulus and Scorpio, leading to differences in the form and size of internal organs, is that presented by the much greater size of the cephalothorax in Limulus. Among the Eurypterine Limuloids we find, however, genera, in which the cephalothoracic carapace has precisely the quadrangular shape and small relative size, as compared with the abdomen, which is noticed in Scorpion (fig. 20). It cannot be doubted that the packing of the viscera was correspondingly affected, and there is great probability that the liver was connected by more numerous ducts with the intestine in these forms (as in Scorpion) than it is in Limulus. It is also probable in the very highest degree that the generative glands were developed in these Eurypterina posteriorly to the genital pores, and not anteriorly, as in Limulus.

Further, the disposition of the eyes on such a quadrangular carapace as that of Slimonia (fig. 20) is singularly like that seen in the Scorpion. Centrally are two small simple eyes, *oc′*, and precisely in the position which they occupy in Scorpion, viz. at the anterior lateral margin of the carapace, right and left, are groups of eyes, *oc*. In the Eurypterina, as in Limulus, these groups are close set in composition, so as to form what is called a compound eye, whereas in Scorpio the individual members of the group are separate.

The individual factors of the compound eye of Limulus are more archaic in their histological structure than are the simple eyes of spiders, but at present we do not know the minute structure of the grouped eyes of Scorpio. It is possible that they may show closer agreement with the Limulus eye than do those of Spiders; or, again, it is not difficult to suppose that from a loose aggregation of very simple marginal eyes, which existed in the common ancestor of Limulus, Eurypterines, and Scorpio, there has been developed, on the one hand, by coalescence, the compound eye of the former; and on the other hand, by individual elaboration, the separate eyes of the modern Arachnid.

Lastly, in regard to that element of the sternum which in *Buthus* is the pentagonal "thoracic metasternite," and in Limulus forms the "chilaria" or paired metastoma, the Eurypterinesserve to tie Limulus more tightly to the Scorpion. The duplicate character of the chilaria of Limulus renders it at first difficult to admit that they are represented by a single median plate in Scorpio. This right-and-left character even led M. Alphonse Milne-Edwards to ignore the position of the genital apertures and to identify the chilaria of Limulus with the pectens of Scorpio. The Eurypterines show clearly enough (even in the absence of embryological evidence) the sternal nature of the King Crab's chilaria, for they possess, just where the chilaria of Limulus are found, a single broad oval plate, which rises up from the surface in such a way as partly to cover in and work as lower lip to the four pairs of coxal jaws in front of it (see woodcut, fig. 19 *ch*). This single metastoma, or chilarium, is readily understood also as the equivalent of the single pentagonal sternite of Scorpio, which is dwindled in size and pushed away in that animal from the functional jaws by the large ankylosed coxæ of the fifth and sixth pairs of cephalothoracic appendages.

D. REVIEW OF OPINIONS OF MODERN AUTHORITIES AS TO THE AFFINITIES OF LIMULUS.

So far in preceding pages my object has been to point out definite points of special resemblance between Limulus and Arachnids, especially the Scorpion. I have not paused to insist upon the absence of any such special agreements between Limulus and the Crustacea. I propose briefly to do this now by examining the statements of those who have asserted that any such special agreements exist.

Clearly between Limulus and any other Arthropod there must exist agreements which are the common characters, more or less, of all Arthropods. It may also be possible to find structural features which are exhibited only by Limulus and by Crustaceans, one feature finding its parallel in one Crustacean and one in another. But I think it must be definitely conceded (1st) that there is no one Arthropod in which anything like so large a number of the structural features found in Limulus are paralleled as the Scorpion, and (2nd) that there are several structural features exhibited by Limulus which have no parallel in the Crustacea at all, but are common to Limulus and the higher Arachnida.

Putting together Limulus and the Eurypterines we may briefly summarise their agreements with Arachnida and disagreements with Crustacea as follows:

1. Limulus and the Eurypterines (the one supplementing the other) agree precisely with the Scorpion in the existence of eighteen segments expressed in the structure of their bodies, and in the distribution of these segments into three groups of six each, viz.: a leg-bearing cephalothoracic region, an anterior abdominal region, in which each segment carries lamellate appendages, and a posterior abdominal region devoid of appendages, ending with the anus and a postanal spine. No Crustacean presents this number and grouping of its constituent somites.

2. Limulus and the Eurypterines agree with the Scorpion precisely in the position of the genital aperture beneath an opercular plate formed by the coalescence of the seventh pair (in Eurypterines the actual sixth pair of appendages). No Crustacean has the generative orifice so far forward, and in none is there a genital operculum of the kind having such relations of position to the genital apertures.

3. They agree with the Scorpion in the character and position of the mouth and upper lip.

4. They agree with the Scorpion in possessing a metathoracic sternite, in the possession of a fibro-cartilaginous entosternite, and in the precise form and relations of that organ. No Crustacean possesses an entosternite or any structure resembling it.

5. They agree with the Scorpion in the disposition of central (single) and lateral (grouped) eyes on the cephalothorax. No Crustacean has an identical arrangement of single and grouped eyes.

6. Limulus agrees with the Scorpion in the form of the alimentary canal and its lateral outgrowths (liver), which are more than one pair. In Crustacea it is very exceptional to find more than one pair of such diverticula, though a single pair may carry numerous secondary branches.

7. It agrees with the Scorpion in possessing a supra- or circum-medullary (spinal) artery, which arises from the dorsal aorta by two arches embracing the œsophagus. No Crustacean has such a supra-spinal artery so originating.

8. It agrees with the Scorpion in the form of the generative glands. No Crustacean has its generative glands in the form of an anastomosing network.

9. It agrees with Scorpio in possessing vibratile spermatozoa. No Crustacea except Cirrhipedia are known to have vibratile spermatozoa.

10. It agrees with Scorpio and Spiders in having a brain

which (like that of the embryo Scorpion and Spider) supplies only eyes and integument with nerves, and not any appendage. In all Crustacea, except some Phyllopoda, such an archicerebrum does not exist; but even in young stages the brain is found to supply at least one pair of appendages as well as the eyes.

11. It agrees with Scorpio in the concentration of the origins of nerves supplying the anterior part of the abdomen, in the cephalothorax in the form of a nervous collar, perforated by the pharynx. Such a nerve-collar has its parallel in Crustacea among the brachyurous Decapoda, which, however, are in other respects the Crustaceans which least resemble Limulus.

The points in which Limulus agrees with the Crustacea and differs from Arachnida are three only. They are as follows:

1. Limulus agrees with many Crustacea, and differs from Arachnida, in that its respiratory organs are adapted to an aquatic in place of an aërial medium.

2. Limulus agrees with Crustacea, and differs from Arachnida, in that it possesses a pair of groups of eyes, in which the association of the individual eyes of each group is so close as to constitute a compound eye.

3. Limulus agrees with Crustacea (excepting some Isopoda ?), and differs from Arachnida, in *not* possessing glandular cæca (the Malpighian tubules) growing out from the proctodæum.

