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MEMOIRS.

On the Hinged Teeth *of the* Common Pike. By Charles S. Tomes, M.A. (With Plate I.)

Notwithstanding the activity of biological research in almost every other direction, it has happened that the teeth, more especially of reptiles and fishes, have received but little attention, and thus many most interesting peculiarities, although met with in creatures exceedingly common, have escaped notice. The angler (*Lophius piscatorius*) has long been known to possess hinged teeth, capable of being bent inwards towards the mouth, but by virtue of the elasticity of the hinge at once resuming the upright position when pressure is removed from them; this arrangement was formerly supposed to be peculiar to the angler, but Professor Owen ('Anatomy of Vertebrates,' 1866) added Anableps and Pœcilia to his list of fish with movable teeth.

In the course of last year I found that the Hake, a voracious predatory fish, and in a less degree other Gadidæ, were possessed of hinged teeth; and shortly afterwards I found a similar condition in certain regions of the mouth of the common pike ('Proc. Royal Soc.,' No. 179, 1877). The structural peculiarities in the attachment of the teeth of the Gadidæ having been briefly described in the paper alluded to, now in course of publication in the 'Philos. Trans.,' I will confine myself in the present communication mainly to the teeth of the pike, merely noting some points of difference.

The hinged teeth with which I am acquainted have certain characters in common; they are all capable of being bent down by very slight pressure, but in a single direction only; to force applied in any other direction, they are rigidly immovable. This direction, with certain variations to be described, is inwards and backwards towards the gullet, so as to facilitate the ingress and the swallowing of food; on the removal of the pressure they rebound to their upright position. It appears to me very probable that adequate

examination will discover the existence of hinged teeth in many other predatory fish, as it has long been overlooked in fish so common as the hake and the pike; meanwhile, it is interesting to note the occurrence of an adaptive modification involving a considerable degree of specialisation occurring in fish so dissimilar in other respects as the angler, the hake, and the pike.

And what is still more significant is the fact that whilst the result attained is pretty much the same in the three fish selected for comparison, the details of the mechanism by which it is attained differ markedly, especially in the last two. In the angler and in the hake the teeth which are hinged form the inner and larger of two rows of teeth set upon the margins of the jaws; their mobility is therefore serviceable in the way of offering no obstacle to the ingress, but opposing the egress of prey. In the mouth of the pike, on the other hand, the marginal teeth are rigidly anchylosed, and the hinged teeth situated on the vomerine and palatine bones are useful, not in the catching, but in the swallowing of the prey.

In the pike the margin of the upper jaw is toothless (with the exception of a few almost rudimentary teeth in front); the lower jaw is furnished in front with small teeth, but at the sides with exceedingly long, sharp, piercing teeth, firmly anchylosed to the bone. Looking into the mouth three nearly parallel bands of teeth are seen upon the palate; in the central band (upon the vomer) the largest teeth are in front, while in the lateral (palatine) bands the largest teeth are those occupying the innermost position along the band, though in these also there is some diminution in size towards the back of the mouth. All the teeth which form these three bands are set upon hinges (with the possible exception of the very smallest), and are capable of being bent down in certain determinate directions until they assume a nearly horizontal position.

The teeth which lie upon the median line of the vomer bend directly backwards; those upon the sides of the vomer backwards and a little outwards (see diagram fig. 1, Plate I).

The teeth upon the two palatine bones bend backwards and inwards, along a line forming an angle of 45° with the median line of the mouth; it is to be noted also that the palatine teeth, especially at the back, descend to a lower level than the median (vomerine) teeth. A moment's consideration will show the *modus operandi* of these hinged teeth with their mobility restricted to a single direction. It

is the habit of the pike to prey upon other fish, often of relatively large size, and these can only be swallowed when they are conveyed to the gullet in a longitudinal direction, either head or tail foremost. The fish is taken into the mouth of the pike either uninjured or but slightly maimed by having been seized by the large marginal teeth; the mouth is then tightly closed, and the prey held up against the palate by the elevation of the tongue and floor of the mouth. In this position the movement of the prey is rendered all but impossible, save in one direction; so long as it lies longitudinally along the median line, between the two palatine bands, its passage backwards to the throat is unobstructed, the hinged teeth giving way before it; but movement in any other direction is checked by its becoming caught upon the sharp points of teeth rigidly fixed against it. Thus the very struggles of the prey are probably utilised in bringing it into, and arranging it along, the median line of the mouth so that it can be easily swallowed; during this process, which, unless the prey be small, lasts some minutes, showers of detached scales issue from beneath the gill-covers of the pike, thus giving evidence of the employment of the teeth within its mouth.

The facts to which I have so far called attention might have been more appropriately detailed elsewhere than in the pages of a microscopical journal, but it seemed desirable to preface an account of the structure of the teeth with a few words describing their manner of use.

The marginal anchylosed teeth of the pike are familiar microscopic objects, and I need only recall one or two points in their structure. They consist externally of a very thin, apparently structureless layer, probably enamel; inside this comes a tolerably well-marked layer, in which tubes like those of ordinary dentine run out towards the surface, and this is generally described as a layer of hard or unvascular dentine; inside this, solidly filling up the interior of the tooth, in which there is no axial pulp cavity, comes a coarse calcified tissue permeated by many longitudinal canals (as in fig. 2), the vaso-dentine of Owen, which for reasons detailed elsewhere ('Proc. Royal Soc.,' No. 179, and forthcoming 'Phil. Trans.') I prefer to call osteo-dentine.

It is necessary to briefly describe the development of this internal core, as without so doing one of the most striking peculiarities of the hinged teeth would be unintelligible. In the calcification of the dentine papilla the outer shell of hard dentine (*d*) is first formed; from its interior rods of calcifying tissue shoot down through the whole substance of

the pulp, and by their interlacing form the central bony core of the tooth, which, when completed, cannot be said to have any pulp cavity. The tooth is attached to the subjacent bone by a continuation of these calcifying rods down to the bone, with which they coalesce, the shell of hard dentine thinning down to nothing at the base of the tooth. The core of the tooth and the subjacent bone are structures so similar that it is absolutely impossible to find a line of demarcation between the two in a completed tooth.

The principal characters of the hinged teeth may be understood by an inspection of figs. 3 and 4. It will be seen that the teeth are not solid, like the anchylosed tooth represented in fig. 2, but that there is a sort of pulp-chamber (*b*), not wholly filled up by calcified tissue, and that the teeth are, as viewed by a low power (figs. 4 and 5), or in dried specimens, connected with the subjacent bone only by means of a hinge of fibrous tissue (*e*, fig. 4), the base of the tooth being received upon a little pedestal of bone (*f*). The central cavity is not, however, wholly empty, nor is it solely occupied by soft pulp tissue; from the interior of the shell there run down rods of calcified tissue (*h*), which are roughly parallel with the long axis of the tooth, and which become slender and more transparent as they go down.

In a favorable section the thin parallel strings into which they dwindle may be seen to pass right down to the bone, and as they approach the bone they become again thicker, and blend insensibly with it (see *g*, fig. 5). These filaments were at first a source of great bewilderment to me; running from the interior of its dentine cap straight down to the bone, they would, if inelastic, tie the tooth down (see fig. 5), so that unless they were broken the hinge would be a useless superfluity, and their absolute straightness, apparent rigidity, and high refractive index, made them look exceedingly brittle; they were often broken, but never curved nor bent in the sections. However, reiterated examinations have demonstrated that they are not calcified in their whole length; they are calcified where they start from the dentine cap, and apparently again where they blend with the bone, but the intermediate portion remains soft; and the resumption of the upright position by the tooth, after being bent down, is wholly due to the elasticity of these fibrils; if they are carefully divided, the hinge being left wholly uninjured, the tooth remains in any position in which it is left. They are thus capable of great extension, and are very perfectly elastic, a depressed tooth springing back to its upright position with an audible snap.

If we compare the fully developed hinged tooth with an anchylosing tooth of the same fish which has not as yet advanced to complete calcification, the true nature of this curious bundle of elastic strings is at once apparent.

It has already been mentioned that in the case of a tooth destined to be attached by anchylosis an outer cap of hard dentine is formed upon the surface of the pulp after the ordinary fashion of dentine formation, but that the tooth is then completed by a different process, rods of calcification shooting down through the pulp, and by their increase in size obliterating all central pulp chamber, while by their extension downwards to, and final blending with, the bone they fix the tooth in place.

Arrest the progress of calcification in these rods at a stage when they are not thick enough to collectively block up and obliterate the pulp cavity, and we have the elastic bands of the hinged tooth; in fact, the same structure which is made use of to rigidly fix the one is, by arrest of its development at a certain point, made to do duty as an elastic spring in the other.

During the period of their active development osteoblast-cells thickly and regularly coat each one of the rods; whilst, in places, these osteoblast-cells are elongated instead of being spherical (*o*, fig. 6). But in a completed tooth these osteoblast-cells have vanished; the rods, if examined tolerably high up within the dentine cap, are calcified; they are quite rigid, brittle, breaking with a sharp fracture, and no cell structures are to be distinguished. The space between the parallel rods is not, however, empty, but the sole remainder of the cellular vascular pulp is a filmy, cobwebby-looking tissue, equally impossible to describe and to draw. I have attempted to delineate this web in fig. 7, but no drawing can convey any idea of its exquisite delicacy and filmy transparency.

The nature of the attachment of one of the rods to the bone of the jaw is shown in fig. 6; its relation to the dentine cap is sufficiently indicated in figs. 4 and 5. In many places bundles of parallel fibres may be found springing from the same eminence of bone (*i*, fig. 8), and these in their further development would coalesce into a single thicker rod. Thus the development of the osteo-dentine in this situation would seem, so far as it goes, to confirm Von Ebner's view as to the fibrous basis of all bone; but I have not been successful in demonstrating this fibrous structure at a period subsequent to full calcification.

There is little to be said as to the structure or the develop-

ment of the hinge; in its complete condition it consists of many well-defined, wavy bands of fibrous tissue, continuous at one end with processes of bone, at the other with processes of osteo-dentine (see fig. 5). Like the far finer bands, which have already been described as conferring upon the tooth its resiliency, they appear to be continuations of the calcifying rods of osteo-dentine, in which calcification has stopped short and has left them soft; they distinctly belong to the dentine, and are derived from its formative tissues, and not from any portion of the dental sac, as was supposed to be the case with the hinges of the teeth of Lophius.

Attention has already been drawn to the fact that hinged teeth have been found in three fish so dissimilar as to preclude any idea of near genetic relationship, i.e. the angler, the hake, and the pike; but while the same mechanical result is arrived at the means are slightly different. In the case of the angler and the hake the elasticity resides solely in the tissue of the hinge, so that, everything else being severed, the tooth is as resilient as ever; in the pike the hinge is not in the least endowed with elasticity, but the bundles of fibres proceeding from the interior of the dentine cap are exceedingly elastic.

Again, the tooth of the hake is furnished with a living pulp, richly vascular, the vessels of which enter through the hinge so as not to be put upon the stretch by the movement of the tooth, while the hinged tooth of the pike is a comparatively pulpless structure.

Of this incompleteness of the communication I am very fully aware; but as it may be long before I am able to enter more thoroughly into the study of these particular structures it has seemed better to offer these observations, imperfect as they are, than to withhold all notice of an adaptive modification which appears to me peculiarly suggestive.

Note on the Movements *of the* Vibracula *in* Caberea Boryi, *and on the supposed* Common Nervous System *in the* Polyzoa. By the Rev. Thomas Hincks, B.A., F.R.S.

The theory of a common or colonial nervous system in the *Polyzoa*, first propounded by Fritz Müller, must be regarded as still *sub judice*. The question may be approached on two sides, the histological and the physiological. Müller's attention seems to have been first drawn to the subject by the behaviour of the polypides in certain cases which, appeared to point to the existence of a system of nerves apart from the individual cells, by which the members of the colony are to some extent controlled, and brought into relation. He refers specially to the energetic movements of the peduncle of *Pedicellina*, after the fall of the body, and to the simultaneous movements of the cells in *Mimosella gracilis*, a fact which I had previously observed and recorded. He was thus led to investigate the stems and branches of some of the *Ctenostomata*, and demonstrated the existence of the (supposed) ganglia and trunks of the colonial system. Smitt adopted his views and made similar observations on the *Cheilostomata*.

Their conclusions have been criticised by Reichert and Nitsche, and more recently by Joliet. These writers are agreed in regarding F. Müller's doctrine as erroneous. Viewing the subject anatomically and histologically they arrive at the same result, that the structure which Müller and others have described as a colonial nervous system, has in reality has a very different significance. Reichert regards the cords and network of threads that occur in the stems of the *Ctenostomata* as a medium of communication between the polypides of a colony, and as a channel by which stimuli applied to the cœnœcium may be transmitted and diffused, but denies that they have the character of a true nerve-tissue. Nitsche takes much the same ground with reference to the *Cheilostomata*. Joliet, as the result of histological investigation chiefly, gives a decided verdict against F. Müller's interpretation. He also states that he has cut in two the supposed nerve-trunk pervading a branch, and that the polypides expanded on the same branch did not retract themselves, a fact which seems to tell with as much force against Reichert's view as against Müller's. He identifies the supposed nerve-threads passing to the body of the polypide from the stem with the *funiculus*.

I do not propose to enter at all into this branch of the subject in the present note, but merely to direct attention to a physiological fact, which naturally leads us to infer the existence of some such colonial nervous system as Müller has described, and of which indeed some such system seems to offer the only explanation. I refer to the *simultaneous movements* of the *vibracula*, which have been noticed in one of the Cheilostomatous genera, and which probably occur in others. This remarkable fact has hitherto attracted very little attention. About four years ago I had the opportunity, in Guernsey, of studying the *Caberea Boryi*, Audouin, in the living state, and was much surprised to find that the highly-developed vibracular appendages with which it is furnished, instead of acting independently, as these organs do in other *Polyzoa, moved together with perfect regularity*. I was under the impression at the time that the observation was as new as it was undoubtedly interesting; but I have since ascertained that the fact had not escaped Mr. Darwin's notice, but is mentioned briefly and quite incidentally in his work on the 'Origin of Species.' In that great storehouse of fact and observation it seems to have lain *perdu*, and I have never met with a reference to it in any writer on the *Polyzoa*.

Mr. Darwin has not given any detailed description of the species on which his observation was made; but it is evident from his brief notice of it that it was a *Caberea*, and it may very probably have been the *C. Boryi*, which is a cosmopolitan form.

In the genus *Caberea* the *Vibracula* are enormously developed, and give a very distinctive appearance to the zoarium. In *C. Boryi* they are long and slender and serrated along one side, while the grooves into which they fall when at rest stretch completely across the posterior surface of the cells. After a short interval of quietude all the *vibracula* on a shoot are seen, as if moved by one and the same impulse, to start into sudden activity, swinging themselves round simultaneously to the front of the cells, and then sweeping backwards again and resuming their former position. After another interval the same synchronous and perfectly regular movement takes place, and so on continually. The action is as orderly as that of a machine.[1] There is something positively startling, after the perfect quiet, in the sudden, simultaneous rush of the whole host of *vibracula* into energetic activity.

In this genus then the setiform appendages act not indi-

[1] This remark is intended to apply to the movements only and not to the intervals between them, which vary in length.

vidually but in companies, obedient to a common impulse; and in such a case it seems impossible to doubt that there must be some special nervous arrangement, apart from the zooœcia, by which the vibracular zooids are brought into relation and their synchronous movements determined.

I have said that similar phenomena may probably have a place in the history of other genera. Amongst the family *Selenariadæ*, Busk, the *vibracula*, we know, are developed to an extraordinary extent, and attain a larger size than in the genus *Caberea*.

Their remarkable character, coupled with the fact that the members of this group are free in their adult state, led Mr. Busk to conjecture that these appendages might have a locomotive function. And he informs me that this conjecture has been verified by actual observation, and that some of the *Selenariadæ* at least do actually move about by means of their *vibracula*. If this be so, it is in the highest degree probable that those belonging to each colony act in concert, and that their movements are, as in *Caberea*, simultaneous.

However this may be, the case of *Caberea Boryi* seems to be conclusive, as far as it goes. It may be considered to prove that a nervous system, distinct from that of the individual polypides, by which certain zooids in the colony are brought into relation and common action, exists in one instance, at least, amongst the *Polyzoa*.[1] And this not unnaturally leads to the inference that a similar system, though perhaps in a less highly specialised form, may probably occur more widely in the class.

It is not my purpose however to theorise, but merely to direct attention to a very remarkable fact which has been strangely overlooked, and its relation to the interesting question raised by Fritz Müller's investigations.[2]

[1] It will be noticed that this goes much beyond the kind of communication between the various elements of the colony, which is supposed by Reichert to exist, and for which his "communale Bewegungsorgan" provides.

[2] Since the foregoing was written I have seen Joliet's later researches on the (supposed) Colonial nervous-system. ['Comptes Rendus,' Aug. 13th, 1877.] He finds it present in all the Polyzoa he has examined, and very largely developed. It is composed of fusiform cells. At the expense of this tissue, the polypide with its muscles is developed; and in its bosom the ova and the mother-cells of the spermatozoids are formed. He regards it as a distinct, constituent tissue of the Polyzoon, which he proposes to call the *Endosarc*. Its special function is the production of the polypides or the reproductive elements. It is itself derived from the Endocyst. If these conclusions are confirmed, they will form a very important addition to our knowledge of the structural and physiological history of the Polyzoa. But they do not affect the significance of the fact to which I have directed attention in my paper.

The Development *of the* Cranial Nerves *in the* Chick.

By A. Milnes Marshall, D.Sc., B.A., Fellow of St. John's College, Cambridge. (With Plates II and III.)

I have elsewhere shown[1] that the mode of development of the nerves, both cranial and spinal, of the chick is in all essential points the same as that first described by Balfour[2] in the case of the spinal nerves of Elasmobranchs, and subsequently extended by him so as to include the cranial nerves also.[3]

At the time of writing my previous account embryos of forty-three hours were the earliest of which I had prepared satisfactory specimens illustrating the development of the nerves, and I had "not determined the exact date of the earliest appearance of the nerves."[4] Since that time I have continued my investigations, and have succeeded in preparing specimens illustrating the development of both cranial and spinal nerves from their very earliest appearance.

In the present paper I propose, firstly, to describe these earliest stages fully, so as to complete my previous account; and, secondly, to give some further details concerning the mode of development of several of the cranial nerves. I shall also take the opportunity of correcting some statements in my former paper that now appear to be wholly or in part erroneous.

The majority of my specimens have been prepared by immersion for three to five hours in Kleinenberg's preparation of picric acid, and then transferring to alcohol of about 30 per cent., which was gradually increased in strength till absolute; such specimens were subsequently stained with Kleinenberg's solution of hæmatoxylin. I have also employed, with very good results, weak solutions of chromic acid—$\frac{1}{4}$ to $\frac{1}{2}$ per cent. —to which a few drops of a 1 p. c. solution of osmic acid were added. In this solution the embryos were left for about twenty-four hours, and then transferred to alcohol. They have the advantage over picric acid specimens of not requiring staining; but are liable to become brittle and difficult to cut.

The exceedingly close correspondence between specimens prepared in these two ways is highly satisfactory as testimony of their trustworthiness; the correspondence being so close that

[1] 'Proceedings of the Royal Society,' No. 179, 1877; and 'Journal of Anatomy and Physiology', vol. xi, part iii, 1877. It is to the latter paper, which contains a much fuller description, with figures, that I shall refer in future.

[2] 'Phil. Trans.,' vol. 166, part i.

[3] 'Journal of Anatomy and Physiology,' vol. xi, part iii. 1877.

[4] Loc. cit., p. 504.

I have had to abandon as impracticable any attempt to distinguish between them in my drawings.

A great part of my work was done during the past summer at the zoological laboratory of the University of Cambridge; the remainder at St. Bartholomew's Hospital, London.

I am indebted to Mr. Balfour for kind and valuable assistance of many kinds received during the course of my investigations.

The earliest stage in the development of the nerves is shown by an embryo of nominally twenty-seven hours, but really corresponding to a typical twenty-two hours' chick.[1] In this specimen the medullary folds have arched over towards one another, and nearly met in the region of the head and neck, but have nowhere coalesced completely; while none of the protovertebræ have yet been definitely established.

Plate II, fig. 1, represents a transverse section through the middle cerebral vesicle of this specimen. The medullary folds have just met one another, but have not coalesced. The external epiblast (*ep*) is thin, and consists of but a single layer of cells; the wall of the cerebral vesicle itself (*m*, *b*) consists of somewhat elongated cells, about three deep, closely apposed to one another, and placed with their long axes perpendicular to the surface of the vesicle. Towards the summit of the vesicle these cells become more spherical and rather less compactly arranged; and at the angle where the external epiblast turns in to form the neural canal there is a small, but evident, outgrowth of these spherical cells, forming on either side a projection that in transverse section is somewhat conical in shape (*m*).

This outgrowth (*m*) we shall find from its subsequent history constitutes the earliest stage in the development of the nerves in the chick. On examining the sections in front of and behind that figured, the outgrowth is found to form on either side a longitudinal ridge, which is most prominent in the section figured, and gets rapidly smaller in both directions, disappearing completely at the constriction separating the middle from the anterior cerebral vesicle, and extending very little further in a posterior direction. This ridge I propose to speak of in future as the *neural ridge*.

A word of explanation is here necessary. Foster and Balfour state[1] that "for a brief period," after closure of the medullary folds, "the calibre of this tube (neural canal) is uniform throughout." This is not the case. Before the neural canal is closed at any point in its length the anterior cerebral vesicle is a con-

[1] Foster and Balfour, 'Elements of Embryology,' part i, p. 53.
[2] Op. cit., p. 58.

spicuous dilatation, separated by a slight, but evident constriction from the middle vesicle, which is separated by a less marked constriction from the posterior vesicle; and before closure of the anterior vesicle is effected the optic vesicles are already prominent objects. Coalescence of the medullary folds occurs at these constrictions before it is effected in the dilated vesicles themselves. The point is one of very minor importance, but it was necessary to give this explanation in order to justify the language used in the preceding description.

Thus, the earliest indication of nerves in the chick occurs about the twenty-second hour in the form of a longitudinal ridge of spherical cells on either side at the angle of inflection of the epiblast to form the neural canal. This ridge is but slightly marked, and extends along the mid brain, and a short distance down the hind brain, being most prominent opposite the widest part of the mid brain. The points of chief interest appear to be—

1. The neural ridge appears before closure of the neural canal is effected, so that the ridges of the two sides are primitively independent of each other.

3. The ridge is not developed directly from the external epiblast or from the neural canal, but from the re-entering angle between the two.

3. The ridge appears first in the mid brain.

The next stage I propose to consider is illustrated by Plate II, figs. 2, 3, 4, and 5, representing transverse sections through various parts of the brain of a twenty-four hours' chick embryo.

Fig. 2 passes through the anterior part of the fore brain (*f b*). The section appears at first sight somewhat anomalous, inasmuch as it consists of two halves completely separated from one another. This may be due simply to projection forwards of the anterior lips of the medullary folds, but, I believe, is really to be attributed to cranial flexure, which, instead of commencing "towards the end of the second day,"[1] really begins before the neural canal is closed in. Cranial flexure is practically due to hypertrophy of the dorsal wall of the neural canal, which, during the first few days, grows much faster than the ventral wall, so causing the well-known flexure. If this relatively rapid growth commences before closure of the canal is effected—and we have already seen that there is hypertrophy of the anterior end of the neural canal from its very earliest appearance—fig. 2 becomes at once intelligible; and it is seen that the lower part of the figure, as well as the upper, consists of the as yet unclosed lips of the neural canal.

[1] Foster and Balfour, op. cit., p. 78.

Two other points in this figure require notice—

1. That the whole section consists of epiblast.

2. That there is no trace of the neural ridge.

Fig. 3 passes through the posterior part of the optic vesicles (*o v*), which are very prominent, though the medullary folds have not yet coalesced. The section, which is a somewhat imperfect one, owing to the brittleness of the embryo from which it was cut, passes through the fore gut, of which the roof only (*hy*) is seen in the figure. The neural ridge forms a very prominent outgrowth (*m*) on either side of the summit of the canal.

Fig. 4 passes through the widest part of the mid brain, *i. e.* the same spot as fig. 1, but at a slightly later date. Complete coalescence of the medullary folds has occurred, and the external epiblast (*ep*) now forma a continuous layer across the top of the canal. The neural ridges (*m*) are much more prominent than in fig. 1, and are fused and continuous with one another.

A section taken through the hind brain is given in fig. 5, in which the canal is not yet closed, while the neural ridge (*m*) forms a conspicuous object on either side. It will be seen that the notochord (*n*) is represented as in continuity with the hypoblast of the fore gut (*f g*). I hope to refer to this point in detail on some subsequent occasion, and mention it here merely to point out that it is so represented purposely, and not through carelessness. In the section figured there is perfect continuity between the two structures.

On comparison with the preceding stage we find the neural ridge considerably increased, both in longitudinal extent and in actual size at any given point. It now extends from about the middle of the optic vesicles to the posterior part of the hind brain; its greatest lateral extent is at the point at which it first appeared, *i. e.* the centre of the mid brain. We notice also that the ridge tends to become specially prominent at certain points, and we shall subsequently find that these prominences are the first definite commencements of individual nerves. Thus, at the slight constriction separating the mid and hind brains the ridge is not so large as it is either opposite the middle of the mid brain (fig. 4), or a short way further back in the hind brain (fig. 5).

Fig. 4 shows, further, that when closure of the neural canal is effected the neural ridge remains in connection with the neural canal, but separates completely from the external epiblast, so that all trace of the original connection is permanently effaced.

The extension forward of the neural ridge to the fore brain, as shown in fig. 3, is one of the most remarkable and interesting

points that I have succeeded in bringing to light, and one to which I shall have occasion to refer subsequently.

Figs. 6 to 10, representing transverse sections through the head of a twenty-nine hours' chick, illustrate my next stage.

The section from which fig. 6 is drawn passes through the anterior part of the optic vesicles. It has a superficial resemblance to fig. 3, but is taken much further forward than this latter, and should rather be compared with fig. 2. The section, like that drawn in fig. 2, consists entirely of epiblast; there is no mesoblast or hypoblast in it at all. On the dorsal surface the medullary folds have met, but not coalesced; on the ventral surface coalescence has occurred; but there are still indications, in the shape of two slight prominences of the external epiblast, of the stage preceding coalescence, represented by fig. 2.

The neural ridge forms a conspicuous outgrowth (*m*) on either side of the summit; its characters and relations are precisely the same as those it possessed further back in the brain at an earlier period.

Fig. 7 passes through the constriction (*fm b*) separating the fore brain from the mid brain; it passes also through the fore gut (*f g*), the notochord (*u*), and through a small cellular rod underlying the notochord, and lying between this latter and the hypoblastic roof of the fore gut; this appears to be the same structure as that described by Balfour and Götte as the "sub-notochordal rod." The medullary folds have completely coalesced, and the neural ridge (*m.*) has attained a great size, forming a very prominent feature in the section.

Fig. 8 is taken through the widest part of the mid brain (*m b*). The neural ridge (*m*) is very prominent, and slightly larger than in the preceding figure. Though the neural ridge is in contact peripherally with the mesoblast, the outlines of the ridge are clear and well defined, and there is at this stage very little difficulty in distinguishing between the small spherical, rather closely compacted cells composing the neural ridge, and the larger, irregular, branching, and loosely aggregated mesoblast cells.

Fig. 9 passes through the middle of the hind brain and through the hinder part of the fore gut (*fg*). The medullary folds have not yet coalesced, but the neural ridge (*m*) is very conspicuous on either side.

Fig. 10 is taken from the same embryo, a short way posteriorly to fig. 9, and still in the hind brain. The external epiblast is seen to be thickened laterally, forming the commencement of the auditory epithelium (*aud*). The section passes through the anterior part of the mid gut (*m g*); the neural canal is as yet unclosed, and the neural ridge (*m*) is very small.

We find, then, that at twenty-nine hours the neural canal is closed along the posterior part of the fore brain, along the whole length of the mid brain, and along the most anterior part of the hind brain. The neural ridge does not extend quite to the anterior extremity of the brain. The first few sections show no trace of it, and closely resemble fig. 2; it commences, however, in front of the optic vesicles, and extends backwards nearly to the end of the hind brain. It is exceedingly prominent along the whole length of the mid brain, and is nearly as large at the constriction separating the mid from the fore brain, as it is opposite the widest part of the mid brain. The last point to be noticed is that the mode of development of the ridge is precisely the same as it was at its earliest appearance. This is obvious on comparing fig. 10 taken through the hind brain with fig. 1, which passes through the mid brain of a chick about seven hours younger.

The next stage I propose to consider is furnished by a forty-three hours' chick, and constitutes the earliest described in my former paper. As I have already given a detailed description, with figures, of this stage,[1] I shall only notice it very briefly here.

The neural canal is closed along the whole length of the brain, and along a portion of the spinal cord corresponding to the anterior three or four protovertebræ.

The neural ridge is still recognisable on the summit of the optic vesicles, but is not so conspicuous as before. Along the mid brain it is very much reduced in size, and appears in transverse sections as a very thin rod of cells, still connected with the summit of the canal. It is very slender, and in some specimens I have found it impossible to distinguish it satisfactorily from the surrounding mesoblast cells, which are smaller and more closely compacted than in the preceding stage. At the constrictions separating the mid brain from the fore and hind brains the ridge has apparently completely disappeared. In the anterior part of the hind-brain it is present, but is much more slender than before. Further back it again becomes very conspicuous, but is no longer uniform, presenting a strong development just in front of the auditory pit, and another just behind it, while in the intervening part it is much smaller. It also extends a short distance down the spinal cord.

To *recapitulate*. About the twenty-second hour a small outgrowth of cells appears along the mid brain on each side, at the angle between the external epiblast and the neural canal—the *neural ridge*. This rapidly extends both forwards and backwards: forwards, as far as the anterior part of the optic

[1] Loc. cit., pp. 491—497, and Plate XX, figs. 1—6.

vesicles; backwards, along the whole length of the brain and a certain distance down the spinal cord. Its first appearance precedes the closure of the neural canal, but after about the fortieth hour the closure of the canal proceeds backwards more rapidly than the growth of the neural ridge, so that in the greater part of the length of the spinal cord the ridge is developed as an outgrowth from the summit of the cord itself, and never has any connection with the external epiblast. The ridge early attains a great prominence in the region of the mid brain, but shortly afterwards undergoes a great diminution in size, and becomes almost indistinguishable.

The early appearance of this ridge, its perfect continuity along the whole length of the brain and part of the spinal cord, its rapid growth and almost as rapid shrinking in certain parts, are very remarkable features from a morphological point of view. Some of these I shall have occasion to discuss further on. One point I may notice here. The perfect continuity of the ridge along the whole length of the brain appears to be a very powerful argument against the existence of any ancestral perforation of the central nervous system by the œsophagus either in the neighbourhood of the fourth ventricle, as suggested by Dr. Dohrn, or at any other point in its length. The morphological significance of the neural ridge is by no means evident. Balfour describes it as existing in the body and hind brain of Elasmobranchs,[1] and remarks concerning it that "there can be little doubt that it is some sort of remnant of an ancestral stucture in the nervous system, and would appear to indicate that the central nervous system must originally have been formed of a median and two lateral strands."[2]

I have now brought the history of the neural ridge of the chick up to the point at which my previous account commenced. I have already[3] shown how from this ridge the rudiments of certain of the cranial nerves, and in the body the posterior roots of the spinal nerves, are developed; and have pointed out how exceedingly closely these processes correspond with those occurring in Elasmobranchs, as described by Balfour.

Balfour describes the cranial nerves of Elasmobranchs as being developed successively from before backwards, the first to appear being the fifth nerve. He does not mention any extension of the neural ridge forwards in front of the auditory capsule, an extension that may, however, very possibly occur at an earlier stage than he has noticed as yet. His descriptions do not show whether the ridge makes its first appearance in Elasmobranchs before or

[1] 'Journal of Anatomy and Physiology,' vol. xi, part iii, p. 458.
[2] Loc. cit., p. 426.
[3] Loc. cit.

after closure of the neural canal; but some of his figures strongly suggest that, like the similar structure in the chick, the neural ridge does appear before closure of the neural canal.[1]

I propose now to take the several cranial nerves in order, and describe their development as far as I have been able to elucidate it.

The *olfactory nerve*.—I have already shown[2] that at the seventy-fifth hour the olfactory nerves bear a very close resemblance to the other cranial nerves; they are solid, with no indications whatever of an "olfactory vesicle;" they arise from the fore brain itself, and not from the commencing cerebral hemispheres; their histological structure differs in no appreciable respect from that of the hinder cranial nerves, and their general relations are such as to "strongly suggest that they are strictly comparable" with the other cranial nerves, and that "the olfactory nerves are really the first pair of true cranial nerves." I have since studied the early stages of the olfactory nerves very carefully, and my observations, though I can hardly consider them as conclusive, yet tend to strongly confirm my previous suggestion.

At the twenty-ninth hour the neural ridge extends forwards to the anterior part of the fore brain, in front of the optic vesicles (vide fig. 6, *m*). There is not the slightest doubt whatever that the ridge at this part of the brain is in all respects the same structure, having the same morphological significance as that which, further back in the brain, we shall find gives origin to the rudiments of the fifth, seventh, and other cranial nerves (*vide* figs. 9, 10, *m*), and in the body to the posterior roots of the spinal nerves. This neural ridge is a perfectly definite structure, and must have the same morphological significance at whatever part of the body it occurs. Its subsequent history in the hinder part of the brain and in the body is made known to us by Balfour's researches on Elasmobranchs, which are confirmed in many points by my former investigations on the chick. The ridge becomes more prominent at certain points and shrinks up, or (?) disappears completely, at the intervening points. These prominences are the nerve rudiments. As to the intervening parts between successive nerve rudiments we know this much definitely, that wherever their subsequent development has been traced they are found to develope into nerves of a commissural nature. Balfour's and my own researches agree

[1] Vide 'Phil. Trans.,' vol. 166, part i, plate xvi, figs. B1, B2, B3, and D*a*. In the last figure certain cells marked y are referred to in the description of the figure as "cells left behind on the separation of the external skin from the spinal cord."

[2] Loc. cit., pp. 511—513, and plate xxi, figs. 13—15.

completely in showing that *the sole structures to which the neural ridge gives origin are nerves.*

I see, therefore, no escape from the conclusion that *the presence of the neural ridge at any part of the brain implies actually or potentially the presence of a nerve,* or of a commissure connecting two nerves[1] Therefore there must be in the chick some nerve or nerves arising far forwards from the summit of the anterior part of the fore brain, and strictly equivalent, if embryological evidence is at all trustworthy, to the fifth, seventh, or hinder cranial nerves, or to the posterior root of a spinal nerve; otherwise the presence of the neural ridge in fig. 6 is utterly unintelligible.[2]

We have now to consider what nerve or nerves these can be. There are only two which can have the slightest claim to be considered in connection with an outgrowth from the anterior part of the fore brain—the optic and the olfactory. Of these we may dismiss the optic nerve for the present, merely noticing that even should the outgrowths from the optic vesicle ever be proved to have any connection with the optic nerve it would not be sufficient to account for the whole of this part of the neural ridge, for the ridge extends forwards in front of the optic vesicle, and almost to the extreme anterior end of the fore brain; so that there would still remain a certain part to account for. This can only be explained by supposing it to be the rudiment of the sole remaining nerve—the olfactory. The last remaining possibility, that the extreme anterior part of the neural ridge represents one of the "intervening parts" destined to shrink up or (?) disappear is, I think, completely disposed of by the consideration that in all cases where we are able to trace their ultimate fate these intervening parts become commissures connecting together successive nerves, or rather pairs of nerves. Now, that the extreme anterior part of the neural ridge should be a "commissure" is simply incomprehensible.

We thus see that there is *à priori* evidence of, to my mind, an exceedingly strong nature, that the olfactory nerve is developed from the extreme anterior end of the neural ridge, and that it is strictly comparable to the hinder nerves. Let us now turn to its actual development in the chick.

At fifty hours there is a small outgrowth of spherical or slightly fusiform cells, arising on either side from near the top of the fore brain, just at the slight constriction separating it from the optic vesicle of either side. This forms a small process which may

[1] This reservation will be fully explained in the sequel.

[2] The assumption that the chick has preserved merely the earliest stages of development of some ancestral nerve, of which all subsequent traces have been lost, is so unnecessary and unwarranted that I have not thought it worthy of serious consideration.

be traced for a short distance in successive sections running downwards and slightly outwards, lying close to, but perfectly independent of, the external epiblast. At this period there is a hardly perceptible thickening of the external epiblast at the spot where the olfactory pit will shortly afterwards appear; it is towards this point that the outgrowth is directed. These outgrowths have exactly the same histological appearance as the other cranial nerves at a corresponding stage of development. Their position, relations and subsequent history leave no doubt in my mind that they are the olfactory nerves in an early stage of development. We shall find that they resemble the auditory nerves in attaining a certain size before the corresponding involution of the external epiblast has commenced.

The next point, What is the relation of these nerves—for so we may now call them—to the anterior part of the neural ridge as shown in fig. 6, is not so easy to determine. We shall find that all the other nerves soon lose their attachment to the extreme summit of the neural canal, and travel down the sides a certain distance; so that the fact that the attachment of these nerves is not quite to the summit, speaks in favour of, not against, their being derived from the neural ridge. I have observed a mass of spherical cells occupying nearly the same position as that just described, but not extending so far down, in embryos of thirty-six, forty, and forty-six hours; and I believe that these represent intermediate stages.

I wish, however, to state distinctly that though I myself have but little doubt that the "olfactory nerves" of a fifty-hours' chick are really derived from the anterior extremity of the neural ridge, yet I am fully aware that I have not proved my case. To do so it would be necessary to trace their development hour by hour, inasmuch as the relations of the parts change so quickly and to so great an extent about this time, owing to the rapid growth of the fore brain, and the rapidly occurring cranial flexure, that it is exceedingly difficult, and in some cases impossible to make perfectly satisfactory comparisons of sections taken through the same parts at so short an interval as four or five hours.

I have not figured the stage just described; but a very good and fairly exact idea of the appearance can be gained from figs. 19 and 20 by supposing the olfactory nerves (I, in fig. 19) to be rather nearer one another, and the dilatations of the forebrain (*c f b*) in fig. 20 to be not the cerebral hemispheres, but the optic vesicles; the former not having yet appeared.

The later stages are easier to work. The fore brain grows forwards in front of the optic vesicles, carrying with it the olfactory nerves; it then bulges out laterally to form the cerebral hemispheres. I have already figured the condition of the parts at

seventy-five hours,[1] at which period the olfactory pits are deep and conspicious depression, and the olfactory nerves, which are in continuity with the walls of the pits, arise from the front of the fore brain just at its juncture with the commencing cerebral hemispheres. By the subsequent growth of the brain the olfactory nerves are separated somewhat from one another, while the growth of the hemispheres upwards and forwards soon causes the olfactory nerves to spring from their ventral surface.

Figs. 17—20 illustrate the condition of the olfactory nerves and surrounding parts in a ninety-three-hours' chick. The plane of the sections is readily understood by referring to the figure of a four-days' chick given by Foster and Balfour.[2] If a line be drawn through the centre of the optic lens, parallel to the sides of the page, it will indicate almost exactly the line along which the section in fig. 17 is taken. Such a section passes through the olfactory pits (*olf*); the fore brain (*f b*); the centre of the optic cup (*o c*); and lens (*l*); the infundibulum (*inf*); the forward diverticulum from the mouth involution to form the pituitary body (*pit*); the ophthalmic branch of the fifth (V); the notochord (*n*); and the anterior part of the hind brain (*h b*).

Fig. 18 is taken a little to the right of the line indicated, and passes through the lens near its upper margin; it passes above the olfactory pits, but through the olfactory nerves (I). The fore brain bulges slightly laterally, forming the commencement of the cerebral hemispheres, with which the olfactory nerves are in close relation. The section also passes through the constriction (*mh b*), separating the hind from the mid brain.

Fig. 19 represents part of one of the same series of sections, in a plane slightly dorsad of fig. 18; it passes through the points of origin of the olfactory nerves (I) from the fore brain, or, more strictly, from the ventral surface of the cerebral hemispheres (*c h*).

From this period the olfactory nerves gradually acquire the length and relations characteristic of the adult. I have followed the changes as far as the eighth day, at which period the nerves are still short, and have also carefully examined the nerves in the adult.

I have previously shown that "there is no trace whatever of an olfactory vesicle in the early stages:"[3] this statement can now be extended: *there is no trace of an olfactory vesicle at any period in the life of a chick.*

Thus the direct embryological evidence, though, as I have already pointed out, not absolutely conclusive, yet speaks very

[1] Loc. cit., plate xxi, figs. 14, 15.
[2] Op. cit., fig. 46, p. 142.
[3] Loc. cit., p. 512.

strongly in favour of the view which we have seen to be supported by such very strong *à priori* evidence, viz. that the olfactory nerves are to be viewed as the most anterior pair of true cranial nerves. It is most important to notice that at whatever stage I have definitely recognised the olfactory nerve, its appearance and relations are precisely such as they must inevitably be *supposing* the nerve really developes in the manner suggested by the *à priori* evidence; *i. e.* in the same manner as the other cranial nerves.

I have dwelt at length on this subject because my results are directly opposed to the generally, I might almost say, universally received opinion expressed by the leading authorities in our text-books of anatomy and embryology. The olfactory nerve is stated and assumed to be of a totally different nature to the other cranial nerves; a nature so different that its existence is usually ignored in morphological discussions based on the arrangement and distribution of the cranial nerves.

The olfactory nerve is generally stated to differ from all the other nerves, except the optic, in two main points:

1. *It is an outgrowth of the brain,* and not a mesoblastic structure. This objection need not be considered further; it having been sufficiently shown[1] that *all the nerves, whether cranial or spinal, are outgrowths of the neural canal.*

2. It is a *hollow* outgrowth: in other words, an olfactory vesicle is present. That this is not a universal feature is shown by its absence in the chick; and as it has been wrongly assumed to exist in the chick,[2] so it may prove to have been wrongly assumed to exist in other vertebrates.

I will not allude to the literature on the subject further than to notice the account given by Balfour[3] of the development of the olfactory organ and nerve in Elasmobranchs. The earliest stage he describes corresponds, so far as one can judge, with a five or six-day chick. At this period there is a pair of "lateral outgrowths," or "olfactory lobes" from the cerebral hemispheres: "from the peripheral end of each olfactory lobe a nerve similar in its histological constitution to any other cranial nerve makes its appearance;" "on the root of this nerve there is a large development of ganglion cells. I have not definitely observed its origin, but have no reason to doubt that it is a direct outgrowth from the olfactory lobe, exactly similer *in its mode of development* to any other nerve of the body." A little later on, "the olfactory lobes have become much more pronounced. The

[1] Vide Balfour, loc. cit., and self, loc. cit.

[2] Foster and Balfour, 'Elements of Embryology,' part i, p. 117.

[3] 'Journal of Anatomy and Physiology,' vol. xi, part iii, pp. 444, 450 and 481; and Plate XVIII, figs. 2 and 8 *a*.

root of the olfactory nerve is now very thick, and the ganglion cells it contains are directly prolonged into the ganglionic portions of the olfactory nerve." Balfour also states that "no rudiment of an olfactory nerve" appears till after the olfactory pit has been developed for some time, and its lining membrane raised into the Schneiderian folds. Finally, in discussing the bearing of the cranial nerves on the question of the segmentation of the head, he says: "Although it has been shown above that the olfactory nerve develops like the other nerves as an outgrowth from the brain, yet its *very late appearance* and *peculiar relations* are, at least for the present, to my mind sufficient grounds for excluding it from the category of segmental cranial nerves."[1]

But little comparison is possible between our two accounts, since Balfour's only begins at about the point at which mine leaves off. I would notice, however, that if "the olfactory nerve of Elasmobranchs is a direct outgrowth from the olfactory lobe," then, instead of being "exactly similar in its mode of development to any other nerve in the body," it differs fundamentally from all the other nerves in the body with which we can compare it[2] in the following important points:

1. It does not arise from the mid dorsal line of the neural canal.

2. The two nerves are not at first directly continuous with one another across the top of the neural canal.

3. There is no shifting downwards of the point of attachment of the nerve to the neural canal.

4. The date of first appearance is *very much later* than that of the other nerves.

A good deal of the apparent contradiction between our accounts may very possibly be removed when we know the limits of extension of the neural ridge in Elasmobranchs, and the *earliest stages* of development of their olfactory nerves. On one point there appears to be direct opposition—the presence of an olfactory lobe, *i.e.* a hollow diverticulum of the cerebral hemisphere from which the olfactory nerve arises, and even here we must wait for further evidence, for in a fifty-hours' chick there is a well-marked olfactory nerve *before the cerebral hemispheres have begun to appear, and therefore long before an olfactory lobe could possibly be present.*

As to the "very late appearance" of the olfactory nerve, if my description is correct, the first rudiment of the olfactory nerve appears in the chick before there is any trace of any of

[1] Loc. cit., p. 481. The italics are mine.

[2] This reservation is to exclude the optic nerve, the anterior roots of the spinal nerves, and any cranial nerve that may prove to resemble these latter in their mode of development.

the spinal nerves, or of the vagus or glosso-pharyngeal; the olfactory nerve, in fact, being *one of the first nerves in the body to appear.*

In conclusion, I venture to hope that my account of the development of the olfactory nerve may throw some light on that favourite problem of morphologists—the composition of the vertebrate head; and this at what has hitherto proved its most impregnable point—the extreme anterior part of the head.

If the olfactory nerve really prove to be, as suggested above, the most anterior true cranial nerve, it must be intimately connected with the most anterior cranial segment. Each of the other cranial nerves, as is well known, forks over a visceral cleft. Have we any trace of a cleft in connection with the olfactory nerve? In connection with this point, which I hope to discuss fully in a future paper, I will here only mention that while working at Dr. Dohrn's zoological station at Naples in the spring of 1875 I devoted considerable attention, at Dr. Dohrn's suggestion, to the olfactory organ of fish; one of the results of my investigations being a firm conviction, based solely on anatomical and histological grounds, that there was some very close relation between the olfactory organ and the gills of fish. At that time, however, the ordinary descriptions of the development of the olfactory nerve appeared almost conclusive against such a view.

The optic nerve.—About this I have very little to say. The existence of the neural ridge along the whole length of the optic lobes at an early period is a point of considerable interest. I have not succeeded in tracing the ultimate fate of this ridge, and indeed have not recognised it in specimens later than thirty-six hours; I have failed also to prove that any part of this ridge is any way concerned in the development of the eye.

It would appear, therefore, that the optic nerve is in no way comparable to the other cranial nerves, from all of which it would be sharply distinguished as well by its origin as a hollow diverticulum of the brain, as by its perfect independence of the neural ridge. In view, however, of the extremely complicated character of the vertebrate eye it is quite conceivable that the optic nerve, or some part of it, may have been primitively of the same nature as the other cranial nerves, and that all vestiges of this similarity have been gradually effaced during the evolution and perfection of the organ.

The third nerve.—In the adult the third nerve arises from the ventral surface of the mid brain close to the median line.

We have already seen that the neural ridge makes its first appearance in the widest part of the mid brain (fig. 1, *m*). By the twenty-fourth hour it has grown very considerably at this point (*vide* fig. 4, *m*); and at the twenty-ninth hour it forms a very prominent outgrowth (fig. 8, *m*), the neural ridge at this period, as in all the earlier ones, being considerably larger in the mid brain than at any other part of the neural canal.

Hence we see that, just as in the case of the olfactory nerve, there is a very strong *à priori* probability in favour of this outgrowth developing into one of the cranial nerves; and since the third nerve is the only nerve that arises from the mid brain in the adult, the probability is very little less that it is the third nerve into which this outgrowth developes.

Fig. 22 represents a longitudinal section through the head of a ninety-six-hours' chick, passing a little to the left of the median longitudinal plane. A large nerve (III) is seen arising from the base of the mid brain (*m b*), and running downwards and backwards to a point a little posterior to the optic nerve (II) where it terminates in a ganglionic swelling. The nerve is also ganglionic at its base, which is widened, and somewhat conical in shape.

This nerve, I may state at once, is conclusively shown by its later history to be the third nerve. Its point of origin, its directions, relations, and distribution, at a slightly later period, to certain of the eye muscles prove this absolutely.

In figs. 17—20 we have somewhat different views of this nerve at about the same period—ninety-three hours. The plan in which these sections are taken has been already explained. In fig. 17 the third nerves (III) are seen to lie near the eyes, and well to the inner side of the ophthalmic branch of the fifth nerve (V); to this latter point I shall refer again later on. Fig. 20 passes through the points of origin of the nerves (III). They are seen to arise from the ventral surface of the mid brain (*m b*), near to the median ventral line, so that the nerves of the two sides are in very close proximity to one another, a very characteristic feature of the adult nerves.

Longitudinal sections at sixty-seven hours show the nerve (III) in very much the same condition as in fig. 12, but not quite so long. At sixty hours the nerves are more slender, and their roots do not arise so near the median ventral line, so that the nerves of the two sides are rather wider apart.

In younger specimens the nerves are very difficult to recognise satisfactorily. This is due in part to the rapid growth and shifting relations of the cerebral vesicles, and partly to the fact that the nerve consists in these early stages of cells that are very difficult to distinguish from the mesoblast cells, which latter are

much more compactly arranged than they were in the earliest stages. At forty-three hours there is a slender outgrowth from the extreme top of the widest part of the mid brain on either side; this passes downwards, lying in close contact with the walls of the mid brain, for a short distance; its outlines are very difficult to distinguish from the mesoblast cells. There is no doubt, however, that this is identical with the outgrowth observed at the twenty-ninth hour (fig. 8, *m*). At fifty-four hours there is a rather larger mass of cells, which appears to be connected with the mid brain about half way down its sides.

On considering all these facts—the *à priori* probability that the outgrowth from the mid brain of a twenty-nine-hours' chick should develope into the third nerve; the condition of what is unquestionably the third nerve at the sixtieth hour; and the evidence furnished by such intermediary stages as I have been able to observe, of which that furnished by my forty-three-hours' specimens is perfectly definite, I am led to the belief that the third nerve is developed directly out of the outgrowth (*m*) from the top of the mid brain shown in fig. 8, and that at some period between the forty-third and sixtieth hours its attachment shifts down from the top of the mid brain to the lower part of its sides.

The necessary assumption of this shifting having occurred is the most serious difficulty in the way of the above view. It is very considerably diminished by the consideration that at a corresponding stage of development all the other cranial nerves and the posterior spinal roots undergo a precisely similar shifting of their points of attachment. I propose to notice in detail the different stages of this process in the case of the seventh nerve. It is true that the change is rather greater in the case of the third nerve than of the others—the third nerve being brought ultimately nearer to the median ventral line than the others; but this, I think, is explicable by its very early appearance and the enormous growth of the mid brain, which exaggerates the causes which we shall see lead to the shifting down of the other nerves.

Moreover the seventh nerve exceeds the posterior root of a spinal nerve in the amount of displacement it undergoes far more than the third exceeds the seventh; so that a view that regards the third nerve as originally arising from the mid dorsal line is not so paradoxical as it appears at first sight.

From the ganglionic termination of the third nerve seen in fig. 22 two branches arise. Of these the smaller and later developed one is very short, and runs forward above the ophthalmic branch of the fifth to the superior rectus (*vide* fig. 26, *r s*). The posterior and larger branch, which is also the earlier developed,

continues the course of the main trunk, crosses the ophthalmic nerve (fig. 26, V) *nearly at right angles*, lying to its inner side (fig. 17, III), and then curves slightly forwards, passing in front of the rectus externus (fig. 26, *re*), behind the rectus internus (*r i*), and behind and below the optic nerve (II). This is the precise course of the third nerve and its branches in the adult.

According to the description given above, the third nerve must be a true cranial segmental nerve. It resembles the other cranial nerves in arising from the neural ridge; in shifting downwards towards the mid ventral line at an early period; in being ganglionic at its origin; in having a distal ganglionic swelling, from which two branches are given off, of which the posterior is the larger; and, lastly, in its primitive straightness and general direction. It will be seen by comparing figs. 21, 22, 23, and 26 that the posterior cranial nerves run parallel to one another, but that in front of the ear, owing to the distortion produced by cranial flexure, their distal extremities converge. The direction of the trunk of the third nerve will be seen to be *precisely that which a true segmental nerve springing from the mid brain would necessarily have.*

With the exception of a very brief notice in my former paper[1] I am not acquainted with any previous account of the development of the third nerve.

Foster and Balfour state:[2]—"It is worthy of note that of the third, fourth, and sixth nerves no such early rudiments appear; and there are reasons for thinking that these are in reality intercranial branches, the third and fourth of the fifth, and the sixth of the seventh nerves."

Huxley[3] is "greatly disposed to think" that the motor nerves of the eye "are really the motor portions of the nerves of the orbito-nasal cleft, the third and fourth appertaining to the inner divisions of the ophthalmic, the sixth to its outer division."

Allen Thomson holds that[4] "the third, fourth, and sixth pairs of nerves are of subordinate importance, and may be considered as related, the two first to the fifth pair, and the last to the facial nerve. Their peripheral parts are developed in connection with the muscles of the eyeball, but the mode of the formation of their roots in connection with the nervous centres has not been ascertained."

None of these accounts are based on direct embryological evidence. It is obvious from my descriptions and figures that the third nerve has nothing whatever to do with the fifth, and

[1] Loc. cit., p. 510.
[2] Op. cit., p. 138.
[3] 'Anatomy of Vertebrated Animals,' p. 73, note.
[4] 'Quain's Anatomy,' 8th edition, vol. ii, p. 761.

that it is very far from being a nerve of "subordinate importance," while, if my account of its development is confirmed, it is a point of some interest to notice that *in the chick the third nerve is the very first nerve to be developed in the whole body.*

I have shown above that the third nerve resembles the hinder cranial nerves in giving off from a ganglionated distal extremity two branches, of which the posterior is the larger. I would suggest that the branches of the third are strictly comparable with the branches of such a nerve as the glosso-pharyngeal which stand astride of a visceral cleft. Fig. 26 shows that *the optic nerve is situated in the angle between these two branches.* Whether we have in the eye any modified remnant of a visceral cleft I cannot here discuss; but it is important to notice that this relation is a very strong argument against the possibility of the optic nerve being in any way a modified segmental nerve. One segmental nerve could hardly be so modified as to be, from the very earliest date at which such an arrangement is recognisable, between the primary branches of another segmental nerve.

Balfour's important discovery in Elasmobranchs of what he has termed head-cavities[1] seems to prove the existence of at least one premandibular segment in the vertebrate head. It is peculiarly unfortunate from my point of view that he has not described the relation of the third nerve to this most anterior or first head-cavity. If the course of the third nerve in the shark is in any way comparable to that in the chick there must be a very intimate relation between these structures. In one of Balfour's figures[2] a small bit of nerve, drawn but not described, has the appearance and relations of the third nerve in a four-day chick:[3] *its course is directed straight towards the first head-cavity.* This is strong independent evidence in favour of the third nerve being a true cranial segmental nerve.

I may add that some observations I have recently made on the early stages of development of the salmon, of which I hope shortly to publish a full account, support in a most remarkable and unexpected manner the views advanced above as to the morphological significance of the olfactory and third nerves.

The *fourth nerve.*—I have devoted considerable time and attention to attempting to ascertain the mode of development of this nerve, but as yet have completely failed to recognise it in any section at any period up to the end of the fifth day, beyond which date my investigations do not extend. This I greatly

[1] 'Journal of Anat. and Phys.,' vol. xi, part iii, pp. 481, 482.

[2] Loc. cit., plate xviii, fig. 1 *a*.

[3] I learn from Mr. Balfour that he believes this to be the third nerve, and believed it to be so at the time of writing his paper.

regret, inasmuch as the fourth nerve is in some respects the most remarkable of all the cranial nerves. It is the only one arising in the adult from the dorsal surface of the brain; and since all the other nerves arise primitively from the neural ridge, *i.e.* from the mid dorsal line, it might be argued that the fourth nerve is the only one which has preserved its primitive connection with the dorsal surface. It is very difficult, however, to conceive how the fourth nerve could so preserve its relations while the nerves immediately in front of and behind it lose their attachment and shift down nearly to the mid ventral line. It is just possible that the fourth nerve arises primitively from the constriction between the mid and hind brains, at which point the primitive relations undergo *comparatively* little change, and which is *comparatively* unaffected by the rapid growth of the other parts. I am not at all disposed, however, to adopt this view, inasmuch as my investigations tend very strongly to prove that all the nerves arise primitively from the widest parts of the dilated vesicles, whether of the brain or cord, and never from the intervening constrictions.

In the adult chick the fourth nerve runs parallel to, and dorsad of, the ophthalmic branch of the fifth, from which it is separated by the rectus superior. The muscle it supplies—the obliquus superior—is, at its origin in the chick, as in the skate, the *most anterior* of all the eye-muscles. Now, since it arises posteriorly to the third nerve, and supplies a muscle in front of those supplied by the third, it must cross this latter nerve, as indeed it is readily seen to do in the adult chick. Therefore, *if the origin of the fourth nerve in the adult is its primitive origin*, then the third and fourth nerve cannot be morphologically equivalent; and if the third nerve is a true cranial segmental nerve, in favour of which we have seen that evidence of a very strong nature exists, then the fourth nerve *cannot possibly be one also*. It is impossible for two segmental nerves to cross one another in the manner in which the third and fourth nerves do.

Such suggestions are perhaps, in the absence of direct embryological evidence, not of much weight, but I have thought it worth while to record them, because it is of very great importance to determine, by any method we can, the relations of the eye-muscle nerves to the other cranial nerves and to the head segments.

The fifth nerve.—At twenty-four hours the neural ridge has extended backwards from the mid brain a certain distance down the hind brain (fig. 5, *m*). By the twenty-ninth hour the hind brain is divided by a series of slight constrictions (cf. figs. 9 and 10) into a series of vesicles, of which the most anterior one is the largest. At its centre or widest part the neural ridge (fig. 9, *m*)

is rather more prominent than it is either just in front of or just behind this point. This prominence is the earliest rudiment of the fifth nerve.

In my previous account I have described and figured it at forty-three hours as "an outgrowth of very slight vertical thickness from the summit of the anterior dilatation of the hind brain."[1] This slender outgrowth reaches about half way down the side of the hind brain, with which it is still connected at the extreme dorsal summit only.

A little later on the attachment begins to shift, and the nerves travel down the sides of the hind brain in a manner that will be fully described under the seventh nerve. At the same time the roof of the hind brain thins out very considerably (fig. 15). The nerve increases rapidly in size, and about the fiftieth hour divides distally into two branches, the ophthalmic and the inferior maxillary.

About the end of the fourth day the fifth nerve has the appearance shown in fig. 21, which represents a longitudinal section through the head of a ninety-six-hours' chick, taken from the same specimen as fig. 22, but a little further from the median line. The proximal part of the nerve is dilated into a large oval ganglionic swelling. This springs from the floor of the hind-brain (*vide* also fig. 15), but the connection between the two is not so evident as in the earlier stages, owing to the white matter which now invests the central ganglionic matter of the brain. The nerve divides almost immediately into two branches, each of which is swollen and ganglionic at its base. Of these the anterior (fig. 21, V 1) passes at first forwards, then bends slightly downwards, crossing the third nerve almost at right angles, and lying in a more superficial plane than this latter (fig. 17, V and III). It then passes under the rectus superior (fig. 26, *r s*) but dorsad of the other eye-muscles and of the optic nerve (fig. 26, II). I have traced it forward at this stage into close proximity with the olfactory nerve and organ. This description leaves no room for doubt that this branch is the rudiment of the ophthalmic branch of the fifth in the adult.

The posterior branch of the fifth passes downwards and backwards to the anterior border of the mandibular arch, along which it runs (figs. 21, V 3, and 23 V 3). Close to its distal extremity it gives off a small branch forwards, which runs down the hinder edge of the maxillary process (figs. 23, V 2 and 21). This latter branch is the rudiment of the superior maxillary nerve of the adult, while the main branch develops into the inferior maxillary division of the fifth.

The fifth nerve therefore arises as a single root, and is to be

[1] Loc. cit., p. 509, and plate xx, fig. 4.

regarded as a single segmental nerve, of which the superior and inferior maxillary nerves are the primary branches—the branches which stand in relation with the corresponding cleft.

Since the ophthalmic branch of the fifth nerve crosses the third at right angles, it is at once obvious that these two nerves cannot be morphologically equivalent, and that if one of them is a segmental nerve the other is something of a totally different nature. The claims of the third nerve to be considered a segmental nerve have already been fully discussed. What, then, is the ophthalmic nerve?

The answer that first suggested itself to me is that it is the ramus dorsalis of the fifth nerve, whose primitive direction has been altered by cranial flexure. This is a somewhat tempting view; it accords well with the fact of the ophthalmic nerve lying superficially to the third, and is greatly strengthened by the fact of its having been already brought forward, *on totally different grounds*, by Gegenbaur[1] and by Balfour.[2]

However, there appear to be very serious difficulties in the way of accepting this explanation. In the first place, the only cause that we are acquainted with to which we could ascribe such a change in the direction of the ramus dorsalis of the fifth nerve is cranial flexure. Now, a glance at figures 21 and 22 shows that *cranial flexure does not affect the part of the brain from which the fifth nerve arises to any appreciable extent*, and is totally inadequate to produce a change of direction of about 130°; and yet such a change must have occurred if this view be true. Secondly, *no amount of cranial flexure*, or of any other process that I can conceive of, *could possibly cause the ramus dorsalis of one segmental nerve to pass right across the segment next in front of that to which it belongs;* and if the third nerve is a segmental nerve, the ophthalmic not only does this, but actually crosses over into the next segment (the olfactory), and reaches almost to the extreme anterior end of the head. Moreover, no explanation is offered of the remarkably close connection between the ophthalmic and olfactory nerves. These considerations seem to me to render the ramus dorsalis hypothesis untenable.

There is one other view which I would venture to suggest here, though I do so with great diffidence, inasmuch as I have no *direct* embryological evidence to offer on its behalf. We have seen above that the nerves do not arise separately from the

[1] "Ueber die Kopfnerven von Hexanchus," 'Jenaische Zeitschrift,' vi, 1871, pp. 508, 545 *seq.*

[2] 'Journal of Anat. and Phys.,' vol. xi, part iii, p. 480. The comparison suggested itself to me quite independently, simply from a consideration of the relations of the ophthalmic and third nerves at an early stage.

neural canal, but that they arise as *outgrowths from a continuous longitudinal neural ridge*. What becomes of the intervening parts of the neural ridge between the successive pairs of nerves? The neural ridge itself is such an exceedingly definite structure that it would be a most remarkable circumstance if these intervening parts, which early attain a very considerable size (*vide* fig. 7), were destined to disappear without undergoing further development. We shall see shortly that there is direct evidence of a very strong nature, tending to prove that the intervening parts of the neural ridge form commissures connecting together the posterior roots of the spinal nerves in the body, and in the hinder part of the head connecting the roots of the vagus with the posterior root of the first spinal nerve behind, and with the root of the glosso-pharyngeal in front.

Seeing, therefore, that in all cases in which their subsequent development has been traced these intervening parts of the neural ridge develope into commissural nerves, I would suggest—

1. That the intervening parts persist in the anterior part as in the posterior part of the brain and the body; and that, as in the latter, they persist in the form of a longitudinal commissure connecting the several segmental nerves together.

2. That the ophthalmic nerve is the persistent commissure connecting the fifth with the third and olfactory nerves.

On this view we should get a ready explanation of several otherwise perplexing points, such as the course of the ophthalmic nerve and the bend it makes near its root; the connection of the ophthalmic nerve with the third nerve at the ophthalmic ganglion; and the remarkably close relation between the ophthalmic and olfactory nerves in the olfactory organ of most vertebrates.

Further, if we assume that the commissure between the fifth and seventh nerves persists in like manner, we shall at once get a clue to the otherwise perplexing variations presented by the ramus ophthalmicus superficialis and ramus ophthalmicus profundus, which might be explained as due to the commissure splitting along its length into two or more branches, which may unite together again. Finally, it is just possible that the fourth nerve may be a part of this commissure, whose relations have been altered by the growth of the brain; the origin of the fourth nerve from one of the intervening parts of the brain supports this suggestion.

As to the other branches of the fifth nerve there can, I think, be no doubt that V 3 is the segmental nerve belonging to the anterior border of the mandibular arch. V 2 arises from V 3 in exactly the same manner that the anterior branch arises from the posterior in each of the other segmental nerves. If this be so, then the cleft between the maxillary and mandibular arches must be

a true visceral cleft; and, therefore, the maxillary process ought to be viewed as a separate arch, and not as a bud of the mandibular.

Finally, to return to the ramus dorsalis hypothesis of the ophthalmic, it is worthy of note that "in the tadpole and some Urodeles the fifth nerve gives a cutaneous branch to the dorsum of the head."[1] Is not this the true ramus dorsalis of the fifth? Similarly in Elasmobranchs there are branches of the fifth which would appear to be true rami dorsales.[2]

The sixth nerve.—I have made some observations on the development of the sixth nerve, which, though incomplete, I think worth while recording here, inasmuch as I am not aware that it has been treated of by any previous writer.

Fig. 25 represents part of a longitudinal section through the head and neck of a five-day chick, the section being taken a very little to one side of the median line. It passes through the investing mass (*i v*), the downward prolongation of the fore brain to form the infundibulum (*inf*), the mouth with its pituitary diverticulum (*pit*), the fore gut, and mandibular and hyoid arches.

At VI a nerve is seen arising from the floor of the ~~(mid-)~~brain hind by a large number of slender roots, extending over a considerable longitudinal extent. This nerve runs forward without any ganglionic enlargement in any part of its course, parallel to the floor of the brain, and just above the investing mass.

By tracing this nerve forwards it is found to run straight to a muscle (*r e*, fig. 26), which, from its relations to the third and ophthalmic nerves, can be none other than the rectus externus, or, as it would be more properly called, rectus posterior. This nerve is, therefore, the sixth. Its course is perfectly straight, and it gives off no other branch.

The sixth nerve differs from all the other pre-auditory nerves in several important points:

1. It arises by a series of small slender roots instead of by a single large ganglionic root. In this respect it resembles the anterior spinal roots of the chick[3], to which it closely corresponds in position and relations.
2. It is very much more slender than any of the others.
3. It does not branch.
4. Its direction is at right angles to the segmental nerves.
5. It appears much later than the others. On this point I cannot speak definitely, as I have failed to observe its early

[1] Huxley, 'Encyclopædia Britannica,' vol. i, art. "Amphibia," p. 767.

[2] Jackson and Clarke, "The Cranial Nerves of Echinorhinus spinosus," 'Journal of Anat. and Phys.,' vol. x, p. 83, and plate vii, fig. 2, *d d*.

[3] Loc. cit., p. 505.

stages. At one hundred, ninety-six, and ninety-three hours it has very much the same appearance and relations as at 122 hours (fig. 25). I have not yet detected it in any specimen earlier than ninety-three hours.

6. A transverse section of the brain passing through its roots passes also through that of the seventh nerve. Such a section shows that the two sixth nerves arise very close to the mid ventral line, and are separated from the roots of the seventh by a considerable interval.

From a careful investigation of the relations of this nerve I have been led to the conclusion that the sixth nerve bears the same relation to the seventh that the anterior root does to the posterior root of a spinal nerve. And since we know the seventh to correspond to the posterior root of a spinal nerve we may speak of the sixth as the anterior root of the seventh. However, as I have not noticed the early stages of development of this nerve, I do not wish to speak too confidently, especially as my conclusions are in direct opposition to Balfour's definitely expressed opinion,[1] that no anterior roots exist to the cranial nerves. My own decided opinion is that the sixth is an anterior root, but in the absence of direct embryological proof I must leave the question open.

There are great difficulties in the way of the sixth belonging to the seventh nerve, the chief of which is the extension forward of the former to the rectus externus. My sections, however, seem to leave no doubt on this point; the hindmost of the roots of origin of the sixth is but a very short distance in front of the auditory capsule; however, the foremost of these roots appear to be situated in front of the seventh, so that it is possible that the sixth represents the combined anterior roots of the seventh and fifth, in which case the difficulty just noticed vanishes.

Lockhart Clarke[2] describes and figures the sixth nerve as arising *in the adult* from the same nucleus as the seventh, mentioning also that the sixth nerve has a large number of roots, strongly suggesting comparison with an anterior spinal root. Meynert[3] has also pointed out and figured the same thing. We are as yet ignorant of the relations between the nerve-nuclei of the adult and the primitive embryonic roots of origin of the nerves, but the above statements manifestly lend support to there being some close connection between the sixth and seventh

[1] 'Phil. Trans.,' vol. 166, part i, p. 189, note, and 'Journal of Anat. and Phys.,' vol. xi, p. 459.

[2] 'Phil. Trans.,' 1868.

[3] Stricker's 'Histology,' English translation, vol. ii, fig. 254, p. 493, also Quain's 'Anatomy,' 8th edition, vol. ii, p. 513, fig. 359.

nerves, and that of precisely the nature I have been led to suspect on embryological grounds.

The seventh and auditory nerves.—In my former account I mentioned that both auditory and facial nerves arose from a single root.[1] This is in accordance with Balfour's account of the development of the nerves in Elasmobranchs, and is confirmed by my later work. The common outgrowth very speedily divides distally into an anterior branch—the facial, and a posterior—the auditory.

Like all the other nerves the facial and auditory undergo a considerable change in their point of attachment to the brain. At their earliest appearance they are, as simple prominences of the neural ridge, connected with the hind brain at its extreme summit (fig. 10); so that the nerves of the two sides are directly continuous with one another across the top of the neural canal. This attachment is preserved for a short time only; in the later stages the attachment is to the side of the brain, so that the nerves of the two sides are widely separated from one another.

This change is effected in the same way in all the nerves in which it occurs, and the following description of the several stages will apply alike to the cranial nerves and to the posterior roots of the spinal nerves.

Fig. 11 represents a transverse section through the hind brain of a forty-seven hours' chick, passing through the middle of the seventh nerve, and through the anterior edge of the thickening auditory epithelium. The nerve (VII) has attained a great size, and is in close contact with the sides of the hind brain for about a third of its circumference. This contact is especially close at the point *a*, the lowest point at which it occurs. Still, however, the outline of the brain is clearly and uniformly defined, and the attachment of the nerve is still to the extreme summit of the brain only, though that attachment has become very much more slender than it was in the earlier stages.

Fig. 12 is a transverse section through the same region in a fifty hours' chick. The section passes on the left side through the auditory pit (*aud*), on the right a little further forward, through the combined seventh and auditory nerve (VII). The hind brain has grown very considerably, and its roof, which was before of some thickness, is now very thin indeed. The nerve (VII) is still attached by a very slender process (*b*) to the extreme summit of the brain, but it has also acquired a secondary connection with the brain at the point (*a*).

At fifty-four hours the connection at *a* is somewhat more marked, while the original connection at *b* is lost completely;

[1] Loc. cit., p. 509.

so that at this stage the nerve is attached to the brain, not by its apex, but by its side, and the nerve presents a conical projection sticking up beyond its point of attachment.

This conical process gradually shrinks, and by sixty-seven hours is no longer recognisable (fig. 14, VII). Owing to the roof of the brain growing more rapidly than its sides and floor the nerves appear to be gradually driven down towards the base of the brain. This further change is due entirely to relative rate of growth; there is no further shifting of the nerve attachment. Fig. 16 shows the relation of the parts at ninety-three hours; the rapid growth of the roof of the brain is evidenced by its extreme thinness.

The above description applies also, as already stated, to the other cranial nerves and to the posterior spinal roots. In my former paper I was unable "to determine with certainty" the mode in which the shifting of the roots occurred. Having now traced all the stages, I am perfectly satisfied that the original attachment is lost and a new one acquired. This, I may notice, can hardly be a primitive state of things.

This shifting of the point of attachment has been noticed also by Balfour in Elasmobranchs. He, however, does not describe a secondary attachment, but considers the shifting as due[1] entirely to growth of the cells in the median dorsal line of the neural canal. Consequently he finds the stage described above at fifty-four hours, where the nerve is attached by its side to the brain, and projects up above the point of attachment, very puzzling. The occurrence of this stage in Elasmobranchs seems to me to prove that a secondary attachment is acquired in Elasmobranchs in the same manner as I have just described in the chick.

In figs. 21 and 23 the relations of the facial and auditory nerves are well seen. The facial (VII) is derived from the anterior part of the common root; it passes downwards and forwards, swelling out into a ganglion just behind the upper end of the hyo-mandibular cleft. From this ganglion two branches arise—a larger posterior one, which arches somewhat backwards, and then passes downwards in the hyoidean arch, and a smaller anterior one, which arches over the top of the cleft and then runs down along its anterior wall.

The part of the combined root which becomes the auditory nerve (VIII) is considerably larger than that from which the facial is derived. It passes downwards and backwards as a large ganglionic mass closely applied to the anterior wall of the auditory vesicle (figs. 26, 23, and 21).

It is worthy of notice that the nerve comes in contact with the auditory epithelium at a very early period—about fifty hours;

[1] Loc. cit., p. 425.

and that the two epiblastic formations—nerve and sensory epithelium—completely fuse together. This complete fusion at an early period, before any definite histological differentiation has set up in the nerve, is a point of considerable interest in connection with the much debated question of the continuity of nerve fibres with epithelium cells in the adult. If complete fusion of nerve and epithelium occurs at a very early period, how are we to determine whether a given cell in the adult is nervous or epithelial in nature or origin ?[1]

I have devoted considerable attention to the early stages of the seventh and eighth nerves, and am perfectly confident that they do arise, as described above, as one outgrowth ; and as the seventh nerve is a segmental nerve I regard the auditory as a branch of it. Even if the auditory had proved to have a separate origin it could hardly be a segmental nerve, since there is no room for one, as far as visceral arches and clefts are concerned, between the facial and glosso-pharyngeal.

I have not noticed an anterior branch to the seventh, like that described by Balfour in Elasmobranchs,[2] but have suggested above that such a branch, when present, is not to be considered as a ramus dorsalis, but as part of the commissure connecting the seventh with the nerves in front of it.

The glosso-pharyngeal nerve.—I have already shown[3] that the glosso-pharyngeal and vagus nerves are developed from a single outgrowth on either side from the neural ridge in the posterior part of the hind brain, and behind the auditory involution. The roots of the two nerves remain in connection with one another, but at an early period the outgrowth divides distally into an anterior branch—the glosso-pharyngeal, and a posterior one—the vagus.

In fig. 22, IX, the condition of the glosso-pharyngeal is shown at the ninety-sixth hour. It is a slender nerve running downwards behind the auditory capsule to the upper part of the angle of the second visceral cleft, where it expands into a ganglionic swelling. From the ganglion two branches are given off—a posterior larger one, which runs downwards along the anterior border of the first branchial arch, and an anterior smaller one, not shown in this figure, which arches over the top of the cleft, and runs down along the posterior border of the hyoid arch.

In fig. 23 the same parts are shown at the one hundredth hour on a slightly larger scale. The visceral arches and clefts are rather more clearly shown here than in the preceding figure.

[1] *Vide* also Balfour, 'Journal of Anat.,' loc. cit., p. 411.
[2] Loc. cit., p. 465, *seq.*
[3] Loc. cit., p. 508.

Fig. 26, which represents a longitudinal section through the hind brain and mouth of a chick of 122 hours, shows the glosso-pharyngeal (IX) with its ganglion and its two branches, with their relations to the second visceral cleft, very clearly.

A point of considerable interest, shown in figs. 22, 23, and 26, is that the glosso-pharyngeal is not attached to the hind brain by a single root like the fifth and seventh, but by a variable number—four or five—of small roots which spread out in a fan-like manner and enter the brain separately. In this respect they resemble the vagus roots and the posterior roots of the spinal nerves, and differ from the anterior cranial nerves, which would appear to be, in this respect at least, more primitive. In figs. 23 and 26 the anterior of these roots is seen to stretch forwards over the auditory capsule, so as to bridge over to a great extent the gap between the glosso-pharyngeal behind and the facial and auditory in front. I regard this as an indication of a commissural connection between these nerves.

It is worth noticing that in the frog there is a dorsal commissural cord connecting the roots of the facial and glosso-pharyngeal nerves together. This, I would suggest, is part of the longitudinal commissure derived from the neural ridge.[1]

The vagus.—This is derived from the posterior of the two branches into which the outgrowth common to it and the glosso-pharyngeal divides. It is at first rather smaller than the glosso-pharyngeal, and even as late as the 122nd hour (*vide* fig. 26, IX, X) there is hardly any appreciable difference in size between the trunks of the two nerves. Its root soon acquires the multiple character noticed in the glosso-pharyngeal (figs. 22, 23, and 26).

The condition of the vagus at 122 hours is shown in fig. 16, X, where the nerve is seen to run downwards and backwards parallel to the glosso-pharyngeal for a certain distance. It then expands into a very large fusiform ganglion, which overlies the third and fourth visceral clefts. Beyond the ganglion the nerve is continued, as the intestinal branch, in close connection with the walls of the alimentary canal.

The anterior root of the vagus is continuous, without entering the brain, with the posterior root of the glosso-pharyngeal (figs. 23 and 26).

The auditory capsule in its early stages of development lies very close to the hinder end of the brain, and its hinder border is separated from the first protovertebra by an interval not exceeding the width of a single protobertebra. If we hold that the

[1] *Vide* Huxley, 'Encyclopædia Britannica,' vol. i, Art. "Amphibia," fig. 25, p. 766; also 'Proc. Zoological Society,' 1874, p. xxxi, fig. 5, *Sy.*

cranial and spinal nerves are in any way comparable, then we must either consider—(1) the whole vagus and glosso-pharyngeal as together equivalent to a single spinal nerve—this is obviously unsatisfactory—or (2) regard the glosso-pharyngeal and each of the main branches of the vagus as equivalent to so many spinal nerves.

On the latter hypothesis, which we may consider as almost demonstrated by the relations of the branches of the vagus to the hinder visceral clefts, it is obvious, when we consider the very short length of the neural canal from which the whole of the vagus is derived, that very considerable concentration must have occurred at the hinder end of the brain. Since the concentration occurs in the chick in the very earliest stage of its development, it is probable that approximation and fusion of the several vagus roots, with accompanying concentration of the hinder end of the brain, was gradually acquired by the ancestors of the chick; while in the chick this fusion and concentration have been thrown back to the very earliest ontogenetic stage at which they could possibly occur, *i.e.* at the very first appearance of the nerve rudiments. At the opposite end of the brain the opposite process seems to occur, judging from the distances between the roots of the segmental nerves, and instead of concentration we have expansion.

Transverse sections through the part of the hind brain from which the vagus arises show, about the end of the fourth day, a number of small outgrowths from the ventral surface of the brain, close to the median line on either side. These are separated by a considerable interval from the roots of the vagus, from which they are perfectly distinct. In appearance, position, and relations, these small paired outgrowths are precisely similar to the anterior roots of the spinal nerves, to which I believe them to be strictly equivalent. The earliest date at which I have observed them is the sixty-seventh hour. I have not succeeded in tracing their ultimate fate. They extend forwards nearly as far as the auditory capsule. Balfour suggests that some similar roots noticed by himself and by others in adult or embryonic Elasmobranchs are "ventral roots of spinal nerves whose dorsal roots have been lost."[1] I cannot accept this suggestion, which has no direct evidence in its favour, as far as the roots I have just described are concerned. These roots belong, unquestionably, to the vagus; and the description I have given above of the sixth nerve shows that there is every probability that we have true anterior roots considerably further forwards in the brain.

The multiple character of the roots of the glosso-pharyngeal

[1] 'Journal of Anatomy and Physiology,' loc. cit., p. 471.

and vagus I regard as a secondarily acquired feature, which possesses some interest from the fact that both the anterior and posterior roots of the spinal nerves of the chick are similarly multiple. As far as I have been able to determine, these roots, which vary considerably in size, are not constant in number. They appear to increase in number and complexity with age.

The cranial nerves and the posterior roots of the spinal nerves arise as outgrowths of the continuous longitudinal neural ridge. I propose, in conclusion, to give the history, as far as I have succeeded in determining it, of the portions of the neural ridge that intervene between successive pairs of nerves.

Fig. 27 represents a horizontal section through the cervical region of a ninety-three hours' chick close to the dorsal surface; it shows the muscle-plates, of which that marked (*m p*) is the second. It passes through the spinal cord, and just touches the hind brain. The cord is slightly constricted opposite the centres of the protovertebræ, and slightly dilated opposite the intervals between successive protovertebræ.

On the right side the section passes through the point of attachment of the posterior root of the third spinal nerve (*s p*) to the cord; the nerve is seen to be ganglionic, and is attached to the cord by several roots, not shown in the figure; it lies opposite the anterior half of the corresponding muscle-plate.[1] The ganglion (*sp*) is connected by a nervous commissure (*com*) with the ganglion of the nerve in front of it.

In fig. 28 these relations are still better seen. The section is taken from the same embryo as fig. 27, but a little further back, passing through the fifth, sixth, and seventh protovertebræ. *Sp* is the ganglion of the seventh cervical nerve on the left side, at the point of attachment of the posterior root to the cord. The commissure (*com*) is well seen; it is dilated opposite the anterior halves of the protovertebræ.

The posterior roots of all the spinal nerves are connected together in this way by a longitudinal commissure. At the stage figured the nerves have acquired their secondary attachment to the sides of the cord. From the accounts I have already given there can, I think, be no doubt that the commissure is formed from the intervening portions of the neural ridge between successive pairs of spinal nerves.

This commissure was first described by Balfour[2] who justly

[1] In my former paper I erroneously stated that the posterior roots are situated opposite the *posterior* halves of the corresponding protovertebræ. Figs. 24, 27, and 28 show conclusively that they lie opposite the *anterior* halves.

[2] 'Phil. Trans.,' vol. 166, part i, and 'Journal of Anat.,' loc. cit., p. 424.

regards its discovery as "one of the most remarkable results of his researches upon the Elasmobranch nervous system." I am not aware that it has been noticed by any other observer.

In figs. 23 and 26 a nerve (*com*) is seen running backwards from the posterior root of the vagus close to its point of origin from the brain. This runs backwards, and becomes directly continuous with the longitudinal commissure just described as connecting together the posterior roots of the spinal nerves; it is manifestly an anterior continuation of this latter.

It is shown very well in fig 24, which represents a longitudinal section through the hinder part of the head and the cervical region of a ninety-six hours' chick. The commissure (*com*) presents two ganglionic swellings (IX, X) at the points of origin of the glosso-pharyngeal and vagus nerves. It also presents smaller swellings, not shown in the figure, corresponding with the posterior roots of the spinal nerves. It is shown also in fig. 25 (*com*).

The existence of this commissure is a very remarkable fact. I have not followed it late enough to know its ultimate fate. In Elasmobranchs, according to Balfour, "it becomes gradually thinner and thinner, and finally ceases to be observable . . . I can only conclude that it gradually atrophies, and finally vanishes without leaving a trace."[1]

I have failed to trace the commissure further forwards; but the forward extension of the anterior root of the glosso-pharyngeal, already noticed, is of interest as indicating a probable continuation, and I have already suggested that the ophthalmic branch of the fifth nerve is very possibly the part of the commissure in front of the fifth nerve. If this is so, it is the only part that we know to persist in the adult condition.

Since the intervening portions of the ridge persist, at least for a time, in the posterior part of the head as a definite structure, there is certainly a presumption in favour of a similar persistence of the anterior part of the ridge, which is at places of enormous size in its early stages (fig. 7, *m*). Its enormous size at this part of the brain may, however, be due in part to the general hypertrophy to which the anterior part of the neural canal is subject.

[1] Loc. cit., pp. 471—426.

A Contribution *to the* History *of the* Embryonic Development *of the* Teleosteans. By Edouard Van Beneden, Professor in the University of Liège. (With Plate IV.)

During my stay at Villafranca, in August and September, 1874, I had occasion to make some observations on the development of an osseous fish. I applied myself chiefly to the study of the segmentation and endeavoured to elucidate the much discussed question of the origin and mode of formation of the embryonic layers. I found daily, in the midst of the produce of my fishing with Müller's net small pearl-like bodies, colourless and perfectly transparent, floating freely near the surface of the water; the diameter of these little hyaline spheres scarcely exceeded that of a large Noctiluca, and it was only after I had examined them with a lens that I discovered that I had before me the embryos of an osseous fish.

Several times the fishermen brought me masses of a gelatinous appearance, taken on the surface of the sea and formed by hundreds or thousands of agglutinated eggs. These eggs presented all the characters of those of which mention has just been made; they had the same transparency, and the same composition. All the eggs in the same mass were always found to be in the same stage of development. This circumstance greatly facilitates the study of the successive phases of development. The eggs die very rapidly on the microscope-stage and consequently one cannot witness the progress of the development under one's eyes in one and the same egg. But since all the eggs of a given mass develope simultaneously, it is always possible to determine the time which has been necessary for the production of modifications which have arisen since the moment when the last eggs examined were detached from the agglomeration.

In all the masses of eggs which were brought to me the eggs were always in course of cleavage or else they contained very young embryos. I have never found embryos on the point of hatching nor even embryos provided with primordial vertebræ. On the other hand, I have never found in the free isolated condition eggs in course of segmentation nor even embryos young enough to be utilised in the study of the formation of the germ-layers.

It seems therefore probable that the agglutinated eggs come from the same fish as those which were taken isolated. It appears that the eggs laid in mass remain for some time adherent one to another and afterwards separate and then float free from all adhesion on the surface of the sea.

I ought, however, to add that having preserved some groups of eggs in jars in order to follow step by step the modifications which are produced, I never saw the eggs become detached from one another. But it is not possible to keep them indefinitely in a living state under these conditions. Although I took care to renew the water several times a day, I found it impossible to preserve my embryos alive for more than from twenty-four to thirty-six hours.

Although I had no intention, when I went to Villafranca, of occupying myself with the embryology of fishes and the desire of arriving at a solution of the various questions relative to the organisation and development of the Dicyemida left me but little leisure, yet I could not resist the temptation of utilising the beautiful material for study which I found placed in my hands. None of the difficulties which are usually met with in the study of the development of fishes present themselves in this case. The capsule of the eggs is very thin and of perfect transparency. The deutoplasm is constituted by an albuminoid globule which is perfectly homogeneous, hyaline, free from all granulation and limited by a very sharp and perfectly regular contour. In the protoplasm of the egg, whether of the germ or of the protoplasmic mantle which clothes a part of the vitelline globe, there exists neither fat-globule, nor vesicle, nor formed element of any kind; nothing, in a word, which one could confuse with a cell or a cell-nucleus.

Very similar pelagic eggs, belonging probably to a closely-allied species, were observed by Haeckel (1) during his last visit to the coast of Corsica. He has published his researches on the history of their development in the second part of his work 'Die Gastrula und die Eifurchung der Thiere.' He found the same eggs at Nice in 1876. Haeckel did not succeed any better than I have done in determining with precision the species to which the eggs which he had under observation are to be attributed. Basing it on the description given by Retzius of the eggs of *Gadus lota*, Haeckel puts forward the opinion that the fish, the development of which he studied, is a Gadoid allied to the Burbot, perhaps a *Motella*.

The eggs which have furnished me with the observations of which I am about to give an account present a very great resemblance to those studied by Haeckel. Found under the same conditions, at the same part of the Mediterranean coast, they have very nearly the same dimensions, the same appearance, and the same composition. At the period of deposition they are agglutinated in masses of various volume and form. The quantity of matter which holds them together is very small indeed, so that one cannot say of my eggs as Haeckel says of

his, that they are embedded in a gelatinous substance. It is not possible to isolate fragments of the cementing substance —scarcely possible to see it between the eggs.

My eggs have a diameter of 0·80 to 0·85 millimètres. They are colourless, and present the transparency of crystal. The membrane, which is very thin, shows neither canalicular pores nor punctation of any kind; it is homogeneous, of considerable resistency, and very elastic. It is very difficult to tear it without injuring the contained egg.

The youngest eggs which I had under observation showed the disc segmented into two spheres. They were brought to me one morning about seven o'clock. I received two other masses, the eggs of which were at the end of their segmentation; they had also been collected very early in the morning. It is probable that the deposition of the eggs takes place during the night or in the morning about sunrise. In all these eggs the form was that of an ellipsoid, nearly a sphere, the major axis being scarcely a sixth longer than the minor. At one of the extremities of the major axis (animal pole) is situated the germinal disc or germ. This rests on the vitelline globe, which has the same form as the egg itself, only the ellipsoid is truncated at one of the extremities of its major axis along a surface, concave at its centre, convex at its margin. From this results the formation of a polar chamber, limited externally by the membrane of the egg, internally by the vitelline globe. It is in this space that the segmented germ is lodged.

The vitelline globe is formed by a hyaline substance, which is perfectly homogeneous, colourless, little refringent, and devoid of all structure. It holds in suspension a single solitary structural element. This is a brilliant spherical mass, with very dark contour, and occupies constantly the same position in the globe. Excepting for this, the deutoplasm is absolutely devoid of any granulation, of any vesicle or element which could be mistaken for cell or nucleus. The substance which composes it is an albuminoid matter; it is coagulated by alcohol or osmic acid, and is rendered turbid by acetic acid. The refringent sphere held in suspension in the deutoplasmic globe is a drop of oil or of fat. It is coloured black by osmic acid, and dissolves in ether. Haeckel observed this same "Oelkügel" in the eggs which he studied, but the drop instead of being suspended in the vitelline globe occupied the vegetative pole of the egg, and was simply embedded in a spheroidal depression of the surface of the vitelline globe. This is a characteristic which separates the eggs studied by Haeckel from those observed by me. Owing to the circumstance that the specific gravity of the oily drop is less than that of the substances which compose the other parts of the egg, the

eggs studied by Haeckel took in the water the same position invariably. The animal pole was always directed downwards, the vegetative pole upwards. I ascertained that in my eggs the position of the oil-drop was quite constant. It is always placed eccentrically and invariably occupies a position in the vegetative hemisphere, but is immersed in the albuminoid substance which surrounds it on all sides. I have in vain endeavoured to explain to myself this fact by some peculiarity of structure in the deutoplasm. I entirely failed to discover any trace of filaments connecting the oil-drop either with the surface of the vitellus or with the germinal disc. Van Bambeke (2) has made out in the fecundated egg of the Tench, the presence of pseudopodia which penetrate the vitelline sphere radiating from the base of the germinal disc. These pseudopodia which are visible before the commencement of segmentation have the function of bringing up to the disc certain elements previously disseminated. Ransom (3) had already seen granular currents comparable to those to which Van Bambeke attributes the characters of pseudopodia. I have not observed anything comparable to these.

The difference between my eggs, then, and those which Haeckel had under observation, has reference (1) to their mode of aggregation. Haeckel says, "Diese Laich bildet kleine weiche Gallertklumpen in welche zahlreiche, kleine, vollkommen durchsichtige Eier eingebettet sind." I cannot say so much of my eggs, which adhered one to the other, but were certainly not embedded in a sort of jelly. (2) The eggs of Haeckel were spherical, and measured 0·64 to 0·65 mm. in diameter. (3) The position of the oil-drop constitutes a third differential character.

It seems to me, then, certain that we have not studied the eggs of the same species; but the differences are so trivial that I think one must ascribe the eggs studied by me and by Haeckel to allied species, if in any case the affinities of Teleosteans can be judged by the characters presented by their eggs.

As I have stated above, the youngest eggs which I observed had the germ segmented into two. I have represented one of these eggs in Plate IV, fig. 1. The segment spheres are convex externally, adherent one to another by a nearly plane surface, and terminated on the side in contiguity with the nutritive vitellus by a convex, well-marked line, which is, however, less obvious than the lines which mark their lateral limits. They are formed of a very clear and perfectly homogeneous protoplasm; it is not possible to discover in it any trace of a nucleus. The segment spheres do not rest immediately on the vitellus; they are separated from it by a layer of a substance which is finely

granular, but devoid of any globule. I took the very greatest pains to ascertain whether there exists in this layer any structure which might be considered as a cell-nucleus. Neither on examination of the fresh, living eggs, nor any more after having treated them with osmic acid and colouring matters (picro-carmine, Beale's carmine, hæmatoxylin), did I find any trace of anything of the kind. Acetic acid of 1 per cent. dilution gave me no better results. This layer forms for the deutoplasmic globe a continuous investment of such a sort that at no point do the segmentation spheres rest directly on the deutoplasm. It extends in every direction beyond the margin of the segmented disc, and everywhere is seen to be closely spread upon the vitellus. It is not easy to see the limit of this protoplasmic mantle, so fine does it become at its margin. It does not present the same thickness in every part. At the centre beneath the segmented germ it forms a biconvex lenticular body which occupies all the space between the vitelline globe, which is depressed at this point and the deep face of the segmentation spheres. In addition it presents a considerably increased thickness beneath the margins of the germ. This thickening forms all round the germ a circular wall, triangular in section. One side of the triangle is adjacent to a segmentation sphere; it faces upwards and inwards towards the animal pole of the egg; the second side is adherent to the nutritive vitellus, and concave. It faces downwards towards the vegetative pole of the egg. The third side is free; it faces directly outwards, and is slightly convex. It is separated from the membrane of the egg by a space filled with a clear and hyaline liquid. This layer is homologous, as I shall show further on, with that which Van Bambeke has described in the Roach by the name of *intermediate layer*. This name is appropriate on account of its interposition between the germ and the deutoplasmic globe, and has the great advantage of not in any way prejudging its morphological value. I shall then designate it by this name: I shall call the thickening which occurs at its centre beneath the germ the *median lens* (lentille); with Van Bambeke I shall call the circular thickening subjacent to the margins of the segmentation disc the *peripheral welt* (bourrelet).

I was able to see the successive phases of the segmentation by examining at small intervals new eggs removed from the mass which was brought to me at seven o'clock in the morning, and in which the eggs were at that hour provided with two segmentation spheres. About half-past eight all the eggs exhibited the disc segmented into four; so that at least an hour and a half, and probably more, must elapse between the appearance of the first groove and the moment when the two first spheres

divide in their turn. I have ascertained that the time which elapses between two successive phases of cleavage is shorter and shorter as the cells diminish in volume and in consequence of such diminution. I pointed out the same fact after having studied the segmentation of the egg of *Gammarus locusta*, and it is remarkably evident in the Rabbit. I will not delay over a detailed description of the segmentation. This phenomenon has been often described and figured, and, moreover, I was not able to study either the order or mode of appearance of the successive grooves with sufficient completeness to be able to add anything to what is already known. All my attention was concentrated on the *intermediate layer*, and I endeavoured to see as exactly as possible the modifications which it underwent during the earliest period of embryonic development. I have represented in the Plate two phases of the segmentation properly so called. Figure 2 shows the stage at which the development had arrived about eleven o'clock. At this moment I could not discover any trace of nuclei in the cells of the germ whilst still living; but on treating the eggs with osmic acid, then by weak alcohol, colouring them subsequently by means of picro-carmine, I was able to demonstrate the existence in each of the segmentation spheres of a fine spherical nucleus, homogeneous and devoid of nucleoli. When the eggs are allowed to die on the object-slide the protoplasm of the spheres becomes cloudy and finely granular, taking at the same time a slightly brownish tint. There appears then at the centre of each sphere a large clear spot, ill-defined and homogeneous in appearance. These spots are simply the nuclei. Acetic acid of 1 per cent. also renders them very obvious. At this phase of development a very sharp line separates the segmented germ from the "intermediate layer." The latter has retained precisely the same contour as that seen in the phase previously described. It shows clearly its median lens and its peripheral welt. I could not discover in any part of this layer the least trace of a nucleus. Neither during life nor after the death of the egg, when the intermediate layer shrinks somewhat and when nuclei make their appearance in the segmentation spheres, nor indeed by means of osmic acid, nor by picro-carmine, nor by acetic acid, nor by hematoxylin, could I succeed in causing nuclei to appear in this layer. I think myself, then, entitled to affirm that at this phase of the development of the egg there exists no trace of nucleus in the intermediate layer.