The first of these agreements is purely one of functional adaptation. The lamelligerous organs of Scorpio and the Spiders act upon atmospheric oxygen, as might be expected in animals living on dry land. The fact that the corresponding organs of Limulus respire the oxygen dissolved in sea water, as do the gills of Crustacea, does not even remotely tend to establish a morphological agreement between Limulus and Crustaceans. All attempts to associate organisms in one genealogical group on account of an agreement in the ultimate mode of performing such functions as respiration and locomotion, without reference to the exact nature of the organs by which those functions are performed, are liable to serious error. We cannot, as a principle, associate in genealogical classification all animals that breathe air, or all animals that breathe water, or all animals that fly, or all animals that swim, or all animals that walk. On the contrary, we must hold the actual structure and anatomical relations of organs to be the only guide to the genetic affinities of the animals which possess them, quite irrespective of the special adaptations of those organs to an aquatic or aërial mode of life.

The second agreement, viz. that as to the existence of compound eyes, is more apparent than real; for it is quite obvious that a coming together of simple eyes might at any stage in the evolution of Arthropods produce a compound eye, whilst further in the actual details of structure of its compound eye, Limulus is altogether unlike the Crustacea. The resemblance of the compound eyes in the two cases is a superficial one, due to homoplasy.[1]

The third agreement is of a purely negative character. Limulus and the Crustacea may have independently lost the Malpighian tubules which were perhaps possessed by the earliest ancestral Arthropods; or, on the other hand, these organs may have developed for the first time in the terrestrial Arachnida, and have been derived from them by the other Arthropoda which possess them (Hexapoda, Myriapoda); or, again, the latter may have also developed such organs *de novo*. In any case their absence from Limulus is no evidence of affinity to Crustacea. It is to be noted that the smaller terrestrial Arachnida are also devoid of these organs.

It will now be convenient briefly to point out and criticise some of the views which have recently been expressed as to the affinities of Limulus.

Dohrn (1), in 1871, whilst pointing out at some length the affinities of Limulus and the Eurypterina, originally suggested by MacCoy and placed on a firm basis by Hall, and also whilst demonstrating some of the relationships of the larvæ of Limulus to Trilobites, proposes to unite these forms in one group—Gigantostraka (a name originally proposed by Haeckel for the Eurypterina alone), and to place this group near the Crustacea, not absolutely within that class.

Although Dohrn cites the views of Straus Durkheim, he does not support them, and definitely states that we are not in a position to say what may be the relationships of Gigantostraka to Arachnida.

Dohrn holds that the first pair of appendages of Limulus, though not the second, is innervated from the cerebral ganglion, but he is free from the erroneous conception of the post-anal spine of Limulus as representing a series of segments. At the same time he failed to be struck with the exact identity in the number and disposition of the segments which is revealed when Limulus and the Eurypterina taken

---

[1] See 'Annals and Mag. of Nat. Hist.,' July, 1870, on the use of the term "Homology."

together, on the one hand, are compared with Scorpio, on the other.

Claus (14), as late as 1881, adopts exactly Dohrn's view of the systematic position of Limulus. He accepts the group Gigantostraka (including Merostomata and Xiphosura), and places it as a division of the class Crustacea, in opposition to the Eucrustacea, consisting of the great subclasses Entomostraca and Malacostraca. Of the relationships of the Gigantostraka to Arachnida, Claus says nothing.

Owen (7), in his monograph on the King Crab, discusses Dohrn's views and brings to the question a large mass of anatomical and palæontological fact. His conclusion that Limulus exemplifies "that lower condition of the Crustacea which has been expressed by the term Entomostraca," is vitiated by the fact that although one of the first to recognise that the "chilaria" are sternal elements and not appendages, he yet seeks for the representatives of missing body segments in the postanal spine, and, above all, it is falsified by his adhesion to the opinion of Van der Hoeven, that two pairs of appendages are innervated from the cerebral ganglion. That no appendages are so innervated is now demonstrated by the dissections of A. Milne-Edwards which I have confirmed Accordingly, Professor Owen would now probably be amongst the first to admit the affinities of Limulus with the Arachnida, since he observes: "If it were a fact that in Limulus only the foremost pair of limbs was innervated from the superœsophageal ganglion, the rest deriving their nerves from the abdominal ganglionic chain, the advocate for its elimination from the Crustaceous class would have an argument of weight for the affinity of Limulus and its extinct allies with the Scorpion and the Spider."

Huxley (16), who has at various times approached the question of the affinities of Limulus, holds that it has relationships, on the one hand, through the Eurypterina to the Copepod Crustaceans, and on the other hand, to the Phyllopoda through the Trilobites, and again independently to the Scorpion. At the same time he definitely places it in the class Crustacea in the order Merostomata, together with Eurypterina and the Trilobites. Presumably this implies that Limulus is a nearly related representative of an ancestral form which gave rise to the Copepods as one branch, to the Trilobites and Phyllopoda as another, and to the Arachnida as a third.

Without discussing for the moment the possibility of any close connection between the Phyllopoda and Trilobites, I may remark that the connection of Limulus and the Eurypterina with the Copepoda appears to me to have only the support of a certain resemblance of general form in its favour, such resemblance of general form being one which frequently recurs in the Arthropod series, and has the significance merely of a homoplastic agreement, *i.e.* is a like moulding of readily modifiable parts brought about quite independently in the cases compared by the operation of like adaptive causes. Other examples in relation to the Eurypterina have been previously cited by Professor Huxley ('Lectures on Nat. Hist.,' 1857), *e.g.* the Cumacea and the Zoea of some Decapods. I cannot find, on comparing a Copepod, on the one hand, with the full organisation, on the other, expressed by a combination of the characters of Limulus and the Eurypterina, any points which appear to me indicative of close affinity; the agreements are such as either are common to the majority of Arthropods or are agreements of general form, of a nature similar to those which exist between the macrurous Arachnida and the macrurous Decapod Crustacea. Such agreements as exact coincidence in the position of the genital apertures, in the number, form, and grouping of the appendages, in the disposition of the eyes, in the development of sternal plates, and over and above the individual agreements such intimate connection as is implied by the multiplied significance of the combined occurrence of two, three, or more of these agreements, cannot be established as between the Copepoda and Limulus.

Between Eurypterina and such Copepoda as Cyclops, there is a general resemblance of the form of body. We find a broad carapace covering segments bearing five pairs of limbs, followed by a tapering series of segments, of which the anterior carry limbs, and may be distinguished as a separate region from those which follow. But whilst the Copepod body terminates in a characteristic furcal postanal process, the Eurypterina present, like the Scorpion and King Crab, a single spine or plate. The number of segments succeeding the carapace in the Copepoda is at most ten; in the Eurypterina it is, as in the Scorpion, twelve. Most significant is the position of the genital apertures, which in Limulus (and presumably in the Eurypterina) is placed on the first segment succeeding the six-segmented carapace, whilst in the Copepods the whole series of five segments, bearing swimming feet (which would be compared to the lamelligerous feet of Limulus), intervene between the carapace and the genital segment. In structure and position the eyes on the carapace of Copepods have no resemblance to the central and lateral eyes of Limulus, the Eurypterina, and the Scorpions.