At five o'clock in the afternoon the eggs had arrived at the stage which I have represented in fig. 3. The cleavage-disc presents, taken as a whole, the form of a plano-convex lens; it

rests by its plane face on the intermediate layer with which it is seen to be everywhere in immediate contact. It is composed of polyhedric cells which are very clear and in each of which it is easy to distinguish, even in the living state, a large, spherical or slightly ellipsoidal nucleus. The cells are not, however, pressed one against the other in every part: here and there among them small spaces of varying form and dimensions can be observed. But neither at this stage of development nor at any moment during segmentation does there exist within the thickness of the disc any 'segmentation cavity.' It is well known that this cavity, discovered first of all by Lereboullet (4), in the Perch and the Pike, was re-discovered by Van Bambeke in the common Roach. The superficial cells are polyhedric as are the subjacent ones and also those which limit the blastodisc inferiorly. The cleavage mass or blastodisc does not therefore at this moment exhibit any trace of delamination. The only difference which the cells of the disc present is relative to their dimensions; the volume of the cells increases somewhat in passing from the surface to the deeper parts.

The intermediate layer has undergone important modifications. It is possible now to ascertain the existence, throughout the extent of this layer, of a great number of nuclei, generally oval, with very sharp contours and provided with one sometimes with two punctiform nucleoli. All these nuclei have very nearly the same dimensions, they are a little smaller than those of the cells of the blastodisc. If the focus of the microscope is arranged so as to give a surface-view of the deutoplasmic globe and if the region which immediately fringes the blastodisc be examined, a finely granular zone is distinguished there in which nuclei disposed regularly and at equal distances are scattered. It is impossible to make out any delimitation of cell-areas, but around each nucleus a small zone of a more granular character is seen in which a very manifest radial striation is apparent. The latter becomes much more obvious after the action of 1 per cent. solution of acetic acid. The perinuclear zones are separated one from another by clear spaces devoid of all granulation. These spaces together form a network in the meshes of which the nuclei with their radial haloes are disposed. It is clear that it is this part of the intermediate layer, namely, the part situated outside the blastodisc, which has been observed by Kupffer (5) in *Spinachia* and *Gasterosteus*, and which has received from this excellent observer the name of "Körnerzone" (nuclear zone). I have also been struck with the remarkable resemblance between the objects which I have just described and that cell-layer which appears in the Cephalopoda at the surface of the deutoplasm and is formed by the elements discovered and de-

scribed by Ray Lankester, from whom they have received the name of "autoplasts" (6).

In that part of the intermediate layer which extends beyond the blastodisc the nuclei form a single layer and are arranged with very great regularity. But if the focus of the microscope is so altered as to give an optical section of the egg, it is discovered that in the median lens and also in the peripheral welt the nuclei occur in various planes and that they appear to be disseminated without order in the protoplasm of the intermediate layer.

The simultaneous apparition of a great number of nuclei, surrounded each from the first with a granular radiated zone, in a layer which up to that moment had presented no trace of nuclear elements, can only be explained by admitting an endogenous generation of cells in the protoplasm. The regular grouping of the granules of the protoplasm around each of the nuclei as soon as they make their appearance indicates a subdivision of the protoplasm into so many cell-territories. It is not to be concluded from the fact that we cannot distinguish the limits of the cells, that there is no individualisation of the elements. The radial striation of the protoplasm around the nuclei proves that we have not here to do merely with a genesis of nuclei, but, in fact, with a formation of cells; nuclei and cell-bodies appear simultaneously.

I was unable to follow further the development of the eggs whose history I had watched from the morning onwards; the following morning most of them were dead. In others the blastodisc, considerably extended, covered in a great part of the deutoplasmic globe, and already exhibited the first traces of the embryonic rudiments in the widened portion of the marginal welt. A few hours later all my embryos had ceased to live, in spite of the care which I had taken to renew the water frequently in the course of the day.

But a short time before this, I had been led to study a stage of development very close to that which I have just described. Figures 4 and 5 represent an egg in this stage, one seen in optical section (fig. 4), the other seen from the surface (fig. 5). The blastodisc, a little more flattened than in the previous stage, is also more extended. It is in immediate contact throughout its inferior surface with the intermediate layer. There exists no trace whatever either of segmentation cavity or of germinal cavity (Keimhöhle).

The disc is formed of clear and nucleated polyhedric cells. The only important character in which it differs from the pre-

ceding stage consists in the differentiation of the superficial cells. These have become flattened, and form a sort of simple pavement epithelium, which limits the blastodisc externally. In section these cells appear lenticular, their external face is nearly plane, their deep face is regularly convex or presents facettes, by which these cells are moulded to the subjacent elements. The deepest cells present nothing particular.

The blastodisc has then divided itself by delamination into an enveloping lamella (Umhüllungshaut of Reichert (7), epidermoid layer of Vogt (8) and Lereboullet, Deckschicht of Götte (9), and a deeper mass, which is *the primitive external layer*.

The intermediate layer has not changed; the only peculiarity which I observed, was that in optical section, there may be distinguished, both in the peripheral welt and in the median lens, certain rounded cells which appear to be definitely individualised, though immersed in the protoplasm of the intermediate layer (fig. 7). I believe that these cells form the origin of a layer, which in the next stage will be found to have made its appearance between the blastodisc and the intermediate layer.

To the stage just described succeeds that represented in fig. 6. The eggs of this mass, which in the morning about ten o'clock presented the characters above indicated (figs. 4 and 5), had arrived in the afternoon about three o'clock at the stage which I am about to describe.

The blastodisc has extended considerably, and forms now a cap fitted to the truncated portion of the deutoplasmic globe, which has become slightly convex. The blastodisc has become flattened out and notably thinner. Between it and the intermediate layer has appeared an eccentrically placed cavity, and in the disc itself we can distinguish two regions: the one, central and thinner, forms the roof of the germinal cavity; the other, peripheral and thicker, forms a marginal welt to the blastodisc. This welt is in the form of a ring, broader at one side than the other, and thus brings about the eccentricity of the cavity.

The cellular constitution of the blastodisc has become notably modified, and first of all it is observable that the cells have multiplied and become much more voluminous. We may distinguish in the disc (assigning to it all which is placed out of the region of the intermediate layer, properly so-called):

1st. An enveloping lamella formed by a single layer of flat cells.

2nd. A layer constituted by polyhedric cells, pressed one against the other, clear and transparent, with a nearly homogeneous protoplasm and nuclei devoid of nucleoli. This layer forms the greater part of the blastodisc; it has its maximum

thickness in the broadest part of the marginal welt, so that in optical section it presents, taken altogether, the form of a comma, the head of the latter corresponding to the widened part of the peripheral thickening.

3rd. A layer formed by round cells, but little adherent to one another, finely granular, and provided with nuclei having punctiform nucleoli. This layer delimits the blastodisc inferiorly, but it does not exist throughout the area of the blastodisc; it is absent from the vault of the roof of the germinal cavity, or, at any rate, is only represented in this region by a few isolated cells. It is, on the other hand, fairly thick within the limits of the marginal welt of the blastodisc, which rests by its intermediation on the intermediate layer.

The intermediate layer has also undergone some modifications of importance. The median lenticular thickening no longer exists, but in its place are found on the floor of the germinal cavity certain cells which resemble in every respect the cells which line the inferior face of the blastodisc, and which form what we have called its third layer. It is manifest that the cells which rest on the floor of the germinal cavity are derived from the "median lens" of the intermediate layer; for we find by the side of completely isolated cells other cells, which, whilst projecting into the germinal cavity, are still partly implicated in the intermediate layer.

The peripheral welt has preserved very nearly the same condition as in the preceding phases, and its form is scarcely at all modified. Rounded cells, very like those which rest on the floor of the germinal cavity, are found embedded in the welt. Throughout the extent of the intermediate layer can be distinguished in contact with the deutoplasm a very regular range of flattened oval nuclei. The limits of the cells are too indistinct to justify one in saying that they form a simple pavement epithelium; but it appears as though an epithelial layer, formed by a single range of flat cells, were in the course of detaching itself from the deeper part of the intermediate layer. This epithelium rests directly on the surface of the deutoplasm.

Such is the series of the phases of this development, which I am able to describe with sufficient completeness to render it possible to draw positive conclusions from them The observations which I have made on the ulterior stages are too imperfect for publication. It now remains for me to compare the facts which I have above set forth with what is actually known relative to the formation of the germ-layers in Teleostean Fishes.

I. What comes out most strikingly in the first place from my

observations, with the fullest evidence, is this:—That the germ-layers do not proceed exclusively from the segmented germ, that part which at the conclusion of the cleavage-process forms what Lereboullet calls the *blastoderm*, and what I have denominated, with Haeckel, the *blastodisc*. A part of the germ-layers is derived directly from a protoplasmic layer which covers in the deuto-plasmic globe, and takes no part whatever in segmentation. It is this layer which Lereboullet designated under the name of "*membrane sousjacente au germe*," or at other times "*feuillet muqueux*." It was called "*vitelline membrane*" by Œllacher (10), "*Rinden-schicht*" by His (11), "*membrane intermediaire*" by Van Bambeke. It does not contain before the segmentation of the blastodisc any trace of cellular elements nor of nuclei; there develops in it, towards the conclusion of the segmentation period, by endogenous generation, a very large number of cells, and their mode of formation recalls in every respect the well-known phenomena of the formation of the blastoderm in Insects.

This intermediate layer was not recognised by Haeckel, which leads him to declare, "so können nur die Furchungszellen einzig und allein die Grundlage des enstehenden Fischkörpers bilden," and he attributes the formation of the endoderm to the invagination of the margin of the blastodisc. Is it possible to admit that this layer is absent in the eggs which Haeckel had under his eyes, and that the development of certain layers is brought about by invagination of the margin of the blastodisc in some Osseous Fish, and, on the other hand, in others at the expense of a special layer, which takes no part in the segmentation? I think not, when the identity of the constitution of the eggs is admitted, and I imagine that Haeckel himself will not feel tempted to adopt such a supposition. There are, moreover, several particulars in the drawings published by Haeckel which appear to me to prove that the intermediate layer existed in his eggs. In figures 55 and 56 of his pl. iv Haeckel figures the segmentation-spheres prolonging themselves outwards by means of a sort of tail applied to the surface of the albuminous globe (Eiweiss-kügel). If these figures are compared with figure 1 of my plate, it will be recognised that this tail corresponds to that part of the intermediate layer which extends outwards beyond the margin of the blastodisc.

This first conclusion from my observations gives support to the results arrived at and maintained in reference to various Osseous Fishes by Lereboullet, by Kupffer, by Owsjannikow (12), by Klein (13), by His, and by Van Bambeke.

I am, however very far from wishing to give any support to the famous "Parablast-theory" of Professor His, which is defended in so far as Osseous Fish are concerned by Owsjannikow.

The fundamental idea of this theory is that a part of the tissues of the embryo are not derived from the egg-cell but proceed from maternal cells, which immigrate into the vitellus and are destined to give rise to connective and vascular tissues. The embryo on this theory would be related to the maternal organism by a double connection; through its archiblastic tissues derived from the egg-cell it would be descended from parental archiblastic tissues; through its parablastic tissues it would be derived directly from the mother's connective and vascular tissues. Not only has this theory not been demonstrated by Mr. His, but the idea appears to me to be in formal contradiction to the facts which are the outcome of comparative embryology. But it is equally erroneously that Rathke (14), von Baer (15), Stricker (16), Reineck (17), Weil (18), and Œllacher have maintained that the various germ-layers are formed in the Osseous Fishes by a process of delamination at the expense of that part of the egg alone which takes part in the cleavage process. Whilst I cannot admit the view adopted by Götte and Haeckel—according to which the internal layer is formed by invagination of the margin of the blastodisc, and is accordingly derived like the external layer from the segmented disc—it by no means follows that I should deny that the phenomenon of invagination does take place in the Teleosteans. The following is the mode in which it seems to me necessary to interpret the phenomena which characterise the commencement of development. Directly after fœcundation the egg of the Osseous Fish divides into two very unequal cells, very dissimilar, differing in constitution and significance; the one is the germ which segments and from which the blastodisc is derived; the other is formed by the deutoplasmic globe—clothed, at least partially, by a thin layer of protoplasm forming "the intermediate layer." This cell is the origin of the endodermic layer of the future embryo; it has a constitution analogous to that of a fat-cell. This never proceeds to segmentation, but there appears in it at the conclusion of the segmentation of the other cell (the germ) a great lot of cells, which take their rise by "free-cell formation." Hence results the apparition of a cellular layer subjacent to the blastodisc. The latter is the homologue of the ectoderm of other Vertebrates, the former is the homologue of the endoderm. It is ascertained that the blastodisc extends its area little by little and tends to cover in by "epiboly"—the deutoplasmic globe. The observations of Van Bambeke have rendered it very probable that the intermediate layer makes a simultaneous progressive movement. The upshot of this is that the development of the Teleosteans commences by the total cleavage of the vitellus into two cells; one of the two continues to segment whilst the other

retains its characters and remains undivided. The fact of a greater indisposition to segment on the part of the cells destined to give rise to the endoderm is very frequent, not only among the Vertebrates but also in the other divisions of the animal kingdom. It is exhibited always in the case of the formation of a gastrula by epiboly ; and, indeed, it is after this mode that the development of the gastrula of the Teleosteans is carried out. The "discogastrula" has, then, no existence as the result of a special mode of gastrulation ; "discoidal segmentation" differs in no essential respect from the "inequal segmentation"(Inequale Fürchung) of Haeckel.

An objection to these views may perhaps be made on the score of the peculiar mode of formation of the cells of the intermediate layer, which do not result from segmentation. I may, in reply, call to mind that Strasburger (19) has demonstrated that the endogenous generation of cells is not a primordial form of cell-generation but a secondary mode derived from division pure and simple. It is connected to division by a complete series of intermediate forms. The simultaneous formation by the endogenous mode of a great number of cells in a single cell is an abbreviation or condensation of successive divisions which have become less and less complete. It is clear that the formation of the blastoderm of Insects must be interpreted in this manner, as well as the existence in the group of the Arthropods, chiefly among the Crustacea, of a series of transitional forms. This view receives in the present case a new application ; the formation of the endoderm in the Osseous Fish has the same relation to the development of the layers by regular segmentation, such as is presented, for instance, by the Acrania, by the Cyclostoma and Batrachia, which the formation of the blastoderm in insects has to the total cleavage observed in many Crustacea.

II. On comparing my observations on the constitution and development of the intermediate layer with those of my predecessors I find that Kupffer is the only one who has recognised and described in Osseous Fishes the development of cells on the surface of the deutoplasmic globe, beyond the area of the segmented germ. The "nuclear zone" of Kupffer, observed in the genera *Gasteroseus* and *Spinachia* evidently corresponds to that part of my intermediate layer which lies outside the blastodisc. The part of this layer situated under the blastodisc corresponds to the "*membrane formed of vitelline granules*," to the "*membrane underlying the blastoderm*," or to the "*mucous layer*" of Lereboullet. I find in it the essential characters attributed by Van Bambeke to his intermediate layer. The peripheral welt, triangular in section, on which rests the margin of the blastodisc, was perfectly described by Van Bambeke. As to the

"median lens" of my eggs, it appears to be absent in other Osseous Fishes. It even appears, according to some observers, that only the peripheral welt exists at first, and that secondarily by growth from the periphery towards the centre the intermediate layer becomes complete beneath the blastodisc.

Kupffer has put forward the opinion that his "nuclear zone" is something quite distinct from the "feuillet muqueux" of Lereboullet. It is clear from my observations that the two authors have had under their observations simply different parts of one and the same layer, the extent and characters of which vary in all probability from one genus to another. It is this same layer which Œllacher described under the name of 'vitelline' membrane. This excellent observer has been unfortunate in the choice of a name for the layer and has been led into error in assuming that the cells which he found therein at the conclusion of segmentation are cells derived from the blastoderm, having fallen from the roof of the germinal cavity.

Klein not only recognised this same layer in the Trout, but he demonstrated that cell-nuclei appear in it in great quantity towards the end of the period of segmentation. He gives to this layer the name of parablast. His, who has seen the same objects in the Salmon, gives to this intermediate layer the name of *Rindenschicht*, the peripheral welt is called by him *Keimwall*, and the cells observed therein at the conclusion of cleavage are designated by him *Parablastisch* or *Nebenkeim Zellen*.

III. At no period of development have I found any trace of that cavity which Van Bambeke calls the "*cavité de segmentation.*" Kupffer, who was not able to demonstrate any such cavity in living eggs, found it in eggs of *Gobius niger*, which had been previously hardened in a solution of sulphuric acid. Lereboullet found, when he made the eggs of the Perch and the Pike coagulate, that the blastoderm, is at a given moment, "a hollow vesicle." Van Bambeke himself could only persuade himself of the existence of his "segmentation cavity" by the examination of sections cut from hardened eggs. This cavity has not been asserted to exist in the Trout by Reineck, nor by Weil, nor by Stricker, nor by Klein, nor by Œllacher, nor by His. No more did Haeckel find any trace of it. I may then venture to affirm, on the testimony of these various observers and on the strength of my own researches, that the presence of a cavity in the substance of the blastodisc is not a constant fact; even more than this, no author has been able to prove its existence in a living egg; it is therefore possible that in the cases where it has been asserted to exist it is an artificial product. The absence of any regular disposition of the cleavage spheres

around this cavity tends to confirm this view, as Kupffer has pointed out. I am anxious to insist, moreover, upon the fact that the name of "segmentation cavity" given to this cavity is not at all appropriate to it.

The segmentation cavity, as known in Amphioxus, the Cyclostomes, the Batrachians, and a host of invertebrates, is invariably situated between the ectoderm and the endoderm. It is limited on one side by the concavity of the ectoderm, on the other by that part of the primitive vesicle which will, after its invagination is accomplished, constitute the endoderm. If we once admit that the intermediate layer of Osseous Fishes is the homologue of the endoderm of the other Metazoa, it is clear that the cavity which develops between the blastodisc and the intermediate layer alone deserves this name. This cavity, which existed in my eggs, and which the majority of embryologists have observed in Teleosteans, is generally designated by the name of germinal cavity (Keimhöhle). It is indispensable to modify our terminology in accordance with the preceding observations. I propose, then, to designate under the name of *cavity of Lereboullet* the cavity pointed out by this author in the midst of the blastodisc in the Perch and the Pike, by Van Bambeke in the Roach, and by Kupffer in *Gobius niger*. Balfour (20) has discovered a homologous cavity in the Elasmobranchs.

It is necessary, on the other hand, to give the name of *cavity of von Baer*, *segmentation cavity*, or *blastocœl*, to the space which appears at the conclusion of egg-cleavage, between the blastodisc (ectoderm) and the intermediate layer (endoderm). It is this cavity which was described in the Trout by Stricker, by Reineck, by Weil, by Œllacher, by Klein, by His, and by Götte, and which has been called sometimes *Fürchungshöhle*, sometimes *Keimhöhle*. In common with Œllacher I consider this cavity as the homologue of the germinal cavity of the chick. It is for this reason that I think the name of Keimhöhle may be retained equally with the other names for this cavity. The existence of this cavity appears doubtful in the Roach and in the genera Gasterosteus and Spinachia, if we may judge by the observations of Van Bambeke and Kupffer.

IV. A final question which I wish to enquire into relates to the ultimate destination of the two primordial layers of the embryo of osseous fishes—the blastoderm on the one hand, and the intermediate layer on the other. At the phase represented in figure 6 of my plate the embryo is composed, if we except the enveloping lamella, of three cellular layers well defined in the marginal welt. The external one, limited by the enveloping lamella, is evidently derived from the blastodisc; the internal

one is only the intermediate layer, in the deepest part of which the cells are disposed as a simple pavement epithelium. Between the two exists an incomplete layer of cells which are very different. This layer is absent from the vault of the roof of the segmentation cavity, and its thickness diminishes progressively from without inwards. It is evidently this layer which Haeckel considered to be formed by the invagination of the margin of the blastodisc and as representing the endoderm. What is the origin of this layer? Is it derived from the blastodisc, or, on the other hand, is it composed of cells derived from the intermediate layer? I believe that it is composed of cells which originate in the peripheral welt of the intermediate layer. I base this opinion on the following considerations :

1st. The cells of this layer have the same dimensions, the same form, the granular texture, the same oval nuclei, sometimes irregular and always nucleolated, which we observe in the cells found in the substance of the peripheral welt, and which are, without doubt, of endodermic origin. They are, on the other hand, very different from the cells of the blastodisc.

2ndly. The cells which rest on the floor of the segmentation cavity, and which are formed at the expense of the "median lens" of the intermediate layer, possess the same characters as those which form the middle layer.

3rdly. The blastodisc remains all this time very sharply delimited inferiorly, and in no part is there a passage from one to the other. In no part have I found the slightest indication in favour of invagination.

According to my view, the intermediate layer not only furnishes the epithelium of the digestive canal, but it intervenes largely in the formation of the middle layer, to which it probably furnishes the connective and vascular elements. In the phase represented in fig. 6 we must then distinguish—

1st. The enveloping lamella.

2nd. An ectodermic layer derived from the blastodisc, and destined to subdivide subsequently into sensorial lamella and external mid layer (first and second secondary blastodermic layers of Haeckel).

3rd. Of an internal mid layer, of endodermic origin, destined to furnish the vessels and the connective tissues (third secondary blastodermic layer of Haeckel).

4th. Of an internal layer, destined to furnish new cells to the internal mid layer and to give rise to the epithelium of the digestive tube (fourth secondary layer of Haeckel).

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On the Homologies *of the* Suspensor.[1]

By Sydney H. Vines, B.A., B.Sc., Fellow and Lecturer of Christ's College, Cambridge. (With Plate V.)

The first definite account of this structure seems to have been given by Mirbel.[2] He says, "un fil très-délié, le suspenseur, descend du sommet de l'ovule dans la quintine, et porte à son extrémité un globule qui est l'embryon naissant." Robert Brown also observed it in his investigation of the development of the embryo of Orchids.[3] He describes it as "a thread consisting of a simple series of short cells .... the lowermost joint or cell of which is probably the original state of what afterwards, from enlargement and deposition of granular matter, becomes the opaque speck or rudiment of the future embryo." In his paper on "The Plurality and Development of the Embryos in the Seeds of Coniferæ,"[4] he states that in *Pinus sylvestris* "this filament or funiculus consisted generally of four series of elongated transparent cells or vessels, usually adhering together," and further on he adds, "the opaque granular extremity of the funiculus is evidently the rudiment of an embryo." Hartig[5] also described and figured the suspensors of Conifers so early as 1840.

Mettenius[6] was apparently the first to detect the presence of a similar organ in the developing embryo of a cryptogamic plant. In his account of the changes taking place in the central cell (oosphere) of the archegonium of *Selaginella involvens*(?) in consequence of fertilisation, he mentions that a suspensor (Embryoträger) is formed from its upper half. This observation was confirmed by Hofmeister in his "Vergleichende Untersuchungen",[7] and more recently the whole subject of the development of the embryo of Selaginella has been investigated by Pfeffer,[8] with results which will be discussed hereafter.

It is usually believed that the suspensor which is found in Selaginella, in Gymnosperms (Archisperms), and in Angiosperms (Metasperms), is a special organ, no trace of which is to be de-

[1] *Suspenseur*, Mirbel. *Embryoträger*, *Keimträger*, *Keimstrang*, *Chorda embryonalis*, Schleiden and others. *Keimschlauch*, Unger. *Vorkeim*, Hofmeister.

[2] 'Ann. d. Sci. Nat. Jour.,' xvii, 1829.

[3] 'Trans. Linn. Soc.,' vol. xvi, 1833. This paper is contained in the 'Collected Writings of Robert Brown,' published by the Ray Society.

[4] 'Ann. and Mag. of Nat. Hist.,' 1844. This paper is contained in the 'Collected Writings of Robert Brown,' published by the Ray Society.

[5] 'Naturgesch. d. först. Culturpflanzen,' 1840. (Explanation of plate xxv.)

[6] 'Beiträge zur Botanik.,' Heft 1, 1850.

[7] Leipzig, 1851.

[8] In Hanstein's 'Botanische Abhandlungen,' Bd. i, 1871.

tected among the lower Cormophytes. Recent embryological investigations suggest, however, that the suspensor of the higher members of this group is not so completely isolated an organ as at first sight it appears to be, but that true homologues of it are to be found in the developing embryos of the lower. A comparison of the principal facts in the embryology of different members of the group of Cormophytes will, I think, suffice to show that there is ample evidence for the support of this view.

The development of the suspensor in the embryo-sac of an Angiosperm may be briefly described as follows.[1] As a consequence of fertilisation the germinal vesicle (oosphere—central cell) is divided, as a general rule, by a horizontal wall into two cells, a superior and an inferior. The upper of these two cells is attached to the wall of the embryo-sac, and undergoes numerous divisions, both transverse and longitudinal, in consequence of which it gives rise to a mass of tissue of an elongated form, the suspensor, which bears at its inferior end the embryo which has been formed from the lower of the two cells into which the germinal vesicle primarily divided. In that portion of the suspensor which immediately abuts upon the embryo a large well-defined cell is formed, which is termed the hypophysis, and which, by repeated division, gives rise to the tissues which complete the layers of that portion of the embryo from which subsequently the primary root is formed (fig. 1, A, B, C).

This account is generally applicable to the process of development in all Phanerogams. Modifications of the process certainly occur in various plants. In the Grasses, for instance, the suspensor consists of a mass of cells, which is larger, in the early stages, than the embryo itself, and to its apical portion the special name has been given of "embryonic appendage." (*Keimanhang*, Hanstein.)

It may be generally concluded, therefore, that it is from the superior (*i.e.* the one nearer the micropyle) of the two cells formed by the division of the germinal vesicle that the suspensor is derived, and further, that the lowest cell of the suspensor, the hypophysis, contributes to the embryo the tissues which go to form its primary root.

We may now pass on to consider the embryology of the Conifers, as being a group representative of the Gymnosperms, which will be found to differ in many important respects from that of the Angiosperms. In so doing it will be advisable at the outset to accept the

[1] The facts here mentioned are based upon the following researches:—Hofmeister, 'Entstehung. d. Emb. d. Phanerogamen,' Leipzig, 1849. Id., "Neuere Beobacht.," in Pringsheim's Jahrbüch., vol. i, 1858. Hanstein, 'Botanische Abhandlungen,' vol. i, 1870. Fleischer, 'Beit. zur Embryologie,' Regensburg, 1874.

view of Strasburger,[1] in accordance with which the whole central-cell of the corpusculum is to be regarded as being the true oosphere, and therefore as the homologue of the germinal vesicle of Angiosperms, as opposed to that of Hofmeister,[2] according to which a formation of secondary germinal vesicles takes place within the central cell, one of which is fertilised and gives rise to the embryo. Strasburger considers the secondary germinal vesicles described by Hofmeister to be merely vacuoles.

One of the principle changes to be observed in the oosphere of *Pinus sylvestris* in consequence of fertilisation is the disappearance of its nucleus, and this is immediately followed by the appearance of four new nuclei in that portion of its protoplasm which is most distant from the neck of the corpusculum (archegonium). Each of these nuclei exercises an influence upon the protoplasm which surrounds it, in consequence of which the granules of the protoplasm are arranged in lines radiating from the nucleus. Intermediate between any two nuclei there is observable a vertical row of large granules, which defines the limits of each cell and the position of the future cell-wall, and a horizontal row (in section) of similar granules marks off the whole mass of protoplasm in which the four nuclei lie from the remaining protoplasm of the oosphere. These nuclei then divide, and four new cells are formed, which lie in one plane above the preceding, from which they are separated by a cellulose wall. The four upper cells then divide, and in this way three layers, each consisting of four cells, are formed. The cells of the uppermost layer remain as a rosette in the base of the oosphere, those of the middle layer elongate considerably and form the suspensors, and those of the lowermost layer give rise each to an embryo (fig. 2).

The similarity between the process of the development of the embryo of Pinus and the general account previously given of that of Angiosperms is at once apparent. In both cases the cell, from which the new individual is to be developed divides primarily into two, from the upper of which the suspensor is developed, from the lower the embryo itself. The principal difference is that in Angiosperms, it is the whole oosphere which thus divides into two, whereas in Gymnosperms the dividing cell has been derived, by a process which is essentially one of free-cell formation, from the oosphere.

In the further development of the embryo, there is one particular in which the process occurring in Gymnosperms differs from that in Angiosperms, and that is the mode of origin of the

[1] 'Befruchtung der Coniferen,' 1869, p. 18, also 'Die Coniferen und Gnetaceen,' 1872, p. 279, and 'Zellbildung und Zelltheilung,' 2te Aufl., 1877, p. 18.

[2] 'On the Higher Cryptogamia,' p. 412, Ray Society, 1862.

root. In the latter case, as has been already pointed out, the root is derived from the hypophysis, *i.e.* from a cell belonging to the suspensor. In the former case it appears, from the researches of Strasburger, that the root is entirely developed from the tissues of the embryo itself.

In passing from the Gymnosperms to the higher Cryptogams we come first to the class of the Dichotomeæ. Of the embryology of the members of one subdivision of this class, that of the Lycopodiaceæ, comparatively little is known, whereas that of the members of the other subdivision, the Ligulatæ, has been to some extent investigated. Pfeffer[1] has given a very full account of the development of the embryo of Selaginella, one of the two genera included in the group Ligulatæ. According to him the first division of the fertilised oosphere of the archegonium takes place in a horizontal plane, and thereby two cells are formed, an upper and a lower. The upper cell, usually after having undergone divisions, becomes elongated and forms the suspensor. The lower cell is divided by a septum, 2 (fig. 3), which is nearly at right angles to the plane of the first septum, into two equal segments, (A and B), and of these two segments one (A) is further subdivided by the formation of a wall, 3, which extends from about the middle of the height of wall, 2, to the external wall of the cell. By this means a wedge-shaped segment (C) is formed, which is the apical cell of the embryonic axis. In the remaining portion of segment A a wedge-shaped cell is formed, which is immediately adjacent to the segment C, and a similar cell is formed in a corresponding position in segment B. These two cells are the apical cells of the two first leaves (cotyledons) fig. 3, A. The remainder of segment A contributes to the formation of the hypocotyledonary axis, as does also that portion of segment B which lies immediately behind the apical cell of the leaf which arises from it (fig. 3, B). From the cells still further back, the structure to which Pfeffer gives the name of "foot," is developed, and lying back between the foot and the suspensor are some cells from which the root takes its origin.

On comparing the development of the embryo of Selaginella with that of the embryo of an Angiosperm, it is at once apparent that the suspensor in each case is developed in the same manner, and the one is therefore the homologue of the other. There appears to be a development, in Selaginella, of a new organ, the foot, which has no representative in the embryo either of a Gymnosperm or of an Angiosperm. The propriety of applying the term "foot" to this structure will be discussed hereafter. Further, the mode of development of the root of Selaginella differs from that obtaining among Angiosperms, and resembles that above de-

[1] Loc. cit.

scribed as occurring in Gymnosperms, in that it is developed, not from a cell belonging to the suspensor, but from the tissue of the embryo itself. It appears that in Selaginella we have the first indication of that mode of the development of the root which is more evident in Gymnosperms.

The embryology of Isoëtes, the other genus included in the group Ligulatæ, although it has been investigated by Mettenius,[1] by Hofmeister,[2] and quite recently by Bruchmann,[3] is by no means so clearly made out as that of Selaginella. Enough is known, however, to show that there is a wide difference between the modes of development of the embryo in the two plants. According to Bruchmann the oosphere of Isoëtes is divided into two, as is that of Selaginella, by a horizontal wall. The upper cell grows rapidly towards the neck of the archegonium, and forms the cotyledonary portion of the embryo. The lower cell grows slowly downwards into the spore, and forms the hypocotyledonary portion of the embryo. At the lower part of the anterior surface of the cotyledon, a cell grows out into a prominence, from which the "ligula" is subsequently formed. The cells lying immediately at the base of this organ, give origin to the growing point of the stem. From that part of the hypocotyledonary portion of the embryo which is diametrically opposite to the growing point of the stem, the first root is developed, and the remainder of the hypocotyledonary portion goes to form a mass of tissue to which the name "foot" is given. From this brief account it appears that the development of the embryo of Isoëtes differs from that of Selaginella in two principal particulars, viz. (1) that one of the two primary cells gives rise, in the former, to a root and to a foot, whereas in the latter a suspensor is developed from the corresponding cell; and (2) that Isoëtes possesses only one primary leaf (cotyledon), whereas Selaginella possesses two. A discussion of these points of difference will follow in the concluding paragraphs of this paper.

The next group of plants to be considered is that of the Rhizocarpeæ, and of the plants included in it Marsilia and Salvinia are those whose embryology has been most fully investigated. From the observations of Hanstein[4] upon Marsilia, it appears that the oosphere is first divided by a nearly vertical wall into two cells, one anterior,[5] the larger, the other posterior, and then each of these cells is divided by a septum at

[1] Loc. cit.

[2] 'Beit. z. Kennt. d. Gefässkryptogamen,' i, 1852.

[3] 'Jenaische Zeitschrift,' 1874.

[4] "Befr. u. Entwick. d. Gattung Marsilia," Pringsheim's Jahrb., iv, 1865.

[5] The word anterior implies that this cell is the nearer to the apex of the prothallus.

right angles to the first. Of the four segments thus formed (fig. 4), Hanstein regards the anterior superior as giving rise to the first leaf, the anterior inferior to the apical cell of the stem and to a part of the foot, the posterior superior to the root, the posterior inferior to the larger part of the foot. He points out that the apparent origin of the stem and of part of the foot from the same segment is contrary to expectation. It seems, however, to be unnecessary to regard any portion of the segment from which the apical cell of the stem is derived as forming part of the foot. The apical cell is certainly the one by the divisions of which the stem of the plant is formed, but the cells belonging to the same segment are as much representatives of the stem, in the early stages of development, as is the apical cell. Hanstein himself suggests that this portion of the anterior inferior segment should be regarded as a "paracotyledonary" portion of the axis.

In Salvinia (fig. 5), according to Pringsheim,[1] the oosphere is divided by a nearly vertical wall, and the anterior of the two cells formed is divided by a septum at right angles to the first. The superior half of the anterior cell forms the first leaf (cotyledon), and the inferior half the stem. The whole of the posterior cell forms the foot (stielchen—caulicle). The process of development resembles that of Marsilia, but differs in that the posterior of the two primary cells is not differentiated into a root-segment and a foot-segment. In consequence of this Salvinia is entirely destitute of roots.

It appears that in Ferns the plane of the first division of the oosphere intersects the vertical at a variable angle, but that this angle is always less than a right angle. The division of the two cells thus formed varies in different individuals. Hofmeister[2] points out that in *Pteris aquilina* the septum dividing the anterior of the two first formed cells is inclined to the first septum at an acute angle, whereas in *Aspidium filix-mas* the angle between the new septum and the first one is nearly a right angle. The septum dividing the posterior cell, in Pteris, is inclined at nearly a right angle to the primary septum, in Aspidium the angle between the corresponding septa is acute. In his account of the development of the embryo of *Ceratopteris thalictroides*, Kny[3] makes no exact estimate of the angles between the primary and the two secondary septa, but it appears

[1] "Zur Morphologie der Salvinia natans," Jahrbüch. f. wiss. Bot., iii, 1863.

[2] 'On the Higher Cryptogamia,' Ray Society, p. 200.

[3] "Keimung und Entwickelungsgeschichte von Ceratopteris," 'Bot. Zeitg.,' 1874. Also, "Die Entwickelung de Parkeriaceen," 'Nov. Act. Leop. Carol. Akad.,' 1875.

that they are nearly right angles. The result of these divisions is that the embryo consists of four cells. Of these, according to Hofmeister (fig. 6), in *Pteris aquilina*, the anterior inferior cell is the one from which the first leaf (cotyledon) is developed, as also the apical cell of the stem, the posterior inferior gives rise to the root, and the two superior cells together form the foot. He considers that in *Aspidium filix-mas* the anterior inferior cell gives rise, as in Pteris, to the first leaf and to the apical cell of the stem, that the root is developed from the posterior superior cell, the foot from the anterior superior cell, and that the posterior inferior cell only grows to a slight extent, and does not form "a detached portion of the germ-plant, but forms the cortical portion between the back of the first frond and the first root."

Hofmeister's account above given of the development of the embryo of Pteris presents the difficulty which has already been met with in the consideration of Hanstein's account of the development of the embryo of Marsilia, viz. the origin of a portion of the foot from the anterior of the two cells into which the oosphere primarily divides. The account given by him of the development of the embryo of Aspidium is apparently incomplete, and presents the same difficulty as the preceding. Kny's recent investigations, to which allusion has already been made, afford some clue to a more correct interpretation of the facts. Kny finds that the oosphere of Ceratopteris gives rise by division to four cells, which lie in a plane parallel to that of the prothallus, two of these cells being anterior and two posterior. The two anterior cells produce the first frond, and later, the apical cell of the stem. From one of the posterior cells the root arises, from the other, the foot.

Applying these results to the observations of Hofmeister, it may probably be correctly stated that the two anterior cells of the embryo of Pteris give rise to the first leaf and to the apical cell of the stem, and that of the posterior cells, the superior forms the foot, the inferior the root. In the case of Aspidium it might equally be said that the two anterior cells give rise to the first leaf and to the apical cell of the stem, and that probably the foot is here really formed by the posterior inferior segment, to which Hofmeister assigned no special function, the root being derived from the posterior superior segment.

In the Equisetaceæ the first division of the oosphere is in a plane, which is somewhat inclined to the longitudinal axis of the archegonium,[1] and septa at right angles to the primary wall subdivide the two cells. At this stage the embryo consists of four cells arranged as the quadrants of a sphere. From the two lower the

[1] Hofmeister, loc. cit,. p. 300.

foot takes origin, and from one of the upper quadrants the stem is developed. Sadebeck,[1] in a recent paper, has given an account of some observations upon the embryology of Equisetum. Unfortunately I am only able to become acquainted with them by means of a short abstract. It appears from this that the most superficial segment (*i.e.* the anterior superior) developes into the stem, the most deeply placed into the root, and that the two lateral segments together form the first leaf-sheath, no foot being developed.

The development of the embryo of the Mosses[2] begins with a division of the oosphere in a plane, which is nearly or quite at right angles to the axis of the archegonium, and of the two cells thus formed the superficial forms the sporogonium, the deeply placed, the seta and foot. According to Kienitz-Gerloff,[3] however, the sporogonium of the true Mosses is formed, not from the whole of the upper segment of the oosphere, but from a portion only of it, the remainder of the segment remaining undeveloped. Leitgeb[4] had already noticed in Anthoceros that one half of the upper segment became more developed than the other, a condition which may have become more fully expressed in Mosses.

The first division of the oosphere of the Hepaticæ (fig. 7) takes place in a plane, which in the lower forms intersects the axis of the archegonium at an acute angle, but in the Jungermannieæ at right angles. The more superficial of these two cells—the one, that is, which more immediately underlies the neck of the archegonium—undergoes numerous divisions, by means of which the tissue of the future sporogonium is formed. The tissue resulting from the frequent division of the more deeply placed cell forms, according to Kienitz-Gerloff,[5] the seta, the lower expanded portion of which is termed the foot. The only exception to this mode of development is to be found in Riccia, the oosphere of which simply gives rise to a sporogonium.

It appears, then, that with this solitary exception, the first

[1] Read at Hamburg, Sept. 26, 1876.

[2] The embryology of mosses is treated of in the following works :— Hofmeister, 'On the Higher Cryptogamia,' Ray Society, 1862. Schimper, 'Rech. anat. et physiol. sur les Mousses,' 1848. Kühn, 'Entwick. d. Andreæaceen,' Bot. Mittheil., v. Schenk und Luerssen, Bd. i, 1874. Vouk, "Entwick. d. Sporogoniums von Orthotrichum," Sitzber. d. Wien. Akad., 1876.

[3] 'Sitzber. d. Gesell. Naturf. Freunde zu Berlin.' März, 1876.

[4] 'Unters. ueb. Lebermoose,' Heft. ii, 1875.

[5] "Vergl. Unters. ueb. Entwick. d. Lebermoos-sporogoniums," Bot. Zeitg., 1874-75, see also Leitgeb, "Entwick. d. Kapsel. v. Anthoceros," Sitzer. d. Wien. Akad., 1876.

stage in the development of the embryo of all Cormophytes is the division of the oosphere (central cell—germinal vesicle) into two cells, one of which is more particularly devoted to the formation of the embryo, and may be specified as the "embryonic cell," the other forming a structure which maintains for a time the connection between the embryo and the structures in which it is embedded, and to this the name "embryophore" may be given. Among the lower Hepaticæ the plane of this first division is nearly vertical, but in the Jungermannieæ and in the true Mosses this plane is horizontal. The relation existing between these segments may be made evident by supposing the oosphere of the lower Hepaticæ to be rotated through 90° in such a way that its embryonic or anterior cell may come to occupy the same position as that of the corresponding cell of the Mosses. The relations existing between the segments of the oospheres of ferns, of Marsilia, and of Salvinia, and those of the oosphere of Isoëtes, may be indicated in a similar manner, and it will be found that the anterior cell of the embryo of the former plants correspond to the superior cell of the embryo of the latter. In Selaginella, in Conifers, and in Angiosperms, the embryonic cell occupies the inferior position. In order to compare the embryo of one of these plants with that of a Fern, for instance, it would be necessary to imagine the oosphere of the Fern to have been rotated through 90° in a direction opposite to that in which it had to be rotated in order to be comparable with the embryo of Isoëtes.

Whatever may be the relative position of the embryonic cell to the embryophore, whether it be anterior to it, or above it, or below it, the organs to which it gives origin suffice to indicate its true nature in all cases. In the Liverworts and Mosses it gives rise to a mass of tissue, from which the sporogonium is formed, and which is morphologically a thallome. In the higher Cormophytes this is the case in the early stages, but at a later period differentiation takes place into caulome and phyllome, and apparently, in some cases, such as Selaginella and Conifers, roots also may be developed from it. In Mosses and Liverworts the embryophore forms the seta upon which the sporogonium is borne, but no differentiation takes place. This is the case also in some of the higher forms. In Salvinia the foot is formed from the whole embryophore, and apparently the suspensor of Selaginella and of Conifers is formed in the same way. These facts justify the conclusion that the suspensor of Selaginella and of Conifers is completely homologous with the foot of Salvinia and with the seta of Mosses

[1] Selaginella and the Conifers (Gymnosperms?) must be regarded as possessing no true primary roots. Such roots are developed only from a segment of the embryospore.

and Liverworts. The mass of cells in the embryo of Selaginella, to which Pfeffer gives the name "foot," is certainly not homologous with the foot of the other Cryptogams, nor is it analogous to it. The function of the foot appears to be to serve for a time as a means of connection, though not of organic connection, between the embryo (sporophore) and the prothallus (oophore). It is quite evident that this function is discharged in Selaginella, not by the so-called foot, but by the suspensor. The word foot can, therefore, be no longer rightly applied to the dilated hypocotyledonary portion of the stem of Selaginella.

The products of the division of the embryophore of ferns, of Marsilia, and of Isoëtes, are differentiated at an early stage, one portion giving rise to the foot, the other to the root, and a similar differentiation also takes place in the case of Angiosperms, but at a later stage. The *complete* homology of the suspensor of Angiosperms with the foot of Ferns, of Marsilia, and of Isoëtes, is thus made apparent. The analogy between these organs is also clear. In these Cryptogams the foot serves as a connection between the embryo and the prothallus. In Angiosperms the only representatives of the prothallus are the antipodal cells. In consequence of this rudimentary nature of the prothallus the embryophore becomes attached, not to it, but to the wall of the embryo-sac (macrospore), and developes into the suspensor.

It has now been shown that the seta and foot of Mosses and Liverworts, the foot of the vascular Cryptogams,[1] the suspensor of Selaginella, of Gymnosperms, and of Angiosperms, are derived from that cell, produced by the division of the oospore, to which, for the sake of clearness, the name of embryophore has been given. These organs may, therefore, be regarded as being truly homologous, and this view is not invalidated with by the fact that the suspensor or the foot is developed, in some cases, from the whole of the embryophore, in others from a part only of it. This fact merely renders the homology incomplete in certain cases.

[1] In consequence of the existing uncertainty with regard to the mode of development of the embryo of Equisitaceæ, this generalisation must not be regarded as applying to that group.

The Red Vascular Fluid *of the* Earthworm *a* Corpusculated Fluid. By E. Ray Lankester, M.A., F.R.S.

In describing the anatomy of the Earthworm in the sixth volume of this Journal some years since, I made the statement, which was in agreement with the current opinion, that the red vascular fluid of that animal is free from corpuscles. This statement, like several others contained in the same essay, is erroneous. I am happy in this case to be able myself to furnish a more correct account of a feature in the organisation of the Earthworm which, however small and insignificant in point of fact, has yet been the subject of much discussion and speculation.

Our positive knowledge of the significance of the red vascular fluid of Chætopodous worms was materially advanced by Nawrocki's demonstration in 1867, that the colouring matter of this fluid in the Earthworm is hæmoglobin—a discovery which I independently confirmed and extended by spectroscopic observation of other Chætopoda ('Journal of Anatomy,' 1867, p. 114; ibid., 1869, p. 119, and 'Proe. Roy. Socicty,' 1873, No. 140.) The fact, however, that abundant corpuscles are present in this same fluid in the case of the Earthworm (and as appears very probable in all similar fluids) has hitherto escaped detection, owing to the difficulties of observation which small corpuscles floating in a deeply coloured liquid present, and also to the fact that the method by which they may be rendered apparent has not been applied to them by the various observers who have occupied themselves with this matter.

The gifted and laborious investigator of the anatomy of the Chætopoda, Edouard Claparède, so far anticipated the observation which I have made in the case of Lumbricus, as to discover in the red vascular system of Cirrhatulus, Ophelia, and Staurocephalus (identical in its general anatomical features with that of Lumbricus), floating histological elements or corpuscles. He says in his introduction to 'Les Annélides Chétopodes du Golfe de Naples,' p. 19, "L'existence de corpuscles du sang dans les vaisseaux de certaines Annélides est ajourdhui indubitable. M. de Quatrefages, dans son *Histoire des Annelés*, en admet trois exemples; les Glycères, les Phoronis, et les Syllidies. Ce dernier seul a de la valeur. En effet chez les Glycères, les corpuscles rouges appartiennent au liquide de la cavité periviscerale, et quant aux Phoronis, elles ne pourront guère conserver leur place parmi les Annélides. Mais, sans parler d'une ancienne observation de Rud. Wagner relative à une Terebelle, observation d'ailleurs confirmée par M. Kölliker, on

peut en citer d'autres exemples. Dans ce mémoire, ou trouvera des corpuscles sanguins proprement dits, décrits chez les Ophélies, chez les Cirratuliens, chez les Staurocéphales."