When we examine the appendages, one striking resemblance is seen between the males of some free-living Copepods, on the one hand, and Limulus and Pterygotus, on the other. The first pair of appendages is in these forms prehensile. No other Arthropods except Arachnida have such a form of the first appendage. But many Eurypterina have non-chelate anterior appendages (see fig. 20), and the comparison of appendages in various Crustacea shows clearly that such a modification is readily acquired and readily suppressed. In one other respect some appendages of some Copepoda appear to resemble those of Limulus, viz. in the union of the basal portions of the swimming feet. In Limulus, however, this union is effected rather by the upgrowth of a median sternal process than by the coalescence of the bases of the appendages themselves.

In other respects the appendages of Copepoda are quite unlike those of Limulus and the Eurypterina in form, and they do not agree with them in number. Those near the mouth have jaw-like coxæ, as in all Crustacea, but they, have the usual Crustacean elements of endopodite, exopodite and epipodite more or less clearly developed, and are not simple rami, as are those of the Eurypterina. What may have been precisely the character of the limbs on the segments following the carapace in Eurypterina we do not know, but there is reason to suppose them to have been lamelligerous, and that is their distinguishing feature in Limulus. No such lamelligerous appendages are known in Copepoda, but in the region which might be compared to that carrying the genital operculum and the five lamelligerous appendages of Limulus —were it not for the fatal difference indicated by the reversed position of the generative orifices—we find four or five pairs of simple biramose swimming feet.

In internal organisation there is nothing in the characters of the nervous, digestive, reproductive, or circulatory organs (such as are present) of the Copepoda to suggest an alliance with Limulus, whilst the presence in the former of the characteristic Entomostracous shell-gland marks a special divergence between them. It is true that Packard has assimilated a brick-red coloured structure occurring at the base of the cephalothoracic limbs of Limulus to a shell-gland, or to a renal organ. In this I cannot agree with him. It is not even apparent, at present, that this brick-red organ, which I have examined, is of a glandular nature at all.

In his 'Manual of the Anatomy of Invertebrate Animals' Professor Huxley has recognised the possibility of the relationship of Limulus to Scorpio as well as to Copepoda, and has also instituted a comparison between the appendages of Limulus and those of the Podophthalmous Crustacea.

He considers only one pair of appendages of Limulus to be innervated from the cerebral ganglion, and regards the chilaria as the seventh pair of appendages, whilst he associates the genital operculum (his eighth pair of appendages) with the cephalothoracic carapace instead of with the abdominal carapace. In these respects recent investigations have necessitated a change of view (as I have explained at some length above), and accordingly the comparisons based upon the earlier view of the facts are erroneous. Thus, Huxley identifies the first pair of appendages of Limulus with the antennules of Astacus, and regards it as absent in Scorpio. The second pair he identifies with the antennæ of Astacus and with the cheliceræ of Scorpio, the third pair with the mandibles of Astacus and with the great chelæ of Scorpio, the fourth and fifth with the two pairs of maxillæ of Astacus and with the two first walking legs of Scorpio, the sixth (the digging leg) of Limulus with the first maxillipede of Astacus and the third walking leg of Scorpio. The chilaria or metathoracic sternites, which are considered by Huxley as the seventh pair of appendages, he identifies with the second maxillipedes of Astacus and with the fourth walking leg of the Scorpion, whilst the genital operculum is identified with the third pair of maxillipedes of Astacus and with the genital operculum of Scorpio.

The comparison of Limulus with the Podophthalmous Crustacean appears to me one which, in reality, it is not possible to carry out so as to establish any identities, or plausible points of contact. Even when we reckon the "chilaria" as appendages we find divergence and difference as the result of the comparison; but when these are removed from the series there is an absolute want of any relation in the grouping of the appendages compared. Not so with the Scorpion. Professor Huxley, in consequence of his view as to the nature of the chilaria, is obliged to assume that the cheliceræ of the King Crab are something over and above what is present in the Scorpion, and thus, eventually, in counting down the segments, he brings the genital operculum of the one into coincidence with that of the other. But when the chilaria are removed from the series offered by Limulus there is no need to assume an existence of extra appendages in front in that animal; the whole series in the two animals compared, viz. Limulus and Scorpio, are found, on inspection, to be identical in general form and relation from one end of the body to the other.

It is not possible, it should be observed, to maintain both positions. If the identification with the parts of the Scorpion is maintained, then all assimilation of the appendages and regions of the body of Limulus to those of a Podophthalmous or of a Copepodous Crustacean must be abandoned. There is no contact whatever between Limulus and Astacus until a common ancestral form is reached which exhibited in the most generalised condition the segmentation and appendages which are the common inheritance of all Arthropoda.

It appears to me quite impossible to assume that this ancestral form had the characters of the Podophthalmous Crustacea. Such differentiation and numerical grouping of appendages as are seen in that highly developed Crustacean order are of late appearance, and accordingly such forms as Astacus and Homarus should not be made use of as standards of comparison representative of the Crustacea, but less differentiated examples must be sought. On the other hand, when we find it possible to establish a series of agreements between a form of doubtful affinities, such as Limulus, and a highly differentiated Arthropod, such as the Scorpion, the closeness of the genealogical connection thereby proved is greater in proportion as the differentiation of the forms compared is high, and as the number of points of agreement are numerous.

The two authors who have had the facts in reference to Limulus and Scorpio most fully before them (since some of the more important were established by their own researches), and yet have not drawn the conclusion from those facts to which it seems to me that they necessarily lead, are MM. Alphonse Edwards and Dr. A. S. Packard. M. Alphonse Milne-Edwards, although he showed that the cerebral ganglion of Limulus was unlike that of the Crustacea, could not admit of its assimilation to that of the Scorpion, not being acquainted, apparently, with Metschnikoff's observations on the development of the latter animal; and although he recognises the similarity of the perineural arterial system of Limulus to the supraneural or "spinal" arterial system of Scorpio, yet he is led away from the assimilation of the two animals by holding to the strange notion that the chilaria of the King Crab placed *just in front* of its genital operculum are the homological equivalents of the pectiniform appendages of the Scorpion placed *just behind* its genital operculum. M. Milne-Edwards places the

Limuli neither with the Arachnida nor with the Crustacea, but in a group apart. The fact that this investigator did not attempt a complete study of the skeleton of Limulus, and a comparison of that and other organs with the corresponding parts of the Arachnida, Eurypterina, and Crustacea, sufficiently explains the conclusion at which he arrived. He confessedly made use of but few data, and those such as he himself brought to light in the case of Limulus. The value of his contributions to our knowledge of the anatomical structure of the King Crab are not in any way diminished by the vulnerability of the conclusion which he based upon them.

With regard to the conclusions of Dr. A. S. Packard, it is difficult to avoid an expression of surprise. We owe to Dr. Packard the important observation of the late appearance of the chilaria, and other observations as to the segmentation of the telsonic region in the young Limulus, and the primitive connection of the genital operculum with the so-called abdomen rather than with the cephalothorax. He has probably seen more of young and old King Crabs than any other naturalist, and yet, writing in 1880 (No. 9), with all the literature before him, with all the facts under his hands, he still maintains that the Limuli are Crustacea, examines the aphoristic statements of Van Beneden to the effect that they are Arachnida and rejects them. Dr. Packard simply adopts from Dohrn the group of Gigantostraca as Claus has done, and as Gegenbaur has done; but whilst Gegenbaur uses for it the old term Pœcilopoda, Packard thinks it necessary to bestow upon it the new name Palæocarida.

An examination of Dr. Packard's latest memoir on Limulus will, I think, show that he clings to the notion that Limulus is a Crustacean, and is unable to perceive that its true place is among Arachnida, because he entertains certain erroneous preconceptions as to the value of the various parts of an arthropod body as indicative of genetic affinity. A respiratory appendage, however, unlike in structure to anything seen in Crustacea, is, if it acts as a branchia, to be considered as "of the Crustacean type" according to Dr. Packard. This is a simple confusion of logical categories. It is true that many Crustacea have branchial appendages, but it does not follow as a consequence that all branchial appendages are borne by Crustaceans, or that such appendages are of "the Crustacean type." So too Dr. Packard speaks of "true antennæ" and a "true mandible," "a thorax," and "an abdomen," as though these were recognised and definable elements of arthropod structure, instead of being as they are descriptive terms devoid of homological significance. Really what Dr. Packard has to deal with is a series of segments and a series of appendages, and he can only compare those of one animal with those of another by taking them in numerical sequence. When an author allows himself to set up such intangible criteria as are involved in Dr. Packard's distinction between "true" and "false" antennæ, he clearly opens the way to any conclusion he may fancy, and may colour a picture as he may choose by the use of these epithets.

Dr. Packard's estimate of the significance and import of parts in the attempt to determine the affinities one with another of various Arthropods, is, it seems to me, fallacious, owing to the fact that it is based upon an old-fashioned morphology. Though he makes use of the phraseology of the doctrine of evolution, and constructs genealogical trees, he has "the doctrine of types" at heart, and meets a matter of fact question in morphology by the use of such phrases as the "crustacean type," the "tracheate type," and the "hexapodous type." With such phrases no critic can possibly deal, for no one can say what Dr. Packard means by these "types." We are told by him that the Arachnida have their mandibles and maxillæ "on hexapodous type," whilst the Merostomata (Limulus) have "only their morphological equivalents (Guathopods)." This is meant to appear as though a wide divergence between the Scorpion and King Crab were being in so many words established, and to Dr. Packard so it may really appear. To me it seems that in the statement quoted, phrases of doubtful meaning are being used in such a way as to vaguely assert the opposite of one of the most obvious facts, namely, that the first and second pairs of appendages of a King Crab are far more like the first and second pairs of appendages of a Scorpion than those of either are like the mandibles and the maxillæ of hexapod insects.

Dr. Packard summarises his views as to Limulus and the Crustacea thus: "The facts that seem to us to point to the Crustacean nature of Limulus and its allies are: (1) the nature of the branchiæ, those of Limulus being developed in numerous plates overlapping each other on the second abdominal limbs; those of the Eurypterida being, according to H. Woodward, attached side by side, like the teeth of a rake; while the mode of respiration is truly Crustacean; (2) the resemblance of the cephalothorax of Limulus to that of Apus; (3) the general resemblance of the gnathopods to the feet of the Nauplius or larva of Cirripedia and Copepoda; (4) the digestive tract is homologous throughout with that of Crustacea, particularly the Decapoda, there being no urinary tubes, as in Tracheata; (5) the heart is on the Crustacean type as much as on the Tracheate type, and the internal reproductive organs (ovaries and testes) open externally, at the base of and in the limbs, much as in Crustacea."

To this series of statements I would reply categorically—(1) the "nature of the branchiæ" is *not* such as is found in any Crustacean, but is only paralleled in the lamelligerous appendages of Arachnida. Other animals have branchiæ besides Crustacea. The mode of respiration is neither truly nor falsely Crustacean, but is simply 'branchial.'

(2) The cephalothorax of Limulus does *not* resemble that of Apus, but differs from it as much as it does from any Arthropodous cephalothorax, as, for example, in the overlapping of posterior segments by the free posterior margin of the carapace of Apus; in the excavation of the carapace in Apus by the shell-glands; in the widely different position of the first and second pair of appendages in relation to the cephalothoracic margin; in the total difference of the eyes; and, above all, in the totally different form, number, and arrangement of the gnathites.

(3) The gnathopods have *no* "general resemblance to the feet of the Nauplius" which calls for remark. They have a *general* resemblance to the feet of any Arthropod, but *less* to the feet of the Nauplius than to many other varieties of Arthropod feet, owing to the fact that the former are biramose, non-chelate, natatory, and feebly chitinized, which those of Limulus are not.

(4) The digestive tract is homologous throughout, not only with that of Crustacea, but with that of all other Arthropods. How Dr. Packard can suppose that it is homologous, particularly with that of Decapoda, I am unable to comprehend, unless he proposed to himself, when writing this passage, to associate Limulus genealogically in a special branch with the Decapoda. Unless this is the case Dr. Packard makes use of the word 'homologous' with a meaning which is unusual and unknown to me.

(5) That "the heart is on the Crustacean type as much as on the Tracheate type" I will not dispute, for I do not feel sure that I know what Dr. Packard means, and he appears to take up a neutral attitude, in regard to the heart at any rate. I will, however, remark that, putting types aside, there is no heart of a Crustacean which so closely resembles the King Crab's as does that of the Arachnid

Scorpion, and there is no heart which so closely resembles the Scorpion's as does that of the King Crab.

That the internal reproductive organs should open externally in the neighbourhood of limbs is certainly not a peculiarity of Crustacea. The relation of the openings to limbs is *not* 'much as in Crustacea,' but quite unlike anything seen in Crustacea. In no Crustacean does a pair of limbs in front of the genital apertures unite to form with a median lobe carrying those apertures—a broad plate, as in the King Crab. A genital operculum of this nature is found only in the King Crab, the Eurypterina, and the Scorpion.

The extreme anterior position of the generative apertures has no parallel among Crustacea nor among Arthropods, excepting the Arachnida, where it is identical in position. Even the chilognathous Myriapods do not exhibit so forward a position of the genital orifices.

E. CONCLUSION ; LIMULUS AND THE ANCESTRY OF TRACHEATE ARTHROPODA.

The nature and degree of intimacy of the relationship between Limulus and the Scorpion—which is indicated by the facts and arguments set forth in the preceding essay—have yet to be considered. It is one thing to establish the fact that a closer relationship obtains between Limulus and Scorpio than between Limulus and any Crustacean, and another thing to estimate more precisely the affinity between the two animals.

A brief consideration of the facts is sufficient to show that the points in which Limulus agrees with Scorpio and Mygale include those structural features on which we have to rely in attempting to characterise the class Arachnida. At the same time it must be admitted that all attempts at limiting classificatory groups by simple definition are hopeless, provided that the groups are intended to express degrees of genealogical affinity, and not merely arbitrary categories, held together by more or less obvious class marks. The real question which we have to attempt to answer, in assigning Limulus and the Arachnida their place in a genealogical classification of the Arthropoda is not, "How may groups be defined which shall give due expression to the structural likenesses and unlikenesses of these forms?" but, "How may groups be arranged so as to exhibit the probable history of ancestral development in relation to these forms?" Owing to the occurrence of degeneration, and to the suppression in some forms of structural features which were the distinguishing characteristics of their immediate ancestry, we find that frequently genealogical groups do not admit of strict definition in terms of structure. And, further, we find that, even in order to arrive at a clear notion with regard to the relationships of a limited portion of a large group—such a portion as are Arachnida in regard to the Arthropoda—it is necessary to consider the genealogy of the whole series included in the larger group.