The same zoologist, however, in his classical 'Histologische Untersuchungen über den Regenwurm,' published in 1869, explicitly affirms the absence of such corpuscles from the vascular fluid of the Earthworm. Speaking of the remarkable spherical dilatations of the blood-vessels which occur on the walls of the segmental organs or nephridia and in other parts, he writes what is here translated, "The structure of these dilatations of the vessels is of such a nature that it is not possible to regard them as accidental. Gegenbaur, moreover, says that he has always seen them filled with a red coagulum enclosing blood-corpuscles. Lankester also saw a granular matter within them. In point of fact I find in them constantly a quantity of nuclei, which in all probability are derived from the division of an ordinary nucleus of the vessel's wall. Such nuclei I am unable to regard with Gegenbaur as blood-corpuscles, *since it is a well-known fact that blood-corpuscles are absent from the Earthworm.*"

The statement made by Gegenbaur, and here referred to, occurs in his article "Ueber die sogenannten Respirations-organe des Regenwurms" in the 'Zeitsch. fur Wiss. Zoologie,' 1852, vol. iv, p. 227. He says "Das Lumen dieser Anschwellungen stellte sich mir fast immer mit einem rothen, Blutkörperchen einschliessenden coagulum ausgefüllt dar." The blood-corpuscles thus recorded by Gegenbaur are those only of the vascular dilatations which differ in character from those which I shall describe below, and though clearly entitled to rank as blood-corpuscles or corpuscles of the red vascular system, are peculiar and apparently confined to these dilatations.

By various writers the absence of corpuscles from the vascular fluid of the Chætopoda has been considered a sufficient objection to the use of the word 'blood' in reference to that fluid, and it has been spoken of as a 'pseud-hæmal,' as distinguished from a true 'hæmal' fluid. A variety of views have also been put forward at different times as to the relationships of this fluid and the vessel which contain it, and of the perienteric or peritoneal corpusculated fluid, to the 'blood' of Mollusca, of Arthropoda and of Vertebrata. Gegenbaur, in his 'Grundzüge der vergleichenden Anatomie,' 2nd edition, 1870, p. 231, in describing the existence of a fluid contained in a distinct vascular system, and of another fluid occupying the body-cavity in the Nemertine worms says, "We shall speak of this latter as chylus, of that contained in the closed vascular system as blood." Further on (p. 233) he applies the same nomenclature to the similar fluids of the Chætopoda, with which the somewhat dif-

ferent vascular apparatus and fluid of the Hirudinea is associated. Taking thus a comparative view of the blood and blood-vessels of the Chætopoda, Gegenbaur was led, especially by a consideration of the condition of the Leeches, where (with the exception of Branchiobdella) the vascular system and perienteric system are in open communication, to attach little importance to the recorded presence or absence of corpuscles in the blood (vascular fluid) which he held to be only a portion of the general liquid of the body cavity which had been gradually differentiated.

Professor Huxley, in his 'Manual of the Anatomy of Invertebrated Animals, 1877,' p. 219, writing of the oligochætous Chætopoda, says, "In addition (to the colourless corpusculated fluid of the perivisceral cavity) there is a system of pseud-hæmal vessels like those of the leeches, provided with contractile walls, and containing a red, non-corpusculated fluid. No communication has been ascertained to exist between these vessels and the perivisceral cavity; but there can be little doubt that, as in the case of the leeches, they must be regarded as a specially differentiated part of the general system of the perivisceral cavity." Further on (p. 223), in the course of an admirable description of the anatomy of the Earthworm, he say, "A colourless fluid, containing colourless corpuscles, and answering to the blood of other invertebrated animals, occupies the perivisceral cavity; but, in addition to this, there is a deep-red fluid, devoid of corpuscles, which fills a very largely developed system of pseud-hæmal vessels."

In a book entitled 'Forms of Animal Life,' published at Oxford in 1870, by Dr. George Rolleston, Professor of Physiology in that University, there are two references to the presence and absence of corpuscles in the vascular system of Annulate worms. At page 124 the writer states that the vascular system of the Earthworm "is called 'pseud-hæmal,' because, though the fluid which it contains is coloured and probably respiratory in function, it is not corpusculated, and, therefore, not morphologically blood." The morphological definition of 'blood' here assumed is clearly another one than that adopted by Gegenbaur. By Gegenbaur the specialisation of a portion of the general body-cavity (cœlom) and its contained fluid for respiratory or nutrient functions or both, in the form of a distinct system of vessels coexistent with the undifferentiated portion, is regarded as the formation of 'blood-vessels' and 'blood.' By Dr. George Rolleston the presence of corpuscles in the differentiated fluid is held to be necessary in order that it may rightly be called 'blood.' The second reference to this subject in this physiologist's treatise is on page cxxix, and scarcely tends to explain the importance which he attaches to a strict morpholo-

gical definition of the term blood. He says, "In a few annelids, again (*Syllidea armata*, the *Opheliæ*, the *Cirratulida*, and the *Staurocephali* and *Branchiobdella*), the so-called pseud-hæmal system contains corpusculated blood, and communicates with the perivisceral cavity, so as to form a lacunar circulation." At first sight this passage would appear to be based upon the statements of Claparède,[1] above quoted, with the omission of the important case of Terebella and the addition of Branchiobdella. So far as the fact of the presence of corpuscles is concerned, this appears probable enough.

The entirely original introduction of Branchiobdella into the list of Annulata with corpusculated "pseud-hæmal fluid" is difficult to explain, since from what follows it seems unlikely that Dr. George Rolleston had made himself acquainted by actual observation with any of the genera to which he alludes. On page 238 of the second edition of Gegenbaur's 'Grundzuge' a somewhat awkwardly introduced reference to Dorner's paper on Branchiobdella might lead an unwary reader to suppose that the statements there given on the authority of Kupffer and Leydig, with reference to the proliferation of blood-corpuscles from the valves of the vessels in other leeches (Piscicola and Clepsine), have reference to Branchiobdella, which they have not. A glance at Dorner's excellent memoir on Branchiobdella would, however, suffice to satisfy a conscientious bookmaker that the vascular fluid of Branchiobdella has not yet been shown to contain corpuscles, and that it notoriously differs from the vascular fluid of true leeches, in that it most certainly does not "communicate with the perivisceral cavity, so as to form a lacunar circulation." The introduction of Branchiobdella into the list

[1] The observation of corpuscles in the blood of *Syllidia armata* is due to M. de Quatrefages. The hasty appropriation of a citation of one author made by a second, is liable to lead a third author into error. The curious information as to a lacunar circulation in Syllidia, which is imparted to students in the passage extracted from the 'Forms of Animal Life,' is a direct contradiction of the statement of M. de Quatrefages himself, whose accuracy with regard to the corpuscles appears nevertheless to be admitted, since his statement on that matter is made use of by the writer. It does not appear possible that the writer of the 'Forms of Animal Life,' can have read the original statement of M. de Quatrefages quoted below. He appears to have been content to avail himself of a passing reference made by a third person, viz. M. Claparède. M. de Quatrefages writes ('Histoire des Annelés,' tome ii, 1865, p. 15) in reference to *Syllidia armata*:—"J'ai vu nettement deux gros vaisseaux contractiles, à parois assez irregulières, placés sur la ligne médiane, l'un au-dessus, l'autre au-dessous du tube digestif. Ils sont mis en communication par des branches latérales à la hauteur de chaque pied; mais je n'ai pu distinguer de ramifications proprement dites. Si elles avaient existé, elle ne m'auraient probablement pas échappé, car, comme je l'ai déjà dit, par une exception fort rare chez les Annélides, le *sang* renferme ici des globules bien caracterisés."

given by Dr. George Rolleston is, it would seem, due to his having misunderstood the German authors.

The statement that "the pseud-hæmal system communicates with the perivisceral cavity so as to form a lacunar circulation" in *Syllidia*, the *Opheliæ*, the *Cirratulida*, and the *Staurocephali*, is more difficult to account for than is that relative to *Branchiobdella*, since whilst there is here also no foundation whatever for such a statement in fact, the description and figures of Claparède with reference to two at least of these genera are admirable in clearness and detail. We are driven to the conclusion that Dr. George Rolleston has acquainted himself with the introduction, without having consulted the body, of Claparède's work.

The blood vascular system of Syllidia, Ophelia, the Cirratulida, the Staurocephali, and the Terebellæ, is a closed system and contains blood in which float corpuscles. These corpuscles are colourless and are not to be confused with the colourless corpuscles existing in the open vascular system of the true Leeches, nor with the corpuscles coloured red by hæmoglobin which exist in the perienteric fluid of the anangian genera Glycera and Capitella. Corpuscles similar to these last are met with in the vascular fluid of some few Nemertines, in Phoronis, in the single blood-lymph fluid of the Lamellibranchs, Arca and Solen (one species), and in the blood of Vertebrates.

Whilst I regret to find myself unable to accede to the statements in the text-book which I have quoted above, I may point out that the errors therein contained are not traceable to any attempt on the author's part to make original observations in the domain of morphology, but are rather due to a failure to observe accurately the contents of books.

The corpuscles of the red blood of the Earthworm are abundant in the larger and even in the finest branches of the vascular system. They are flattened, fusiform bodies, usually somewhat broader at one end than the other, sometimes nearly circular. They vary in size from the $\frac{1}{3500}$th to the $\frac{1}{2000}$th of an inch in long diameter, but by far the majority are of a uniform length of about $\frac{1}{3000}$th of an inch. The corpuscles have a clean, sharp outline, but occasionally what appears to be a small quantity of ragged protoplasm is seen beyond this sharp contour. They are colourless, but stain feebly after treatment with dilute osmic acid followed by picrocarmin. A small centrally placed granule receives, when the corpuscles are thus treated, a deep staining. From a comparison with the structures presented by the walls of the vessels in which these corpuscles occur, it is clear that they are the nuclei of the endothelial cells set free

from the walls of the vessels, whilst the granule which takes a deep staining from picrocarmin is the nucleolus.

In fresh blood-vessels of the Earthworm I have not succeeded in observing the blood-corpuscles. Their small size and delicate character suffices to conceal them in the red-coloured fluid where they float. I first detected them in specimens of the tissues of the earthworm which had been treated with a $\frac{1}{10}$th per cent. solution of osmic acid for half an hour, washed with dilute alcohol, and then stained whilst still under the covering glass by a solution of picrocarmin, and subsequently clarified by glycerin. After such treatment, the finer vessels of the muscular septa and of the walls of the testicular sacs of the earthworm exhibit very clearly the histological elements of their walls, whilst the coagulum within the vessels is seen to contain numerous free corpuscles of the form and appearance above described. The corpuscles occur in the vessels in masses; frequently a large portion of a vessel will be found free from them, whilst an adjacent segment is choked with an abundance.

In order to observe the blood-corpuscles of the Earthworm in the fresh condition it is necessary to remove on to an object-slide a portion of a large vessel by means of two pairs of forceps, and to allow its contents to escape on to the slide. It is not possible in this manner to avoid all admixture with the perivisceral fluid, the corpuscles of which are very abundant and adhere tenaciously to the tissues bathed by that fluid. It is, however, quite easy to distinguish the blood-corpuscles or corpuscles of the vascular fluid from the lymph-corpuscles or corpuscles of the perienteric fluid by their shape and size. A cleanly prepared drop of perienteric fluid shows large, colourless, vacuolated corpuscles, with a ragged outline, often produced into filaments, and provided with a large nucleus; but in such a specimen none of the peculiar oblong, flattened, homogeneous (saving the granule) corpuscles peculiar to the blood or vascular fluid will be found. Accordingly, when a quantity of the vascular fluid is taken, even though it be contaminated by a few lymph-corpuscles, it is quite easy to recognise the small and peculiar blood-corpuscles.

It is my intention to figure the blood-corpuscles of the Earthworm now described, in connection with a description and illustration of a few other points relative to the histology of that animal, which Mr. D'Arcy Power has worked out in the histological laboratory of Exeter College.

The Contractile Filaments *of* Amanita (Agaricus) muscaria *and* Dipsacus Sylvestris. By Francis Darwin, M.B.

The contractile filaments of *Amanita* (*Agaricus*) *muscaria* were discovered by Professor Hoffmann, of Giessen, and described by him in 1853, and again with a figure in 1859.[1] In the latter of the above papers Hoffmann remarks that the filaments have received but small attention from physiologists, and since 1859 the only mention of them which I know of is in a paper by Professor de Bary.[2]

Professor Hoffmann was so good as to call my attention to the existence of the filaments, with the suggestion that they might be of interest to me in relation to my observations on the contractile filaments of the teasel.[3]

From want both of time and material, I only made a few observations on the filaments of Agaricus, and I have but little to add to the observations of Hoffmann and de Bary.

Amanita (*Agaricus*) *muscaria*, the Fly Agaric, is well known to all from its conspicuous crimson pileus covered with little white warts. The ring on the stem hangs down freely, and blends with the surface of the stem itself; the upper surface of the ring or outer surface of the stem is covered with a delicate, creamy coating of whitish-yellow colour. On removing a small portion

Figs. 1 and 2.—Portions of the external surface of the stem of the fly agaric teased out in water (× 430), showing the interlacing cells bearing the contractile filaments. Drawn with Zeiss' camera (objective, Hartnack, No. 9).

[1] "Ueber contractile Gebilde bei Blatterschwämmen," 'Botan. Zeitung,' 1853, p. 857; 'Botan. Zeitung,' 1859, p. 214, taf. xi, fig. 17.

[2] "Die neuere Arbeiten über die Schleimpilze," &c., 'Flora,' 1862, p. 264.

[3] 'Quarterly Journal of Microscopical Science,' July, 1877.

with a needle and teasing it out in water, one sees that the cells, of which the layer is composed, cross and interlace in a felt-like manner. The cells are covered more or less with a yellowish substance, which forms little warts projecting from the surface.

The contractile filaments project in the same way from the cell walls,[1] and immediately arrest the attention from the fact of their being in continuous trembling movement. Various forms of filament are shown in figs. 1 and 2; they consist of a translucent and highly refracting substance, which is certainly the same as that of the little wart-like projections above mentioned.[2] They are usually of a simple cylindricial form, clubbed at the free end, and vary extremely in length and diameter; they also present curious dumb-bell shaped forms. Both Hoffmann and de Bary describe a hollow canal running up the centre of some of the filaments. I saw appearances which might be so interpreted, but I was not sure that it was not a deceptive optical appearance. In many cases, as in fig. 1, the cells are incrusted with dirty-looking accretions, which produce an irregular or untidy appearance. They seem to consist of the same material as the translucent projections, but to have become opaque and granular.

On the whole, the filaments of Agaricus present the closest and most striking resemblance to those of Dipsacus; I repeatedly remarked that I could not have distinguished one from the other, although in other cases I noticed a difference in aspect.

The behaviour of the filaments with reagents has been partly investigated by Hoffmann. But unfortunately he does not mention the strengths of the solutions which he employed. He found that rapid contraction was produced by solution of caustic potash (kalilauge), by ammonia, spirit of wine, oxalic and nitric acids, chloride of calcium, and sulphuric ether. Chloride of sodium produced slow contraction; carbonate of potassium, contraction preceded by lively extension and bending to and fro; sugar solution gradually added produced contraction. He states that the juice squeezed from the stem of the fungus itself produces contraction, though "monads and other infusoria" are not affected by it. Hoffmann describes a phenomenon which I have often seen in the teasel, namely, the swelling up in water of a contracted filament, and the formation of transparent bladders. This is the appearance which I have compared to the formation of soap bubbles, and which often results when a filament has been killed by heat, acids, &c.[3]

The following experiments show that, as in the case of

[1] Their size may be judged of by the drawings, fig. 1 and 2, which are × 430. A moderately long filament measured about ·07 mm.

[2] Only a few of these are seen in the figs.; they are shown in Hoffmann's taf. xi, fig. 17.

[3] Loc. cit., p. 250-51, fig. 5, plate xix.

Dipsacus, dilute solutions of certain reagents produce contraction. A preparation was irrigated with 2 per cent. solution of carbonate of ammonia, which produced contraction. The same result followed irrigation with 2 per cent. acetic acid. Aqueous solution of sulphate of quinine (about $\frac{1}{10}$ per cent.) produced contraction. Aqueous solution of camphor (about $\frac{1}{10}$ per cent.) produced contraction. Methylated spirit produced contraction. In their sensitiveness to very dilute solutions of quinine and camphor we have another striking point of resemblance between the filaments of Agaricus and those of Dipsacus. Hoffmann found that water at 30° C. causes violent contraction. Warm water at various temperatures under 57° C. produces contraction of the Dipsacus filaments. Again, as in Dipsacus, strong pressure makes the filaments run together into lumps. (Hoffmann, p. 862.)

The question—What do the filaments consist of, and what is the nature of their movements? presents precisely the difficulties which I have attempted to discuss in the case of Dipsacus.[1] The opposing theories being, as in that case—(1) that the filament contains irritable living matter; in other words, that it is protoplasmic; and (2) that the movements are mechanical, and due to the loss and gain of water. In the later of the above-cited papers Hoffmann concludes that it is a pure "imbibition phenomenon, although of the most remarkable kind." This does not seem to have been his first impression, as he says Bt. Zng., 1853, p. 863), "It seems to me that we have here undoubtedly the contractile substance, or a substance closely related to it, whose very general occurrence in the animal kingdom Dujardin demonstrated, and which was called by him "sarcode."

On the other hand, De Bary is strongly of the opinion that the filaments are not protoplasmic, and that the movements are due to the imbibition or discharge of water.

In his opinion[2] they are similar to the movements of myelin, as described by Beneke.[3] He states that the substance of which the filaments are composed is nearly all soluble in alcohol and ether, but that a small remnant is left which stains yellow in iodine. If the slimy coating of an entire stalk is extracted with absolute alcohol and evaporated the residue consists of irregular granules and spheres of a substance melting easily, burning with a bright flame and feeling sticky, but giving no oily stains to paper. It may therefore be called resinous. He believes that

[1] Loc. cit., p. 264.

[2] 'Die Myxetozoen,' p. 113.

[3] Myelin is prepared by evaporating an alcoholic extract of the yolk of hens' eggs. When treated with water remarkable changes occur, filaments growing out and exhibiting a species of movement. The filaments, as figured

the part which remains after the resin has been removed by alcohol is capable of swelling in water, and is the cause of the movements. He supposes that the resin is intimately blended with the water-absorbing substance. As far as my observations go on Agaricus, they confirm the view that the filaments consist of two parts, one soluble, the other insoluble in alcohol.

If, instead of a water-absorbing substance, we substitute living matter intimately blended with the resin, the above description agrees with my idea of the constitution of a teasel filament, viz. a large quantity of resin, animated by a small quantity of living or irritable matter.

From the small number of observations which I had time to make on the Agaricus, I am not able to bring forward all the arguments which convinced me that the filaments of Dipsacus are protoplasmic.

The one fact that such weak solutions as $\frac{1}{10}$ per cent. of sulphate of quinine and camphor produce contraction seems to me far more in favour of the view that the filaments of the Agaric are protoplasmic than of the contrary belief. From the strong similarity between the filaments of the teasel and the Agaric, it is impossible not to give some weight to experiments performed on the teasel alone, although such application of their results may not be strictly correct. If it were proved that the movements of the Agaric filaments were an imbibition phenomenon, I should be quite ready to grant that the same is almost certainly true of Dipsacus. The two cases must sink or swim together.

As to what the physiological meaning of the filaments in the Agaric may be I can form no conjecture. I have tried to show that the filaments of the Dipsacus are a resinous protoplasmic secretion; whether the filaments of the Agaric are in any way homologous to a secretion I cannot pretend to say. Professor De Bary to whom I addressed a question on this subject was so kind as to inform me by letter that probably the resinous matter results from the disorganisation of the compact tissue which in the young state fills up the space between the stalk and the lamellæ ;

by Beneke ("Studien über Vorkommen, &c., der Gallenbestandtheil, &c." Giessen, 1862), certainly resemble those of the teasel or Agaric to a certain extent. One difference between these filaments and those of Dipsacus or Agaricus is that they are not affected by weak reagents (Virchow, in 'V.'s Archiv,' 1854, p. 562). Beneke states ('Archiv für Wiss. Heilkunde,' 1865, p. 380) that weak acids do not hinder the movement, but actually causes the production of good forms. This is quite different from the behaviour of teasel filaments. When it is a question of strong solutions, Beneke finds ('Studien,' &c.) that acids retard while alkalies promote the movement. This certainly agrees with the behaviour of teasel filaments. On the other hand, it partially agrees with the behaviour of undoubted protoplasm. De Barry describes ('Mycetozoen,' p. 50) the changes of form produced by alkaline solutions in the plasmodia of the Mycetozoa.

on the other hand, he considers that the possibility of its being an active secretion is not excluded.

As to the function of the filaments I can say nothing. In the case of Dipsacus, I made the suggestion that the filaments serve to absorb the putrifying fluid which collects in the cups, and the ammonia from the dew and rain which falls on the seedling leaves. I was inclined to take this view by the wish of suggesting some function for the filaments; it is however, quite speculative and should have been kept, perhaps, more distinct from the purely physiological part of the paper. And since the fact of the occurrence of contractile filaments in so different a position as a stalk of an Agaric, will naturally throw doubt on my view of the functions of the filaments in Dipsacus. I may take the opportunity of stating that the speculations as to function should be considered as resting on a different footing from the main question—Are the filaments protoplasmic or not? It seems to me necessary to state this clearly, as in more than one notice of my paper the absorption of nitrogenous matter has been described as though it were the best grounded of my conclusions.

Finally, the very fact that contractile filaments occur in such different plants as an agaric and a teasel lends great probability to the idea that the phenomena is of more general occurrence than our present knowledge would have us believe.

The following observations relate to the teasel only, although they are of interest in relation to the filaments of the Agaric.

The experiments were made on teasel plants growing in pots out of doors and which were removed to the workroom. The leaves used were the very young ones growing in the centre of the rosette of leaves on seedling, or rather second-year plants, which from having failed to produce a flowering stem, retained the condition of first-year plants. Thin transverse sections were cut with a razor, the specimens mounted in distilled water under cover glasses, and irrigated with the various reagents in the usual way.

Irrigation with 1 per cent. solution of common salt (NaCl) produced no effect on the filaments; after allowing the filament to remain in the salt solution for a quarter of an hour the preparation was irrigated with $\frac{1}{10}$ per cent. of sulphate of quinine, and contraction followed immediately. A filament irrigated with sugar, 1 per cent., did not contract in seven minutes, but did so in half a minute with $\frac{1}{10}$ per cent. camphor solution.

On a subsequent occasion sections were mounted at 10.31 a.m. in 8 per cent. sugar solution, and when filaments were protruded they were irrigated with $\frac{1}{10}$ per cent. sulphate of quinine solution at 11.20, and contraction of a filament took place slowly at 11.25. These experiments, which are fully confirmed by subsequent trials on salt and sugar solutions, struck me forcibly,

for they seemed to show very clearly that the contraction is not due to a mechanical withdrawal of water. The following experiments also point to the same conclusion:—10.5 a.m. irrigated a preparation with 1 per cent. solution of caffein with no effect; at 10.11 added a drop of 1 per cent. nicotin solution, and instantly the filament contracted. Nicotin does not seem to be a simple poison, but to cause much excitement in the filaments. For in another experiment irrigation with 1 per cent. solution produced rapid shooting out of filaments followed by contraction and writhing movements; ultimately the filaments became transparent as if treated with ammonia. The same effect of nicotin was again obtained in another case. These results agree with the effects of thein and nicotin observed by my father on Drosera.[1]

In this connection it is worth giving the results of some comparative experiments made with thymol and carbolic acid on the filaments of Dipsacus.

Thymol.—I am indebted to the kindness of Dr. Burdon Sanderson for some of this substance. It is extremely fatal to low organisms, being said to be about 200 times as poisonous to bacteria as carbolic acid. It is easily dissolved by adding a trace of alcohol to the water used for the solution. A centigram accurately weighed was dissolved in ·39 gram absolute alcohol; it was then made up to 10 c.c. with distilled water and a little alcohol. Its strength was nearly $\frac{1}{10}$ per cent. thymol and 10 per cent. alcohol. As a control solution 10 per cent. (by bulk) of absolute alcohol was used. 11.50 a.m., irrigated with 10 per cent. alcohol, which produced no effect; 11.55, irrigated with $\frac{1}{10}$ per cent. thymol; 11.55½, contraction of filament. A similar experiment gives the same result. I subsequently mounted specimens in 10 per cent. solution of absolute alcohol and plenty of filaments were protruded. My father has shown[2] that dilute spirit of wine is not poisonous to Drosera.

The above thymol solution was diluted with an equal bulk of water and used to irrigate two preparations, in both which it caused the filaments to contract. The poison was here present in the proportion of 1 to 2000. On another occasion a solution containing $\frac{1}{10}$ per cent. thymol and 4 per cent. alcohol was prepared. The above experiment of irrigating thoroughly with control solution of 10 per cent. absolute alcohol, and then substituting the thymol solution, was repeated with the same results. In many of the later experiments contraction did not take place nearly so rapidly—not till irrigation with thymol had been continued for several minutes.

[1] 'Insectivorous Plants,' p. 203-4.
[2] Ibid., p. 78.

Carbolic Acid.— Pure crystallised acid sold as "absolute phenol" was employed, and a 2 per cent. solution made with distilled water. This proved fatal at once, causing contraction of the filaments. The same effect was caused by 1 per cent. solution, and specimens mounted in 1 per cent. produced no filaments. On the other hand, $\frac{1}{2}$ per cent. is certainly innocuous. Thus, in one of several experiments a preparation mounted at 3.5 p.m. in water was thoroughly irrigated with $\frac{1}{2}$ per cent. carbolic acid; at 3.35 there were many extended filaments. The preparation was then irrigated with $\frac{1}{10}$ per cent. thymol solution, and the filaments contracted in three minutes. These results agree with the above statement that thymol is far more poisonous to low organisms than is carbolic acid. Moreover, the fact that $\frac{1}{2}$ per cent. is not poisonous to the filaments is what might be expected on the assumption that they are protoplasmic. For Dr. Baxter has shown[1] that contagia, microzymes, &c., are not destroyed by $\frac{1}{2}$ per cent. solution of carbolic acid. Thus with vaccine less than 1 per cent. has no effect, 2 per cent. is fatal; infective inflammation, less than 1 per cent. no effect, 1 or more per cent. fatal. Virus of charbon, less than 1 per cent. no effect (Devaine). Dr. Baxter also proves the same thing for the virus of glanders and for microzymes in Cohn's fluid. It should be added, however, that the spores of Penicillium are killed by $\frac{1}{10}$ per cent.

The results of the experiments with sugar solutions are perhaps worth noting. I was surprised to find that filaments were protruded in 12 per cent. solution of sugar. I therefore mounted other sections in water, and irrigated (5.30 p.m.) with 20 per cent. of sugar solution. At 5.40 I saw well-extended filaments on three glands, and there was no shrinking of the gland-cells. I then added more sugar to the solution, so as to make it up roughly to 30 per cent. At 6.14 irrigated with 30 per cent. of sugar. At 6.17 the gland-cells slightly shrunk, but filament not contracted. At 6.21 all the four glands under observation have their *cells* shrunk; one filament quite, another nearly contracted, and two still extended. At 6.47 irrigated with 35 per cent. (roughly). At 7.2 irrigated with 40 per. cent. (roughly). At 7.6 two filaments still uncontracted. At 7.14 preparation irrigated with *syrup* of sugar, and contraction took place in a few minutes.

The following experiment was made with sugar solution of between 44 and 45 per cent. A preparation was mounted in water at 3.43 p.m., and irrigated with the sugar solution. At 3.56 the filament under observation contracted, and at 4 no extended filaments were to be found in the preparation. A solution of 45

[1] 'Report of the Medical Officer of the Privy Council,' new series, vi, 1875, pp. 225, 237, 245, 249, &c.

per cent. is therefore strong enough to cause contraction though not very rapidly.

The effects of salt solutions are very different to the above results. At 11.3 a.m. irrigated a preparation with 10 per cent. solution of chloride of sodium, and contraction took place. As far as I could see, the protoplasm lining the gland-cells began to shrink and the filament to contract simultaneously. The preparation was then washed with distilled water, and the gland-cells rapidly recovered their form. I could not watch the filament any more, as a floating object was washed on to the top of the gland. Observation was transferred to a neighbouring gland, on which a crumpled, half-contracted filament was seated. At 11.17 I found that normal filaments were protruding alongside of the crumpled filament. At 11.42 the crumpled filament had almost recovered, and normal filaments were found on a neighbouring gland.

In some other experiments made with salt solution the gland recovered its normal appearance on being washed with distilled water, and fresh vigorous filaments were protruded; yet the actual filaments which had been made to contract retained their spherical figure as long as I watched the preparation. These results agree with De Vries'[1] observations, who points out (p. 8) that vegetable cells, after being treated with very strong solutions of harmless salts, recover perfectly if washed in water.

As before noticed, irrigation with 10 per cent. solution of chloride of sodium produced shrinking of the wall-protoplasm and contraction of the filament nearly simultaneously. The same fact was observed with 5 per cent. solution. Here the filament contracted first, and then almost immediately the protoplasmic lining of the stalk-cell of the gland. The similarity in behaviour between the filaments and the cell-protoplasm was shown again by irrigating with 2·5 per cent. NaCl, which neither produced cell- or filament-contraction. Again, a preparation was treated with 4 per cent. NaCl; the filament contracted slowly, and then the stalk-cell and the whole gland.

Nevertheless, the behaviours of the filaments and of the cell-protoplasm are not always precisely similar; for on two occasions irrigation with 1 per cent. chloride of potassium and chloride of sodium produced contraction of filaments, while it caused no shrinking of the cell-wall protoplasm. And the converse case has already been noticed, in which 30 per cent. solution of sugar produced shrinking of the gland-cells, but not contraction of the filaments. The remarkable difference between the strengths of

[1] 'Bot. Zeitung,' 1877, No. 1, and 'Untersuchungen über die Mechanischen Ursachen der Zellstreckung,' Leipzig, 1877.

sugar and salt solution required to produce contraction of the filaments agrees with De Vries' results. He remarks[1] that about 25 per cent. of sugar solution is required to produce the same amount of shrinking as is caused by 4 per cent. solution of chlorides of sodium or potassium.

If we compare the effects of dilute acetic acid and of salt solution on the wall-protoplasm of the glands we find that both cause shrinking, but that the results of the two reagents are clearly distinguishable by subsequently irrigating with water. If salt solution has caused the shrinking the cells rapidly recover, but the shrinking caused by a poison is permanent. In the case of the cell-protoplasm we clearly distinguish therefore between the action of a poison and the mechanical withdrawal of water. But in the case of the filaments precisely the same difference is found to exist between the action of salt solution and acid—when tested by subsequent irrigation with water.[2] Therefore the effects of salt solution being certainly mechanical, the effect of the acid seems to be probably poisonous, using poisoning to mean a specific injurious action on living matter.

Note *on* Atmospheric Bacteria. By G. F. Dowdeswell, B.A. Cantab.

It has been observed both by Dr. Burdon Sanderson, in this country, and by Prof. F. Cohn, in Breslau, that when atmospheric air is drawn through a nutrient fluid in wash bottles, no Bacteria are developed. To elucidate the cause of this, a series of experiments has been made, in which the method adopted was to put about 100 c. c. of Cohn's normal cultivating solution[3] in each of several wide-mouthed bottles, previously superheated, then boiling the solutions, covering the bottles with watch glasses, and placing them in the incubator at 35° C. for some days, to ascertain if they were free from organisms, as tested by their contents remaining pellucid; they were then fitted with caoutchouc stoppers, through which glass tubes were inserted, to draw air through the solution, each tube being bent once at a right angle, excepting the egress tube of the last bottle, which was straight, plugged with cotton wool at both ends, and connected

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

[2] It not only cannot recover its normal extended condition, but swells up and forms a "soap-bubble" mass which can be made to contract and swell out again by alternations of salt solution and water. This effect, which is, of course, mechanical, is quite different from the extension and contraction of a filament which has not been treated (killed) with acid, quinine, &c.

[3] Pot. Phosp., 1·0, Mag. Sulph., 1·0, Ca. Cl., 0·1, Amm. Tart., 2·0, and Aq., 200.

with two large water jars by a caoutchouc tube. The wash bottles were then connected by tubing which was previously subjected to prolonged boiling, as were the stoppers ; the glass tubing and the cotton wool was heated in an oven to over 100° C. for a considerable time. The aspirator formed by the water jars was then set in motion, and upwards of 100 Lts. of air drawn through the wash bottles, at the rate of about 2 Lts. an hour. The aspirator being only worked mornings and evenings, this occupied between two and three days. The experiments were made in an ordinary sitting-room, the temperature of which varied between about 50° F. and 70° F. An uncovered bottle of the solution was placed near the wash bottles for comparison. When the requisite quantity of air had passed through, the bottles were removed, the entrance tube of the first wash bottle was rinsed out into a fresh bottle of the solution by a stream of water from an ordinary wash bottle, previously boiled, and cooled with precautions against contamination ; the cotton wool at the bottom of the exit tube of the last wash bottle was pushed into the fluid of its own bottle by a thin rod previously heated. All the bottles were then covered with watch-glasses, and placed in the incubator at 35° C. The result was that in twenty-four hours the fluid in the uncovered bottle was found to be slightly turbid, containing bacterioid growth, the same with the bottle into which the entrance tube of the first wash bottle had been rinsed, while that of the last bottle, into which the cotton wool had been dropped, was distinctly more turbid ; but the solution in the first wash bottle remained free from Bacteria, and continued so for several days, as long as observed. A microscopical examination showed that the numbers of Bacteria present in the different bottles corresponded to the macroscopical appearances mentioned above.

From these observations it is concluded, that when atmospheric air is drawn through wash bottles containing cultivation fluids, part of the Bacteria present are entangled in the tube by which the air enters, as might have been anticipated from the well-known experiments of M. Pastern and others ; and that part of them are "washed out" by the current of air, as conjectured by Cohn ;[1] which latter, in these experiments, were caught by the plug of cotton wool.

The above observations refer only to Bacteria ; no mention is made of other organisms or bodies, of which, and of the different species of Bacteria present, with their relative numbers, an account will be given in a subsequent communication ; and further details of the experiments, with the results of observations on other points which have suggested themselves.

[1] 'Beit. z. Biol. d. Pflanzen,' 3 H., 146 S., 1875.

REVIEW.

An Account of Reichenbach's Researches on the early Development of the Fresh-water Crayfish.[1] By T. Jeffery Parker, Associate of the Royal School of Mines. (With Plate VI.)

Up to last year three important papers had appeared on the development of *Astacus:* Rathke's classical monograph in 1829; Lereboullet's 'Recherches' in 1812, and Bobretsky's Russian paper in 1873. In all these highly important results are obtained, and these results are now supplemented, some of them confirmed and others corrected, by the elaborate account of the earlier developmental stages, published during the course of last year, by Heinrich Reichenbach, of Frankfort.

Of the process of segmentation Reichenbach gives a very imperfect account—an unfortunate circumstance, as the most detailed description of this process, that of Lereboullet, leaves many important points to be settled, and is by no means easy to connect with the existing accounts of the same process in other animals.

According to Lereboullet, the germinal vesicle, after impregnation, travels from the centre to the surface of the egg, becomes flattened, and ruptures. The egg is then seen to be covered with a delicate envelope composed of brilliant hyaline corpuscles (corpuscles plastiques) and constituting the formative yolk; the remainder of the egg consists of food-yolk. The "plastic corpuscles" before long unite to form a white disc, which, shortly after the egg is laid, breaks up into smaller and smaller fragments, a continuous investment to the whole vitellus (membrane génératrice) being eventually produced. This membrane again breaks up, forming stellate masses, which become vacuolated, the portions surrounding the vacuoles then detaching themselves and becoming converted into rounded masses, multiplying by division, the "globes générateurs." While these are being formed, a network appears on the surface of the egg, the polygonal

[1] "Die Embryonenlage und erste Entwicklung des Flusskrebses." Von Heinrich Reichenbach. 'Zeitschrift für Wiss. Zool.,' xxix Bd., 2 Heft, July, 1877.

meshes of which surround the rapidly multiplying "globes générateurs," until each of the latter is enclosed in a polygonal area. The process of division still continues, until, at last, the network ceases to be distinguishable, and the egg is covered with a pavement of closely fitting polygonal blastomeres. The food yolk, in the meantime, has become aggregated into pyramidal masses (Fig. 1, *y. p.* woodcut), the base of each pyramid corresponding accurately with one of the division masses, and its apex pointing towards, but not quite reaching, the centre of the egg.

At this stage Reichenbach's observations begin. He confirms Lereboullet's discovery of the yolk pyramids, and shows that, in addition to the finely granular substance of which they are composed, there are two other deutoplasmic constituents, globular fatty bodies, and rounded vacuolated masses—the white yolk elements (fig. 1, *w. y.*)—which disappears at an early period. He states, however, that when the pyramids are first formed they are completely surrounded with protoplasm, which, as development proceeds, retreats to the surface and there forms the pavement-like cells of the blastoderm. The formation of the pyramids is evidently a process of yolk-division, the egg, in this stage, being a modified morula (perimorula), and the withdrawal of the protoplasm to the surface of the egg being quite analogous to the formation of a cleavage cavity and the production of a periblastula.

At first the protoplasm forms merely the bases of the yolk pyramids, and is only distinguished from them by difference of texture, and by the greater readiness with which it takes up colouring matters. Before long, however, the protaplasmic base of each pyramid is completely separated off as a distinct pavement cell.

This account of the appearances observed by Reichenbach seems clear enough as far as it goes, but then one is led to ask, what is the meaning of the curious and repeated ebb and flow of formative yolk observed by Lereboullet? It is, at any rate, certain that the Crayfish's egg does not undergo a regular division into two, four, eight, &c., blastomeres, like that of Penæus or Palæmon, and it seems probable that the process of the formation of the blastoderm is similar to that observed by Ed. van Beneden in Gammarus, in which "a number of isolated cells (exactly comparable in origin to the Cephalpod's *autoplasts*) rise to the surface of the yolk and then proceed to divide, and so form a complete Perimorula."

Another point, as to which no explanation is offered, is the presence in the centre of the egg, at this stage, of a sharply defined globular body (fig. 1), of considerable size, and containing vacuoles and fatty yolk elements. There is no evidence as to the origin of this anomalous structure, nor has the time or manner of

its disappearance been observed. Reichenbach compares it to the "Dotterkern" of Arachnida.

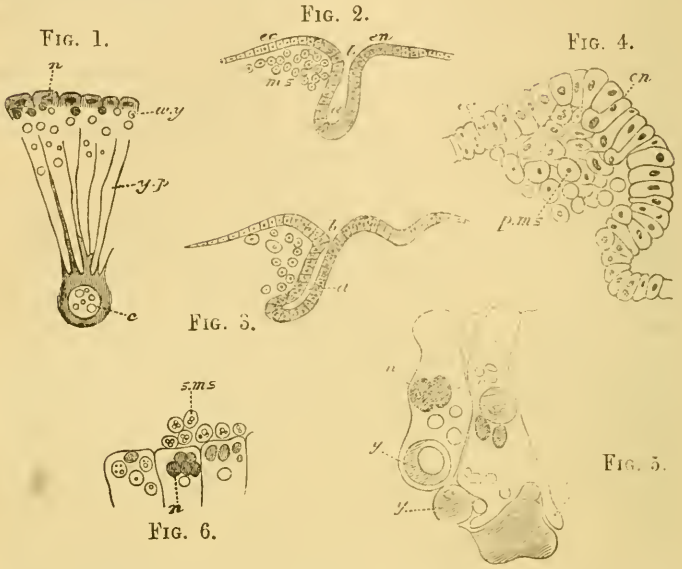


Fig. 1.—Part of a section of the Perimorula of *Astacus*: the protoplasm is distinguished from the deutoplasm by shading. *c.* Central globular body. *y. p.* Yolk pyramids. *w. y.* White yolk elements. *n.* Nucleus of one of the blastoderm cells.

Fig. 2.—Longitudinal section of an embryo in the first stage (Pl. VI, fig. 1); the endoderm is distinguished from the ectoderm by shading. *a.* Archenteron. *b.* Blastopore. *ec.* Ectoderm. *en.* Endoderm. *ms.* Mesoderm.

Fig. 3.—Longitudinal section of an embryo in the second stage (Pl. VI, fig. 2), showing the forward extension of the archenteron, and the subsequently invaginated plug of endoderm, immediately to the right of *b.*

Fig. 4.—Anterior edge of blastopore, in the third stage, showing the origin of the primary mesoderm (*p. ms.*).

Fig. 5.—Two of the endodermal cells from the hæmal side of the archenteron in the fourth stage. *n.* Nucleus. *y.* Yolk spheres. *p.* One of the pseudopodial processes.

Fig. 6.—Three of the endodermal cells from the neural side of the archenteron in the sixth stage, showing the endogenous origin of the secondary mesoderm. *n.* Nuclei. *s. ms.* Secondary mesoderm.

(Figs. 1—6, after Reichenbach, somewhat diagrammatised.)

After this preliminary account of the appearances presented by the egg immediately after the formation of the blastoderm, Reichenbach describes six developmental stages, in the first of which the formation of the archenteron has commenced, while in the sixth the Nauplius-form is assumed.

In the first stage (Pl. VI, fig. 1), the surface of the blasto-

derm which is turned towards the mother is raised up into an oval area: at about the hinder third of this is the blastopore in the form of a depression, semilunar at first, but soon becoming horseshoe-shaped, and with its convexity turned towards what will afterwards become the anterior extremity of the embryo. Immediately in front of the blastopore is a perceptible swelling of the blastoderm (A), marking the spot where the future thoracico-abdominal process is destined to appear.

A longitudinal section of the blastoderm at this stage (fig. 2, woodcut) shows that the archenteron (*a*) is already a considerable cavity, having its long axis directed forwards from the blastopore (*b*), so that its front wall makes an acute angle with the anterior part of the blastoderm. The endoderm cells (*en.*) lining it are columnar, as also are those of the ectoderm (*ec.*) covering the embryonic area: the latter pass insensibly into the pavement cells of the general surface of the blastoderm.

The mesoderm has already appeared in the form of a number of globular cells (*ms.*) confined, at present, almost entirely to the central portion of the embryonic area, beneath the thoracico-abdominal process and in front of the archenteron.

In the second stage (Pl. VI, fig. 2) the blastopore has undergone a notable change: from being horse-shoe-shaped it has become irregularly circular, its ends having united behind, and enclosed a raised plug-like area, the surface of which is at first on the same level as that of the blastoderm generally. The archenteric cavity has increased considerably, and its distal end has begun to curve forwards, or towards the head-end of the embryo. (Fig. 3, woodcut.)

In the third stage (Pl. VI, fig. 3) far more important and extensive changes have taken place. The embryonic area is no longer oval, but has taken on a sort of rough heart-shape, being divided anteriorly into two well-defined lobes, the procephalic processes. The blastopore (G) has decreased greatly in transverse diameter, and the endodermal plug (H), which in the last stage nearly filled the aperture, has undergone a marked diminution in size, and has sunk so far below the line of the blastoderm that the gastrula-mouth is converted into a definite oval opening.

Extending from the depression between the procephalic lobes to the anterior end of the abdominal elevation is a shallow median furrow (R); this may be called the medullary groove, as the central nervous system is subsequently produced from the cells lining it.

There is no evidence to show whether this groove is formed, as in Amphioxus, from behind forwards: the existence of the thoracico-abdominal process entirely prevents the possibility of a communication between its posterior end and the blastopore.

A circular thickening of the ectoderm has taken place in each of the procephalic processes, forming a pair of discs (Kopfscheiben, K), the centres of which are depressed in such a manner as to form two small rounded pits (V). The cells of these, which may be called the optic fossæ, are afterwards converted into the optic ganglia, and probably into the retinal elements, so that, as in Cephalopods, the organ of sight originates as an invagination of the epiblast. No previous observer seems to have noticed these structures: both Rathke and Lereboullet describe the eyes as originating *after* the antennæ and mandibles in the form of ridges; in other words they overlooked the first appearance of the eye itself, and saw only that of the ophthalmic peduncle.

A point of great interest, brought out by section of embryos in the first three stages, is the origin of the mesoderm, which is conclusively shown to arise as a direct product of the endoderm.

The cells of the ectoderm are, as has already been mentioned, regularly columnar, passing insensibly at the edge of the embryonic area into the ordinary pavement cells of the blastoderm; they also rarely contain more than one nucleus, and evidently proliferate very slowly. The endoderm cells, on the other hand, are very variable in form, and frequently contain two or even three nuclei. These characters, an evident sign of active multiplication, are most marked on the anterior steep edge of the blastopore, the boundary region between ectoderm and endoderm; here the multiplication of the cells (see fig. 4, woodcut) is very evident; some are seen to be dividing longitudinally, forming cells similar to themselves; others are dividing transversely, the pinched-off inner moieties assuming the form of mesoderm cells. These latter do not, as yet, form a continuous tissue, but lie scattered about the deutoplasm; they vary a good deal both in form and size. This may be due either to pressure or to amœboid movements; very probably the latter, as they are already found a considerable distance from their place of origin.

It becomes now of great importance to decide the question as to the real nature of this boundary region: ought it to be considered as belonging to the ectoderm or to the endoderm? A transverse section through the blastopore in the third stage shows in a very instructive manner how the diminution in diameter of the gastrula-mouth is brought about, namely, by the active proliferation and consequent ingrowth towards the middle line of the cells forming its lateral edges. The cells as they grow inward constantly undergo division, and thus by one and the same process the blastopore is closed and the mesoderm added to. An examination of later stages shows that, by this process of closure, the whole of the cells forming the front slope of the blastopore come to stand at the edge of

the aperture, and are finally forced within it. Counting, therefore, as endoderm, not only those cells which actually form the lining of the archenteron, but also those which will, at a later period, constitute a portion of that lining, it is perfectly certain that the whole of the mesodermal cells at present existing are derived by a process of division from the endoderm. This important fact was first observed by Bobretsky.

In the fourth stage (Pl. VI, fig. 4), the thoracico-abdominal process has increased considerably, forming now a well-defined, transversely-oval elevation (A), just in front of the blastopore. The latter (G), has decreased to a third of its original dimensions, and the endodermal plug, which had already begun to diminish in the third stage, has almost disappeared.

This last observation corrects in a very important particular the statements of Rathke and Lereboullet. Both these authors observed the blastopore, but thought that the "plug" was converted into the thoracico-abdominal process, the anus appearing in its centre.

Sections of the embryo at this stage show the great forward extension of the archenteron, the lining cells of which are now, at the anterior boundary of the blastopore, sharply distinguished by their form and size from the ectoderm, while on its posterior boundary they still pass insensibly into the latter membrane.

Many of the endoderm cells, especially those immediately beneath the abdominal process, have increased to twice their former size, and exhibit certain very remarkable characters. They contain, in many cases, two or even three nuclei, the shape of which may be round, oval, biscuit-shaped, or semilunar. Besides the finely-granular protoplasm, accumulated mostly round the nuclei, they contain large, often vacuolated spheres of deutoplasm, of precisely similar character to the spheres of which the food-yolk itself is now made up, and upon which these endoderm cells abut. But the most interesting point of all has regard to the shape of the cells. Under a low power they seem to have the ordinary columnar form, but when highly magnified (see fig. 5, woodcut) it is seen that the ends of them, which are turned towards the yolk, are irregularly lobed, and give off more or less fine threads of protoplasm (*p.*), which pass between, and in some cases surround, the yolk spheres (*y.*) These processes have, in fact, all the characteristics of pseudopodia, and it seems perfectly evident that the endoderm cells absorb the nutritive matter of the yolk, not by a passive process of diffusion, but by an active process of ingestion, the food particles being immediately "plunged into the living protoplasm of the cell," and there digested. It is extremely interesting to see so archaic a mode of nutrition as this retained in the embryo of so highly organised an animal as the Crayfish,

at a time, too, when it is, in no sense whatever, morphologically equivalent to a Protozoon, but has taken on all the characters of a triploblastic Enterozoon. I may mention that this active ingestion of yolk by embryonic cells was first observed by Professor Ray Lankester in the Cephalopod's egg.

The mesoblastic cells, the origin of which was described above, have, in this stage, wandered as far forward as the optic fossæ. Amongst them, and evidently taking part in the formation of the middle layer, is a number of globular sharply-contoured bodies, of variable size, filled with finely granular protoplasm, and containing vacuoles and nucleus-like structures. These are called by Reichenbach the *secondary mesoderm cells*, to distinguish them from the *primary mesoderm cells* formed in the process of closure of the blastopore. The origin of the former will be better discussed under the sixth developmental stage, when their true relations are more clearly seen than at present.