The Arthropoda form a very large branch of a great phylum to which I have applied the name 'Appendiculata'—cœlomate animals with more or less distinct metameric segmentation of the body and possessed of lateral lobes or processes of the body itself which serve primarily as locomotor organs. Besides the Arthropoda the phylum Appendiculata includes the Rotifera and the Chætopoda. Each of these three great branches of the Appendiculata has its special developments, but it seems to be probable that they all started from a common ancestry which had characters intermediate to those of such a Rotifer as Pedalion and of such a Chætopod as Syllis. Probably the Arthropoda were developed from an ancestry resembling the Chætopoda, but devoid of the chætæ carried by the appendages of the latter.

The distinguishing motive of the development of the Arthropod branch of the Appendiculata is the adaptation of one or more pairs of the appendages proper to the segments succeeding the mouth, to the purposes of the prehension and mastication of food. Hence it would be well to substitute the term Gnathopoda for Arthropoda. All Arthropoda are not arthropodous, that is to say, do not exhibit a jointing of the exo-skeleton of the appendages. Peripatus though truly a Gnathopod is not an Arthropod or Condylopod. The disappearance of such jointing in connection with a softening of the integument and a scavenger mode of life amongst rotten wood, is one of those changes which it is probable might occur as an adaptation, and accordingly it is very doubtful whether we should regard the non-arthropodous condition of Peripatus as a retention by it of the soft-bodied character proper to the Chætopod-like ancestry of the Arthropoda.

The structure of its eye, the presence of two lateral nerve-cords in place of a double ventral cord, the limitation of the jaw-feet to a single pair, the existence of paired nephridia in each segment of the body, the peculiar histological structure of the muscular tissue, seem to me to be conclusive evidence in favour of the view that Peripatus is a representative of an exceedingly primitive grade of Arthropod development, corresponding to a period when the Arthropod branch had advanced but little on its special lines of differentiation.

.At the same time Peripatus is specialised and adapted to a terrestrial mode of life. It possesses no remnants of branchial organs but a peculiar tracheal system, air being admitted to the fine vessels formed by its vasifactive tissue through irregularly scattered gland-like pits of the integument.

Its specialisation as a terrestical organism has, it is impossible to doubt, affected in Peripatus the locomotor appendages also, so that much important information is wanting to us, which, on the contrary, an aquatic form belonging to the phase of development indicated by the eyes, nerve-cords, nephridia, and gnathites of Peripatus, could have furnished.

It appears to me that we have no such aquatic representative form, and that Peripatus stands as a specialised terrestrial off-shoot at a much lower point in the Arthropod family-tree than that at which we find outgrowths of existing branchiate Arthropoda.

The antennæ of Peripatus probably are identical with the similar organs of Chætopoda (cf. Spio and Phyllochætopterus), and are *not* originally post-oral appendages which have become præoral by adaptational shifting of the oral aperture, but are actual lobes or processes of the primitive prostomium, like the tentacles on the head of a snail, and innervated by the archicerebrum or original prostomial ganglion.

In the interval between the giving off of Peripatus and the production of the Phyllopod-like ancestors of the Crustacea from the aquatic Pro-Arthropoda, a vast change had to be effected in regard to appendages as well as in the fusing of the nerve-cords, abolition of nephridia, production of a compound eye, striation of muscular tissue, &c. The prostomial antennæ disappeared and their place was taken first by one, then by two pairs of post-oral appendages, which gradually acquired a præ-oral position as actually occurs in their individual growth in the embryo at the present day; eventually the simple prostomial ganglion (archicerebrum) became complicated by the fusion with it of ganglionic material proper to the two shifting appendages, though in the existing Phyllopod Apus it still retains its original purity and independence.

The other appendages probably all acquired at one stage a development of their basal portion which served as an accessory organ for the purpose of bringing food to the mouth and in some degree in crushing such food (as seen in Apus), but this development was specially carried out and localised in two pairs of appendages posterior to the one already so differentiated in Peripatus.

The segments, each with its pair of appendages, were indefinite in number and frequently exceeded one hundred. The definite Crustacean character was attained when two pairs of appendages had become præ-oral, at least three pairs specialised as jaws and no longer locomotor, whilst the remaining appendages retained locomotor, manducatory, and respiratory functions to be subsequently specialised in the further development of the Crustacean stem.

It appears to me probable that the Merostomata, including under this head the Xiphosura (Limulus), the Trilobita, and the Eurypterina, diverged from the main stem[1] of the Arthropod pedigree at a point between that indicated by the grade of organisation of Peripatus and that occupied by the Pro-Phyllopoda or earliest Crustaceans.

Probably none of the known Merostomata suffice to give us a true picture of the structure of the ancestral Merostomata from which they are all derived. Probably these ancestral Merostomata were devoid of the prostomial antennæ—the non-appendicular antennæ. At the same time none of their post-oral appendages had become definitely prœ-oral in position and nerve supply, though not less and probably not more than six pairs of pediform appendages were closely set round the mouth, their bases acting as powerful manducatory organs. To this group of appendages, of which the corresponding segments were more or less completely fused with the prostomium (forming the *prosoma*), succeeded a mid-region of the body (the *mesosoma*), consisting of numerous segments carrying biramose, probably pediform appendages, the bases of which were beset with respiratory lamellæ.

The generative apertures were situate in the first or one of the anterior segments of this mid-region of the body. A third region of the body (the *metasoma*), also consisting of numerous segments, was probably distinguished by the form and smaller size of its appendages and by a tendency of the segments to fusion. Posteriorly to the anus was a median plate or spine. Probably the eyes placed on the dorsal sur-

---

[1] I have treated the line of descent leading to the Crustacea as the main stem of the Arthropod family-tree; it is obviously a matter which may be determined by convenience as to whether one or other of the branches of a genealogical tree shall be treated as the main line of the family.

face of the anterior region of the body were simple eyes, but arranged in two lateral groups and a central group.

From such a form the Xiphosura were derived by retention of the full number of the appendages of the prosoma, the limitation of the segments of the mesosoma to six, and their specialisation as plate-like organs serving as genital operculum, branchiæ and swimmerets, further by the limitation of the segments of the metasoma, first of all to six, and their subsequent fusion and partial disappearance even from embryonic expression, and the atrophy of the appendages proper to them. At the same time the lateral groups of simple eyes were replaced by a peculiar form of compound eye.

The Eurypterina diverged from the Xiphosura after most of these features had been elaborated, but so as to retain the six free segments of the metasoma, whilst at the same time they lost one pair (probably the most anterior) of the appendages of the prosoma, and possibly the three hindermost of the appendages of the mesosoma.