In the fifth stage (Pl. VI, fig. 5) a depression (*Oe.*) has made its appearance in the medullary groove, about half way between the emarginate anterior boundary of the procephalic lobes and the abdominal process; this is the commencement of the ectodermal invagination which afterwards becomes the foregut (*stomodæum*, Ray Lankester). The thoracico-abdominal process (A) has taken on a roughly pentagonal shape, and in its centre is a shallow depression (*an.*), the commencement of the hindgut (*proctodæum*). The mouth and anus, therefore, appear contemporaneously, or nearly so.

The blastopore appears, at first sight, to have wholly disappeared, but a close examination of favorable specimens shows that the cells immediately posterior to the thoracico-abdominal process have a peculiar arrangement. Their long axes are turned towards the observer, and they are so disposed as to form a sort of funnel-like depression (G). This depression marks the place of closure of the blastopore.

The first of the appendages to make their appearance—the mandibles—are seen at this stage as a pair of elevations (*Md.*), well-defined posteriorly but shading off into the general surface of the blastoderm in front, situated one on either side of the middle line, just in front of the thoracico-abdominal process. Behind and at the sides of the same process, and separated from it by a furrow, has arisen an indistinct semilunar fold (B), the rudiment of the carapace.

A longitudinal section at this stage shows the complete separation which has taken place between the ectoderm and endoderm, the archenteron being now a completely closed cavity—the midgut. It is evident, too, that the anus is not formed until after the closure of the blastopore, for, while there is a distinct

hindgut invagination, all trace of the gastrula aperture and of its connection with the midgut has disappeared. The thoracico-abdominal process has increased to such an extent that its front wall now makes an acute angle—the tail-fold—with the surface of the blastoderm.

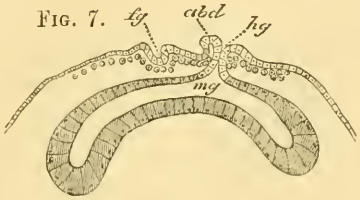


Fig. 7.

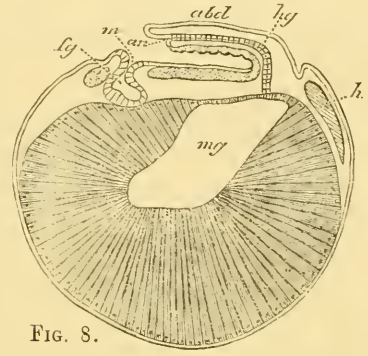


Fig. 8.

Fig. 7.—Longitudinal section of an embryo in the sixth (Nauplius) stage (diagrammatised after Bobretsky). The endoderm is distinguished from the ectoderm by shading. *abd.* Thoracico-abdominal process. *fg.* Foregut. *mg.* Midgut. *hg.* Hindgut.

Fig. 8.—Longitudinal section of a more advanced embryo, showing the increase of the endoderm at the expense of the food-yolk (diagrammatised after Bobretsky). The supra-œsophageal ganglia and the nerve-chain are distinguished by dotting. *m.* Mouth. *an.* Anus. *h.* Heart. The other letters as in fig. 7.

In the sixth stage (Pl. VI, fig. 6) the Nauplius-form is completely assumed. The mandibles (*Md.*) have become well-defined elevations, and two other pairs of processes have appeared in front of them, the rudiments respectively of the antennules (*At. I*) and antennæ (*At. II*). The mouth (*Oe.*) has taken on a semilunar form, its convexity being divided backwards, and an elevation immediately in front of it (*lb.*) is the rudiment of the labrum. The anus (*an.*) has assumed a definite circular contour, and is now of considerable size. The medullary groove has entirely disappeared in front of the mouth, but between that aperture and the abdominal process it is perfectly distinct (*R.*), and at one

spot, just between the mandibles, is so deep that in transverse section it has all the appearance of the medullary groove of a chick at the end of the first day of incubation. Immediately behind the abdomen, between it and the carapace (B), is an insignificant swelling (N.) marking the position of the heart.

A longitudinal section (fig. 7, woodcut) shows that the archenteron or midgut (*mg.*) has now become a very considerable cavity, extending, forwards and backwards, beyond the limits of the embryonic area; its cavity is filled with a coagulated substance of a finely granular appearance. The foregut (*fg.*) is still a blind pouch separated from the archenteron by a layer of mesoderm, but the hindgut (*hg.*) is completely formed, and has established a communication with the midgut at some distance from its posterior end. The cells lining the latter cavity are, on its neural aspect, of the ordinary columnar character, but on its hæmal aspect—that is, on the side turned towards the main mass of yolk—they have increased greatly in size, and are very full of ingested vitelline spheres; they have, in fact, by the method already described, eaten their way for a considerable distance into the food-yolk, and attained a correspondingly gorged and hypertrophied appearance.

The optic fossæ (Pl. VI, fig. 6, *V*) have become, in this stage, deep invaginations, lined with large columnar cells, and in connection with two longitudinal cords, formed by the inward proliferation of the cells forming the lateral parts of the medullary groove. These cords—the commencement of the central nervous system—can be traced as far back as the caudal fold; anteriorly they curve round the gullet, and unite in front of it. Their præ-oral portions, formed by lateral ingrowths of the ectoderm—a virtual not an actual medullary groove—constitute the lateral cords (Seitenstränge) from which part of the supra-œsophageal ganglion is produced. The remainder of the ganglion is formed, partly from the ingrowth of cells from the optic fossæ, and partly from a row of cells (Mittelstrang) lining a groove which is formed between the lateral cords at the period when the ambulatory limbs make their appearance.

The origin of the secondary mesoderm cells, mentioned above, is well seen in this stage, in Reichenbach's figure of a transverse section through the optic fossæ. A smaller portion of this section is reproduced in fig. 6 (woodcut), which represents the upper (neural) ends of the endodermal cells from the neural side of the midgut. The protoplasm is mostly accumulated round the nucleus (*n.*) at the upper end of the cell, the remainder of which is filled with vacuolated deutoplasm. A very large proportion of the cells have two or three nuclei, and in some (*e.g.* the middle cell in fig. 6) the division process has been so rapid that

a sort of mulberry mass of nuclei has been produced. Occasionally the nuclei are altogether absent. Most of the nuclei contain a number of nucleoli, and their form as well as that of their nuclei is excessively inconstant, varying from globular to semilunar.

In many of the cells, instead of, or in addition to the nuclei, are found globular, sharply contoured bodies, containing finely-granular protoplasm, vacuoles, and deeply-staining structures resembling in some cases the nuclei, in others the nucleoli of endodermal cells. The structures in question (well shown in the cell to the left in fig. 6) have, in fact, all the characters of the secondary mesoderm cells (*s. ms.*) scattered about the neighbouring yolk. Some endoderm cells were observed which were ruptured at the upper end, a number of these globular cells being congregated round the aperture then formed. This appearance, however, may have been artificially produced.

The exact mode of origin of these remarkable structures is uncertain, but the appearances observed seem to show that the nuclei of the endodermal cells surround themselves with accumulations of protoplasm, become vacuolated, and so produce a well-defined globular body, which passes out of the cell into the surrounding yolk, acquiring a nucleus either before or after becoming free. This endogenous method of cell formation is one of the most interesting and unexpected points brought out in Reichenbach's paper.

Towards the end of the Nauplius stage a considerable aggregation of mesoderm cells takes place beneath the medullary groove, producing a median cellular cord, which extends from the anterior boundary of the procephalic lobes to the abdominal process, dividing to pass round the gullet. This structure bears a considerable resemblance to the "Wurmchorda" figured by Semper in *Nais* and *Chætogaster*, but as it is very variable in different embryos, and extremely transient, disappearing by the time the maxillæ have appeared, it is quite possible that it may be, as Reichenbach suggests, a mere accidental accumulation of mesodermal cells.

As to the further fate of the secondary mesoderm, Reichenbach considers that it has to do with the formation of the blood. It has, however, in embryos past the Nauplius stage, quite lost its specific character.

Reichenbach gives no figures or detailed descriptions of any stages past the Nauplius. He gives, however, a short account of the subsequent change in the yolk-devouring endoderm cells. These continue their depredations upon the food material until they have, as shown in fig. 8 (woodcut), completely eaten up the

whole of the available yolk, and have come to rest by their peripheral ends against the single-layered ectoderm of the hæmal side of the embryo. They thus acquire, relatively to the other cells of the body, a perfectly gigantic size, and bear a curious resemblance to the yolk pyramids of the periblastula.

With regard to later stages, it is a remarkable circumstance how little of the adult Crayfish's alimentary canal is archenteric in origin. As every one who has dissected the animal knows, the canal consists first of a short gullet and capacious stomach, lined with chitin, then of a short, thin-walled, easily-ruptured, small intestine, not more than an eighth of an inch long, and devoid of a chitinous lining; and lastly of a widish large intestine, separated from the preceding division by an annular ridge, raised internally into several papillose ridges having a slight spiral twist, and lined throughout with a delicate layer of chitin. Now, it seems tolerably certain that only this small intestine is derived from the embryonic midgut, the œsophagus and stomach arising from the foregnt, and the large intestine from the hind-gut; so that almost the whole alimentary canal is ectodermal and not endodermal in origin.

It is curious, too, to notice the great difference in this respect between two animals so closely allied as the Crayfish and Lobster. In the latter the non-chitinised small intestine extends to within an inch and a quarter of the anus, the large intestine, which has all the characters of the corresponding structure in Astacus, forming not more than one sixth instead of fifteen sixteenths of the post-gastric portion of the alimentary canal.

NOTES AND MEMORANDA.

Studies of the Microscopic Images of Medullated Nerve Fibre. By Professor Franz Boll ('Reale Accademia dei Lincei Roma,' June 4th, 1876.)—The author of this important paper commences by referring to the researches of Zawerthal, Schmidt, and Lantermann, who independently published in 1874 descriptions of the discontinuity of the medullary sheath. The first named, however, confuses the medullary sheath with the sheath of Schwann, to which latter he refers the discontinuity which really belongs to the former; the second considers the appearance of discontinuity to be produced by folds of the medullary sheath, and to be of slight importance; only Lantermann, in his preliminary publication ('Centralblatt,' 1874, p. 706), has accurately recognised and described the discontinuity in question. Boll first recognised this particularity of structure independently, in the autumn of 1875, in the electric nerves of the torpedo, and continued to study the subject further, using exclusively the sciatic nerve of adult *Rana esculanta*. His descriptions of this and other points are as follows:—

A.—*Nerve Fibre in Salt Solution, ·75 %.*

When examined in this "physiological" fluid, the medullary sheath appears to be constituted of separate medullary segments, of which there are from twenty to thirty in each nerve-segment (*i.e.* part of the nerve comprised between two Ranvier's nodes). The dimension of these medullary segments varies, the shortest being often not longer than the transverse diameter of the nerve-fibre, while others are from twelve to fifteen times that length. Each of these segments is tubular, surrounding the axis-cylinder, and they fit into each other by their free ends. One segment may thus embrace, or be embraced by another, or it may embrace at one extremity and be embraced at the other. This latter is the usual arrangement, and the segments then appear imbricated, like tiles on a roof. It also rarely happens that a medullary segment, instead of ending in a sharp edge, as is the rule, terminates by a channelled margin (appearing bi-

furcated in optical section), in which the edge of the neighbouring segment is locked as in a vice. The free edges of the segments next to Ranvier's nodes show a more or less marked curvature. Changes in the appearance of the preparation soon take place, and these are always most marked near the cut ends of the fibres and near Ranvier's nodes. Four stages may be noticed. *First stage.* The fibre looks like a band of uniform width, bounded laterally by sharp and shining double contours, which are parallel and rectilinear. It requires close attention to make out the separate medullary segments, as these are in close contact, and, so to speak, soldered to each other. *Second stage.* The compound nature of the medullary sheath becomes more evident as the segments become slightly detached from each other. The contours do not remain quite rectilinear, but show commencing undulation. *Third stage.* The sharp edges of the segments become split up into fibres, or swell into globose and irregular masses, which mask the discontinuity of the medullary sheath. *Fourth stage.* The whole medullary segments lose their rectilinear arrangement, and appear swollen, coagulated, and contorted. In salt-solution preparations the axis-cylinder is at first indistinctly seen, appearing perfectly clear and homogeneous; but afterwards a nebulous turbidity, at first very pale and indistinct, begins to appear, and finally an irregular coagulum, more visible, but still rather pale, forms within the medullary sheath. This observation, which has hitherto escaped the attention of histologists, demonstrates that the axis-cylinder is originally liquid, and confirms another proof of the same fact, viz. molecular movement within the fresh electric nerve of the torpedo, previously described by Boll. This, however, was not seen in the frog. The nerve nuclei also are satisfactorily seen in these preparations; they occupy alveolar depressions on the surface of one, or sometimes two, medullary segments. They are surrounded by a zone of protoplasm, which, however, never extends over two neighbouring medullary segments, so that in the adult frog there is no delicate protoplasmic layer between the sheath of Schwann and the medullary sheath. The sheath of Schwann can hardly be seen in salt-solution preparations.

B.—*Nerve Fibre in Distilled Water.*

In distilled water rapid changes take place. At first the medullary segments lose the appearance of shining bands, becoming wider and duller; they also lose their homogeneity, splitting into a number of concentric tubes and layers, which appear as longitudinal striæ in optical section. This change

starts from the periphery, and advances towards the axis-cylinder. The segments at the same time swell up by imbibition, and their discontinuity ceases by their edges becoming confluent. The medullary sheath is thus converted into a continuous layer formed of concentric coats, and increased in diameter. This increase of volume in the medullary sheath, being accompanied by loss of refraction, brings more distinctly into view the sheath of Schwann and the axis-cylinder. The former appears as a delicate outline bounding the altered medullary sheath, usually in contact with it, but sometimes separated by a fine interval filled by fluid. A similar separation often takes place between the internal surface of the medullary sheath and the axis-cylinder, which appears as a pallid and homogeneous streak bounded by two contours, and is of unequal breadth. It appears to contract somewhat, and to form a rather compact coagulum. The appearances above described soon become notably changed. Owing to the continually increasing absorption of water, the altered medullary sheath, which is at first solid, becomes viscous, then semi-fluid, and finally completely liquid. At the same time, its laminated structure is dissolved, and gives place to a material of a frothy appearance. This material escapes from the cut ends of the fibres in considerable masses of irregular or rounded shape, remaining at first connected with the nerve fibre, but they soon become detached and float away. A current of this frothy matter becomes set up with increasing rapidity, running between the axis-cylinder and the sheath of Schwann. Consequently the latter must form a completely closed tube, not interrupted in any part of its course, as the liquefied material always remains in the above narrow channel, not escaping even at Ranvier's nodes; so that at this point also the sheath of Schwann must be regarded as continuous, which had not been hitherto proved with certainty. Another interesting point concerning the structure of the sheath of Schwann and Ranvier's nodes may be observed; the current of the liquefied medullary sheath may be observed to stagnate for a moment at these points, and afterwards to pass the narrow channel with increased velocity. There must have been, therefore, a considerable resistance to the stream at this point, the sheath of Schwann being not only narrowed, but also specially resistant. The axis-cylinder continues to appear as a longitudinal band of variable breadth after the medullary sheath has begun to liquefy; but soon the axis-cylinder is itself altered in a similar manner, gradually liquefying and escaping from the cut ends of the nerve fibre in the form of

drops. From the manner in which these escape and become detached, Boll thinks it most probable that the axis-cylinder is provided with a special sheath of its own.

c.—*Nerve Fibre in Picrocarmine and Concentrated Picric Acid.*

If nerve be teased in a drop of concentrated picrocarmine the medullary sheath becomes very soon of a greenish-yellow tint. During the progress of the coloration similar results are produced to those effected by distilled water, but more slowly. The medullary sheath swells and becomes wider, its segments become fused together, and finally it becomes laminated, but does not liquefy. The axis-cylinder becomes tinted red, but very slowly, hours, and even days, being necessary for completion of the process. It then appears as a homogeneous red filament of nearly uniform breadth. Concentrated picric acid acts on the medullary sheath in quite the same way as picrocarmine, but more energetically, the nerve fibres being almost immediately coloured yellow. The medullary segments become fused into a continuous mass, but this, instead of being laminated, has a more or less granular appearance. The axis-cylinder appears as a filament of uniform breadth.

d.—*Nerve-fibre in Osmic Acid.*

If nerve fibre be teased in 1% solution of osmic acid, the first changes are that the medullary segments become brown and broader than before, nearly double their previous breadth. At the same time they lose their brilliancy and homogeneity, becoming granular and turbid. By a longer action of the reagent, the medullary sheath becomes darker and darker, and folded upon itself, forming longitudinal and transverse wrinkles. The medullary segments lose their sharp edges, and afterwards become completely fused together. If small nerves be examined after being kept for days or weeks in osmic acid, we find the medullary sheath of an inky-black colour, and the transverse diameter of the nerve fibres is invariably diminished; they look dessiccated, and have evidently lost a considerable quantity of water. This shrinking must be chiefly produced at the expense of the axis-cylinder, as the medullary sheath appears dilated and granular, and usually much wrinkled and folded. The medullary segments can usually still be recognised as distinct, but so altered that the discontinuity might easily be attributed to accidental lesions in the process of hardening. If nerve be treated

with a more dilute solution, *e.g.* ·1%, we often obtain images very closely resembling those produced by distilled water, from which they are only distinguished by their brownish colour, we again find a widening and swelling of the medullary segments, which lose their brilliancy; they then become liquid and fused together into a frothy mass, which, after prolonged action of the solution, becomes of a black colour. The splitting takes place not only longitudinally, but in all directions. Sometimes a special appearance is produced in the altered medullary sheath of dark striæ, at right angles to the longitudinal axis of the medullary segments, which then appear to consist of short and very delicate rods, extending from the axis-cylinder to the sheath of Schwann. Lantermann, who first described these appearances, inclines to consider them as the expression of natural structure; but Boll does not share his opinion, as no trace of rods can be seen in the fresh sheath, and, moreover, it is not to be seen in all osmic acid preparations.

E.—*Nerve Fibre in Chromate of Ammonia*, 2%.

McCarthy ('Q. J. M. S.,' 1875, p. 380) has described a formation of rods as being displayed in the medullary sheath by this reagent. Boll, however, in teased preparations in a drop of this liquid found the results exactly the same as in ·75% salt solution, the changes of the axis-cylinder being even better seen, as it takes a slightly yellowish tint. If nerve be kept in the solution for several days, the fibres shrink very much, and the medullary sheath, of which the segments can no longer be made out, becomes wider and irregularly striated; but Boll could find no trace of rods. He supposes that special conditions of diffusion must have been set up in the larger anatomical preparations exposed to the fluid by McCarthy, whereas he used only isolated nerves. But as this reagent preserves perfectly for a long time the characteristic image of fresh nerve, if much later any rod-like structure should appear, it must be due to post-mortem decomposition.

F.—*Nerve Fibre in Salt Solution*, 10%.

Immediately after making the preparation the medullary segments can still be distinguished, but are no longer separate, being fused by drops at their points of contact. The whole nerve fibre appears occupied by a very large number of very fine and strongly refracting granules, of which it is impossible to determine whether they are placed within the axis-cylinder or on its surface, or perhaps in the

substance of the medullary sheath. Later on every trace of separation between the medullary segments disappears; the granules become larger, perhaps by confluence, so that the fibres appear to be studded with shining drops. It was, unfortunately, not possible to determine whether they were formed exclusively from the axis-cylinder, or were also products of the decomposition of the medullary sheath. They are characteristic not only of 10% salt solution, but of all liquids which harden by extracting water, and may be seen in nerve kept in picric acid, 1% osmic acid, 2% chromate of ammonia, alcohol, and Müller's fluid.

Boll draws the following conclusions from his examinations:

1. The axis-cylinder is liquid, or at least semi-liquid; it certainly does not possess the fibrillar structure commonly assigned to it. This liquid substance is contained in a special sheath.

2. The medullary sheath is not continuous from one Ranvier's node to another, but formed of a greater or less number of distinct medullary segments which are, so to speak, grafted on each other. Their substance is perfectly homogeneous in the fresh state, and very strongly refracting. The modifications induced by reagents cannot be interpreted as showing a pre-existent structure.

3. The sheath of Schwann is a completely closed tube without solution of continuity. At Ranvier's nodes its substance is thickened in an analogous manner to the sheath of connective-tissue bundles at the points of the so-called "spiral fibres."—E. Klein.

Bizzozero and Salvioli on the Structure and the Lymphatics of Human Serous Membranes.—Part I. "On the Structure of the Diaphragmatic Peritoneum" ('Archivio per le Scienze Mediche,' vol. i, No. 3, 1876).—After referring to the description of Bizzozero, in 1873, of an extremely delicate connective-tissue layer in human serous membranes beneath the endothelium, forming a membrana limitans, the authors proceed to divide the human diaphragmatic peritoneum into the following layers: — 1. Endothelium. 2. Membrana limitans. 3. Supporting layer. 4. Basement layer, or body of the serosa, subdivided into a more superficial, reticular, and a deeper compact portion. 5. Subserous layer. These layers are not absolutely separated from each other, but are joined together by a few bundles of fibrils.

Endothelium.—Each cell forms a homogeneous plate, to which is applied the nucleus surrounded by a zone of proto-

plasm. The cells vary in size, and in general the smaller ones are arranged in groups, which correspond to lymphatic lacunæ in the basement layer of the peritoneum, while the larger ones correspond to the connective-tissue bundles forming the walls of the lacunæ. Stomata were not generally found in the endothelium, the cells being closely apposed to each other. Only in old persons and those who have died from wasting diseases apertures may not uncommonly be found between the cells; but these the authors consider to be caused by an atrophic process, and not to be a physiological peculiarity. Apertures between the cells are not necessary for the passage of granules or migratory cells, as these may push between the edges of neighbouring cells, thus causing a temporary opening, which closes after their passage.

Membrana limitans.—This membrane appears homogeneous, finely granular, or finely fibrillar, swells up, and becomes invisible in acetic acid, and contains no trace of nuclei. It is perforated by numerous small apertures in those parts which cover subjacent lymphatic lacunæ in the basement layer.

The *supporting layer* underlies the membrana limitans, and is formed of wide and flattened connective-tissue bundles, which are collected together into shining cords, separated from each other so as to leave free intervals, and form bridges over the lymphatic lacunæ of the basement layer, and immediately under the perforated portions of the membrana limitans, to which they afford a support.

Basement layer.—The reticular stratum consists of rather shining fibrous bundles, whose general direction is from the peripheral parts of the diaphragm to the central tendon. They diverge from each other and again unite, so as to leave intervals (lymphatic lacunæ), which communicate freely with each other. From the lacunæ bundles pass obliquely upwards to the membrana limitans, where they spread out and form a network of fibres, which becomes fused with those of the supporting layer. The reticular stratum contains large and small blood-vessels, which have a close relationship to the lymphatic lacunæ, into the lumen of which they often project more or less distinctly. The compact stratum forms a comparatively continuous layer beneath the reticular layer, and forms the wall of the lacunæ which separates them from the subserous layer. It contains blood-vessels and lymphatics, and consists of horizontal connective-tissue bundles surrounded by numerous elastic fibres.

The *subserous layer*, which fixes the peritoneum to the diaphragm, consists of loose connective tissue, and contains

flattened connective-tissue corpuscles. The above arrangement of the layers of the peritoneum holds good for the peritendinous zone of the diaphragm. In the peripheral (muscular) parts it has no lacunæ, or they are very sparing. Over the central tendon it is thinner, and its various layers are more intimately fused together, the lacunæ being represented by a series of canals forming a network, with dilatations here and there.

Lymphatic vessels.—These were studied by absorption of Indian ink suspended in salt solution, and also injected by puncture with Prussian blue. On examination it was found that the granules of Indian ink occupied the lacunæ which occur in the reticular stratum of the basement layer, and from which lymphatic vessels of two kinds start. The first kind pass *over* the walls of the lacuna from which they start, and open into neighbouring lacunæ, often after anastomosing with neighbouring lymphatics. They thus form a very superficial network. The second kind of lymphatic vessels are larger, and starting from the lacunæ pass into the depth of the compact stratum of the basement layer, where they become ampullated, and give origin to the large lymphatic trunks of the serosa. The Indian ink enters the lacunæ through the apertures of the membrana limitans, around which its granules are found to accumulate. The walls of the lacunæ are lined by endothelium, which appears to occlude the apertures in the membrana limitans; but this point was found difficult to settle, owing to the subjects of study not being perfectly fresh.—E. Klein.

The Lymphatics of the Bones. By Dr. Albrecht Budge. ('Archir. f. microsc. Anatomie,' Bd. xiii. Edited by V. La Valette St. George, and W. Waldeyer.) By injections with Berlin blue by "puncture" into the periosteum of metatarsus of calf and cow, it can be proved that the blood-vessels of Haversian canals are surrounded by perivascular lymphatic vessels (injected). The larger Haversian canals may be shown to possess special lymphatic vessels with proper walls, which (vessels) can be injected directly from the lymphatics of the periosteum. The injection further proves that the bone lacunæ (bone corpuscles) are directly connected with the perivascular lymphatics by their canaliculi. Budge thus shows by direct injection that the radicals of the lymphatics of bone are identical with the bone lacunæ (bone corpuscles), these lead by their canaliculi into the perivascular lymphatics of the Haversian canals, and these empty themselves into the lymphatics of the periosteum.—E. Klein.

PROCEEDINGS OF SOCIETIES.

Dublin Microscopical Club.

21*st June*, 1877.

Spurious Isinglass.—Professor W. R. McNab showed specimens of spurious isinglass sent him by Dr. B. Wills Richardson with the following note:—"This isinglass was imported into London, and I believe was used for mixing with the genuine article. Samples of it were sent to Mr. Harry Draper, who, recognising their vegetable nature, asked me to make sections. Two of the specimens having been swollen by submersion in water for some hours, were then placed in the freezing microtome and the sections made; when stained they were mounted in glycerine-gum. Each piece has a nucleated cortex, as will be best seen by an examination of the cross-sections." This spurious isinglass consists of altered starch, a few granules being easily separated from the mass, which, all but the peculiar cortex, colours blue by application of iodine. No trace of animal matter exists in the substance. The cortex probably consists of dextrine and gives no reaction with iodine. Dr. McNab failed to find any structures in the cortex to which the name nuclei was applicable.

Chlorochytrium Cohnii (Perceval Wright) *parasitic on other algæ besides Diatoms.*—Professor E. Perceval Wright showed fresh examples of his new Chlorochytrium, now parasitic on a green alga, seemingly seated externally upon, yet incorporated with, the host. Hence the parasite had a good deal of the aspect of a "fruit" or "spore" of some kind, and quite possibly it may actually ere now have been so interpreted.

Living Plants of a Species of Hypopterygium, shown—Dr. Moore showed a living plant of the curious genus of mosses, Hypopterygium, which has the leaves three-ranked, the third rank being dissimilar to the other two, and closely resembling the amphigastria (stipules) of some Hepaticæ. He stated that plants of this genus might readily be taken for a Jungermannia when found without fruit. The plant exhibited resembled a miniature tree fern, with an upright stem above an inch high, with the branches and leaves radiating in a dendroid form. He supposed the species to be *Hypopterygium filiculæforme*, Bridel, and stated that several of them were received alive from New Zealand, growing on the stem of a plant of *Todea superba*, and

that they continue to grow and increase in the fern-house at Glasnevin, but have not yet produced female fruit.

New Aulacodiscus, shown.—Rev. E. O'Meara exhibited a species of Aulacodiscus, the peculiarities of which he thought had hitherto escaped the notice of observers. The form had frequently come under his notice when examining the Richmond and Maryland deposits, and in other localities, where he found it difficult to assign it to its proper genus. A very careful examination with good illumination, however, recently brought out its latent characters and enabled him to give it its place in the genus Aulacodiscus. When examined in a dry state the form is so dark that its features are quite indistinguishable, and even when mounted in balsam it requires very exact adjustment to bring them out. An areolate surface is ever observable, the areoles being large and distinctly hexagonal, but over the areoles there is superimposed a surface marked with distinctly radiate puncta, and only when this surface is exactly in focus, do the marked features of the genus Aulacodiscus reveal themselves. The nodules are usually six in number, very small, and sub-marginal. The furrows connecting them with the centre are narrow and difficult to discern. Considering the size, the general aspect of this form, as well as the locality, Mr. O'Meara considered it likely that this is the form figured by Ehrenberg as *Coscinodiscus asteromphalus*, 'Microgeologie,' t. xviii, f. 45 *a*, *b*. The central rosette of elongated areoles, so conspicuous in the figure, is in this case absent, but there is an indistinct appearance of it when viewed with a low power. He proposed to name the form *Aulacodiscus areolatus*.

Exhibition of "Moss-copper"—Mr. Crowe showed examples of metallic copper, in the form known as "moss-copper," forming an exceedingly pretty low-power object. This was from a new locality, Glendalough Mines, Co. Wicklow.

Structure of Spines of Diadema mexicanum.—Mr. Mackintosh exhibited sections of the spines of *Diadema mexicanum*, kindly given him by Dr. Günther, F.R.S., British Museum, which presented the peculiarity of having dimorphic spines—a condition which, though characteristic of a closely allied genus Centrostephanus, Peters, he had not met with previously in Diadema. The majority of the spines were of the usual shape, long, cylindrical, and closely verticillate, and, in section presented no difference of any importance from the typical structure described in 'Transactions of the Royal Irish Academy,' vol. xxv, plate 16, 1875. On the actinal (oral) aspect of the corona were a few spines, which externally differed only in size and colour from the fusiform spines of Centrostephanus (loc. cit.), in section also presented a striking resemblance to them, whilst sparsely scattered over the corona were to be seen spines which were exactly intermediate in form and structure between the two.

Abnormal example of Euglypha.—Mr. Archer showed an abnormal example of the test of the Euglypha he had named

Euglypha tincta (*E. brunnea*, Leidy?). This abnormality consisted in the complete fusion of two tests, "Siamese-twin"-like, the openings of each looking at right angles, that is, the longitudinal axis of each test would intersect that of the other at right angles. Hence, although the openings were not directly opposite to one another, the specimen, were it the first ever noticed of this species, had some resemblance to an amphistomatous form, but a second look plainly showed by its complete want of symmetry that it was merely a monstrous growth, but, as such, not devoid of interest.

19*th July*, 1877.

Aulacodiscus Sollitianus, exhibited.—Rev. E. O'Meara exhibited a specimen of *Aulacodiscus Sollitianus* from the Maryland deposit. For this beautiful species, rarely found perfect, he was indebted to Rev. George Davidson, Logie-Coldstone, Aberdeenshire.

Sections of Pacinian Corpuscles, exhibited.—Mr. B. Wills Richardson exhibited oblique and transverse sections of Pacinian corpuscles taken from the pulp of the human finger; he stated that a few years ago he found such difficulty in getting useful sections of these bodies that he gave the task up in despair; but, thanks to the freezing microtome, any tyro can procure dozens of beautiful sections of them. It is not necessary to do more than make thin sections of the frozen skin of the end of the finger, from which, when stained, the sections of the Pacinian bodies are to be removed with needles, and mounted in either glycerine or glycerine-gum.

Podophrya gemmipara, Hertwig.—Dr. E. Perceval Wright exhibited some mounted specimens of a large Podophryan, which he had found pretty common on the ultimate twigs of several species of red algæ. It, apparently, was the *Podophrya gemmipara* of Hertwig, as suggested by Mr. Archer. Originally described by Alder as a new animacule found at Newcastle-on-Tyne, its life-history had only within the last two years been worked out by Richard Hertwig, whose well-illustrated memoir on specimens found at Heligoland forms the second article in volume 2 of Professor Gegenbaur's 'Morphologisches Jahrbuch.' Dr. Wright, having during his investigations of some algæ found it about spring time rather common at Howth, thought that probably some of the members might be glad to learn of its occurrence on our shores.

Zygospore of Staurastrum turgescens, de Not.—Mr. Archer exhibited examples of the zygospore of a Staurastrum, seemingly *St. turgescens*, de Not., which in its form is unique. The Staurastrum itself is commonplace enough; it resembles *St. alternans*, probably, the most. It seems, however, in the unconjugated state, distinct from it and most others coming nearest to it by the arrangement of the contents, which do not form as many pairs of chlorophyll-plates as angles, radiating from the centre to the angles, but rather stellate masses, that is, forming a number of

irregularly, divergent, more or less interrupted, rays emanating from a common centre—in a word, with a Cylindrocystis-like configuration of the contents. But in the present gathering the unconjugated examples showed the contents with a dense and highly granular appearance; it is, however, well known that at times the characteristic arrangement of the contents in many forms is so masked. The zygospore is very remarkable. It is circular, compressed (thus shaped like a round cushion); in the broad view the margin is undulate, undulations nine to twelve, smooth; in the narrow (edge) view the zygospore is oblong-elliptic, sides parallel and straight for a notable distance at the middle, then gradually merging into the broadly rounded extremities, margin smooth; contents at maturity passing into a bright brownish-yellow colour. An inspection of a zygospore in an oblique position, or, still better, of an empty membrane, showed that the undulations at the circumference of the broad aspect were carried onwards over the front surface, and that the elevations converged towards the centre, and at the same time diminished inwards, so as to disappear ere they reached the centre, where the surface appeared flat. The wall at the margins was considerably thickened and colourless. Fresh examples of this curious zygospore formed an extremely and unusually beautiful object.

18*th October*, 1877.

Structure of Spine of Echinostrephus molare.—Mr. Mackintosh exhibited cross sections of the spine of *Echinostrephus molare*, A. Agass., a genus belonging to the family Echinometridæ. The structure of the spines resembles in general plan that of Echinometra, but differs in the much greater development of the solid rays, which, expanding very rapidly, leave but little room for the tubular tissue, which forms so conspicuous a feature in the spines of Echinostrephus; they, moreover, are very slender, the section exhibited being only about $\frac{1}{30}''$ in diameter.

Structure of Scale of the Ganoid, Amia calva.—Mr. Mackintosh also showed a scale of the ganoid *Amia calva* (kindly given to him by Professor Macalister), and called attention to some peculiar lacunæ occurring in it. They were long, linear-lanceolate in shape, and provided with a number of short, rapidly tapering canaliculi, which came off at right angles, and had no tendency to anastomose. They were found grouped together towards the centre of the scale immediately beneath the superficial layer of ganoin. Scattered about through the rest of the scale were numerous lacunæ and canaliculi, like those of *Lepidosteus osseus*, a section of whose scale was shown for sake of contrast.

Plumularia echinulata, exhibited.—Mr. Grant exhibited a specimen of *Plumularia echinulata*, Lamk., which had been taken growing on *Chorda filum*, between tide-marks near Monkstown Co. Dublin. This species, which is remarkable for the extremely small size of the nematophores, occurs but sparingly on the

Dublin coast, and the present specimen was noteworthy on account of the absence of the nematophores generally placed above the thecæ, only possessing those in the axils of the pinnæ, and one under each cup for the hydranth.

Epichloe typhina, shown.—Mr. Pim showed sections of *Epichloe typhina* investing stems of *Dactylis glomerata*, gathered near Clonmel. This was the first time he had met with this curious parasite.

Didymium farinaceum, exhibited.—Dr. W. M. A. Wright showed examples of *Didymium farinaceum* from Powerscourt, where it had occurred abundantly on sticks, rotten leaves, &c.

Botryococcus Braunii. — Dr. Moore showed *Botryococcus Braunii*, remarkable for the vast quantities in which it had occurred in Lough Bray. It is a common production here and there in moorland localities.

Crystals of Phosphorus shown, and their mode of preparation—Mr. R. J. Moss showed crystals of phosphorus. These were obtained by enclosing small sticks of phosphorus in glass tubes, which were then carefully exhausted by means of the Sprengel-pump and hermetically sealed. The tubes having been enclosed while in opaque cases, fitting closely and blackened at the ends surrounding the phosphorus, were exposed for some weeks to diffused day-light. The phosphorus sublimed and condensed on the sides of the tubes in crystals of remarkable beauty. Some of them, which were complex forms of the cubic system, were nearly 3 mm. in diameter. Owing to their great transparency and the high refractive index of the phosphorus, the crystals exhibited very brilliant colour-effects when strongly illuminated.

Antheridia in Griffithsia.—Dr. E. P. Wright exhibited and called attention to some preparations of the antheridia in *Griffithsia setacea*, Ag. Harvey describes the antheridia in this species as "minute oval bodies composed of dense whorls of exceedingly minute glassy filaments, which frequently occupy the place of tetraspores in the involucres." It is not at all easy to understand this description.

Dillwyn seems never to have seen either tetraspores or antheridia on this species.

Derbés and Solier, in their elaborate description of the fructification in *Griffithsia sphærica*, assert that in this genus the spores, antheridia, and tetraspores are all borne on separate plants, and describe the antheridia as the products of a terminal cell, which divides into a number of daughter-cells, which, before arriving at maturity, generally force asunder the cell-wall of the mother-cell, and then present the appearance of a cluster of grapes on the summit of a stalk, each daughter-cell discharging its contents as an antherozoid form. On this subject Agardh throws no light.

The specimens exhibited, however, show that the tetraspore-sacs and the antheridial-sacs are in this species—the typical one of this genus—borne on the same involucre; that this involucre

is the result of a outgrowth of either a terminal cell of a filament or a lateral cell of a filament; that the involucral whorl is itself composed of a series of verticillately-arranged ramuli, which are advanced a good way in their development ere they burst asunder the cell-wall of the containing terminal cell, which ramuli develop on their inner surface a series of bud-like projections, which develop into oval, sac-like forms, the contents of some of which develop into tetraspores, and of others into a series of daughter-cells, which latter equal the antheridial-cells. These daughter-cells discharge their contents by means of an exceedingly well-marked, tube-like prolongation of their cell-wall, which projects out for some distance beyond the cell-wall of the mother-cell, giving this large cell an appearance as if it were filled with a number of Olpidium-like forms.

Such a development seemed to be as yet unnoticed in any of the Florideæ, and seemed to point to rather a high differentiation in this species. The peculiar endings of the ramuli and their trichomes, also to be seen in this species, were also apparently undescribed.

New Species of Stauroneis.—Rev. E. O'Meara exhibited an undescribed form of Stauroneis, found by him in stomachs of Ascidians dredged at Monkstown, Co. Dublin, by Mr. H. W. Mackintosh, of which the following is a detailed description:—

Stauroneis Mackintoshii.—Valve linear elliptical, acuminate towards the ends, stauroform band broad, very slightly expanded towards the margin; striæ obscure, slightly radiate, not extending to the ends; on front view frustule linear, very slightly constricted in the middle; considerably depressed at ends; length ·0036, breadth ·0008.

Euastrum divaricatum, Lundell, *new to Britain, exhibited.*—Mr. Archer exhibited *Euastrum divaricatum*, Lundell, new to Britain. The examples were taken in Rannoch Moor, in Scotland, on a recent tour in company with Dr. Barker. This is an extremely pretty and well-marked little species.

MEMOIRS.

On the Phenomena accompanying *the* Maturation *and* Impregnation *of the* Ovum. By F. M. Balfour, M.A., Fellow of Trinity College, Cambridge.

The brilliant discoveries of Strasburger and Auerbach have caused the attention of a large number of biologists to be turned to the phenomena accompanying the division of nuclei and the maturation and impregnation of the ovum. The results of the recent investigations on the first of these points formed the subject of an article by Mr. Priestley in the sixteenth volume of this Journal, and the object of the present article is to give some account of what has so far been made out with reference to the second of them. The matters to be treated of naturally fall under two heads: (1) the changes attending the ripening of the ovum, *which are independent of impregnation;* (2) the changes *which are directly due to impregnation.*

Every ovum as it approaches maturity is found to be composed (Fig. 1) of (1) a protoplasmic body or vitellus usually containing yolk-spherules in suspension; (2) of a germinal

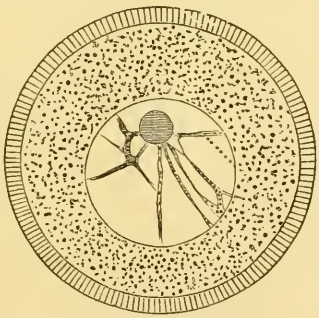


Fig. 1.—Unripe ovum of Toxopneustes lividus (copied from Hertwig).

vesicle or nucleus, containing (3) one or more germinal spots or nucleoli. It is with the germinal vesicle and its contents that we are especially concerned. This body at its full development has a more or less spherical shape, and is enveloped by a distinct mem-

brane. Its contents are for the most part fluid, but may be more or less granular. Their most characteristic component is, however, a protoplasmic network which stretches from the germinal spot to the investing membrane, but is especially concentrated round the former (Fig. 1). The germinal spot forms a nearly homogeneous body, with frequently one or more vacuoles. It occupies an often eccentric position within the germinal vesicle, and is usually rendered very conspicuous by its high refrangibility. In many instances it has been shown to be capable of amœboid movements (Auerbach, and Os. Hertwig), and is moreover more solid and more strongly tinged by colouring reagents than the remaining constituents of the germinal vesicle. These peculiarities have caused the matter of which it is composed to be distinguished by Auerbach and Hertwig as nuclear substance.

In many instances there is only one germinal spot, or one main spot, and two or three accessory smaller spots. In other cases, *e.g.* Osseous Fish, there are a large number of nearly equal germinal spots. The eggs which have been most investigated with reference to the changes of germinal vesicle are those with a single germinal spot, and it is with these that I shall have more especially to deal in the sequel.

The germinal vesicle occupies in the first instance a central position in the ovum, but at maturity is almost always found in close proximity to the surface. Its change of position in a large number of instances is accomplished during the growth of the ovum in the ovary, but in other cases does not take place till the ovum has been laid.

The questions which many investigators have recently set themselves to answer are the two following :—(1) What becomes of the germinal vesicle when the ovum is ready to be impregnated ? (2) Is any part of it present in the ovum at the commencement of segmentation ? According to their answers to these questions the older embryologists roughly fall into two groups : (1) By one set the germinal vesicle is stated to completely disappear and not to be genetically connected with the subsequent nuclei of the embryo. (2) According to the other set it remains in ovum and by successive divisions forms the parent nucleus of all the nuclei in the body of the embryo. Though the second of these views has been supported by several very distinguished names the first view was without doubt the one most generally entertained, and Haeckel (though from his own observations he was originally a supporter of the second view) has even enunciated the theory that there exists an anuclear stage, after the disappearance of the germinal vesicle, which he regards as an embryonic repetition of the monad condition of the Protozoa.

While the supporters of the first view agree as to the disap-

pearance of the germinal vesicle they differ considerably as to the manner of this occurrence. Some are of opinion that the vesicle simply vanishes, its contents being absorbed in the ovum; others that it is ejected from the ovum and appears as the *polar cell* or *body*, or *Richtungskorper* of the Germans—a small body which is often found situated in the space between the ovum and its membrane, and derives its name from retaining a constant position in relation to the ovum, and thus serving as a guide in determining the similar parts of the embryo through the different stages. The researches of Oellacher (15)[1] in this direction deserve special mention, as having in a sense formed the foundation of the modern views upon this subject. By a series of careful observations upon the egg of the trout and subsequently of the bird, he demonstrated that the germinal vesicle of the ovum, while still in the ovary, underwent partial degeneration and eventually became ejected. His observations were made to a great extent by means of sections, and the general accuracy of his results is fairly certain, but the nature of the eggs he worked on, as well as other causes, prevented his obtaining so deep an insight into the phenomena accompanying the ejection of the germinal vesicle as has since been possible. Lovén, Flemming (6), and others have been led by their investigations to adopt views similar in the main to Oellacher's. As a rule, however, it is held by believers in the disappearance of the germinal vesicle that it becomes simply absorbed, and many very accurate accounts, so far as they go, have been given of the gradual atrophy of the germinal vesicle. The description of Kleinenberg (14) for Hydra, and Götte for Bombinator, may perhaps be selected as especially complete in this respect; in both instances the germinal vesicle commences to atrophy at a relatively early period.

Coming to the more modern period the researches of five workers, viz. Bütschli, E., van Beneden, Fol, Hertwig, and Strasburger have especially thrown light upon this difficult subject. It is now hardly open to doubt that while part of the germinal vesicle is concerned in the formation of the polar cell or cells, when such are present, and is therefore ejected from the ovum, part also remains in the ovum and forms a nuclear body which will be spoken of as the *female pronucleus*, the fate of which is recorded in the second part of this paper. The researches of Bütschli and van Beneden have been especially instrumental in demonstrating the relation between the polar bodies and the germinal vesicle, and those of Hertwig and Fol, in showing that part of the germinal vesicle remained in the ovum. It must not, however, be supposed that the results of these authors are fully sub-

[1] The numbers appended to authors' names refer to the list of publications at the end of the paper.

stantiated, or that all the questions connected with these phenomena are settled. The statements we have are in many points opposed and contradictory, and there is much that is still very obscure.

In the sequel an account is first given of the researches of the above-named authors, followed by a statement of those results which appear to me the most probable.

The researches of van Beneden (3 and 4) were made on the ovum of the rabbit and of Asterias, and from his observations on both these widely separated forms he has been led to conclude that the germinal vesicle is either ejected or absorbed, but that it has in no case a genetic connection with the first segmentation sphere. He gives the following description of the changes in the rabbit's ovum. The germinal vesicle is enclosed by a membrane, and contains one main germinal spot, and a few accessory ones, together with a granular material which he calls *nucleoplasma*, which affects, as is usual in nuclei, a reticular arrangement. The remaining space in the vesicle is filled by a clear fluid. As the ovum approaches maturity the germinal vesicle assumes an excentric position, and fuses with the peripheral layer of the egg to constitute the *cicatricular lens*. The germinal spot next travels to the surface of the cicatricular lens and forms the *nuclear disc :* at the same time the membrane of the germinal vesicle vanishes though it probably unites with the nuclear disc. The nucleoplasma then collects into a definite mass and forms the nucleoplasmic body. Finally the nuclear disc assumes an ellipsoidal form and becomes the nuclear body. Nothing is now left of the original germinal vesicle but the nuclear body and the nucleoplasmic body both still situated within the ovum. In the next stage no trace of the germinal vesicle can be detected in the ovum, but outside it, close to the point where the modified remnants of the vesicle were previously situated, there is present a polar body which is composed of two parts, one of which stains deeply and resembles the nuclear body, and the other does not stain but is similar to the nucleoplasmic body. Van Beneden concludes that the polar bodies are the two ejected products of the germinal vesicle. In the case of Asterias, van Beneden has not observed the mode of formation of the polar bodies, and mainly gives an account of the atrophy of the germinal vesicle, but adds very little to what was already known to us from Kleinenberg's (14) earlier observations. He describes with precision the breaking up of the germinal spot into fragments and its eventual disappearance.

Though there are reasons for doubting the accuracy of all the above details on the ovum of the rabbit, nevertheless, the observations of van Beneden taken as whole afford strong grounds for

concluding that the formation of the polar cells is connected with the disappearance, partial or otherwise, of the germinal vesicle. A very similar account of the apparent disappearance of the germinal vesicle is given by Greeff (19) who states that the apparent disappearance of the germinal spot precedes that of the vesicle.

The observations of Bütschli are of still greater importance in this direction. He has studied with a view to elucidating the fate of the germinal vesicle, the eggs of Nephelis, Lymnæus, Cucullanus, and other Nematodes; and Rotifers. In all of these, with the exception of Rotifers, he finds polar bodies, and in this respect his observations are of value as tending to show the widespread existence of these structures. Negative results with reference to the presence of the polar bodies have, it may be remarked, only a very secondary value. Bütschli has made the very important discovery that in perfectly ripe eggs of Nephelis, Lymnæus and Cucullanus and allied genera a *spindle*, similar to that of ordinary nuclei in the act of division, appears close to the surface of the egg. This spindle he regards as the metamorphosed germinal vesicle, and has demonstrated that it takes part in the formation of the polar-cells. He states that the whole spindle is ejected from the egg, and that after swelling up and forming a somewhat spherical mass it divides into three parts.

In the Nematodes generally, Bütschli has been unable to find the spindle modification of the germinal vesicle, but he states that the germinal vesicle undergoes degeneration, its outline becoming indistinct and the germinal spot vanishing. The position of the germinal vesicle continues to be marked by a clear space which gradually approaches the surface of the egg. When it is in contact with the surface a small spherical body, the remnant of germinal vesicle, comes into view, and eventually becomes ejected. The clear space subsequently disappears. This description of Bütschli resembles in some respects that given by van Beneden of the changes in the rabbit's ovum, and not impossibly refers to a nearly identical series of phenomena. The discovery by Bütschli of the spindle and its relation to the polar body has been of very great value.

The publications of van Beneden, and more especially those of Bütschli, taken by themselves lead to the conclusion that the whole germinal vesicle is either ejected or absorbed. Nearly simultaneously with their publications there appeared, however, a paper by Oscar Hertwig (11) on the eggs of one of the common sea urchins (*Toxopneustes lividus*), in which he attempted to show that part of the germinal vesicle, at any rate, was concerned in the formation of the first segmentation nucleus. He believed (though he has himself now recognised that he was in error on

the point) that no polar cell was formed in Toxopneustes, and that the whole germinal vesicle was absorbed, with the exception of the germinal spot which remained in the egg as the female pronucleus.

The following is the summary which he gives of his results, pp. 357-8.

"At the time when the egg is mature the germinal vesicle undergoes a retrogressive metamorphosis and becomes carried towards the surface of the egg by the contraction of the protoplasm. Its membrane becomes dissolved and its contents disintegrated and finally absorbed by the yolk. The germinal spot appears, however, to remain unaltered and to continue in the yolk and to become the permanent nucleus of the ripe ovum capable of impregnation."

After the publication of Bütschli's monograph, O. Hertwig (12) continued his researches on the ova of Leeches (*Hæmopis* and *Nephelis*), and not only added very largely to our knowledge of the history of the germinal vesicle, but was able to make a very important rectification in Bütschli's conclusions. The following is a summary of his results :—The germinal vesicle, as in other cases, undergoes a form of degeneration, though retaining its central position; and the germinal spot breaks up into fragments. The stages in which this occurs are followed by one when, on a superficial examination, the ovum appears to be absolutely without a nucleus; but there can be demonstrated by means of reagents in the position previously occupied by germinal vesicle a spindle nucleus with the usual suns at its poles, which Hertwig believes to be a product of the fragments of the germinal spot. This spindle travels towards the periphery of the ovum and then forms the spindle observed by Bütschli. At the point where one of the apices of the spindle lies close to the surface a small protuberance arises which is destined to form the first polar cell. As the protuberance becomes more prominent one half of the spindle passes into it. The spindle then divides in the normal manner for nuclei, one half remaining in protuberance, the other in the ovum, and finally the protuberance becomes a rounded body united to the egg by a narrow stalk. It is clear that if, as there is every reason to think, the above description is correct, the polar cell is formed by simple process of cell-division and not, as Bütschli believed, by the forcible ejection of the spindle.

The portion of the spindle in the polar cell becomes a mass of granules, and that in the ovum becomes converted without the occurrence of the usual nuclear stage into a fresh spindle. A second polar cell is formed in the same manner as the first one, and the first one subsequently divides into two. The portion of the spindle which remains in the egg after the formation of the second polar cell reconstitutes itself into a nucleus—the female

pronucleus—and travelling towards the centre of the egg undergoes a fate which will be spoken of in the second part of this paper.

The most obscure part of Hertwig's work is that which concerns the formation of the spindle on the atrophy of the germinal vesicle, and his latest paper, though it gives further details on this head, does not appear to me to clear up the mystery. Though Hertwig demonstrates clearly enough that this spindle is a product of the metamorphoses of the germinal vesicle, he does not appear to prove the thesis which he maintains, that it is the metamorphosed germinal spot.

Fol, to whom we are indebted in his paper on the development of Geryonia (7) for the best of the earlier descriptions of the phenomena which attend the maturation of the egg, and later for valuable contributions somewhat similar to those of Bütschli with reference to the development of the Pteropod egg (8), has recently given us a very interesting account of what takes place in the ripe egg of *Asterias glacialis* (9). In reference to the formation of the polar cells, his results accord closely with those of Hertwig, but he differs considerably from this author with reference to the preceding changes in the germinal vesicle. He believes that the germinal spot atrophies more or less completely, but that in any case its constituents remain behind in the egg, though he will not definitely assert that it takes no share in the formation of the spindle at the expense of which both the polar cells and the female pronucleus are formed. The spindle with its terminal suns arises, according to him, from the contents of the germinal vesicle, loses its spindle character, travels to the surface, and reacquiring a spindle character is concerned in the formation of the polar cells in the way described by Hertwig.

Giard (10) gives a somewhat different account of the behaviour of the germinal vesicle in *Psammechinus miliaris*. At maturity the contents of the germinal vesicle and spot mix together and form an amœboid mass, which, assuming a spindle form, divides into two parts, one of which travels towards the centre of the egg and forms the female pronucleus, the other remains at the surface and gives origin to two polar cells, both of which are formed after the egg is laid. What Giard regards as the female pronucleus is perhaps the lower of the two bodies which take the place of the original germinal vesicle as described by Fol. *Vide* the account of Fol's observations on p. 117.

Strasburger, from observations on *Phallusia*, accepts in the main Hertwig's conclusion with reference to the formation of the polar bodies, but does not share Hertwig's view that either the polar bodies or female pronucleus are formed at the expense of the germinal spot alone. He has further shown that the so-called canal-cell of conifers is formed in the same manner as the

polar cells, and states his belief that an equivalent of the polar cells is widely distributed in the vegetable subkingdom.

This sketch of the results of recent researches will, it is hoped, suffice to bring into prominence the more important steps by which the problems of this department of embryology have been solved. The present aspects of the question may perhaps be most conveniently displayed by following the history of a single ovum. For this purpose the eggs of *Asterias glacialis*, which have recently formed the subject of a series of beautiful researches by Fol (9), may conveniently be selected.

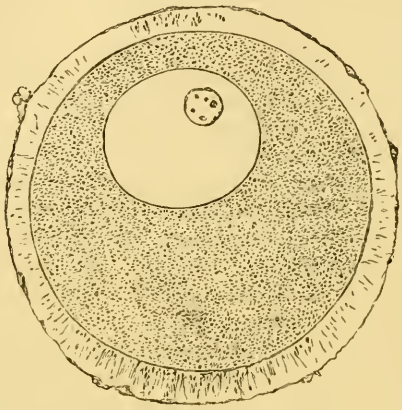


Fig. 2.—Ripe ovum of Asterias glacialis enveloped in a mucilaginous envelope, and containing an excentric germinal vesicle and germinal spot (copied from Fol).

The ripe ovum (fig. 2), when detached from the ovary, is formed of a granular vitellus without a vitelline membrane, but enveloped in a mucilaginous coat. It contains an eccentrically situated germinal vesicle and germinal spot. In the former is present the usual protoplasmic reticulum. As soon as the ovum reaches the sea water the germinal vesicle commences to undergo a peculiar metamorphosis. It exhibits frequent changes of form,

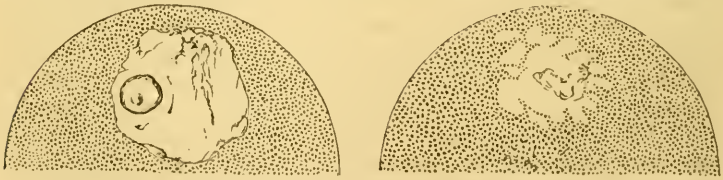


Fig. 3.—Two successive stages in the gradual metamorphosis of the germinal vesicle and spot of the ovum of Asterias glacialis immediately after it is laid (copied from Fol).

its membrane becomes gradually absorbed and its outline indented and indistinct, and finally its contents become to a certain extent confounded with the vitellus (Fig. 3).

The germinal spot at the same time loses its clearness of outline and gradually disappears from view.

At a slightly later stage in the place of the original germinal vesicle there may be observed in the fresh ovum two clear spaces (fig. 4), one ovoid and nearer the surface, and the second

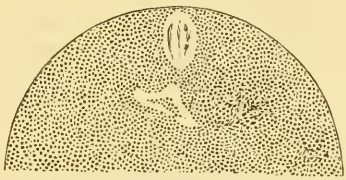


Fig. 4.—Ovum of Asterias glacialis, showing the clear spaces in the place of the germinal vesicle. Fresh preparation (copied from Fol).

more irregular in form and situated rather deeper in the vitellus. By treatment with reagents the first clear space is found to be formed of a spindle with two terminal suns on the lower side of which is a somewhat irregular body (Fig. 5). The second clear space by the same treatment is shewn to contain a round body. Fol concludes that the spindle is formed out of part of the germinal vesicle and not of the germinal spot, while he sees in the round body present in the lower of the two clear spaces the metamorphosed germinal spot. He will not, however, assert that no fragment of the germinal spot enters into the formation of the spindle. It may be observed that Fol is here obliged to fill up (so far at least as his present preliminary account enables me to determine) a lacuna in his observations in a hypothetical manner, and O. Hertwig's (13) most recent observations on the ovum of the same or an allied species of Asterias tend to throw some doubt upon Fol's interpretations.

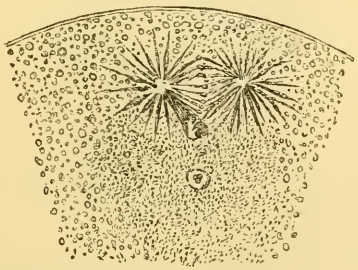


Fig. 5.—Ovum of Asterias glacialis, at the same stage as Fig. 4, treated with picric acid (copied from Fol).

The following is Hertwig's account of the changes in the germinal vesicle. A quarter of an hour after the egg is laid the protoplasm on the side of the germinal vesicle towards the surface of the egg develops a prominence which presses inwards the wall of the vesicle. At the same time the germinal spot develops a large vacuole, in the interior of which is a body consisting of nuclear substance, and formed of a firmer and more refractive material than the remainder of the germinal spot. In the above-mentioned prominence towards the germinal vesicle, first one sun is formed by radial striæ of protoplasm, and then a second makes its appearance, while in the living ovum the germinal spot appears to have vanished, the outline of the germinal vesicle to have become indistinct, and its contents to have mingled with the surrounding protoplasm. Treatment with reagents demonstrates that in the process of disappearance of the germinal spot the nuclear mass in the vacuole forms a rod-like body, the free end of which is situated between the two suns which occupy the prominence of the germinal vesicle. At a slightly later period granules may be seen at the end of the rod and finally the rod itself vanishes. After these changes there may be demonstrated by the aid of reagents a spindle between the two suns, which Hertwig believes to grow in size as the last remnants of the germinal spot gradually vanish, and he maintains, as before mentioned, that the spindle is formed at the expense of the germinal spot. Without following Hertwig so far as this[1] it may be permitted to suggest that his observations tend to show that the body noticed by Fol in the median line, on the inner side of his spindle, is in reality a remnant of the germinal spot and not, as Fol supposes, part of the germinal vesicle. Considering how conflicting is the evidence before us it seems necessary to leave open for the present the question as to what parts of the germinal vesicle are concerned in forming the first spindle.

Fig. 6.—Portion of the ovum of Asterias glacialis, showing the spindle formed from the metamorphosed germinal vesicle projecting into a protoplasmic prominence of the surface of the egg. Picric acid preparation (copied from Fol.).

[1] Hertwig's full account of his observations, with figures, in the 4th vol. of the 'Morphologische Jahrbuch,' has appeared since the above was written. The figures given strongly support Hertwig's views.

The spindle, however it be formed, has up to this time been situated with its axis parallel to the surface of the egg, but not long after the stage last described a spindle is found with one end projecting into a protoplasmic prominence which makes its appearance on the surface of the egg (Fig. 6). Hertwig believes that the spindle simply travels towards the surface, and while doing so changes the direction of its axis. Fol finds, however, that this is not the case, but that between the two conditions of the spindle an intermediate one is found in which a spindle can no longer be seen in the egg, but its place is taken by a compact rounded body. He has not been able to arrive at a conclusion as to what meaning is to be attached to this occurrence. In any case the spindle which projects into the prominence on the

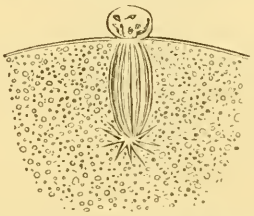


FIG. 7.—Portion of the ovum of Asterias glacialis at the moment of the detachment of the first polar body and the withdrawal of the remaining part of the spindle within the ovum. Picric acid preparation (copied from Fol).

surface of the egg divides it into two parts, one in the prominence and one in the egg (Fig. 7). The prominence itself with the enclosed portion of the spindle becomes partially constricted off

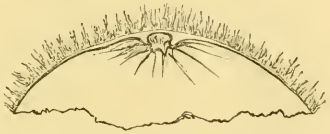


FIG. 8.—Portion of the ovum of Asterias glacialis, with the first polar body as it appears when living (copied from Fol.).

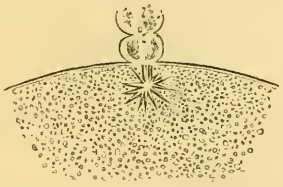


FIG. 9.—Portion of the ovum of Asterias glacialis immediately after the formation of the second polar body. Picric acid preparation (copied from Fol.).

from the egg as the first polar body (Fig. 8). The part of the spindle which remains in the egg becomes directly converted into a second spindle by the elongation of its fibres without passing through a typical nuclear condition. A second polar cell next becomes formed in the same manner as the first (Fig. 9), and the portion of the spindle remaining in the egg becomes converted into two or three clear vesicles (Fig. 10) which soon unite to form a single nucleus, the female pronucleus (Fig. 11).

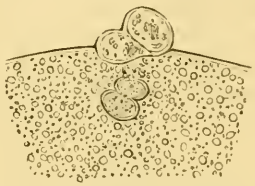


Fig. 10.—Portion of the ovum of Asterias glacialis after the formation of the second polar cell, showing the part of the spindle remaining in the ovum becoming converted into two clear vesicles. Picric acid preparation (copied from Fol.).

The two polar cells appear to be situated between two membranes, the outer of which is very delicate and only distinct where it covers the polar cells, while the inner one is thicker and becomes, after impregnation, more distinct and then forms what Fol speaks of as the vitelline membrane. It is clear, as Hertwig has pointed out, that the polar bodies originate by a regular cell division and have the value of cells.

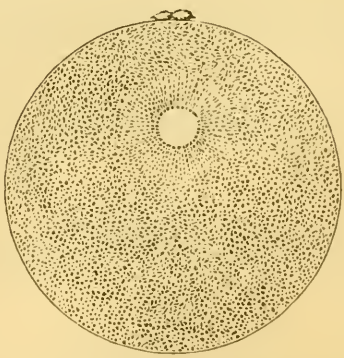


Fig. 11.—Ovum of Asterias glacialis with the two polar bodies and the female pronucleus surrounded by radial striæ, as seen in the living egg (copied from Fol.).

General conclusion.

Considering how few ova have been adequately investigated with reference to the behaviour of the germinal vesicle any general conclusions which may at present be formed are to be

regarded as provisional, and I trust that this will be borne in mind by the reader in perusing the following paragraphs.

There is abundant evidence that at the time of maturation of the egg the germinal vesicle undergoes peculiar changes, which are, in part at least, of a retrogressive character. These changes may begin considerably before the egg has reached the period of maturity, or may not take place till after it has been laid. They consist in appearance of irregularity and obscurity in the outline of the germinal vesicle, the absorption of its membrane, the partial absorption of its contents in the yolk, and the breaking up and disappearance of the germinal spot. The exact fate of the single germinal spot, or the numerous spots where they are present, is still obscure; and the observations of Oellacher on the trout, and to a certain extent my own on the skate, tend to show that the membrane of the germinal vesicle may in some cases be ejected from the egg, but this conclusion cannot be accepted without further confirmation.

The retrogressive metamorphosis of the germinal vesicle is followed in a large number of instances by the conversion of what remains into a striated spindle similar in character to a nucleus previous to division. This spindle travels to the surface and undergoes division to form the polar cell or cells in the manner above described. The part which remains in the egg forms eventually the female pronucleus.

The germinal vesicle has up to the present time only been observed to undergo the above series of changes in a certain number of instances, which, however, include examples from several divisions of the Cœlenterata, the Echinodermata, and the Mollusca, and also some of the Vermes (Nematodes, Hirudinea, Sagitta). It is very possible, not to say probable, that it is universal in the animal kingdom, but the present state of our knowledge does not justify us in saying so. It may be that in the case of the rabbit, and many Nematodes as described by van Beneden and by Bütschli, we have instances of a different mode of formation of the polar cells.

The case of Amphibians, as described by Bambeke (2) and Hertwig (12) cannot so far be brought into conformity with our type, though observations are so difficult to make with such opaque eggs that not much reliance can be placed upon the existing statements. In both of these types of possible exceptions it is fairly clear that, whatever may be the case with reference to the formation of the polar cells, part of the germinal vesicle remains behind as the female pronucleus.

There are a large number of types, including the whole of the Rotifera[1] and Arthropoda, with a few doubtful exceptions, in which

[1] Flemming (6) finds that, in the summer and probably parthenogenetic eggs of *Lacinularia socialis*, the germinal vesicle approaches the surface

the polar cells cannot as yet be said to have been satisfactorily observed.

Whatever may be the eventual result of more extended investigation, it is clear that the formation of polar cells accord into our type is a very constant occurrence. Its importance is also very greatly increased by the discovery by Strasburger of the existence of an analogous process amongst plants. Two questions about it obviously present themselves for solution: (1) What are the conditions of its occurrence with reference to impregnation? (2) What meaning has it in the development of the ovum or the embryo?

The answer to the first of these questions is not difficult to find. The formation of the polar bodies is independent of impregnation, and is the final act of the normal growth of the ovum. In a few types the polar cells are formed while the ovum is still in the ovary, as, for instance, in some species of Echini, Hydra, &c., but, according to our present knowledge, far more usually after the ovum has been laid. In some of the instances the budding off of the polar cells precedes, and in others follows impregnation; but there is no evidence to show that in the later cases the process is influenced by the contact with the male element. In Asterias, as has been shown by O. Hertwig, the formation of the polar cells may indifferently either precede or follow impregnation—a fact which affords a clear demonstration of the independence of the two occurrences.

To the second of the two questions it does not unfortunately seem possible at present to give an answer which can be regarded as satisfactory.

The retrogressive changes in the membrane of the germinal vesicle which usher in the formation of the polar bodies may very probably be viewed as a prelude to a renewed activity of the contents of the vesicle; and are perhaps rendered the more necessary from the thickness of the membrane which results from a protracted period of passive growth. This suggestion does not, however help us to explain the formation of polar cells by a process identical with cell division. The ejection of part of the germinal vesicle in the formation of the polar cells may probably be paralleled by the ejection of part or the whole of the original nucleus which, if we may trust the beautiful researches of Bütschli, takes place during conjugation in Infusoria as a preliminary to the formation of a fresh nucleus. This comparison is due to Bütschli, and according to it the formation of the polar bodies would have to be regarded as assisting, in some as yet unknown

and becomes invisible, and that subsequently a slight indentation in the outline of the egg marks the point of its disappearance. In the hollow of the indentation Flemming believes a polar cell to be situated, though he has not definitely seen one.

way, the process of regeneration of the germinal vesicle. Views analogous to this are held by Strasburger and Hertwig, who regard the formation of the polar bodies in the light of a process of excretion or removal of useless material. Such hypotheses do not unfortunately carry us very far.

I would suggest that in the formation of the 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 (*vide* p. 126). More light on this, as on other points, may probably be thrown by further investigations on parthenogenesis and the presence or absence of a polar cell in eggs which develope parthenogenetically. Curiously enough the two groups in which parthenogenesis most frequently occurs in the ordinary course of development (*Arthropoda* and *Rotifera*) are also those in which polar cells, with the possible exception mentioned above, of the parthenogenetic eggs of Lacinularia, are stated to be absent. This curious coincidence, should it be confirmed, may perhaps be explained on the hypothesis, I have just suggested, viz. *that a more or less essential part of the nucleus is removed in the formation of the polar cells; so that in cases, e.g. Arthropoda and Rotifera, where polar cells are not formed, and an essential part of the nucleus not therefore removed, parthenogenesis can much more easily occur than when polar cells are formed.*

That the part removed in the formation of the polar cells is not absolutely essential, seems at first sight to follow from the fact of parthenogenesis being possible in instances where impregnation is the normal occurrence. The genuineness of all the observations on this head is too long a subject to enter into here,[1] but after admitting, as we probably must, that there are genuine cases of parthenogenesis, it cannot be taken for granted without more extended observation that the occurrence of development in these rare instances may not be due to the polar cells not having been formed as usual, and that when the polar cells are formed the development without impregnation is less possible.

The remarkable observations of Professor Greeff (19) on the parthenogenetic development of the eggs of *Asterias rubens* tell, however, very strongly against this explanation. Greeff has found that under normal circumstances the eggs of this

[1] The instances quoted by Siebold from Hensen and Oellacher are not quite satisfactory. In Hensen's case impregnation would have been possible if we can suppose the spermatozoa to be capable of passing into the body-cavity through the open end of the uninjured oviduct; and though Oellacher's instances are more valuable, yet sufficient care seems hardly to have been taken, especially when it is not certain for what length of time spermatozoa may be able to live in the oviduct. For Oellacher's precautions, *vide* 'Zeit. für Wiss. Zool.,' Bd. xxii, p. 202.

species of starfish will develope without impregnation in simple sea water. The development is quite regular and normal though much slower than in the case of impregnated eggs. It is not definitely stated that polar cells are formed, but there can be no doubt that this is implied. Professor Greeff's account is so precise and circumstantial that it is not easy to believe that any error can have crept in; but neither Hertwig nor Fol have been able to repeat his experiments, and we may be permitted to wait for further confirmation before absolutely accepting them.

It is possible that the removal of part of the protoplasm of the egg in the formation of the polar cells may be a secondary process due to an attractive influence of the nucleus on the cell protoplasm, such as is ordinarily observed in cell division.

Impregnation of the Ovum.

A far greater amount of certainty appears to me to have been attained as to the effects of impregnation than as to the changes of the germinal vesicle which precede this, and there appears, moreover, to be a greater uniformity in the series of resulting phenomena. For convenience I propose to reverse the order hitherto adopted and to reserve the history of the literature and my discussion of disputed points till after my general account. Fol's paper on *Asterias glacialis*, is again my source of information. The part of the germinal vesicle which remains in the egg, after the formation of the second polar cell, becomes converted into a number of small vesicles (Fig. 10), which aggregate themselves into a single clear nucleus which gradually travels toward the centre of the egg and around which as a centre the protoplasm becomes radiately striated (Fig. 11). This nucleus is known as the *female pronucleus*.[1] In *Asterais glacialis* the most favorable period for fecundation is about an hour after the formation of the female pronucleus. If at this time the spermatozoa are allowed to come in contact with the egg, their heads soon become enveloped in the investing mucilaginous coat. A prominence, pointing towards the nearest spermatozoon, now arises from the superficial layer of protoplasm of the egg and grows till it comes in contact with the spermatozoon (Fig. 12 and 13). Under normal circumstances the spermatozoon, which meets the prominence, is the only one concerned in the fertilisation, and it makes its way into the egg by passing through the prominence. The tail of the spermatozoa, no longer motile, remains visible for some time after the head has bored its way in, but its place is soon taken by a pale conical body which is, however, probably in part a product of the meta-

[1] According to Hertwig's most recent statement a nucleolus is present in this nucleus.

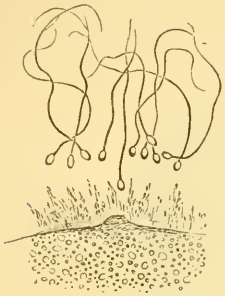


Fig. 12.

Fig. 13.

Fig. 12 and 13.—Small portion of the ovum of Asterias glacialis. The spermatozoa are shown enveloped in the mucilaginous coat. In Fig. 12 a prominence is rising from the surface of the egg towards the nearest spermatozoon; and in Fig. 13 the spermatozoon and prominence have met. From living ovum (copied from Fol).

morphosis of the tail itself (Fig. 14). This body vanishes in its turn.

At the moment of contact between the spermatozoon and the egg the outermost layer of the protoplasm of the latter raises itself as distinct membrane, which separates from the egg and prevents the entrance of any more spermatozoa. At the point where the spermatozoon entered a crater-like opening is left in the membrane (Fig. 14).

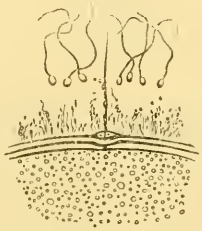


Fig. 14.—Portion of the ovum of Asterias glacialis after the entrance of a spermatozoon into the ovum. It shows the prominence of the ovum through which the spermatazoon has entered. A vitelline membrane with a crater-like opening has become distinctly formed. From living ovum (copied from Fol).

The head of the spermotozoon when in the egg forms a nucleus for which the name *male pronucleus* may be conveniently adopted. It grows in size by absorbing, it is said, material from the ovum, though this may be doubted, and around it is formed a clear space free from yolk-spherules. Shortly after its formation the protoplasm in its neighbourhood assumes a radiate arrangement (Fig. 15). At whatever point of the egg the spermatozoon may have

entered, it gradually travels towards the female pronucleus. This latter, around which the protoplasm no longer has a radial

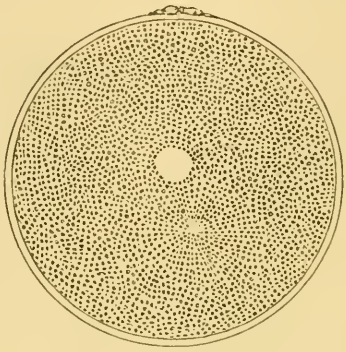


Fig. 15.—Ovum of Asterias glacialis, with male and female pronucleus and a radial striation of the protoplasm around the former. From living ovum (copied from Fol).

arrangement, remains motionless till it comes in contact with the rays of the male pronucleus, after which its condition of repose is exchanged for one of activity, and it rapidly approaches the male pronucleus, and eventually fuses with it (Fig. 16).

Fig. 16.—Three successive stages in the coalescence of the male and female pronucleus in Asterias glacialis. From the living ovum (copied from Fol).

The product of this fusion forms the first segmentation nucleus (Fig. 17), which soon, however, divides into the two nuclei of the two first segmentation spheres. While the two pronuclei are approaching one another the protoplasm of the egg exhibits amœboid movements.

Of the earlier observations on this subject there need perhaps only be cited one of E. van Beneden, on the rabbit's ovum, showing the presence of two nuclei before the commencement of segmentation. Bütschli was the earliest to state from observations on *Rhabditis dolichura* that the first segmentation nucleus arose from the fusion of two nuclei, and this was subsequently shown with greater detail for *Ascaris nigrovenosa*, by Auerbach (1). Neither of these authors gave at first the correct interpretation of their results. At a later period Bütschli (5) arrived at the con-

clusion that in a large number of instances (*Lymnæus, Nephelis, Cucullanus,* &c.), the nucleus in question was formed by the

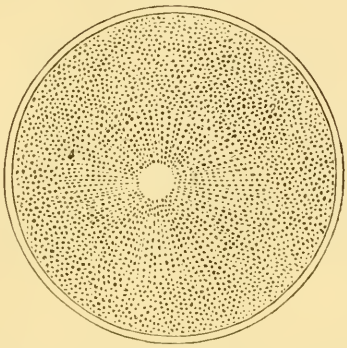


Fig. 17.—Ovum of Asterias glacialis, after the coalescence of the male and female pronucleus (copied from Fol).

fusion of two or more nuclei, and Strasburger at first made a similar statement for *Phallusia,* though he has since withdrawn it. Though Bütschli's statements depend, as it seems, upon a false interpretation of appearances, he nevertheless arrived at a correct view with reference to what occurs in impregnation. Van Beneden (3) described in the rabbit the formation of the original segmentation nucleus from two nuclei, one peripheral and the other central, and he gave it as his hypothetical view that the peripheral nucleus was derived from the spermatic element. It was reserved for Oscar Hertwig (11) to describe in *Echinus lividus* the entrance of a spermatozoon into the egg and the formation from it of the male pronucleus.

Though there is a general agreement between the most recent observers, Hertwig, Fol, Selenka, Strasburger, &c., as to the main facts connected with the entrance of one spermatozoon into the egg, the formation of the male pronucleus, and its fusion with the female pronucleus, there still exist differences of detail in the different descriptions which partly, no doubt, depend upon the difficulties of observation, but partly also upon the observations not having all been made upon the same species. Hertwig does not enter into details with reference to the actual entrance of the spermatozoon into the egg, but in his latest paper points out that considerable differences may be observed in occurrences which succeed impregnation, according to the relative period at which this takes place. When, in Asterias, the impregnation is effected about an hour after the egg is laid and previously to the formation of the polar cells, the male pronucleus appears

at first to exert but little influence on the protoplasm, but after the formation of the second polar cell, the radial striæ around it become very marked, and the pronucleus rapidly grows in size. When it finally unites with the female pronucleus it is equal in size to the latter. In the case when the impregnation is deferred for four hours the male pronucleus never becomes so large as the female pronucleus. With reference to the effect of the time at which impregnation takes place, Asterias would seem to serve as a type. Thus in *Hirudinea*, *Mollusca*, and *Nematodes* impregnation normally takes place before the formation of the polar bodies is completed, and the male pronucleus is accordingly as large as the female. In *Echinus*, on the other hand, where the polar bodies are formed in the ovary, the male pronucleus is always small.

Selenka, who has investigated the formation of the male pronucleus in *Toxopneustes variegatus*, differs in certain points from Fol. He finds that usually, though not always, a single spermatozoon enters the egg, and that though the entrance may be effected at any part of the surface, it generally occurs at the point marked by a small prominence where the polar cell was formed. The spermatozoon first makes its way through the mucous envelope of the egg, within which it swims about, and then bores with its head into the polar prominence. The head of the spermatozoon on the entering the egg becomes enveloped by the superficial protoplasm, and travels inward with its envelope, while the tail remains outside. As Fol has described, a delicate membrane becomes formed shortly after the entrance of the spermatozoon. The head continues to make its way by means of rapid oscillations, till it has traversed about one eighth of the diameter of the egg, and then suddenly becomes still. The tail in the meantime vanishes, while the neck swells up and forms the male pronucleus. The junction of the male and female pronucleus is described by Fol and Selenka in nearly the same manner.

Giard gives an account of impregnation which is not easily brought into harmony with that of the other investigators. His observations were made on *Psammechinus miliaris*. At one point is situated a polar body and usually at the pole opposite to it a corresponding prominence. The spermatozoa on gaining access to the egg attach themselves to it and give it a rotatory movement, but according to Giard none of them penetrate the vitelline membrane which, though formed at an earlier period, now retires from the surface of rhe egg.

Giard believes that the prominence opposite the polar cells serves for the entrance of the spermatic material, which probably passes in by a process of diffusion. Thus, though he regards the

male pronucleus as a product of impregnation, he does not believe it to be the head of a spermatozoon.

Both Hertwig and Fol have made observations on the result of the entrance into the egg of several spermatozoa. Fol finds that when the impregnation has been too long delayed the vitelline membrane is formed with comparative slowness and several spermatozoa are thus enabled to penetrate. Each spermatozoon forms a separate pronucleus with a surrounding sun; and several male pronuclei usually fuse with the female pronucleus. Each male pronucleus appears to exercise a repulsive influence on other male pronuclei, but to be attracted by the female pronucleus. When there are several male pronuclei the segmentation is irregular and the resulting larva a monstrosity. These statements of Fol and Hertwig are at first sight in contradiction with the more recent results of Selenka. In *Toxopneustes variegatus* Selenka finds that though impregnation is usually effected by a single spermatozoon yet that several may be concerned in the act. The development continues, however, to be normal if three or even four spermatozoa enter the egg almost simultaneously. Under such circumstances each spermatozoon forms a separate pronucleus and sun.

It may be noticed that, while the observations of Fol and Hertwig were admittedly made upon eggs in which the impregnation was delayed till they no longer displayed their pristine activity, Selenka's were made upon quite fresh eggs; and it seems not impossible that the pathological symptoms in the embryos reared by the two former authors may have been due to the imperfection of the egg and not to the entrance of more than one spermatozoon. This, of course, is merely a suggestion which requires to be tested by fresh observations. We have not as yet a sufficient body of observations to enable us to decide whether impregnation is usually effected by a single spermatozoon, though in spite of certain conflicting evidence the balance would seem to incline towards the side of a single spermatozoon.[1]

The discovery of Hertwig as to the formation of the male pronucleus throws a flood of light upon impregnation.

The act of impregnation is seen essentially to consist in the fusion of a male and female nucleus; not only does this appear in the actual fusion of the two pronuclei, but it is brought into still greater prominence by the fact that the female pronucleus is a product of the nucleus of a primitive ovum, and the male pronucleus is the metamorphosed *head* of the spermatozoon which

[1] The recent researches of Calberla on the impregnation of the ovum of *Petromyzon Planeri* support this conclusion.

is itself developed from the nucleus of a spermatic cell.[1] The spermatic cells originate from cells (in the case of Vertebrates at least) identical with the primitive ova, so that the fusion which takes place is the fusion of morphologically similar parts in the two sexes.

It must not, however, be forgotten, as Strasburger has pointed out, that ~~that~~ part of the protoplasm of the generative cells of the two sexes also fuse, viz. the tail of the spermatozoon with the protoplasm of the egg. But there is no evidence that the former is of importance for the act of impregnation. The fact that impregnation mainly consists in the union of two nuclei gives an importance to the nucleus which would probably not have been accorded to it on other grounds.

Hertwig's discovery is in no way opposed to Mr. Darwin's theory of pangenesis and other similar theories, but does not afford any definite proof of their accuracy, nor does it in the meantime supply any explanation of the origin of two sexes or of the reasons for an embryo becoming male or female.

Summary.

In what may probably be regarded as a normal case the following series of events accompanies the maturation and impregnation of an egg:—

(1) Transportation of the germinal vesicle to surface of the egg.

(2) Absorption of the membrane of the germinal vesicle and metamorphosis of the germinal spot.

(3) Assumption of a spindle character by the remains of germinal vesicle, these remains being probably largely formed from the germinal spot.

(4) Entrance of one end of the spindle into a protoplasmic prominence at the surface of the egg.

(5) Division of the spindle into two halves, one remaining in the egg, the other in the prominence The prominence becomes at the same time nearly constricted off from the egg as a polar cell.

(6) Formation of a second polar cell in same manner as first, part of the spindle still remaining in the egg.

(7) Conversion of the part of the spindle remaining in the egg after the formation of the second polar cell into a nucleus—the female pronucleus.

(8) Transportation of the female pronucleus towards the centre of the egg.

(9) Entrance of one spermatozoon into the egg.

[1] This seems the most probable view with reference to the nature of the head of the spermatozoon, though the point is not perhaps yet definitely decided.

(10) Conversion of the head of the spermatozoon into a nucleus—the male pronucleus.

(11) Appearance of radial striæ round the male pronucleus which gradually travels towards female pronucleus.

(12) Fusion of male and female pronuclei to form the first segmentation nucleus.

List of important recent Publications on the Maturation and Impregnation of the Ovum.

1. *Auerbach.* Organologische Studien, Heft 2.
2. *Bambeke.* Recherches s. Embryologie des Batraciens. Bull. de l'Acad. royale de Belgique, 2me sér., t. lxi, 1876.
3. *E. Van Beneden.* La Maturation de l'Œuf des Mammifères. Bull. de l'Acad. royale de Belgique, 2me sér., t. xl, no. 12, 1875.
4. *Idem.* Contributions à l'Histoire de la Vésicule Germinative, &c. Bull. de l'Acad. royale de Belgique, 2me sér., t. xli, no. 1, 1876.
5. *Bütschli.* Eizelle, Zelltheilung, und Conjugation der Infusorien.
6. *Flemming.* Studien in d. Entwickelungsgeschichte der Najaden. Sitz. d. k. Akad. Wien, B. lxxi, 1875.
7. *Fol.* Die erste Entwickelung des Geryonideneies. Jenaische Zeitschrift, vol. vii.
8. *Idem.* Sur le Développement des Pteropodes. Archives de Zoologie Expérimentale et Générale, vol. iv and v.
9. *Idem.* Sur le Commencement de l'Hénogénie. Archives des Sciences Physiques et Naturelles. Geneve, 1877.
10. *Giard.* Note sur les prémiers phénomènes du développement de l'Oursin. 1877.
11. *Hertwig, Oscar.* Beit. z. Kentniss d. Bildung, &c., d. thier. Eies. Morphologische Jahrbuch, Bd. i.
12. *Idem.* Ibid. Morphologische Jahrbuch, Bd. iii, Heft 1.
13. *Idem.* Weitere Beiträge, &c. Morphologische Jahrbuch, Bd. iii, Heft 3.
14. *Kleinenberg.* Hydra. Leipzig, 1872.
15. *Oellacher, J.* Beiträge zur Geschichte des Keimbläschens im Wirbelthiereie. Archiv. f. micr. Anat., Bd. viii.
16. *Selenka.* Befruchtung u. Theilung des Eies von Toxopneustes variegatus (Vorlaufige Mittheilung). Erlangen, 1877.
17. *Strasburger.* Ueber Zellbildung u. Zelltheilung. Jena, 1876.
18. *Idem.* Ueber Befruchtung u. Zelltheilung Jena. 1878.
19. *R. Greeff.* Üb. d. Bau u. d. Entwickelung d. Echinodermen. Sitzun. der Gesellschaft z. Beförderung d. Gasammten Naturwiss z. Marburg, No. 5. 1876.

Postscript.—Two important memoirs have appeared since this paper was in type. One of these by Hertwig, 'Morphologische Jahrbuch,' Bd. iv, contains a full account with illustrations of what was briefly narrated in his previous paper (13); the other by Calberla, 'Der Befruchtungsvorgang beim Ei von *Petromyzon Planeri*.' 'Zeit. für Wiss. Zool.,' Bd. xxx, shows that the superficial layer of the egg is formed by a coating of protoplasm free from yolk-spheres, which at one part is continued inwards as a column, and contains the germinal vesicle. The surface of this column is in contact with a micropyle in the egg-membrane. Impregnation is effected by the entrance of the head of a single spermatozoon (the tail remaining outside) through the micropyle, and then along the column of clear protoplasm to the female pronucleus.

Notes *on the* Structure *and* Development *of* Osseous Tissue. By E. A. Schäfer. With Plates VII and VIII.

(*From the Physiological Laboratory of University College, London.*)

I. *The lamellæ of bone.*—That the lamellæ when stripped off from a bone that has been softened in acid but subsequently completely freed from all traces of the acid by long steeping in water or spirit exhibit under the microscope an appearance of intercrossing fibres (the *reticulating fibres* of Sharpey), is a familiar fact to every student of histology in this country. But in spite of its obvious importance and of the ease with which the fact can be demonstrated, it has been, if not actually denied, at least ignored by Continental writers almost without exception.

It is not, however, only in preparations of decalcified bone that these fibres may be observed, nor does the fact of their existence depend upon the appearances seen in a lamella when viewed on its surface; for the assumption of the existence of fibres in the lamella affords the only rational explanation of what is seen in a section of the bony layers made perpendicular to their surface. In such a section, taken we will suppose for simplicity of description across a Haversian system, we find concentric rows of angular dots which are embedded in a homogeneous substance, an appearance of alternating granular and clear zones being thus produced. Sections of the flattened cell-cavities (lacunæ) occur here and there in the clear zones, but except where the lacunæ are present the homogeneous osseous substance is continuous throughout the Haversian system, being only partly interrupted by the concentric rows of angular dots. The question therefore arises, What in the section shall be taken to represent a single lamella?

Before endeavouring to supply the answer, it will be well to consider the opinions of previous authors who have given special attention to the subject. Sharpey described the appearances as follows:[1]—"In a thin transverse section of hard bone the concentric lines, or rather bands, which represent the cut edges of the lamellæ, generally present with transmitted light a dark granular-looking, and a light, transparent, and usually narrower zone. . . . In a decalcified section the dark part shows a multitude of short bright lines running radially across it, with dark angular particles between them . . . the appearance of dark particles seems to be produced by the cut ends of the reticulat-

[1] Quain's 'Anatomy,' seventh edition.

ing fibres of which the lamellæ are made up. A longitudinal section of the bone presents a corresponding appearance, for as the fibres run more or less obliquely to the axis of the bone, they present cut ends in a longitudinal section also. It thus appears that the animal basis of bone is made up of lamellæ composed of fine reticulating fibres. . . . " It would seem, therefore, that although Sharpey notes the existence of clear zones alternating with the granular or fibrous zones, he does not attach sufficient importance to them to reckon them as an integral part of the lamella.

Ranvier[1] describes a section of hard bone as showing "deux espèces de lamelles qui alternent l'une avec l'autre pour former les couches successives; les unes homogènes . . . les autres d'aspect strié. . . . Il est facile de se convaincre que l'aspect strié d'une des espèces de lamelles est dû à de petits ponts à bords sinueux, formés d'une matière semblable à celle des lamelles homogènes.

"Cette structure des lamelles osseuses se voit aussi bien sur des coupes longitudinales que sur des coupes transversales.[2] . . . "

It is clear that this represents little more than a reproduction of Sharpey's original description. The facts are the same, but the deduction as to what constitutes an osseous lamella is different, and there would obviously be in a given thickness, according to Ranvier, twice as many lamellæ as would be enumerated by Sharpey.

In order to arrive at a more exact definition of a lamella, it is necessary to compare the structure of bone with that of the other lamellated tissues to which it is most closely allied in essential structure, especially some of the forms of connective tissue. Of these that composing the cornea affords the most striking analogy in structure to osseous tissue. Thus, the cornea is composed of a number of lamellæ which can be separated from one another, and which are made up of fibrillated bundles united together in the same lamella by a homogeneous ground substance which also serves to bind together adjacent lamellæ. This interlamellar ground substance is partially occupied by the

[1] 'Traité Technique,' p. 314.

[2] Ranvier goes on: "Cela prouve que la striation est réellement produit par des petits ponts, et non pas par des lames longitudinales, comme on pourrait le croire si l'aspect strié ne se montrait que sur des coupes transversales." Is not Sharpey's explanation, that the fact of the same appearance being seen whether the section be transverse or longitudinal is owing to the oblique direction of the intercrossing fibres, a far more reasonable one? But M. Ranvier cannot admit the existence of such fibres: "Nous avons essayé le procédé indiqué par l'auteur (Sharpey), mais nous n'avons pas réussi à voir la texture fibreuse dont il parle!"

cells of the tissue—the corneal corpuscles—contained in special cell-cavities, which are flattened conformably with the lamellæ, and which serve in sections of the cornea to indicate the limits of adjoining lamellæ. Comparing with bone, we find in the latter also layers of fibres united by a homogeneous ground-substance, in which are seen between the several layers cell-cavities or lacunæ containing the cells of the tissue flattened conformably with the layers between which they lie. A further analogy of structure is to be found in those fibres which in the cornea pass obliquely, uniting layer to layer; more especially the almost vertical "binding" fibres which pass through the superficial lamellæ towards the outer surface; for these, from their position and general appearance, may without much effort of the imagination be looked upon as representing the perforating fibres of bone.

The chief point of difference, leaving out of account the chemical constitution of the two tissues, is to be found in the fact that in the cornea the fibres in a lamella run in the same direction, while in bone there are two sets of fibres which intercross with one another and are generally fused together at the places where they come in contact. There is, however, sufficient in common in the structure of the parts to enable a close comparison to be made. Since in sections of the cornea therefore we speak of the lamellæ as being separated from one another by the flattened corpuscles, and in this way define a lamella as a layer of fibrillated bundles closely held together by a homogeneous ground-substance which also covers the surfaces of the layer, so in bone we must similarly define a lamella as a layer of reticulating fibres covered on both surfaces by a homogeneous substance, which extends between the fibres. It is clear that in each case the homogeneous substance will only be distinctly visible in sections across the lamellæ; in strip-preparations in both cases the lamellæ seem almost entirely composed of fibres. Further, since the corpuscles are in neither case epithelioid, *i.e.*, do not fit together by their edges after the manner of epithelium-cells, gaps are left between the adjacent cells, and these gaps are occupied by the homogeneous ground-substance which serves to unite the lamellæ to one another. So that apart altogether from any special fibres that may pierce the lamellæ, or that may pass across from one lamella to another, the lamellæ are never entirely distinct from one another, which they would be were the cells actually epithelioid; as for example, is the case with those covering the connective-tissue tunics of the Pacinian corpuscles. They allow of being stripped away from one another in bone, in consequence of the presence of the cell-cavities (lacunæ) in strata between the lamellæ, in the cornea,

in consequence of the soft nature of the interlamellar ground-substance as compared with the fibrillated bundles of which the lamellæ are mainly composed.

We are led therefore from these comparisons to the conclusion that each lamella of bone consists of a layer of fibres crossing obliquely in two directions, covered on both surfaces by and embedded in a homogeneous ground-substance, which is continuous between the fibres, and also fused with the ground substance of adjoining lamellæ; the planes of separation between the lamellæ being in the fully-formed bone indicated by the presence of the flattened lacunæ.

II. *Nature of the Perforating Fibres of Sharpey.*—It is now generally admitted that the perforating fibres agree in their general appearance and intimate structure with the bundles of tendon or ligament, with the exception, of course, that they have undergone calcification. This view is further strengthened by the fact that many of them are to be traced directly from the fibrous tissue of the periosteum:[1] moreover, as Ranvier has pointed out, wherever a tendon or ligament is inserted into bone, the bundles of fibres of the tendon or ligament are continued into the bone as perforating fibres. On the other hand it is to be remarked that the latter differ from tendon-bundles in the fact that they are not overlaid by flattened cells. In micro-chemical reactions also the ordinary perforating fibres of Sharpey agree with tendon-bundles, swelling up with acids and so on. Heinrich Müller, however, remarked that some of the perforating fibres were of the nature of elastic tissue, basing this opinion chiefly upon the resistance they offered to the action of acids. My present purpose is to show how these elastic perforating fibres can readily be distinguished *in situ*, and differentiated from those of the white variety.

The process consists in staining sections of the softened bone with magenta. This dye has a singular affinity for elastic tissue, and it suffices to mount the sections, after every trace of the acid which has been used to decalcify the bone has been washed away, in a solution of magenta in glycerine and water. The fluid should be only slightly coloured, and the edges of the coverglass must be at once cemented so as to prevent evaporation of the water. This is necessary, because concentrated glycerine redissolves any of the colouriug matter that has been deposited in the tissue, and for a similar reason it is not possible to use an alcoholic solution.

[1] This by no means applies universally, for groups are often to be found which commence near the medullary canal, generally close to the circumference of a Haversian system, and the fibres of which taper off before reaching the periosteum. *Vide* Plate VII., fig. 1, *pf*.

If any of the elastic perforating fibres are present in the section they are brought very clearly into view, being stained of a dark-red colour;[1] whereas the general substance of the bone is merely tinted of a rosy-red, and the white perforating fibres are hardly coloured at all. It is not difficult in this way to make out the number and disposition of those of the elastic variety.

It is seen, in the first place, that they vary considerably in number in different parts. In some sections it is difficult to make out any, in others they are more frequent but run singly, and can only be traced for short distances, whilst in some other sections, as for instance in the one from which fig. 1 Plate VII, is taken, they are very numerous, and run both singly and in groups, generally but not always distinct from those of the white variety, and, like these,[2] being always found piercing the circumferential and interstitial lamellæ, never those of a Haversian system. They are also often traceable directly from elastic fibres in the periosteum.