The Trilobita diverged from the common ancestry of the Xiphosura and Eurypterina probably at a time when the number of six segments to the mesosoma and six to the metasoma had not become a definite limitation, and when appendages were carried by both those regions of the body, differing only from the leg-like gnathites of the prosoma in possessing a second ramus and lamelliform branchial processes. Possibly the compound eye of the Trilobite was inherited from an ancestor common to it and the Eurypterina. According to Walcot (12), a very distinctive feature in the differentiation of the Trilobita was the reduction of the number of appendages of the prosoma from six pairs to four. In all these forms it is important to note that the appendages of the prosoma, whether six pairs in number or less, whether chelate, tactile, ambulatory, or natatory, so far as the 'palp' or chief ramus is concerned, yet all, with the exception of the most anterior pair, continue by means of their enlarged basal joint to act as manducatory organs. As a set-off to the loss of the manducatory functions of their coxæ, the first pair possess, with rare exceptions, nipping or stabbing palps.

The relationship of the Scorpion and other living Arachnida to the Merostomata appears to be this. From an ancestral form, which was nearly related to the common progenitor of the Xiphosura and Eurypterina, which possessed six pairs of appendages to its prosoma, the terga united to form a carapace, six free segments to its mesosoma and six free segments to its metasoma—the metasoma devoid of appendages as in Xiphosura and Eurypterina, the mesosoma provided with a genital operculum (united appendages) on its anterior segment and with five pairs of lamelligerous respiratory appendages on the five succeeding segments—from such a form by a very slight process of change, consisting in adaptation to terrestrial in place of aquatic conditions, the primitive Scorpions were developed. It is probable that the particular form antecedent to the differentiation of Xiphosura and Eurypterina, from which the Scorpions took origin had not developed lateral compound eyes, but still exhibited a primitive condition, which is retained by the Scorpions and other Arachnida, viz. a lateral grouping of simple eyes.

The structural changes necessary to produce a Scorpion from such an ancestral Merostom as has been just sketched are so small that it is not possible to place the Scorpions and the Merostomata in separate classes, if by the use of the division known as a 'class' we are to indicate as nearly as possible, in different parts of the pedigree of animals, an equal break or unrepresented interval of structural change. At the same time the Scorpions, having once been developed, appear to have given rise to the whole series of living Arachnida, to the Pedipalpi first, and through these to the Araneina, and through the Araneina to the Acarina.

Galeodes is probably a special development from the Scorpionina, as in a different direction are the Opilionina and Pseudoscorpions.

This conclusion, if it be well founded, justifies some important inferences of a secondary character. In the first place we have to admit a very extensive process of degeneration in the course of development, leading from the Scorpion to such Acarina as Demodex, or even Hydrachna. In the second place we obtain a definite answer as to the mode of origin of tracheæ, in so far, at least, as the tracheæ of the Arachnida are concerned. The vascular lamelligerous appendages of the Limuloid ancester of the Scorpion became dry and filled with air in place of with blood. From this blood-sinus, converted into an air-sinus, the air appears gradually to have made its way, encroaching upon pre-existing blood-canals, and converting them into air-canals. The highly developed condition of the blood-vascular system in the Scorpions renders it probable that the tracheæ of the tracheate Arachnida are not *new* vessels specially developed as an aërial vascular system, but are the modified and adapted blood-vascular channels, just in the same way as the air-containing lamelligerous appendages of the Scorpion are not new organs, but the modified and adapted blood-containing appendages of a Limuloid ancestor.

The relationship of the groups of Arachnida to one another thus suggested may be best indicated by means of a genealogical tree (see last page). I have also drawn up the names and arrangement of groups suggested in a tabular form.

I have further thrown into the form of a genealogical tree the conclusions to which I am led in reference to the relationship to one another of Peripatus, the Crustacea, and the Arachnida.

In this pedigree of the Arthropoda no place is assigned to the two great tracheate groups of Insecta Hexapoda and Insecta Myriapoda. In the present state of knowledge it appears to be impossible to assign to either of them one position rather than another. We have not even sufficient ground for concluding that they are closely related to one another. The antennæ of Hexapods and of Myriapods *may* be, as probably are those of Peripatus, non-appendicular prostomial antennæ, which would be, in addition to the presence of tracheæ, a reason for considering both to have been developed from such a form as Peripatus. On the other hand, possibly only the Myriapoda are derived from Peripatus-like ancestry, and, probably enough, neither one nor the other. It seems to be in the highest degree probable, and is not difficult of admission, that there is no such a group to be recognised as the Tracheata. Tracheæ have probably developed independently in Peripatus and in the Insecta, and again in the Arachnida. Nevertheless, the view is capable of being defended that all tracheate Arthropoda have a common tracheate ancestor; in which case it will be necessary to derive the Insects, the Myriapods, and, to be consistent, Peripatus also, from the tracheate Arachnida, through such a form as Galeodes. The derivation of Galeodes through the Scorpions, from the branchiate Arachnida, is, relatively speaking, a well-grounded conclusion; and if tracheæ are to have but one starting-point, it is of necessity here that we must look for it.

Insuperable difficulties are, however, found in the derivation of Hexapoda from Galeodes, in spite of curious homoplastic agreements between the two. Such a difficulty is the absence of appendages corresponding to the antennæ of Insects in Galeodes, and in the whole line of its Arachnidan ancestry, which absence has to be recognised if the pincers of Galeodes are identified with the mandibles of an Insect.[1]

---

[1] I do not admit the truth of this identification.

In deriving the Hexapods and Myriapods from Galeodes we should have to suppose the antennæ of the former to arise *de novo*—a supposition which is contrary to one of the fundamental principles of phylogeny, viz. that new organs do not arise *de novo* as new parts, but by the modification of pre-existing parts.

Hence it seems that in any case the tracheate Arachnida must be left apart from the other tracheate Arthropods as the extreme modification of the series originating in the Limuloids.

This conclusion is, however, in opposition to the view that the renal Malpighian tubes are of phylogenetic significance. It is a very striking fact that all well-developed tracheate Arthropoda (except Peripatus) have not only tracheæ as respiratory organs, but also have these Malpighian cæca growing from the proctodæum. Either the Hexapods and Myriapods are closely related to the air-breathing Arachnids or these Malphighan cæca have, like the tracheæ, appeared more than once independently in divergent lines of the Arthropod family-tree.

A minute comparative study of the structure and development of these cæca is wanting; at the same time it appears that certain of the Isopod Crustacea possess organs comparable to them. If this be so, another possible place of attachment for the Hexapods and Myriapods to the Arthropod family-tree is indicated, which, on independent grounds, has much in its favour. Supposing that the antennæ of Hexapods and Myriapods should prove not to be identical with the prostomial antennæ of Chætopods but should be shown by the examination of the development and structure of their connected nerve-ganglia to be like those of Crustacea, originally post-oral appendages, or supposing on any other grounds that the antennæ of these forms could be identified with one pair of the Crustacean's antennæ, then it would not be difficult to conceive of such a modification of the post-oral appendages of an Isopod as would give the disposition characteristic of them in either Myriapods or Hexapods.

And it is to be noted that among existing Isopods, terrestrial forms are known with peculiar lung-like pouches adapted to aërial respiration.

A strong argument in favour of the derivation of the Hexapoda from Crustacea appears at first sight to be afforded by the minute structure of the compound eye of the two series of organisms.