The groups, like those of the white bundles (Gegenbaur), often run alongside blood-vessels (Haversian canals), which pass in from the surface of the bone, and some may even appear to pass into or emerge from such a canal. This may have given rise to the idea that the perforating fibres were to be found piercing the lamellæ of Haversian systems. It should be noticed, however, that these canals near the periphery of the bone are frequently not encircled by systems of concentric lamellæ, but merely lie between the circumferential lamellæ.

The individual elastic fibres are always, in the human subject at least, very much finer than the white perforating fibres.

Like the elastic fibres in connective tissue elsewhere, they constantly tend to branch and to unite by their branches with neighbouring fibres. Their course is never straight but wavy and often irregular through the bony substance; indeed, they are sometimes singularly curled and contorted. This fact with regard to the elastic fibres in bone casts doubt upon the notion that in ordinary connective tissue the elastic fibres in their natural condition always pursue a straight course, an opinion which seems to have been based chiefly upon the examination of stretched specimens.

III. *Origin of the Intercrossing Fibres and of the Perforating*

[1] My attention was first drawn to this by Mr. W. Rushton Parker, one of the students in my class at University College.

[2] Ranvier, 'Traité Technique. So far as my observations have gone they lead to an entire disagreement with Gegenbaur's statements and figures as to the relation of the perforating fibres to the lamellæ of the Haversian systems and to the lacunæ.

Fibres.—I am not aware that it has hitherto been attempted to identify the intercrossing fibres which are seen in the developed osseous tissue with any of the appearances exhibited by bone which is in progress of development. And this is scarcely to be wondered at, considering that most writers have ignored the very existence of those fibres. It seems nevertheless surprising that no one should have, at least so far as my knowledge of the matter extends, offered any conjecture respecting the destination of the osteogenic fibres, which are so characteristic of the true intra-connective ossific process. It is generally assumed that by the extension of the calcific deposit into them and by a deposit of a material similar to that of which they are composed, upon and between them, they become altogether blended, and that as distinct fibres they are entirely obliterated. On the contrary, I would venture to conclude that so far from this being the case *the osteogenic fibres persist as the intercrossing fibres of Sharpey.*[1]

In order to make clear the reasons upon which this opinion is based, it is desirable that the mode of advance of the ossific process into a connective-tissue membrane should be exactly understood. We will therefore first of all consider the details of the process as it proceeds in the membrane-skull and in the perichondrium of the long bones respectively.

a. Ossification of one of the Membrane Bones of the Skull.—The advancing edge of the growing bone is jagged in outline (Plate VIII, fig. 1), the bony matter shooting out into the contiguous membrane in the form of pointed processes, the so-called "spicules," to which the deposit of earthy salts in the form of minute globules gives a coarse granular appearance. Each spicule is prolonged into the adjoining connective tissue by a bunch of long, straight, unbranched delicate fibres, the osteogenic fibres. These themselves show an indication of fibrillation, and on this account and because of the similarity in chemical constitution, they are regarded by Gegenbaur as simply connective-tissue bundles of the fibrous membrane. It is possible that they may pass into true connective-tissue bundles at their further extremity, but this is by no means certain, nor do I think that it has been ever clearly made out. At any rate here, close to the advancing calcification, they are quite different in general appearance from the connective-tissue

[1] Not as the perforating fibres, as Clementi (in a paper which he has published lately, and which he makes an ill-supported claim of priority of the discovery of the perforating fibres on behalf of the Italian anatomists) wrongly makes Gegenbaur assert. Clementi's paper is more fully dealt with in the letter which follows this article. See also farther on for a criticism of Ranvier's views as to the origin of the perforating fibres.

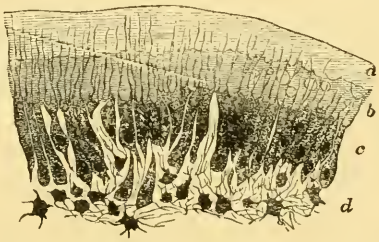
bundles which overlie them, being in fact of a very characteristic aspect (Plate VIII, fig. 2). Their straight course and their stiffness of aspect are alike remarkable; in these respects I can only compare them to the fibres of which the basilar membrane of the cochlea is composed, and which are possibly of a similar nature.

The osteogenic fibres of a bunch diverge from one other, the intervals between them are occupied by the irregularly-shaped osteoblasts. The fibres appear to be angular in shape, and the ends of the osteoblasts are flattened and are applied to the fibres which they lie between.

Since the osteogenic fibres diverge from the end of a spicule, those which are situated laterally come in contact with the similarly divergent fibres of the neighbouring bunches. When this is the case their extremities may meet and blend, and some of the fibres from one spicule may thus pass continuously into those from another spicule (see fig. 2, Plate VIII). But the majority do not thus blend, but pass across one another's direction at a more or less acute angle, and thus present an appearance of lattice-work like that of the intercrossing fibres of perfect bone, except that in their present condition the fibres are more distinct one from another. Meanwhile the calcific deposit gradually extends from the already calcified spicule into the clear and soft osteogenic fibres and also into the osteogenic substance which here cements the fibres together. The earthy deposit may also make its appearance in isolated patches on the fibres, a little in advance of the general area of ossification, but these are soon united to the rest by an extension of the calcification along those parts of the osteogenic fibres which bridge across the intervening space. In this way the first bony lamella becomes formed, and while it is thus becoming extended peripherally it undergoes a process of thickness *pari passu* at its central part by a formation of other lamellæ upon its surface. These, like the first, are preceded by osteogenic fibres which shoot out at numerous points from the surface of the bone which is already calcified; they form in a similar way systems of intercrossing fibres, and the earthy deposit gradually extends itself along them and in the intervening soft osteogenic substance. While in this manner layer upon layer of the flat bone becomes formed, two kinds of spaces are left, those which are to become respectively the lacunæ and the Haversian canals of the bone. The mode in which these are produced has been so often and so carefully described that it is unnecessary to dwell upon it here. It is sufficient to remark that the Haversian canals, large at first and enclosing besides a blood-vessel a quantity of osteoblastic tissue, become gradually narrowed by the deposit of

layer after layer of osteogenic fibres in a concentric manner upon the wall of the space. Some of the osteoblasts, in their containing lacunæ, remain between the lamellæ as the corpuscles of the future bone, and since the lacunæ are at first of considerable size they give the appearance of distinct gaps in the osteogenic substance, which thus appears to form a network upon the walls of the spaces which are to become the vertical Haversian canals. This concentric deposit and the corresponding appearances just noticed commence already upon the sides of the advancing spicule. Hence the osteogenic substance was described by Sharpey as "spreading out at the sides of the trabeculæ and encroaching on the intervening space in form of a bright trellis-work." In reality it is formed here in the same way as elsewhere, namely, in the form of straight or curved, stiff fibres applied to each other layer upon layer, and their interstices occupied by a homogeneous substance which, as well as the fibres, soon becomes infiltrated with calcareous deposit.[1]

b. Periosteal Ossification of a Long Bone.—The mode of advance of the ossific process in the periosteum of a long bone is precisely similar to that seen in the skull-membranes. The



Part of section of developing tooth of young rat, showing the mode of deposition of the dentine. Highly magnified. *a.* Outer layer of fully formed dentine; *b*, uncalcified matrix (*odontogen*) with a few nodules of calcareous deposit; *c*, odontoblasts, with processes extending into the dentine; *d*, pulp. The section was stained with carmine, which colours the uncalcified matrix, but not the calcified part.

[1] It is interesting in comparing the development of dentine with that of bone to note that in this case also the hard substance is preceded by a soft material deposited in layers, and becoming subsequently infiltrated by calcareous deposit in the form of globules (much larger than those of osseous development), which for the most part disappear as the intermediate ground-substance becomes calcified. The main difference in the two processes is to be found in the fact, that in dentine there are no fibres developed in the ground-substance. Since the soft material in which the calcareous deposit of bone takes place is termed "osteogen," that in which the calcification of dentine proceeds might be termed "odontogen."

periosteal bone is quite different in structural appearance and in its behaviour to many staining reagents from the subjacent cartilage-bone, beyond which it is prolonged at either end of the shaft for a little distance over the surface of the cartilage, so that the formation of the periosteal membrane-bone always slightly precedes the extension of the calcification of the cartilage within.

This advanced part of the ossifying periosteum generally lies embedded in a groove on the surface of the cartilage (Plate VII, fig. 2)—the "encoche d'ossification" of Ranvier; and when the ossification has progressed nearly to the end of the bone, the advanced part becomes very much thickened and extends deeply into the cartilaginous head. The appearance presented is as if the periosteal thickening were eating its way into the cartilage which is becoming absorbed before it; but it is possible that it may be produced by the lateral expansion of the cartilaginous head over the end of the ossifying periosteal tube.

Whatever may be the precise mode of its production the appearance is very striking, as exhibited in the longitudinal section of a bone the shaft of which is far advanced in the process of ossification like the one represented in fig. 2. The groove formed by the advancing periosteal ossification is represented more highly magnified in fig. 3. It is here seen that the tissue which occupies the groove is sharply marked off and totally distinct from the cartilage itself. It includes no cartilage-cells at any part, but is chiefly occupied by rounded or irregular granular cells (osteoblasts) amongst which are seen a number of straight tapering fibres, which can be traced below from the already formed periosteal bone. The tissue in question is obviously not fibro-cartilage, as described and figured by Ranvier.[1] It encroaches on the cartilage and may possibly be formed at the expense of the cartilage, but even if this be so, there is no trace of the latter remaining in it. A few of the fibres above mentioned may apparently be traced for a short distance into the matrix of the cartilage, but these are exceptional, and are mostly met with in the superficial parts near the fibrous perichondrium, the fibres of which, it is well known, are generally traceable for a short distance into the subjacent cartilage.

Respecting the delicate straight fibres which are seen in this tissue, it has been observed that they are directly continued from the bony matter which is already formed under the periosteum. From this circumstance, as well as on account of their general appearance and their osteoblast surroundings, it is im-

[1] 'Traité Technique,' fig. 159, p. 450.

possible to regard them as any other than osteogenic fibres. This was the view taken by Sharpey, who was the first to describe the tissue in question, and who was led to the conclusion from the study of preparations torn or sliced from the surface of the ossifying cartilage. In fact, in preparations so made the appearance of the advancing ossification is almost precisely the same as that of the advance of ossification in the parietal bone.[1]

Nevertheless, Ranvier has seen fit to re-christen these subperiosteal osteogenic fibres of the "encoche d'ossification" with the name of "fibres arciformes." Moreover, he describes them as being developed at the expense of the matrix of the cartilage, and curving back from this to abut against the surface of the newly formed periosteal bone, some even becoming embedded in the osseous substance and transformed into the perforating fibres of Sharpey.

How M. Ranvier could have arrived at these conclusions I am unable to conceive, unless he were misled by the examination of sections which did not happen to pass in or near the axis of the bone. The figure (fig. 3, Pl. VII) represents with the utmost fidelity the "encoche d'ossification" of the humerus of a cat-embryo, and it in no way corresponds to Ranvier's description. Moreover, I have seen similar appearances in scores of sections from growing bones of a number of different species of animals.

Sometimes bundles of connective-tissue fibres may be seen in the *encoche* passing from the periosteum and crossing the direction of the osteogenic fibres (see fig. 2, Pl. VII *pf*). It would seem to be these that become the perforating fibres; the others, I have no doubt, form here, as in the parietal, the reticulating fibres of the perfect bone.

The extension of the bony substance by osteogenic fibres which pass in bunches from the ends of the osseous points or spicules and intercross with those from adjacent points can also be seen in the transverse section of a growing bone (Plate VII, fig. 5). Here the fibres spread out in all directions in the osteoblastic tissue; they are totally distinct from the connective-tissue fibres of the periosteum, which occur chiefly near the outer surface of that membrane.

I am indebted for the careful and elaborate drawings from which most of the figures which serve to illustrate this note have been executed to the facile pencil of Mr. John Lawrence, one of the students in my class of histology. To the faithfulness of the drawings as copies of the preparations I can testify; as artistic productions they speak for themselves.

[1] See Quain's 'Anatomy,' fifth edition.

Postscript by Dr. Sharpey.

DEAR SCHÄFER,—As I know you are about to publish the results of inquiries you have lately been making into the structure and growth of bone, I am induced to ask you to give a place at the end of your communication to the following remarks which I desire to make on a note addressed to the Accademia Gioenia of Catania by Dr. Clementi, professor in the university of that city, purporting to show that the "perforating fibres of bone," to which I drew attention in 1856, had been previously described by two Italian writers on the structure of bone, Domenico Gagliardi and Michael Troja.[1]

Gagliardi's work, entitled 'Anatomes Ossium, Pars prima' (no second part followed), was published at Rome in 1689, and his views on the structure of bone have often been referred to by anatomists since his time. He considered that the compact tissue of bone has a foliated structure, which becomes apparent in bones—especially the tabular bones of the skull—which have undergone desquamation by long exposure to the weather. The layers into which they are thus resolved, "squamulæ or bracteæ," are coarse and rugged, and not to be confounded with the fine lamellæ now recognised by aid of the microscope, and Gagliardi describes them as traversed by little osseous nails or pegs—"claviculi"—which pin them together. These nails are held by Dr. Clementi to be identical with the perforating fibres which I have described.

Now, it is not easy to say what these claviculi really are; probably, as suggested by Sappey, they may be little rolls of concentric Haversian lamellæ, which become dislodged from their place; but, whatever be their nature, it is to be noted that they are comparatively large objects, represented by Gagliardi as visible to the naked eye in a figure he gives of a cranium (Tab. i, fig. 1), reduced to a third of the natural size, and no more comparable to the perforating fibres than a broomstick is to a bristle.

With greater justice, however inconsistent with his notion concerning Gagliardi's nails, Dr. Clementi next refers to passages to be found in a work of the Neapolitan surgeon, Michael Troja, which really prove that the perforating fibres had been recognised by that eminent Italian professor. Troja, in the last century, acquired well-merited distinction on account of his admirable experimental 'Essay on the Regeneration of Bone,' published at Paris, in Latin, in 1775, and afterwards in Italian, with important additions, at Naples in 1779. After having been many

[1] La Scoperta delle fibre dello Sharpey rivendicata all' Italia. Nota del Dottor Gesualdo Clementi, Professore pariggiate di Patologia speciale chirurgia, &c., nella R. Universita di Catania, 1875.

years engaged in the practice of his profession, Troja resumed his early work, and in 1814 published a quarto volume, entitled 'Osservazioni ed Esperimenti sulle Ossa, in Supplemento ad un Opera sulla Rigenerazione delle Ossa, impressa nel 1775 e nel 1779.' This volume contains the results of further inquiries on the reparation of bone and observations on various diseases of the osseous system, with finely executed figures, preceded by an account of the intimate structure of bone in general, as studied in the growing bones of the fœtal cranium at different stages, and in adult bones decalcified by means of phosphoric or nitric acid; but no figures are given in explanation of the author's histological descriptions.

Although aware that his distinguished countryman, Scarpa, denied the lamellar structure of bone, Troja maintained its reality as generally understood by preceding and contemporary anatomists, inasmuch as he could split the compact tissue, after decalcification, into layers—"piani fibrosi"—of greater or less thickness, but not identical with the fine lamellæ since recognised. The layers he describes as made up of fibrous bundles, which he distinguishes into two orders—first, those of larger size "fasci fibrosi di prim' ordine"—which run longitudinally in some bones, as the tibia, or radially, as in those of the calvaria, where they form meshes by oblique lateral junction and by lateral offsets, "appendici," an appearance especially well seen in the fœtal head; and, secondly, finer bundles—"fascetti fibrosi di second' ordine." These last cross the larger bundles in the same plane or dip down into deeper strata, an arrangement which he compares to the warp and woof of a web, and describes as visible in the growing parietal of a fœtus of three months, in which the larger bundles appear as if encircled by fine transparent rings formed by those of the second order. Clementi considers these to be undoubtedly the perforating fibres, but to me this is by no means clear. On the other hand, I cannot doubt that Troja did truly recognise the perforating fibres, for he makes unequivocal reference to them in the account he gives (p. 37) of the bands—"legamenti"—by which the different layers are held together. He explains that the different fibrous bundles already described not only join each other laterally in the same layer, but serve to bind together different layers, and thus describes the arrangement. In forcing asunder, he says, with the aid of a probe flattened at one end, the concentric layers in a softened bone, the offsets or appendices of the large fasciculi are seen crossing the probe, and on introducing a finger between a partially detached layer and the bone to which it belongs these appendices, still entire, are seen passing from one surface to the other, but on further pressure with the probe they break across with a faint

crackling noise. The fasciculi of the first order are distinguished by their aspect as well as by the great resistance they offer to the probe, which may need to be aided by a few strokes of a knife. The fasciculi of the second order are distinguishable by their fineness and the readiness with which they break. They may be further recognised on membranous layers which have been entirely detached, and which, though apparently smooth, give to the finger when lightly passed over the surface the sensation of numberless points so closely set together as to feel like a fine brush. Moreover, when viewed with a magnifying power of 40 diameters, they are seen to be perpendicular to the surface of the detached layer, whereas the broken ends of the first order of fasciculi are not only larger, but lie flat on the fibrous layer.

I cannot doubt that the objects here described are really the perforating fibres; at the same time, I cannot well conceive that those I have met with, small and soft as they are, should feel under the finger like the hairs of a brush, however fine.

Respecting Dr. Clementi's note, I have further only to point out that he is in error when he asserts, as he does (p. 12), that I did not notice the existence of the fibres in question in flat or tabular bones; and, in conclusion, I cannot help saying that when I first observed these fibres I had no idea that they had been recognized before, still less did I imagine that the subject of my observation would ever acquire such importance as to lead to a formal claim of priority on the part of Italian science.—Yours faithfully,

W. SHARPEY.

E. A. Schäfer, Esq.,
Dec. 1, 1877.

RECENT RESEARCHES *into the* NATURE *of* LICHENS. By SYDNEY H. VINES, B.A., B.Sc., Fellow and Lecturer of Christ's College, Cambridge.

IN previous volumes of this Journal,[1] Mr. Archer has traced the history of the discussion upon Schwendener's theory from its commencement in 1868 down to the end of the year 1873. I will endeavour to maintain the continuity by briefly alluding to the principal papers which have appeared upon the subject during the intervening years, reserving the more recent publications for a somewhat detailed account.

[1] Vols. xiii and xiv.

The last paper mentioned by Mr. Archer in his résumé is the important one by Treub,[1] published in November 1873. In the 'Flora' for January 1874, J. Müller hastens to combat the results at which Treub had arrived. He regards the question as still an open one, in spite of the recent publications of Treub and Bornet, and maintains firmly the accuracy of his own observations in which he distinctly traced the development of the gonidium from the hypha in *Synalissa Salevensis*. He goes so far as to say that, even if his own observations were inaccurate, the new theory cannot be considered as established until it is ascertained that the spermatia give rise to hyphoid and not to gonidial products. Müller's paper was soon followed by another on the same side from the pen of Nylander.[2] In it are repeated most of the objections which this distinguished lichenologist had already raised to the acceptance of Schwendener's theory, and attention is particularly drawn to the fact that those Algæ which are regarded by the supporters of the theory as playing the part of gonidia in various Lichens—such Algæ, for instance, as Cora, Dichonema, Scytonema, Sirosiphon—are held by him to be themselves of the nature of Lichens. From this point of view, the new theory of the structure of the lichen-thallus is simply absurd.

In the July Number of the 'Popular Science Review,' of the same year, the Rev. J. M. Crombie, comes forward as another opponent of the new doctrine. In his article he gives a brief history of the whole discussion, and sums up strongly in favour of the older views. He is especially severe upon one of Bornet's "strong points," viz., the identity of *Protococcus viridis* with the gonidia of *Physcia parietina*. He admits the similarity existing between these organisms, but cannot recognise it as amounting to identity, for the gonidia of Physcia are larger and multiply less actively, while Protococcus multiplies very rapidly. Some facts, to which attention will subsequently be called, will shew that these differences of habit do not suffice to prove these organisms to be distinct.

Later on in the year an elaborate defence of the older views was published by Körber.[3] He lays down three propositions, (1) that the tissue in which the gonidia of a Lichen are embedded is not of a fungoid nature; (2) that the gonidia are not true Algæ; and (3) that Lichens are not the expressions of a condition of parasitism. In support of his first proposition he recalls the differences which Von Krempelhuber pointed out as existing between the tissues of Lichens and those of Fungi, but

[1] 'Onderzoek. over de Natuur der Lichenen. Leiden.'

[2] 'Flora,' abstracted by the Rev. J. M. Crombie in 'Grevillea,' vol. ii.

[3] 'Zur Abwehr der Schwendener-Bornet'schen Flechtentheorie,' Breslau.

admits that these differences do not hold good in all cases. He states as a fact that there are Lichens, for the most part crustaceous, such as *Secologia abstrusa, Hymenelia affinis, Sarcogyne privigna* and others, which have no hyphæ in their thallus, although asci and paraphyses are present. Such a fact as this he considers to be a crushing proof of the absurdity of Schwendener's theory, for here are Lichens in which only one of the usual constituents is present.

In support of his second proposition he points out that Baranetzky has observed the protrusion of hyphal filaments from gonidia, and adds that he has himself seen this occur in Porocyphus and Collema. Such an occurrence is quite unknown among the true Algæ. Furthermore, it is well known that several forms of gonidia may occur in the same lichen-thallus, as in *Harpidium rutilans, Pannaria granatina, Racoblenna tremniaca,* &c., and Körber suggests that it is extraordinary that a Lichen should require so many forms of Algæ to act as the gonidia of its thallus. He concludes his argument by saying that if the gonidia were true Algæ they would by this time, have been all met with in the free state, whereas this is by no means the case; the gonidia of Nætrocymbe, Phylliscum, Melanormia and others, for instance, have not yet been found elsewhere than in the lichen-thallus. Moreover, he insists that when gonidia are found in the free state, they are not therefore to be regarded as Algæ. Finally, he lays stress upon the apparent fact that the gonidia resemble only such so-called Algæ as reproduce themselves solely by division.

In supporting the third proposition, the author adopts the view of Fries, according to which the term "parasitism" is inapplicable in describing the connection of the "fungus" with the "alga" in a lichen-thallus, and adds that if any "parasitism" exists at all it must be mutual, pointing out that in a thallus there are so many gonidia which are not connected with hyphæ that the relation of the latter to the former must be other than that of a parasite.

In the concluding paragraphs he recalls, as Müller also does in his above-mentioned paper, the old view of the origin of the gonidia from the hyphæ, and goes on to say that if the connection of the gonidia with the hyphæ is not of a genetic nature, it must indicate some nutritive process by which the gonidium obtains from the hypha some of the material which is essential to its existence. From this stand point he ventures upon some remarkable speculations as to the probable details of this nutritive process which need not be considered here.

He enumerates finally the various modes in which a lichen-thallus may be reproduced, six in all, of which two are by means

of spores,[1] and four by gonidia with (soredia) or without hyphæ. The fact that a lichen-thallus may be developed from cells which do, as well as from cells which do not, contain chlorophyll establishes, he considers, the autonomy of the Lichens.

Of the publications which afford evidence in favour of Schwendener's theory the first is that of Borzi.[2] In it an account is given of numerous "culture" experiments made with various Lichens, such as *Parmelia pulverulenta*, *Physcia ciliaris*, and others. The conclusions to which the observations led were the following: (1) that the gonidia stand in no genetic relation to the hyphæ, but that on the contrary they are autonomous organisms, true Algæ, which serve as hosts for the hyphæ; (2) that the relation between the hyphæ and the gonidia is always such as exists between a Fungus and the substratum upon which it lives; and (3) that Lichens consist of ascomycetous Fungi parasitic upon their gonidia, which are true Algæ.

An important contribution to the discussion is the paper by Bornet.[3] He begins by giving some account of cases observed by him in which the gonidia of certain Lichens reassumed their algal condition. In certain old thalli of *Opegrapha varia*—the gonidia of which are furnished by filaments of Trentepohlia (Chroolepus)—he found that the gonidia had here and there regained their normal structure. They had become elongated and had produced numerous sporangia, such as are peculiar to the genus Trentepohlia, and numerous zoospores could be detected moving amongst them. These fertile filaments were distinctly continuous with those which were still acting as the gonidia of the Lichen. Again in *Pannaria triptophylla*, *Nyl. var. nigra*, he frequently found projecting from the tubercles of the thallus which had become ruptured, filaments which were evidently prolongations of the mass of bluish gonidia contained in the thallus of this species. These observations convinced him that the two kinds of gonidia in the thallus of this Lichen, though so different in appearance, are really two forms of the same Alga. The filamentous gonidia are but slightly modified forms, but in the spherical gonidia the original normal algal form is no longer recognisable.

Further, the thallus of Collema, as is well known, becomes covered under certain circumstances with great numbers of small round grains, produced from the extension and development towards the exterior of a fold of the gonidial filaments. Usually

[1] He states that he has observed in Sphæromphale the direct development of gonidia from spores (?).

[2] 'Intorno agli offici dei gonidi de'Licheni, Estr. dalla Scienza contemporanea, Palermo.' Reprinted in 'Nuov. Giorn. bot. Ital.,' 1875.

[3] "Deuxième note sur les gonidies des Lichens," 'Ann. Sci. Nat.,' sér. v, t. xix.

hyphæ penetrate into this excrescence as it forms, and the result is that these microscopic grains possess the two structural elements of a Lichen. It occasionally happens, however, as De Bary[1] has shown, that they have no hyphæ. They are then simply Nostocs which entirely resemble those which are to be found amongst Mosses. Nearly always in such cases the young Nostoc which is attached to the Collema only by a slight gelatinous pedicle, becomes detached.

He goes on to cite other examples of the same kind with the view of proving that the gonidia can easily take on the characters of free Algæ and vice-versa. He concludes this part of his paper by referring to the genus Lichina, expressing an opinion that the gonidia of this genus are formed by filaments of some Rivularia, an opinion which was fully borne out by Kny's researches upon the development of the thallus of *Lichina pygmæa*,[2] which were published later in the year.

Having brought so much evidence to prove the identity of the gonidia of many Lichens with certain of the lower Algæ, he goes on to discuss objections which might be raised against this view, such an objection, for instance, as that raised by Körber (see *antè*), that these Algæ are not autonomous organisms but simply the gonidia of Lichens in a free state. Such an hypothesis, he says, is quite inconsistent with our present knowledge of Algæ, for it is certain that these same Algæ do not multiply merely by division, but also by a fructification of their own which is quite distinct from that of the Lichen. He then briefly gives an account of various investigations which have been made into the life-histories of the gonidia, such as Famintzin and Baranetzky's discovery of zoospores in the *Cystococcus humicola* extracted from the thallus of various species of Lichens, his own observations of the emission of zoospores by Trentepohlia which furnishes the gonidia of *Opegrapha varia*, and draws attention to the similarity, amounting to identity according to Pringsheim, of Phyllactidium which furnishes the gonidial element of *Opegrapha filicina*, with Cœleochæte.[3] Here then are numerous instances of the multiplication of gonidia by certain specialised reproductive processes, and many instances of the kind may be cited as occurring amongst those gonidia which belong to the Phycochromacæ.

He concludes by recounting his experience of separate cultures of gonidia, and of spores of Lichens. The former multiplied immensely but no trace of a hypha could be detected—the latter germinated and grew, but no gonidia were developed.

[1] 'Handb. d. Phys. Bot.,' Bd. ii, Abth. i, p. 290.

[2] 'Sitzber. d. gesellsch. naturf Freunde in Berlin,' Nov., 1874.

[3] See this Journal, vol. xv, p. 334.

In the 'Comptes Rendus' for November, 1874, H. A. Weddell draws attention to the Lichineæ as being very favorable objects for observations of this kind. The gonidia not only form the greater part of the thallus of the Lichens, but they regulate its form. The hyphæ lie in the gelatinous investment and bear the apothecia. Such Lichens have been termed pseudo-algæ. Unfortunately this term has been extended to those Algæ which the opponents of Schwendener's theory regard as imperfect Lichens, that is, as gonidia; such are Stigonema and Scytonema. If it can be proved that there are true Algæ, one of the strongest objections to the theory would be overthrown. He considers that such evidence has now been collected. The researches of Janczewski, and of Thuret and Bornet, upon the reproduction of Nostoc by spores demolish the argument that it is an imperfect or a modified form of Collema; and the researches of Bornet (see *antè*) upon the gonidia of Opegrapha have the same effect. He then brings forward a communication made by Gibelli to the Botanical Congress held at Florence (May, 1874).[1] Gibelli had observed the formation of zoospores in the gonidia of *Lecanora subfusca* while still in the thallus. This observation Weddell says enables us to account for the so-called spontaneous appearance of gonidia in parts of the thallus where none had previously existed. Gibelli also confirms Bornet's experiments on Opegrapha.

At a meeting of the Société Botanique de France, held on November 27th, the physiological aspects of the question were discussed. M. Cornu opened the subject by saying that the peculiar parasitism exhibited by Lichens was not sufficient ground for separating them from other Ascomycetes. Such a parasitism exists also in other families of the same group, as, for example, that of *Sphæria cupularis* upon the red stroma of *Nectria cinnabarina*, and again, of *Asterosporium Hoffmanni* upon *Cucurbitaria macrospora*. M. Weddell pointed out that in these examples the two organisms belonged to the same family group of plants, whereas in Lichens it was a cohabitation of organisms belonging to different classes. The term "parasitism" if used to describe this cohabitation is used in a forced sense, for the host instead of suffering grows the more vigorously.

M. Cornu, in reply, referred to the observations of Bornet, from which it appears that the form of the Algæ is somewhat modified under the influence of the hyphæ. M. Weddell rejoined that in Collema and its allies the hyphæ do not come into contact with the cells of the Nostoc filament, and that, moreover, the modification of the Alga in other Collemaceæ under the influence of the hyphæ is not such as could forbid the assumption that the

[1] 'Atti del. cong. intern. bot. Firenze 1876.'

vegetation of the Alga is under these circumstances more active. M. van Tieghem distinguished two kinds of parasitism among Fungi, (1) necessary and (2) voluntary, examples of which he adduced in illustration. In Lichens it is otherwise, the cohabitation of Alga and Fungus is a reciprocal parasitism, the Alga probably supplying the Fungus with carbohydrates and being, in return, preserved from desiccation by the investing hyphæ.

To the number of this Journal for January, 1875, Archer contributed a paper "On Apothecia in some Scytonematous and Sirosiphonaceous Algæ in Addition to those Previously Known." His researches were suggested by those which led Bornet[1] to remove *Stigonema atrovirens* from the Algæ and to place it among the Lichens under the name of *Ephebe pubescens*; and their object was to discover, if possible, in allied forms the distinctly lichenous fructification—apothecia and spermogonia—which this species of Stigonema had been found to possess. Archer was successful in discovering apothecia in *Scytonema myochrous*, as well as in another unidentified form, in two species of *Sirosiphon* (*alpinus and pulvinatus*), and finally in *Stigonema mamillosum*. He was, therefore, led to assume that these genera and probably the whole of the Scytonemaceæ and Sirosiphonaceæ could be no longer properly accounted Algæ, but should be relegated with Ephebe to the Lichens. Archer was unable to detect any hyphæ in the species which he examined, though Schwendener had found them in Ephebe and Bornet in *Spilonema paradoxum* and in *Lichenosphæria Lenormandi*, nor could he discover any spermogonia.

The next important publication is that of Arcangeli.[1] In the general discussion of the question which precedes the account of his own researches, he points out that Schwendener's theory has in its favour the fact that Fungi are parasitic plants incapable of forming chlorophyll so far as our present knowledge extends. He argues that it is not, however, impossible to imagine the existence of plants which, though of a distinctly fungoid nature, may yet be able to form chlorophyll and to assimilate. Lichens undoubtedly resemble Fungi very closely both in their vegetative structure and in their reproductive organs, why then, he asks, may not Lichens be regarded as plants belonging indeed to the group of Fungi but containing chlorophyll? In the Phanerogams examples occur of natural families, some members of which do, and some do not, contain chlorophyll.

He brings forward some observations in support of this view which tend to prove that chlorophyll may occur in cells of Lichens

[1] "Recherches sur la structure de l'Ephebe Pubescens," 'Ann. Sci. Nat,' sér. iii, t. xviii.

[2] 'Sulla questione dei gonidi, Nuov. Giorn. bot. Ital., 1875.

other than their gonidia. For instance, he believes that he has detected grains of phycochrome in the spores of some Collemeæ and Parmelieæ, and a green substance in the hyphæ of the thallus of *Pannaria triptophylla*.

He regards the researches of Janczewski, of Thuret, and of Bornet, which give us a knowledge of the complete life-cycle of Nostoc as affording strong evidence in favour of Schwendener's theory, but he does not consider that it suffices to prove the autonomy of Nostoc. The well-known heterœcious mode of life of Fungi, as well as their polymorphism, render it quite probable that such phenomena should also be observed in a group so nearly allied to them as that of the Lichens.

Then follows an account of various observations of comparatively small importance which tend to prove the similarity of the tissues of Lichens and Fungi, and of others respecting the mode of connection between the hypha and the gonidia, which he has found, in some cases, to be different from that described by Bornet. With reference to the development of the gonidia he concludes from his observations upon *Usnea barbata*, *Alectoria jubata*, and *Ramalina farinacea*, that in the apices of the branchings of these Lichens gonidia are developed from the hyphæ, for he found them quite isolated in such positions that their origin could not be referred either to a division of previously existing gonidia or to the entrance of zoospores from without. He detected a peculiar mode of development in *Cladonia rangiferina*. At the extremities of the branchings of the thallus he found groups of cells, with ill-defined contours, in which the hyphæ became lost. Near to the apex these cells are colourless, but farther back they are coloured green (gonidia), and between these extremes all intermediate stages occur. The author is inclined to regard these groups of cells as Soredia.

In many foliaceous and crustaceous Lichens, such as *Nephroma levigatum*, *Sticta pulmonacea*, *Peltigera canina*, and others, he detected another mode of development of gonidia. All these Lichens are invested either on one or on both surfaces by a cortical layer of pseudo-parenchyma. A gradual transition can be made out between the colourless cells of this tissue and others, lying internally, with completely green cell-contents (gonidia).

In *Pannaria triptophylla* the author also detected some instructive peculiarities. He found that the tubercles on the surface of the thallus at an early stage contained only one gonidium, or at most a very few, and in this condition they resemble Nostoc-cells or filaments. When these tubercles are mature they usually are ruptured and through the opening filaments of

gonidia protrude which have a basal heterocyst, and closely resemble filaments of Rivularia. These observations agree in the main with those of Bornet already mentioned. The author describes the development of these tubercles as taking place in two ways; in the one, they are formed by a coalescence of the hyphæ of the thallus to form a group of cells in the manner already described in speaking of the development of gonidia in *Cladonia rangiferina*, in the other, they are formed from soredia derived from older tubercles which became invested by hyphæ. The mode of origin of the gonidia in the tubercles is similar to that which takes place in the cortex. From these facts, and from the occurrence of numerous gradational forms both as to shape and as to colour, it appears that the gonidia of this Lichen are in reality all of one kind, a conclusion at which Bornet had also arrived.

Finally, the author points out the improbability of the views held by van Tieghem and by Weddell as to the existence of parasitism in Lichens. He considers that the gonidia do not arise from spores which have recently germinated, but from organs which have a more or less pseudo-parenchymatous structure, a view which finds support in the facts above detailed. He concludes that various Protococcaceæ, Nostocaceæ, and Rivulariaceæ are only certain forms of lichen-gonidia in certain phases of their vegetation, and that therefore the gonidia are organs peculiar to Lichens.

In the 'Flora' for that year, Winter[1] published a paper in which he shews that four of the examples adduced by Körber (see *antè*), in support of his statement that Lichens exists which possess no hyphæ, viz., *Secologia abstrusa*, *Sarcogyne privigna*, *Hymenelia affinis* and *Nætrocymbe fuliginea*, do, as a matter of fact, possess hyphæ. In a second publication,[2] he exposes the inaccuracy of Körber's statements with regard to Sphæromphale and its allies, (1) in that hyphæ do actually exist in this Lichen, and (2) in that the spores on germinating do form hyphæ and do not, as Körber asserts, give rise directly to gonidia. These observations suffice to answer some of the more important objections—because based apparently upon experimental evidence—brought forward by Körber against Schwendener's theory.

'Nature' for January 27, 1876, contains a commmunication from Lauder Lindsay upon the nature of Lichens. In it he criticises severely the views of Schwendener and his followers, and repeats his suggestion for the establishment of intermediate and provisional groups of Algo-lichenes and Fungo-lichenes. He

[1] 'Zur Anatomie der Krüstenflechten.'

[2] 'Ueber die Gattung Sphæromphale und Verwandte, Prings, Jahrb. f. Wiss. Bot.,' Bd. x.

points out that there are several difficulties in the natural history of Lichens with which the Schwendenerians have to deal, as, for instance, the case of Athalline Lichens that have neither hyphæ nor gonidia, that are represented, in fact, only by apothecia—and for examples he refers to Archer's paper which has been considered above. It must be remarked that the author appears to have overlooked the conclusions at which Archer arrived. He finishes his letter by comparing Schwendener with Bayrhoffer!

An interesting paper was contributed later on in the year by Frank.[1] As the result of his observations on the development of the gonidia he states that there two ways in which they are formed in the lichen-thallus; (1) usually they are derived from those already existing in the thallus; (2) they make their way into the growing thallus from without, and then multiply. Another mode of origin of gonidia was described about the same time by Minks.[2] According to his observations the germinating spore gives rise to a mass of hyaline hyphæ from which, together with the coloured secondary hypha (which is probably connected with the development of the apothecia) certain organs are formed,—termed by him Gonangia and Gonocystia—within which gonidia are formed by free-cell formation.

In passing to the consideration of the papers published in 1877 upon this subject it is interesting to recall a sentence which occurs in Lauder Lindsay's letter, already quoted. He says: "If, by artificial cultivation, such a union (*i.e.*, of Fungus and Alga) could be made to produce a Lichen, the theory might he held as proven." Such evidence is afforded by Stahl's[3] paper on the nature of hymenial gonidia, as the following account will show.

The experiments which had previously been made in this direction by Reess, Bornet and Treub, had met with only partial success. In all cases the lichen-spores after being sown upon various Algæ germinated, and their hyphæ formed with the Algæ, a structure resembling in some degree the thallus of a Lichen, but in no case was any fructification ever developed. Stahl's researches upon the hymenial gonidia enabled him to overcome the difficulties which had marred the success of the experiments of his predecessors, and enabled him to trace the whole course

[1] 'Ueb. d. biolog. Verhältnisse des Thallus einiger Krüstenflechten, Cohn's Beiträge,' Bd. ii.

[2] 'Beit. z. Kennt. des Baues und Lebens der Flechten, 1, Gonangium und Gonocystium,' Wien, 1876.

[3] 'Beiträge zur Entwickelungs-geschichte der Flechten, 1877.' 1. "Ueber die geschlechtliche Fortpflanzung der Collemaceen." 2. "Ueber die Bedeutung der Hymenial-gonidien." The first of these papers will be discussed at another time.

of the development of the lichen-thallus from the germination of the spore to the formation of apothecia.

These hymenial gonidia are, as their name denotes, gonidia which occur in the hymenial layer of the apothecium in certain Lichens. Nylander seems to have been the first to observe them, and the subsequent investigations of Fuisting and of Winter, have shewn that they originate from the ordinary gonidia of the thallus. They differ from the gonidia of the thallus in that they are smaller and are of a less vivid colour. The primary object of Stahl's researches was to account for this difference in appear- between these two kinds of gonidia, and to discover the real significance of the presence of gonidia in the hymenium.

The Lichen which he more especially studied was *Endocarpon pusillum* (Hedwig). In its thallus the gonidia, which are of a uniform green colour, form a layer lying between the medullary and cortical parts and extending here and there for a greater or less distance into the cortex. By their mode of division they indicate their connection with the algal genus Pleurococcus. The perithecia present the structure which is usually found in pyreno-carpous Lichens. In the spaces between the asci (Fig. 1), and more or less filling up its whole cavity, is a gelatinous substance which is produced by the swelling up of the membranes of emptied asci, in which lie the numerous pale-green hymenial gonidia. They differ remarkably in size from the gonidia of the thallus, having a diameter of only 0·002—0·004 m.m., whereas the diameter of the latter is from 0·008—0·012 m.m. Each ascus usually con-tains two spores of unequal size, the upper one being rather larger than the lower. When ripe, they are brown, multicellular, pseudo-parenchymatous structures of considerable size. When moistened, the asci and the gelatinous substance surrounding them absorb a quantity of water and swell-up. In consequence of the tension thus produced within the perithecium, the ripe asci burst and expel their spores with such force that they are projected to a distance of several centimeters.

An examination of the spores which have been thus extruded shews that they are surrounded by a number of the pale-green hymenial gonidia. (Fig. 2). If a spore be kept moist, it germi-nates at once after its extrusion, and if it be placed upon a glass slide the whole process can be easily observed. A certain number of the hyphæ which spring from the segments of the spore invest the hymenial gonidia by which the spores is surrounded (Fig. 3), and in a few days the invested gonidia shew that they have undergone considerable change. The previously scanty chlorophyll which gave them a pale-green colour has increased in quantity, so that the enlarged gonidia are uniformly dark-green. A comparison of the free and of the enclosed gonidia shewn in

fig. 3, cannot fail to suggest that the increase in size exhibited by the latter must be attributed to the influence of the Fungus. They have ceased to undergo division, whereas the free gonidia have given rise to numerous individuals. Other hyphæ grow and ramify, and although they come here and there into contact with gonidia, they do not grow round them, but continue to grow in a straight line along the surface of the slide. These hyphæ correspond to those which, on a normal substratum (rhizines) penetrate into it and act as absorbing organs.

The cultures of Endocarpon made in this way, although the plants continued to live for as long as six months, were never so successful as to produce a thallus distinctly differentiated into cortical and medullary portions; apparently the conditions were altogether too abnormal. An endeavour was made to make the conditions more favourable by substituting for the glass-slide, as a substratum, a piece of porous earthenware, the cavities of which had been filled up with soil deposited from suspension in water. Under these circumstance the young thallus presents at an early stage a differentiation into an upper portion which contains the gonidia, and a lower which is subterranean. (Figs. 4 and 5). The structure of the upper portion is at first very simple, consisting of a mass of closely-packed gonidia and hyphæ invested by a layer of pseudo-parenchyma, but gradually a differentiation into a cortical and a medullary part, with an intervening gonidial layer, becomes apparent. The lower, subterranean portion (hypothallus) consists of colourless, septate, branched and anastomosing hyphæ, some of which are isolated and others are aggregated in bundles.

Soon after this stage of development has been attained the first Spermogonia make their appearance. They are ovoid in form and are completely sunk in the thallus; they contain no gonidia. The first appeared within six weeks after the sowing of the spores, and very shortly afterwards the first formation of perithecia became evident. The first spores came to maturity within four or five months.

The history of the hymenial gonidia which did not become invested by hyphæ affords some interesting facts. It has already been mentioned that they divide, and that the products of their division do not exceed in size the mother-cells from which they are derived. The divisions take place in one direction only, namely, at right angles to the longer axis of the cells, so that the Alga ought to be considered in this condition to belong to Naegeli's genus Stichococcus, were it not that further observations shew that these two genera must be united.

Frank has suggested that possibly the gonidia, in consequence of having been for many generations so intimately associated with

hyphæ, have become incapable of active independent vegetation—Stahl does not consider that the facts justify such a view. It is true that the size of the algal cells which are left to vegetate freely is, on an average, much smaller than that of the cells which are acting as gonidia; but the rapid reproduction of the freely vegetating Alga cannot co-exist with imperfect nutrition. The differences in size and appearance must probably be attributed solely to the various mechanical or physical conditions to which the Algæ are respectively exposed.

Observations of a similar nature were made on *Thelidium minutulum* (Körber). This Lichen made its appearance regularly in the cultures of Endocarpon. It could not have made its way thither by means of soredia, and the only means of accounting for its appearance is the assumption that it was produced from spores which, on germinating, availed themselves of some of the extruded hymenial gonidia of Endocarpon to form a thallus. This assumption was proved true by direct experiment. The spores of Thelidium sown in the absence of gonidia gave rise to a mycelium which, so soon as the store of nutriment contained in the spore was exhausted, withered away; but if sown with gonidia of Endocarpon a fructifying thallus was gradually formed. In this case also the influence of the Fungus upon the Alga was made apparent by the increased size of the latter, and in the larger amount of chlorophyll contained within it.

Further interesting facts were obtained by the investigation of *Polyblastia rugulosa* (Massal.). The spaces between the asci of this Lichen (each of which contains usually eight spores) are occupied by hymenial gonidia arranged in rows, derived originally from the gonidia of the thallus—from which they differ in a very marked manner, the former being rod-like, the latter more or less rounded and dividing like the pleurococcoid gonidia of Endocarpon. If some of the thallus gonidia be cultivated on a slide they will be seen to divide in successive planes in the three dimensions of space. The products of this division are not, however, rounded cells like those from which they are formed, but are cylindrical and divide only in a plane at right angles to their length, the segments separating the one from the other. Thus it is possible to obtain from gonidia dividing like Pleurococcus the algal form known as Stichococcus. This Stichococcus is identical in form, in size, and in the mode of its division with the rod-shaped gonidia occurring in the hymenium.

As in Endocarpon so in Polyblastia, a certain number of hymenial gonidia are extruded from the perithecium together with the spore, which became invested by hyphæ when the spore germinates. The rod-shaped hymenial gonidia then gradually lose their cylindrical form, increase in size and assume the rounded

contour which characterises the gonidia of the thallus of the Lichen in question.

The conclusions arrived at as the result of the foregoing investigations are as follows:—(1) in those Lichens which possess hymenial gonidia the thallus is regularly produced by the coming together of the spore of the ascomycetous Fungus and the Alga; (2) the hymenial gonidia when actively dividing derive their nutriment from the constituents of the structure in which they are enclosed: in view of the small quantity of chlorophyll which they contain, it does not seem probable that they give up to the Ascomycete any of the products of their assimilation; (3) if it be suggested that the hymenial gonidia appear to resemble the entophytic algal colonies found in Anthoceros, Azolla, Gunnera and others, it must be remembered that they are of extreme physiological importance in the economy of the Lichen in that they afford to the germinating spores material for the formation of a new thallus.

With this account of Stahl's observations this resumé of the lichen-gonidia discussion must for the present close. It is quite evident that the theory of the structure of Lichens which was suggested originally by De Bary and first tested experimentally by Schwendener is gradually becoming established upon a sure foundation of accurate observations. It will be noticed that the objections brought against it are, on the whole, more of a theoretical than of a practical nature. The theoretical objections may be regarded as silenced by the researches above alluded to of Winter, Frank and Stahl, and if further experimental evidence be required it is to be found in Stahl's paper on the sexual reproduction of the Collemaceæ, which will be discussed hereafter. The practical objections are mainly the observations of Arcangeli and of Minks, which seem to shew that gonidia may be developed from hyphæ. It must be remarked that although these observations tend to prove the same fact, yet they differ widely, and therefore cannot be regarded as being confirmatory the one of the other. It remains to be seen whether or not other observers will in the future be successful in repeating these observations, and if they will endorse the interpretations which have been put upon them.

On the ENDOTHELIUM *of the* BODY-CAVITY *and* BLOOD-VESSELS *of the* COMMON EARTHWORM, *as* DEMONSTRATED *by* SILVER-STAINING. By D'ARCY POWER, Exeter College, Oxford. (With part of Plate X.).

THE application to invertebrata of the methods of histological research devised by students of the organization of vertebrate animals, is likely to yield interesting results. At the suggestion of Prof. Lankester, I have examined the structure of the lining membranes of the body-cavity and blood-vessels of the Earthworm, by aid of the well-known method of silver-impregnation, which has yielded such valuable results in connection with the serous membranes and vascular structures of Vertebrata in the hands of von Recklinghausen and of Klein.