Amongst all the possible points of genetic connection of the Hexapoda and of the Myriapoda with the other large groups of Arthropoda, there is probably more hope of a definite indication being obtained as the result of a critical study and comparison of the structure of the *eyes* than from any other source. The eyes of Arthropods are elaborate in the histological details of their structure, and at the same time have not been inherited from a common ancestor in one and the same elaborate form by all the members of the group, as have been the eyes of craniate Vertebrata for example. Accordingly we may expect that the elaboration of the eye has taken a somewhat different course in different lines of descent within the limits of the Arthropod phylum, and we should be justified in concluding a common line of descent for classes of Arthropods showing identity in numerous details of the optical structure, which details had been ascertained not to be a common inheritance from the primæval Arthropod ancestor.

Whatever may be the conclusion arrived at in the future in reference to the affinities of Hexapoda and Myriapoda, the result of the recognition of the intimate relationship of Scorpio and Limulus must be, I think, to break up the artificial group of "Arthropoda Tracheata" by the separation of the Scorpions, Spiders, and Mites, from any special connection with it.

Phylum.—APPENDICULATA.

Branch 3.—Arthropoda (Gnathopoda).

Class.—Arachnida.

Arthropoda developed from ancestral forms, in which a 'prosoma' formed by the union of the prostomium and six anterior segments was sharply marked off from the rest of the body, both by the confluence of its terga to form a carapace and by the special character and size of its appendages. The six pairs of appendages (including the foremost of the whole series) were arranged round the mouth, and all subservient to the purpose of prehension and mastication of food. In the later developed forms of Arachnida either the number of these appendages may be reduced (Eurypterina, Trilobita), or the functional relation to the mouth of the more posterior of the six pairs may be lost. Whatever their number, the foremost pair is free from a jaw-like enlargement of the coxa. The palps of all six pairs of appendages exhibit a wide range of adaptational form, as prehensile, tactile, ambulatory, natatory, or fossorial organs.

The generative apertures are placed far forward—ancestrally in the first segment of the 'mesosoma' or region following the prosoma, and are covered by a fused pair of appendages, or, when these have aborted, by the corresponding sternite.

The appendages of the mesosoma posterior to the generative apertures carry peculiar respiratory lamellæ, which expose the blood circulating in them to the dissolved oxygen of natural waters in the more archaic members of the group, but are perforated, invaginated in recesses of the ventral integument, and filled with atmospheric air in terrestrial forms (Scorpions, Spiders, &c.), or may be altogether aborted and replaced by tracheæ.

Except in the Trilobita the segments and paired appendages of the mesosoma are not more than six in number, and the same is true of the metasoma or terminal region of the body, which is devoid of appendages (except in Trilobita), and may either have the appearance of a simple continuation of the mesosoma (macrourous forms), or may have its segments fused with one another, but separate from those of the mesosoma (Trilobita); or, again, may be more or less completely aborted and fused with the mesosoma (Limulus), when the segmentation of the mesosoma itself may also become partially (Spiders) or completely (Acarina) obliterated.

In all the larger known forms (Limulus, Scorpio, Mygale) a large free sclerite, the entosternite, is found within the prosoma, giving attachment to muscles inserted into the sternites of the mesosoma.

Tabular view of the Orders of Arachnida.

Grade A.—Hæmatobranchia (= Merostomata):

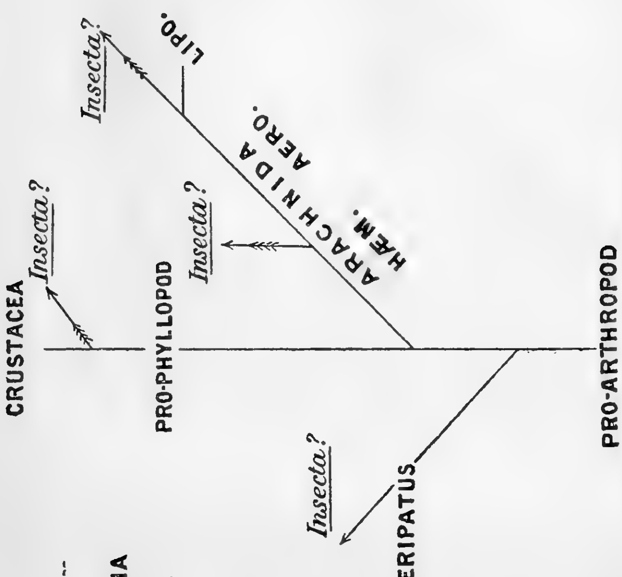
Order 1	Trilobita.
„ 2	Eurypterina.
„ 3	Xiphosura.

Grade B.—Aerobranchia:

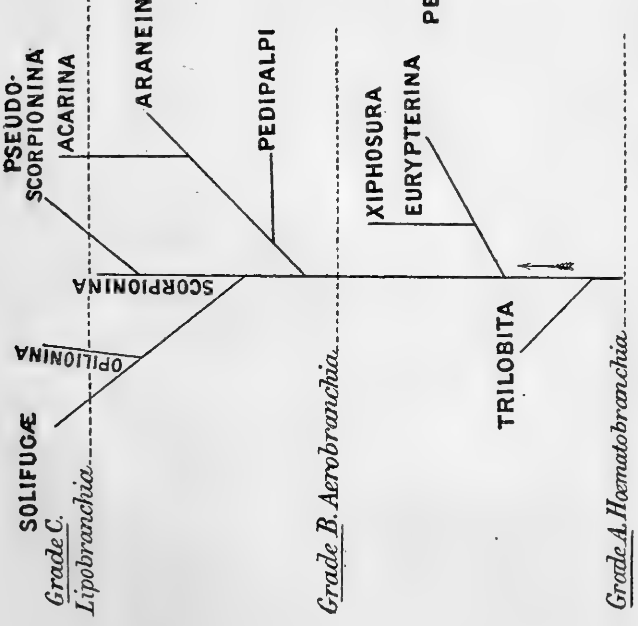
Order 1	Scorpionina.
„ 2	Pedipalpi.
„ 3	Araneina.

Grade C.—Lipobranchia:

Order 1	Solifugæ.
„ 2	Pseudoscorpionina.
„ 3	Opilionina.
„ 4	Acarina.



Genealogical Tree of the Arthropoda.



Genealogical Tree of the Arachnida

NOTES AND MEMORANDA.

Dr. Koch's New Method of Pure Cultivation of Bacteria.—At the recent meeting of the International Medical Congress in London, during August, Dr. Koch, well known by his researches on the life-history of Bacillus anthracis (see this Journal, Vol. XVII, p. 87), gave a series of demonstrations in the physiological laboratory of King's College, which were of the greatest interest and importance.

Dr. Koch has recently been appointed to the charge of a laboratory of experimental research connected with the State Department of Public Health in Berlin, and aided by his two assistants, he brought to London material and instruments for the purpose of exhibiting to the members of the Congress the methods of research into the relation of Bacteria to disease, devised by him. The series of photographs of various forms of Bacteria shown by Dr. Koch were valuable, as affording convincing evidence of the necessity of making use of photography as the means of obtaining and preserving a record of the specific form and character of Bacterian growths. Of great interest also were the cultivations of the Bacteria of blue milk, and of those of blue pus, exhibited by Dr. Koch, and of the septic Bacterium of putrid blood, the toxic effects of which were experimentally demonstrated.

Of most general importance, and in our judgment likely to mark altogether a new era in the study of the relations of Bacteria to certain diseases, and to other fermentative processes, was the demonstration by Dr. Koch of a new and yet absolutely simple and obvious method of obtaining pure cultivations of the species of Bacteria.

It is a well-known fact that there are a large number of species of Bacteria differing from one another in the effects which they produce in the medium wherein they are cultivated. It is also well known that Bacteria are so ubiquitous that the examination of any natural medium attacked by them is almost sure to yield evidence of the presence of more than one species, the various species growing together in inextricable confusion. On this account it has been found a matter of extreme difficulty to determine what effects are due to one species of Bacterium and what to another. And it has indeed been often impossible to determine in such a mixture of forms those which are genetically related to one another, and therefore to distinguish one species from the other forms which are adventitiously associated with it.

To effect the separation of species in a mixture, Mr. Lister employed a method of dilution and division described in his well-known research on the Lactic ferment (see this Journal, vol. xviii, p. 191). Making use of a *fluid* as the nutrient medium of cultivation (as hitherto has been the almost universal practice in such cultivations), Mr. Lister introduced a drop of sour milk containing possibly twenty kinds of Bacteria, and among them the Bacterium of lactic fermentation, into a large quantity of pure water, the dilution and spacing (so to speak) of the Bacteria thus affected being calculated so to render it probable that a *single* drop removed from the diluted Bacterian mixture would contain a *single* Bacterium. Such drops were then removed and placed each into a separate culture-tube containing sterilized fluid nutriment, and thus in a certain number of the tubes a pure cultivation consisting of the progeny of a single Bacterium, and, therefore, unquestionably of but one species, was obtained.

This method is tedious and liable to failure owing to the great care necessary to ensure and maintain sterilization of the cultivation fluid whilst exposed for the purpose of inoculation and again for further examination. Dr. Koch was led to this new method of cultivation, which essentially consists in the substitution of a *solid* for a *fluid* medium of cultivation, by the use of the method known to all mycologists of cultivation, upon slices of potato or beet-root. It is readily observed when slices of boiled potato are exposed in a damp condition to the atmosphere that the surface of the slice becomes the seat of development of various Bacteria and of moulds, the spores of which fall from the atmosphere on to the exposed slice, a fact which struck Dr. Koch as of importance in reference to the slices of potato was this—that the various spores falling on to it remain where they fall, and from the spot where each spore or germ originally fell it proceeds to multiply, producing around it a symmetrical hemispherical growth of perfect purity. In fact, owing to the solid character of the nourishing support the germs and spores cannot get mixed as they do in a liquid, each remains distinct from its neighbour even though in very close proximity, and without any trouble from the resulting growth, which proceeds in a day or two from each germ—new and perfectly pure cultivations may be started in suitable sterilized fluids.

Dr. Koch's method consists in substituting for the potato slice a layer of gelatine which is so saturated with water as just to become solid on cooling. The gelatine liquid is readily sterilized by boiling, and into it can be introduced either Pasteur's salts, peptones, blood-serum, or other nutrient material required by one or other species of Bacterium. The gelatine-medium thus prepared may be kept in a tube and a cultivation thus carried on—on its surface, or (and this is its principal use) it may be spread when liquid on a microscope object-slide and allowed to cool. Then such a gelatine plate may be inoculated by touching its surface with material containing the Bacteria which it is desired to study. The plate is readily protected from the access of accidental atmospheric germs, and maintained at such temperature and degree of moisture (by a glass shade) as the experimenter may desire. The main point of advantage, however, is this—that the point of inoculation on the surface of the gelatine can, owing to its transparency, be readily examined with the highest powers of the microscope and the growth of the Bacteria followed—whilst further, owing to the fact that the medium in which the growth takes place is solid, no mixture of the different kinds which may be present occurs, but each Bacterium produces around it a little spherical nest of its own kind. From these nests, with a sterilized needle-point, individuals can be removed to start new pure cultivations.

But it is obvious that, if the original point of inoculation was very minute, there is no danger of any accidental contamination from atmospheric germs, for these are not likely to fall on the identical spot no bigger than the puncture of a needle's point, where the experimental culture is going on. As a matter of fact, where they fall on to the gelatine there they remain and grow, and fifty such accidental spores may fall on to the gelatine plate without in the least interfering with the purity of the experimental culture.

There is yet, further, a very simple device which enables Dr. Koch to use this gelatine surface as a means of "spacing" and dividing the various species in a mixture of Bacteria. He dips a sterilized needle into such a mixture, and then makes a long shallow streak with the needle's point upon the surface of the gelatine. The Bacteria which were adhering to the needle's point are in this way dropped at intervals along the streak, some nearer some further apart, but all (with rare exceptions) in such a way that their subsequent growth keeps clear of that of a neighbour, and can, with the aid of a low power or even without any microscope, be visited by a sterilized needle point, and thus used to start on another gelatine plate a perfectly pure cultivation.

These pure cultivations, such as Lister aimed at by his method of dilution and division, may be called, in order to indicate to what an extent they are known to be pure, "monosporous cultivations," since the principle which distinguishes them is that all the growth is the offspring of a single isolated germ or spore.

It is only by such monosporous cultivations that we can arrive at solid conclusions in reference to the forms and activities of the Bacteria, *e.g.* as to whether one form can give rise to progeny of another form when its food and conditions of growth are changed, and again, as to whether special fermentative powers can be lost or acquired in the course of generations derived from one parent germ, but subjected to different conditions as to food, temperature, and oxygen.

The method of gelatine cultivation devised by Dr. Koch, places the means of following out these inquiries in the hands of every careful microscopist. Such methods as Lister's were too troublesome and too difficult for general and widespread application; but now that monosporous cultivation of Bacteria has been rendered a comparatively simple and certain affair, we may expect immediate and immense advances in our knowledge of the whole series of phenomena to which the Bacteria are related.

Amongst problems which require immediate investigation by the new method are the distinctive properties of the various kinds of Bacteria which may infest the wounds of surgical practice, and their specific susceptibility to the destructive influence of carbolic acid and other antiseptics; further, the possibility of isolating a specific Bacterium in contagious diseases not yet investigated: and (of great physiological interest) the isolation and investigation of the properties of the specific Bacterium of the ammoniacal fermentation of urine.

Dr. Koch and his assistants will, no doubt, shortly publish a detailed account of the researches which they have been engaged in during the past year, and will give particulars as to the methods of investigation employed by them, which had not (we believe), previously to the meeting of the International Medical Congress, been given to the public.

A remarkable negative result obtained by Dr. Koch, so far as his experiments with the new method of monosporous culture have yet extended, is, that there is no transition of forms amongst, at any rate, the pathogenous Bacteria—a Micrococcus produces Micrococci, and no other form; a Bacillus produces only Bacilli; a biscuit-shaped form (Bacterium proper) only biscuit-shaped forms; a Spirillum only Spirilla. Moreover, the *facies* of the discoidal or spherical mass formed by a growth, as seen with a low power excavating its way in the gelatine is characteristic of species, so that a practised observer can, in some cases, recognise a particular Bacillus or Micrococcus by the naked-eye appearance of the growth alone, or, at any rate, without actually observing the individual units of the growth.—L.

INDEX TO JOURNAL.

VOL. XXI, NEW SERIES.

PRINTED BY J. E. ADLARD, BARTHOLOMEW CLOSE.

25