A large Earthworm, killed by chloroform, is opened along the dorsal median line in a gutta-percha trough, and the body walls are here and there secured to the floor of the trough by pins. A one-half-per.-cent. solution of silver nitrate is then poured into the trough and allowed to cover the opened worm, sufficient solution being used to wash away from the exposed walls of the body-cavity the white coagula formed by the liquid of the body-cavity. After five minutes' action the silver solution is poured off, and an abundance of pure water substituted. Then it is possible to remove with the scissors and forceps portions of the membranous inter-segmental septa and complete segmental organs or nephridia with their attached membranes. The wall of the testicular sacs and portions of the muscular body wall may also be removed for examination. The pieces cut out are separately mounted in glycerine under cover-glasses in the usual way, exposed to the light and then examined with the microscope. It is necessary, if any particular organ is to be exposed to the action of the silver, to so open the worm that the surface of the desired organ shall be freely acted upon by the silver solution, whilst at the same time it is necessary to be very careful not to abraid the exposed surface.

Endothelium of the Body-cavity.—Claparède ("Histologische Untersuchungen über den Regenwurm," in 'Zeitschrift fur wiss. Zoologie,' 1869), has already described the "epithelium-like clothing" of the body-cavity and the organs lying therein. In particular he has figured the epithelial covering of the nerve-cord—the "outer neurilemma," as he terms it, covering in the longitudinal muscles and blood-vessels which surround the nervous tissue. He has also figured (Plate xlviii. fig. 5, loc. cit.) certain knob-like outgrowths of the peritoneal coat which are exactly paralleled by the knob-like outgrowths of the frog's

peritonæum, described by Klein in this Journal (Vol. XII, p. 43.)

Claparède draws attention to the peculiar swollen character which the cells of the peritoneal endothelium (or epithelium) of the Earthworm usually present, and remarks that whilst it may be disputed as to whether they should be considered strictly as an epithelium or as belonging to that "globular-celled form of connective tissue" made known in Mollusca by the researches of Leydig—yet, as a matter of fact, the distinction is not important; for such globular-celled connective tissue actually passes by gradual transition into a more flat-celled epithelium.

At the present day no one would venture to draw a distinction in kind between the cells bounding the surfaces of connective tissue-membranes and those more deeply placed. In the mollusca (for instance in the Lamellibranch's gill) we find the swollen globular cells, which form so much of the connective tissue in some parts, passing over into branched corpuscles forming trabeculæ about the blood-passages of the gill (the "lacunar tissue" described by Peck, this Journal 1877, p. 45), and also proliferating and giving rise to amœboid floating corpuscles. So, too, the connective-tissue cells of the Earthworm present us with a wide range of form, exhibiting in parts, particularly on the intersegmental septa, the character of globular cells (Schleimzellen of molluscan histology), and again where the membrane is subject to tension, as on the surface of the enlarged testicular sac becoming thin, flat, and in fact pavement-like; whilst yet again at other points it seems that these endothelial cells proliferate and give rise to the amœbiform corpuscles which float in the perivisceral fluid.

The very remarkable and special characters assumed by this layer of cells on the surface of the intestine and of the large vessels (forming what has been called, on account of its bile-like colour, the "hepatic" tunic), I am not now prepared to discuss, but I may point out that the yellow granules which here so deeply colour the cells are similar to pigment granules developed in tracts of connective tissue in the Lamellibranchs (see Kollman, "Bindesubstanz der Acephalen," 'Archiv fur Mikr. Anat.,' vol. xiii.)

The observations of Claparède were made without the use of silver nitrate; it is possible without this reagent by means of hæmatoxylin or carmine to bring into view the outlines of the more swollen globular epithelial cells, but the flatter and more delicate tracts of epithelium are missed.

The most striking result of my observations is the very close resemblance which appears to obtain between the epithelium clothing the body-cavity of the Earthworm and that occupying

the same position in Vertebrata. I have traced the silver-outlines on the surface of the following organs—1, The long muscles of the body-wall (elongate cell-outlines with undulations); 2, ovary (polygonal cell-outlines); 3, testicular sacs (large polygonal cell-outlines); 4, outer neurilemma (polygonal); 5, intersegmental septa (interlocking undulated outlines.)

In Plate X. fig. 9 is represented a portion of the tissue which holds the coils of the nephridia to the intersegmental septa. This tissue is not merely an epithelium, but forms an actual membrane comparable to the more delicate examples of mammalian omentum. It is typical "retiform" tissue. Considerable tracts of this fenestrated membrane may be isolated and spread on the glass-slide for observation—that is to say, pieces measuring the one-sixth of an inch in diameter. The "fenestræ" vary greatly in size, as in the omentum, measuring from the $\frac{1}{1500}$th to the $\frac{1}{100}$th of an inch in diameter. In the specimens from which the drawing was made the nuclei of the cells were well preserved, but the silver-outlines were only here and there present in such strength as is shewn in the plate.

Endothelium of Blood-vessels.—The specimens of membranous septa from the earthworm, treated with silver, frequently shewed blood-vessels in which cell-outlines were marked out with all the distinctness which one is accustomed to see in silver-preparations of the vertebrate mesentery.

In Plate X. figs. 7 and 8, I have represented some of these vessels, and in figure 5, a pair of the characteristic vascular globules or swellings so abundant on the renal organs (nephridia) are drawn, one shewing its silver-outlined endothelium.

The endothelial outlines of the two parallel-running vessels drawn in fig. 7 are remarkable on account of the differences which they present, the cell outlines being in the one case such as to indicate elongated cells few in number, whilst in the other case the cells are shorter, more numerous, and more variously interlocked. Such differences are observed in vertebrata between small arteries and veins as between these two vessels, the more elongate epithelium characterizing an artery. It is important to obtain in this way distinct evidence of the differentiation of the vascular trunks of the Earthworm into arterial and venous, afferent and efferent—a differentiation which has sometimes been doubted, but which it would be necessary to assume in the case of such an elaborate excretory organ as the nephridium from which these two vessels are taken, even had we not the structural evidence of the fact which I now submit.

I have not been able in the smaller vessels, such as those drawn in fig. 7, measuring $\frac{1}{1000}$th of an inch or less in diameter, to determine the existence of any muscular coat or of

any adventitia. The vessels larger than this possess an external coat of connective tissue, and I am inclined to think that below this there is only the layer of endothelial cells, which in these larger vessels are modified so as to form incomplete hoop-like or circularly-disposed elements. This appears to be the interpretation which must be put upon the silver-outlines drawn in fig. 8.

I have not at present investigated the structure of the contractile vessels, which should yield interesting results with silver-staining, and also when prepared as transverse sections.

The foregoing observations were made in the histological laboratory of Exeter College, Oxford, being part of a more extended investigation of the structure of the renal tubes or nephridia of the Earthworm.

On the Life History *of* Bacillus anthracis.

By J. Cossar Ewart, M.B., University College, London. With Plate XI.

Through the kindness of Dr. Burdon Sanderson, Superintendent of the Brown Institution, I have been enabled to repeat the well-known inquiries of Cohn and Koch[1] into the life-history of *Bacillus anthracis*. This organism has been described as a motionless, rod-shaped bacterium, which, when kept at a temperature of 32° C., lengthens out into a long, thread-like filament, in which numerous bright, oval spores are formed. The spores, when set free by the disintegration of the filament, develop into rods which, when introduced into the subcutaneous tissue of an animal, increase indefinitely by a process of transverse fission and set up splenic fever.

In repeating the experiments made by Koch and others I have especially directed my attention to the phases through which the rods and spores pass, and, along with Dr. Burdon Sauderson, I have endeavoured to prove that the spores do not, as stated by Pasteur, resist the influence of boiling and of pressure.

In carrying on this investigation I made use of a hot chamber, but found of especial service the warm stage[2]

[1] 'Die Aetiologie der Milzbrand-Krankheit, begründet auf die Entwicklungsgeschichte des *Bacillus anthracis*." 'Beiträge der Biologie der Pflanzen,' Zweiter Band, Zweiter Heft, 1876.

[2] See figure of warm stage in Mr. Schäfer's 'Practical Histology.' The stage may be had from Mr. Casella, 147, Holborn Bars.

introduced by Mr. Schäfer. Through it I was able to keep the same preparation under observation at the same temperature for an indefinite time. Preparations cultivated in the warm chamber were made by fixing a thin glass ring to a glass slip by means of Canada balsam, Brunswick black, or paraffin. The edge of this ring was covered by a thin layer of olive oil, and the cover glass, with the drop of aqueous humour containing the rods, was placed carefully over it. In the same way the cover glass containing the specimen was placed on the warm stage, but when the same cultivation was likely to be under observation for a number of days it was always advisable to fix the cover glass to the brass plate by means of paraffin.

On examining a small piece of spleen or a drop of blood from the spleen of a mouse, which had just died from splenic fever induced by inoculation, in a drop of fresh aqueous humour from a rabbit, numerous motionless rods were seen lying amongst the spleen tissue and blood-corpuscles. The rods varied in length, the shortest being in length nearly twice the diameter of a human red blood-corpuscle; the longer ones were two or three times the length of the shorter, but when carefully examined they were almost invariably seen to be in process of division into two or more segments.

At first the bacilli were absolutely motionless, but in some cultivations, after keeping the temperature at 33° C. for a few hours, a great number of them began to move actively about the field. By watching for several hours the bacilli in a part of the field enclosed by spleen débris and blood-corpuscles it was evident that they all closely resembled each other, that they were alternately at rest and in motion, and that some of them lengthened out into filaments. While at rest they were not altogether without change, for clear lines appearing across them indicated that they were in process of division into segments. Sometimes a number of rods ceased moving, and previous to lengthening out into filaments, arranged themselves into patches of zoogloœa (Plate XI, fig. 19).

When any particular rod was observed for some time it might be seen first, slowly to move backwards and forwards, then the movements gradually increased, until it wriggled in a very active manner from one side of the enclosed space to the other. Having continued active for some time, it would either suddenly or gradually settle down again, as if exhausted, into its former quiescent condition. I have seen rods moving when with the No. X Hartnack they appeared from half an inch to three quarters of an inch in length.

The division into two or more pieces is not always a very rapid process. A rod (Plate XI, fig. 20) which was watched till it divided was at first made up of three pieces, and one of them from the beginning looked as if it might at any moment detach itself from the others; but after six hours' almost constant struggling, though by that time able to remove itself half its own length from the other two segments, it was still connected by a very delicate thread, and before separation, which took place after being under observation for over seven hours, it in a comparatively short time was almost divided into two segments, so that when it did escape from the other apparently inactive pieces it wriggled about the field like two freely movable links of a chain.

After assuming this motile phase for some time the rods lengthened out into spore-bearing filaments (Plate XI, fig. 22). A mouse inoculated with the spores thus obtained died forty-eight hours after, presenting all the usual appearances of splenic fever, and thus proving that the motile rods were none other than a hitherto undescribed phase of *Bacillus anthracis*.

What the conditions are which lead to these movements remain to be discovered. Apparently the same conditions do not always lead to the same results, for several generations may elapse before the motile phase again appears.

The lengthening of the rods into filaments is an extremely rapid process, and is apparently effected by the temperature. In five hours a rod, at a temperature of 32° C. may have increased so as to be from eighty to one hundred times its original length, and in twenty-four hours the filament may be full of spores. If the temperature, however, is kept about 28° C., the spores may not appear till the thirty-sixth or fortieth hour. When the spores have once appeared all the other changes go on at ordinary temperatures, from 12° to 18° C., but not nearly so rapidly, even when the preparation is kept in the sun for a few hours daily, as when artificial heat is applied. On the other hand, a high temperature, 37°—40° C., at once checks all developmental changes.

The filaments differ very much in their arrangement. Sometimes they form a network—indeed a mycelium—made up of bundles of numerous, nearly parallel, unbranched threads, crossing each other at different levels; the threads are sometimes straight but have generally a wavy outline. This condition may obtain throughout the whole preparation, but generally at some parts the filaments are extremely irregular and much convoluted. In some cultivations all the filaments may be so irregular that they may best be

compared to an entangled skein of white silk, and when this is the case beautiful rope works (Plate XI, fig. 23), made up of from two to four regularly twisted loops, radiate for a considerable distance from the edges of the preparation. Still more beautiful are these silken ropes when the threads of which they are composed are studded at irregular intervals with bright refractive oval spores.

The filaments when first formed are perfectly hyaline (Plate XI, fig. 8), but soon the central protoplasmic contents can be distinguished from the gelatinous looking sheath. The protoplasm next divides into numerous short pieces (Plate XI, fig. 9), each being about the size of the original rod out of which the filament was formed. These contract, leaving clear, apparently empty spaces between them, and often again divide to form still shorter masses of protoplasm (Plate XI, fig. 10).

At each side of this transverse line of division minute clear specks appear—the first indication of the spores (Plate XI, fig. 11). These gradually increase in size and in brightness, and as they increase the protoplasm disappears, in fact, the spores seem to be developed out of the protoplasm (Plate XI, fig. 12). This process seems to correspond with the formation of chlamydospores in mucor. The spores thus formed have a regular arrangement, except when only one spore is formed out of the original long piece of protoplasm. Soon after the appearance of the spores the filaments seem to be made up of numerous segments (Plate XI, fig. 12), each segment containing one spore, the spores lying at the adjacent ends of the segments. Then one of several changes may follow. In the first place, the gelatinous-looking envelopes which now surround the spores may so swell that the filament gives way, thus allowing the spores to escape (Plate XI, fig. 15). Again, the filament may break up into long and short pieces, the spores for some time remaining in the detached segments (Plate XI, fig. 16), or the filaments may remain entire, retaining all the spores *in situ* (Plate XI, fig. 14). Whatever happens the filament as it disintegrates gets first granular (Plate XI, fig. 13), and then almost entirely disappears. While in a granular condition minute clear spots are visible at the points recently occupied by the spores (Plate XI, fig. 15).

When the filaments break up into short segments, in many cases containing a spore at one end (Plate XI, fig. 16), they may be easily mistaken for germinating spores. Young rods, however, as long as the remains of the spore are visible in them, are rounded at both ends; whereas pieces

of filaments containing spores have always square or irregular ends. When the filament remains entire, its existence through time can only be inferred by the spores retaining exactly the same position as they occupied when first formed.

Let us now consider the changes through which the spores pass in order to form rods. As a rule the spores begin to escape from the filaments on the third day of cultivation, and only the débris of the filaments is visible on the fifth day. When they escape they are motionless, bright, refractive, oval bodies, surrounded by a thin hyaline, gelatinous-looking envelope, and measuring, according to Cohn,[1] $\frac{1}{25}$–$\frac{2}{000}$ of an inch in their long diameters (Plate XI, fig. 1).

The spores when free, according to previous observers, at once grow into rods, and according to Koch at least, the rod is formed out of the gelatinous-looking envelope surrounding the spore. My observations lead me to believe that the spore does not always at once grow into a rod, but that it divides into four sporules by a process of division, in which the envelope, as well as the spore, takes part. This division I have even seen beginning before the spore escaped from the filament (Pl. XI, figs. 17 and 24), and that it is not a degeneration is certain, for I have watched the sporules thus formed lengthen into rods. This free cell formation I have had verified by many competent observers. It is best seen at a temperature of 20° to 25° C., and may even be seen at ordinary temperatures, and it is generally found when the cultivation has been continued for seven or eight days in the same drop of aqueous humour.

This process of cell division exactly corresponds to what takes place in other cells, that is, the spore elongates (Plate XI, fig. 2), then becomes dumb-bell shaped, and lastly divides into two round bodies, which are at first small and touching each other, and enclosed by a capsule still single. As they increase in size and separate from each other, the capsule also becomes constricted and ultimately divides, and thus we have two perfectly round, bright bodies, surrounded by a thin hyaline capsule, developed from the original oval spore. Each of these round bodies now undergoes the same process, thus producing four sporules, all at first closely adhering to each other, but soon becoming free; and after dancing about in the fluid along with others formed in a similar way, settling down to form a colony (Plate XI, fig. 3),

[1] 'Beiträge zur Biologie der Pflanzen,' ii, 2, 1876, p. 264.

similar to but easily distinguishable from a group of micrococci.

If a particular group of sporules are next watched they may be seen to germinate (Plate XI, fig. 4; Plate XI, fig. 25). Dr. Koch states that the rods are developed from the gelatinous-looking capsule, and not from the bright shining spore. From what I have seen I think there can be no doubt whatever that the capsule takes no active part during the formation of the rod. The sporule first slightly elongates (Plate XI, fig. 4, and Plate XI, fig. 25), and then from one of its poles an opaque process appears, which, as it slowly lengthens, pushes the capsule before it as it would an elastic membrane. The capsule, as this stretching goes on, becomes at last so thin and transparent that it can no longer be distinguished from its contents (Plate XI, fig. 5).

Contrary to one's expectations the remains of the spore or sporule retaining its original brightness persists a considerable time after the rod has been fully formed. Whether it disappears altogether I cannot say. I have often seen in rods a small oval body, which might either be looked upon as the remains of the spore, as a nucleus, or as a vacuole (Plate XI, fig. 7). Very interesting results are often obtained when the same cultivation is kept under observation for twelve or fourteen days. By this time probably both the oxygen in the small amount of enclosed air and the nourishment in the drop of aqueous humour has been exhausted, hence not only do we find apparently degenerative changes taking place in the spores, but the filaments are also modified. In fact, the newly formed rods may scarcely increase at all in length (Plate XI, fig. 26), but even in such rods new spores, as large and as well formed as those of the first generation, may appear. Even when the rods increase from three to ten times in length only a very few spores may be formed, and these at very irregular intervals. That these modifications result from want of proper nourishment we may safely infer, seeing that when fresh aqueous humour is added, the formation of filaments and spores goes on again as actively as in a fresh preparation.

Several experiments were made in order to ascertain whether the presence of other bacteria in any way influenced the development of bacillus.

1. A piece of spleen containing rods was placed on the warm stage. At first the rods began to lengthen out into filaments in the usual way, but when the filaments were with the No. 8 Hartnack apparently two inches in length currents of impure air were admitted under the cover glass

and a small drop of aqueous humour containing ordinary bacteria was added. After this the rods continued to increase until they were apparently three inches in length, but as soon as *Bacterium termo* and other organisms introduced became active all life in the filaments seemed to cease, no spores appeared in them; the filaments, in fact, became granular, and soon went to pieces.

2. In another cultivation the spores were allowed to appear before any other bacteria were added; but, although thousands of micrococci were added, as well as a great number of very active ordinary bacteria, the developmental changes went on unchecked, and when a mouse was inoculated with the spores formed, death took place at the usual time.

3. Pieces of spleen containing rods kept moist at a temperature of 37° C. in free communication with the ordinary atmosphere rapidly putrefied. When examined no vestige of rods or filaments were found, and when pieces so treated were introduced under the skin of mice no symptoms of splenic fever or septicæmia resulted, thus proving not only that the ordinary bacteria had destroyed all the rods and spores, if spores were present, of *Bacillus anthracis*, but also that ordinary bacteria, when introduced under the skin of a healthy animal, are innocuous.

4. Pieces of a spleen containing rods were placed in aqueous humour in blood serum, in Pasteur's solution, in serum from the subcutaneous tissue, and several portions were sealed up in a small test-tube without any fluid being added. These were kept at a temperature of 30° C. In the aqueous humour, blood serum, and in the serum from the subcutaneous tissue, the rods lengthened out into spore-bearing filaments, but no changes occurred in the rods placed in Pasteur's solution nor in the rods in the pieces of spleen simply sealed up in the test-tube.

5. Blood, spleen, or peritoneal fluid, containing rods or spores, which at a moist temperature of 37° C. perish, may be dried at a temperature of 38° to 40° C. and still retain their power to set up splenic fever. The rods in the outer layers of the dried masses are destroyed, but those in the centre retain their activity.

Further, rods or spores in tissues or fluids remain active when sealed up in capillary tubes, and also when kept at a temperature approaching freezing point. Although mice are easily killed by inoculation, they do not seem to be influenced in any way when rods and spores are mixed with their food, unless there is some abrasion about the nose or mouth, neither do they seem capable of being infected by inhaling

the spores. The spores may be found in the alimentary canal of such mice, sometimes as if in process of development into rods and filaments.

Seeing that the rods are constantly dividing, and that the spores divide into sporules, it is impossible to make any definite measurement of either, and it is further scarcely advisable to say that rods and spores apparently somewhat smaller belong to a different species, seeing that the difference in size may be due to the change of habitat, the rods of *Bacillus anthracis* being always longer, for example, in Guinea pigs than they are in mice.

The life history of *Bacillus anthracis*, from what has been said, may be epitomised as follows:—To begin with the well-known resting rod. This rod, if in living tissues, multiplies indefinitely by a process of transverse fission (Plate XI, figs. 5 and 6), but it never seems to lengthen out into a filament; if, however, after death a sufficiently high temperature be maintained and other conditions be favorable, it may lengthen out into filaments the protoplasm of which contracts into spores.

On the other hand, if cultivated on the warm stage under artificial conditions, it may become motile, though rarely; but after being alternately at rest and in motion for some hours it lengthens out into an exceedingly long filament, probably much longer than that found in natural conditions (Plate XI, fig. 22). The protoplasm next divides into numerous segments (Plate XI, fig. 9), which may again divide (Plate XI, fig. 11), and then rapidly contract to form spores (Plate XI, fig. 12).

The spores escape from the disintegrating filaments (Plate XI, fig. 15), and may either at once germinate into new rods or divide into four (Plate XI, fig. 2), not at all unlike Protococcus. These sporules then germinate and form rods similar to those with which we started (Plate XI, fig. 4). Thus the cycle is completed.

The most important morphological conclusion which follows from these and other observations is that micrococcus-forms, bacterium-forms, bacillus-forms, and spore-bearing hyphæ, are in nowise generically distinct, but that they are simply phases of the same life history, a life history doubtless common to all other bacteria.

In the rarely occurring motile phase we find a condition which we constantly find in ordinary bacteria; moreover, the formation of a true aseptate mycelium closely resembles Mucor, an analogy rendered closer when we reflect that the spores of *B. anthracis* are really chlamydospores.

We now have to mention the results following upon boiling and compression of the spores.

Since Pasteur stated that the spores of Bacillus remained active after boiling, much has been said about their wonderful tenacity of life. Having a fair quantity of spores at our command Dr. Burdon Sanderson suggested that we should test the accuracy of the above statement. Accordingly, along with Dr. Sanderson, I added to the aqueous humour containing the spores a small quantity of distilled water, and after carefully stirring the mixture a mouse was inoculated with a few drops, an absolutely clean syringe being used. The rest was boiled for five minutes, and then with a fresh syringe another mouse was inoculated, and so on, after boiling at different periods up to a quarter of an hour.

The result was that in two days the mouse inoculated with the unboiled spores was found dead, but the others remained absolutely unaffected. Again, spores and rods were treated in exactly the same way and with exactly the same results; the spores were rendered inactive after even two minutes' boiling. But thinking newly cultivated spores might be more easily destroyed than old dried ones, we boiled blood containing spores which had been kept dry for five years. The same results followed; the mice inoculated with the solution before boiling died, those inoculated after boiling were unaffected.

It may be mentioned that mice are very susceptible; in no case has the smallest number of spores introduced into the subcutaneous tissue failed to prove fatal.

We next directed our attention to the other fact mentioned by Pasteur, viz. that the spores were not rendered inactive when subjected to a pressure of twelve atmospheres of oxygen. M. Bert repeated M. Pasteur's experiment, but found that the spores were destroyed; hence it was all the more necessary, though, perhaps, a point of little practical importance, to endeavour to make out what influence compression really had. This experiment we were enabled easily to perform through the kindness of Mr. J. Millar Thomson, of King's College, who supplied us with the necessary apparatus.

The spores were suspended in a solution of distilled water in an ordinary test-tube, which was placed in the cylinder, and twelve atmospheres of oxygen allowed freely to come in contact with them. After subjecting them to this pressure for twenty minutes they were introduced under the skin of a mouse, but without producing any indication of splenic fever or any other abnormal condition.

A mouse inoculated with a few drops of the uncompressed solution died, and presented, as before, all the appearances of splenic fever.

Experimental Contribution *to the* Etiology *of* Infectious Diseases *with special reference to the* Doctrine *of* Contagium vivum.

By E. Klein, M.D., F.R.S.

(With part of Plate XI.)

Read before the Royal Society on February 17th, 1878.

The present communication has for its object to bring before the Royal Society the results of an experimental inquiry[1] into the etiology of an infectious disease of the pig, known as Hog Plague, Mal Rouge, Red Soldier, Malignant Erysipelas, or also Typhoid Fever of the Pig. Their are English and continental writers who describe the disease as Anthrax or Splenic Fever of the Pig. I shall show, however, conclusively in my Report to the Medical Officer of the Local Government Board, that it is neither typhoid fever nor anthrax, but is an infectious disease of its own kind, which I propose to call "Infectious Pneumo-Enteritis of the Pig" (*Pneumo-enteritis contagiosa*).

Like other infectious diseases, the "Pneumo-Enteritis" possesses an incubation period, followed by constitutional disturbance and certain anatomical changes. These latter are invariably affections of the lung, of the intestine, and of the lymphatic glands, not only of those of the organs of respiration and alimentation, but also those of the inguinal and lumbar regions. In the lung the changes are those known to pathologists as lobular pneumonia. In the alimentary canal the mucous membrane of the large intestine is chiefly affected, being the seat of smaller or larger ulcerations. There is generally also inflammation of the serous membranes, especially the peritoneum, leading to an exudation of lmph into the serous cavity. The skin is occasionally affected with greater or smaller red patches.

There are hæmorrhagic patches to be found in the lung and serous membranes, the endocard, and the muscle of the heart, the mucous membrane of the intestine (especially duodenum and large intestinc), the tongue, and occasionally also the liver and spleen, only seldom in the skin and kidney.

In anatomical respects, therefore, the Pneumo-Enteritis bears undoubtedly a great resemblance to anthrax or splenic

[1] This being part of a larger research carried out for the Medical Officer of the Local Government Board.

fever. [Pigs are known to be liable to take splenic fever.] There exists, however, a marked difference between the two diseases in the incubation period, the general pathology,[1] and especially in the anatomical character of the spleen and blood. In splenic fever we find the spleen invariably enlarged, being the principal organ of the affection, whereas in pneumo-enteritis it is only occasionally changed. And, likewise, the blood presents entirely different characters in the two diseases; in pneumo-enteritis it is not different in any marked degree from normal blood, whereas in splenic fever it is of dark colour—laky, and does not coagulate at all, or only imperfectly so. Besides, the blood in splenic fever contains the now famous *Bacillus anthracis*, and hence its conspicuous infectious property, whereas in pneumo-enteritis the fresh blood does not, as a rule, contain any foreign matter, and in most instances does not possess any infectious property.

Another disease with which pneumo-enteritis bears a great resemblance on account of certain anatomical characters, viz. inflammation of serous membranes, lung, intestine, and lymphatic glands, hæmorrhage in lung, serous membranes, endocard, muscle of heart, intestinal mucous membrane, and other organs—is specific septicæmia.[2]

The resemblance, however, is not greater than to splenic fever, although the differences are not less well defined. Besides others, there is this great distinction, that in pneumo-enteritis the contagion spreads by simple cohabitation and through th air, which it never does in septicæmia, as in this the virus always requires a broken surface through which to enter a healthy individual. Pneumo-enteritis is occasionally described as malignant erysipelas (mal rouge, red soldier), but this is in so far inadmissible, as the affection of the skin in the former is a very inconstant symptom, and in milder forms of the disease is invariably absent. More recently the pneumo-enteritis has been regarded as tphoid fever of the pig. From a purely anatomical point of view the resemblance between real, *i.e.* human, typhoid fever and pneumo-enteritis is very slight indeed, so slight, in fact, that to mention it requires a total oversight of some

[1] In splenic fever the period of incubation ranges from between a few hours to several days, in pneumo-enteritis it varies from two to five days and more. Splenic fever is easily transmissible to man and the domestic animals, whereas the transmissibility of the pneumo-enteritis is much more limited. Hitherto I have succeeded in communicating it to rabbits, Guinea-pigs and mice, although only with difficulty.

[2] Specific septicæmia as distinct from septic infection. See Dr. Burdon-Sanderson's lectures at the University of London, 1877.

of the most prominent symptoms, *e.g.* inflammation of lung and serous membranes, enlargement of inguinal, lumbar, and bronchial lymphatic glands, hæmorrhages in endocard and muscle of the heart in pneumo-enteritis on the one hand, and swelling and ulceration of the lymphatic glands of the small intestine, swelling and inflammation of spleen in real typhoid fever on the other hand. The resemblance seems to be limited solely to the fact that in both diseases there occurs ulceration in the intestine. But the distribution, the nature, and the development of these ulcerations are totally different in the two diseases.

Having said thus much as a prefatory explanation, I proceed to state the results of the experiments.[1]

The experiments refer to the following series:

1. Experiments showing that the fresh blood of diseased animals does not, as a rule, contain the virus, as it fails to produce the disease when introduced into a healthy animal.

Four animals were inoculated (at different times) with fresh blood of diseased animals. They remained healthy. When subsequently inoculated with virus-containing matter, they became smitten with the disease.

In a fifth instance, however, fresh blood di[illegible] produce infection. [And this same blood proved active after having been kept sealed up in a capillary tube for several weeks.] This blood was obtained from a very severe case with copious peritoneal exudation, in which were found peculiar abnormally large coarsely granular cells; the same cells were also present in the blood; so that it appears probable that the blood became charged, by absorption during life, with matter from the peritoneal exudation. This latter always contains the virus in an active state.

2. Experiments showing that fluid as well as solid lypmh of the diseased peritoneum contains the virus in a very active state.

[1] In all my experiments of inoculation the materies morbi was used in minimal doses, *i.e.* a drop of fluidmatter, or in the case of solids a particle of less than the size of a pin's head. In both cases the materies morbi was diluted or suspended respectively in a few minims of boiled saline solution of $\frac{3}{4}$ per cent. in order to increase its bulk and thus to facilitate its introduction. The inoculation was invariably carried out by injection into the subcutaneous tissue by means of a fine canula of a hypodermic syringe, necessary care being taken that this had been previously thoroughly cleaned and disinfected. After and before inoculation the animals have always been kept isolated and in clean and disinfected places. In order to ensure reliableresults (viz. that the disease in a particular case was really a consequence of the inoculation and not of infection through other sources) care was taken that those who attended the isolated animals were not the carriers of infection.

Six successful inoculations with fluid peritoneal exudation.

There is no difference of activity to be noticed between fresh exudation and one that had been kept sealed up in a capillary tube for several weeks.

Solid lymph obtained from the peritoneal cavity of diseased animals, having been dried at a temperature of about 38° C., proves very active.

3. Experiments showing that parts of the diseased lung, ulcerated intestine, and also diseased spleen, contain the virus in an active state. Diseased parts of lung or intestine, that were dried at a temperature of about 38° C., retain their virulence unaltered.

In all cases of pneumo-enteritis the trachea as well as the bronchi contain frothy blood-containing mucous matter, possessed of infectious property. It must therefore be supposed that the breath of a diseased animal is charged with the poison. On account of the diseased state of the intestine also the dung is to be regarded as infectious.

4. Experiments showing that infection is produced by cohabitation with a diseased animal, or by keeping healthy animals in a place whence a diseased animal had been removed.

5. Several experiments were made to see whether feeding healthy animals on matter obtained from the diseased organs (intestinal ulcers especially) produces the disease. The experiment was always attended with success if a lesion, *i.e.*, abrasion existed in the mucous membrane of the mouth or pharynx; this was usually the case when the matter had to be introduced into the mouth while the animal was being held by assistants.

There were, however, two cases which appear to prove that the disease cannot be produced by simple feeding.

This was, unfortunately, at a time when I was not yet acquainted with the fact that in many animals the disease is of so mild a form that it can hardly be recognised in the living. I have not made any post-mortem examination of those two animals.

But since then I have made two other experiments, in which the virus was brought directly into the stomach by means of an india-rubber tube introduced *per fauces et œsophagum*. In both these instances the animals became diseased and their intestines were most conspicuously affected.

From the last three series of experiments, we may conclude that the principal mode by which contagion of pneumo-enteritis is carried out is through the instrumentality of the air and the food.

6. This series comprises experiments to prove that the virus can be cultivated artificially, *i.e.* outside the body of an animal; in the case of splenic fever it has been successfully done by Dr. Koch.

The experiments are seven in number—(*a*) two refer to cultivations started with fluid peritoneal exudation; (*b*) in the five others the virus had been obtained by cultivation of dried lymph of the peritoneum of an animal suffering from the disease.

(*a*) The cultivation of the virus for the first two cases was carried out thus:

Fluid peritoneal exudation of a diseased animal had been collected and sealed up on November 6 in a capillary glass tube. On the following day there was present a small clot due to coagulation. A minute speck of this clot was removed with the point of a clean needle, and with it was inoculated a drop of fresh aqueous humour of a healthy rabbit. This drop had been placed on a thin covering-glass, which after the inoculation is inverted over a small "cell," made by fixing a glass ring[1] on an ordinary glass slide. The covering-glass is fastened on the glass ring by means of a thin layer of pure olive oil. The preparation was then kept in the incubator for twenty-four hours at a temperature of 32—33° C. After this time it was used to inoculate a new drop of aqueous humour in a similar manner as the one just described. We will call this the second generation.

This new specimen was placed in the incubator and kept there at a temperature of 32—33° C. for further twenty-four hours. In the same manner a third generation was started by inoculating a fresh drop of aqueous humour. After having been kept in the incubator for several days it was used to inoculate two animals at different times. Both animals became smitten with the disease.

(*b*) The other five experiments were carried out with virus cultivated from solid lymph of the peritoneum of a diseased animal. The lymph had been dried at 38° C. (See Series 2). A small particle of dried lymph is crushed into fine powder. With a granule of this a drop of fresh aqueous humour is inoculated in the same manner as above described —First generation.

After having been kept in the incubator for two or three

[1] The glass ring I used is 0·6 to 2 millimètres high, about 2 mm. thick, and about 18 mm. wide. If the preparation is to be observed on the hot stage of the microscope, instead of the ordinary glass-slide, one of only 0·5 mm. thickness is chosen in order to bring the preparation more rapidly up to the desired temperature.

days at a temperature of 39—33° C. it is used to inoculate a second generation, care being taken to use a trace only of the fluid part and not to come in direct contact with the original granule, which may be still discerned in the preparation.

The specimen representing the second generation is kept in the incubator for a day or two. It is then used to inoculate a fresh preparation—Third generation. And, finally, this is used for establishing a fourth generation. After having been kept in the incubator a part of it is used for inoculating *two* animals, the inoculation being carried out at different times. Both these animals became smitten with the disease.

Another portion of this fourth generation was used to start a fifth generation, then a sixth, a seventh, and an eighth generation. With this three animals were inoculated at different times. All three animals became diseased in due time.

In order to correctly interpret the results of this last (6th) series of experiments it is important to mention that inoculation with dried lymph, diluted far less than would correspond to the third generation in the last-named experiments, is followed by a negative result.

The microscopic examination of the cultivated liquids proves that these are the seat of the growth and development of a kind of bacterium, which has all the characters of *Bacillus subtilis* (Cohn). The bacillus in our case is a very fine and delicate rod, thinner than both that described by Professor Cohn in hay-infusion and the *Bacillus anthracis* so thoroughly investigated by Dr. Koch.

Our bacillus differs also in other respects from *Bacillus anthracis,* inasmuch as it possesses a moving stage; the *Bacillus anthracis* described by Dr. Koch is non-moving. Like *Bacillus subtilis* of hay and *Bacillus anthracis,* our bacillus grows under favorable conditions into long leptothrix-like filaments, which occasionally form more or less complex convolutions.

In these filaments highly refractive spores make their appearance. These become free after the disintegration of the original filamentous matrix. The fully-developed spores of our bacillus differ from those of hay-bacillus and anthrax-bacillus by being more distinctly cylindrical and much smaller.[1] According to Professor Cohn ('Beiträge zur Bio-

[1] In the figures accompanying Dr. Koch's paper on *Bacilius anthracis* ('Beitr. z. Biologie d. Pflanzen,' ii, 2, 1876) the spores are represented in many places as more or less spherical in shape.

logie der Pflanzen,' II, 2, 1876, p. 264) the long diameter of the spores of bacillus of hay and also of anthrax—for both are identical in morphological respects (l. c., p. 275)—amounts to 0·0015—0·0022 mm. or $\frac{15 \text{ to } 22}{25000}$ of an inch, whereas the spores of our bacillus are little less than 0·0005 mm. or $\frac{1}{50000}$ of an inch in their long diameter.[1] At first I misinterpreted the spores, regarding them as a kind of micrococci, and only after repeated observations have I succeeded in tracing them through their different stages of development.

After many failures—owing to the introduction and development of *Bacterium termo*—I succeeded at last in obtaining, already in the second generation of original virus, a pure crop of bacillus and its spores. With these I started several separate cultivations, in which the germination of the spores into delicate bacillus, the swarming stage, the rapid multiplication by division, their growth into long apparently smooth filaments, and, under sufficient access of air, the formation of the bright cylindrical spores could be distinctly traced.[1] (No other organisms appeared in these cultivations.) These were again used to inoculate other preparations of aqueous humour, and so on, until I succeeded in obtaining considerable quantities of liquid containing only bacillus and its spores. The last-named animals were infected with liquid of this kind.

[1] In convolutions of filaments the outlines of these latter become gradually lost after the spores are formed. The spores appear now to be embedded in a transparent gelatinous matrix. At the edges of such masses or where they are in a sufficiently thin layer, the linear arrangement of the spores can be still recognised. But there is undoubtedly a transparent jelly present in these masses forming the ground substance for the spores and fibres. Professor Cohn mentions (l. c., p. 263) a similar jelly in convolutions of hay-bacillus. I entirely differ from Dr. Koch with regard to the mode of germination of the spores of bacillus. Koch states (l. c., p. 288, and also in his latest paper on Bacteriæ in 'Biol. d. Pfl.,' 2 Bd., 3 Heft.), that it is not the highly refractive spore which directly produces the bacillus, but that the hyaline gelatinous envelope surrounding each spore elongates so as to form the bacillus, while the bright spore-matter itself gradually diminishes and finally disappears. From *à priori* reason it is impossible to assume that this can be so, viz. that the gelatinous envelope should grow into the bacillus; for Cohn proved beyond doubt that in the case of hay-bacillus the spores germinate even after having been exposed to boiling heat. Surely this gelatinous envelope, if living protoplasm, must become, under these conditions, deprived of its germinating power. Direct observations proves that in my case the spores possess another membrane within that gelatinous envelope and during germination this inner membrane is broken at one pole and the contents of the spore protrudes and grows out as the bacillus. This is also in accordance with the observations of Professor Cohn, for this authority states (l. c., p. 265) "Die Sporen schwollen etwas an und trieben an einem Ende einen kurzen Keimschlauch."

Seeing that splenic fever, pneumo-enteritis, and specific septicæmia possess a great affinity in anatomical respects, and seeing that in splenic fever and pneumo-enteritis the materies morbi is a definite species of bacillus—the difference of species being sufficiently great to account for the differences in the two diseases—we may with some probability expect that also the third of the group, viz. specific septicæmia, is due to a bacillus. This, however, remains to be demonstrated. It seems, finally, justifiable to speculate whether or not we have in these three varieties of disease "a variation of species" in the sense of the evolution theory.

On *the* Nature *of* Fermentation. The Introductory Address *delivered in* King's College, London, *at the opening of the Session, October 1st,* 1877. By Joseph Lister, F.R.S.; Professor of Clinical Surgery in King's College, and Surgeon to King's College Hospital; &c.

[The following report has been revised and corrected by Prof. Lister for publication in this journal.—Ed.]

Gentlemen,—In making my first appearance as a teacher in King's College, I cannot refrain from expressing my deep sense of the honour conferred upon me by the invitation to occupy the chair which I now hold; and, at the same time, my earnest hope that the confidence thus reposed in me may not prove to have been misplaced.

In considering how I could best discharge my duty as the person selected to deliver the Introductory Address of the Medical Session, I have felt that two courses were open to me: either to spend the short but important time at my disposal in an endeavour to convey to the student some sense of the exalted privileges, and correspondingly high responsibilities, of the beneficent calling to which he proposes to devote himself, or to treat on some special subject, in the hope that I might say something which should have interest and, if possible, even instruction, not only for the student, but also for the eminent men whom I have the honour to see around me. The latter is the course which I have decided to follow, and the subject which I have selected is a short account of an inquiry in which I have been engaged in the interval between the cessation of my official duties in Edinburgh and their commencement here. The object of that investigation was to obtain, if possible, some more precise and definite knowledge of the essential nature of a class of phenomena which interest alike the physician, the surgeon, and the

accoucheur. I allude to the changes in organic substances which are designated by the general term *fermentation*.

In medicine, the large class of diseases termed zymotic derive their name from the hypothesis that their essential nature is fermentative. In obstetrics, puerperal fever, the most frequent cause of disaster after childbirth, is now regarded by many of the highest authorities as likewise due to fermentative disorder; and, in surgery, among the various causes which may disturb a wound, we know that by far the most frequent in operation, and the most pernicious in its effects, both upon the wounded part and upon the constitution, is putrefactive fermentation. If this be so, it is clear that to understand the nature of fermentation must be a matter of the very highest importance, with a view to curing or preventing the various evils to which I have alluded.

What, then, do we mean by fermentation? I shall best approach the answer to this question by giving an example. Rather more than a week ago, I witnessed in the north of Italy the time-honoured practice of treading grapes in the wine-vat. I was told that the juice would within twenty-four hours boil, as it was said, over the vats into which it was introduced; in other words, that the sugar of the grape-juice would within that short time be so converted into alcohol and carbonic acid that the carbonic acid gas, by its evolution, would cause sufficient frothing to produce the effect to which I have referred. This conversion of the sugar of the grape into alcohol and carbonic acid is accompanied by the development of a microscopic organism, the yeast-plant, or, to continue the old nomenclature, *Torula cerevisiæ*, consisting of microscopic cells multiplying by pullulation, as indicated in this diagram (not here represented). Now, it is, I believe, universally admitted that the alcoholic fermentation of grape-sugar is due to the growth of the yeast-plant. M. Pasteur thinks that he has traced the origin of the yeast-plant in the juice of the grape to a minute fungus adhering to the outside of the skin of the grape.[1] Be this as it may, it is admitted on all hands that the alcoholic fermentation is caused by the growth of the yeast-plant. So long as the juice of the grape is protected by the skin of the berry, no fermentation occurs; but, as soon as it escapes from that protection, the organism, by its development, induces the fermentation. Nor is it by any means exclusively in the natural juices of fruits that such fermentation occurs. Any sugary solution, provided it contains, besides the sugar, other ingredients requisite for the nutrition of the yeast-plant, will serve as pabulum for the organism, and in that case the yeast-plant will give rise to the fermentation. Here is a glass containing what is termed Pasteur's solution, a solution devised by M.

[1] Vide Pasteur, 'Etudes sur la Bière,' pp. 150 *et seq.*

Pasteur for the very purpose of affording nourishment to the yeast-plant and other minute organisms. This was prepared on August 7th in a flask purified by heat, covered over with a cap of pure cotton wool,[1] which permits the entrance of air, but does not permit the access either of the yeast-plant or of any other form of dust. The Pasteur's solution, containing, besides sugar, ammoniacal and earthy salts for the nutrition of the fungus, was heated to about the temperature of boiling water, so as to destroy any organisms that might exist in the water. The result is, that it continues perfectly unchanged, just as it was on August 7th; but, if we were to add to it a little of the yeast-plant from fermenting grape-juice, we should find that, at the temperature of summer weather, it would very soon be in a state of free fermentation at the same time that the yeast-plant would multiply. This, then, is a typical instance of fermentation. We have an active agent termed the ferment, which ferment is capable of self-multiplication. That I believe to be the essential property of a true fermentation. Now, in this particular case, I have already said it is admitted on all hands that the yeast-plant is the cause of fermentation. Persons may differ as to how the development of the yeast-plant gives rise to the resolution of the sugar into the alcohol and carbonic acid gas; but all now agree that, somehow or other, the organism causes the fermentation. Now, is it the case that all true fermentations are caused by the development of organisms? That, gentlemen, is the question which it is desirable that we should be able to answer.

Take, for example, the case of the putrefactive fermentation of blood. We all know that, if blood be shed from the body into any vessel without special precautions, in a few days it putrefies. The bland nutrient liquid, soon after leaving its natural receptacle, becomes foul, acrid, and poisonous; a change fully as striking as that which grape-juice undergoes in the alcoholic fermentation. Here, on the other hand, we have a vessel (a liqueur-glass) into which blood was received with special precautions. In the first place, the glass, covered, as you see, with a glass cap and a glass shade, with a view of preventing the access of dust, and standing upon a piece of plate-glass, had been heated to about the temperature of 300° Fahr., and cooled with an arrangement which ensured that the air which entered during cooling was filtered of its dust; so that we were perfectly sure that the glass contained at the outset no living organisms.

[1] The cotton-wool was rendered free from living organisms by soaking it with a solution of carbolic acid in one hundred parts of anhydrous ether and allowing the ether to evaporate, leaving the carbolic acid behind in the cotton.

Then, in the second place, the glass had been charged from a flask like this, provided with a spout. It contains, as you see, a glass tube introduced into it; it is stuffed well with cotton-wool between the neck of the flask and the tube, there is a piece of cotton-wool over the end of the tube, and another piece is tied securely over the spout of the flask. The flask so arranged was heated just as the glass had been heated. It is not necessary to heat so high as to singe the cotton. Heat far short of this is adequate, according to my experience, to make perfectly sure that you destroy all living organisms. The flask having been thus prepared, the jugular vein of an ox was exposed and divided, with precautions against the entrance of anything putrefactive,[1] and, the cotton cap having been taken off from the end of the tube, the vein was slipped over the tube and securely tied on, and then the hand of the assistant, who previously restrained the flow of blood, being relaxed, blood was permitted to flow into the flask. Then, before coagulation had time to take place, this and various other similar glasses were charged after the removal of the cotton cap from the end of the spout. Now, the first thing that may strike you is the remarkable fact that this blood-clot has not undergone any contraction. One of the earliest things that your professor of physiology will have to teach the junior students will be that blood, after coagulation, contracts; that the fibrin of the coagulum shrinks and the serum is pressed out. But here no such thing has taken place. There has been no shrinking of this clot, no pressing out of the serum, and I venture to say that there is no one here—at least I think it is unlikely that there is any one here except myself—who has seen such a phenomenon, illustrating how, when the most familiar objects are placed under new circumstances, the most unexpected results may arise. Now, this is a matter of very considerable interest with reference to the behaviour of blood-clots inside the body in wounds and so forth. However, that is not a point on which I wish to dwell on the present occasion.[2] The point to which I wish to draw your special attention is, that this blood, although it has been six weeks in this glass, without any close fitting of the glass shade or the glass cap, and therefore with free opportunity for the access of the gases of the atmosphere, has not putrefied. The air in the glass-shade is perfectly sweet, perfectly free from odour.

Now, gentlemen, this, without going further, is a very important matter. It proves that the blood has no inherent

[1] This was secured by washing the skin and the instruments with a strong solution of carbolic acid (1 to 20) and performing the operation under a carbolic spray.

[2] I desire to guard myself against being supposed to express any opinion here as to the cause of this phenomenon.

tendency to putrefaction. It further proves that the oxygen of the air is not able to cause the blood to putrefy, as used to be supposed. There was a time—the effect is still seen to a certain extent—when the dark venous colour of this blood-clot gave place to the crimson colour of arterial blood in a gradually deepening band from above downwards. We still see some of the red colour remaining, though now the converse effect has begun to take place. That florid redness, gentlemen, showed that the oxygen of the air was in reality acting upon the blood, yet it did not putrefy. Now, if I were to take a little morsel of already putrefied blood, say, upon the end of a needle, and touch with it this clot of blood, putrefaction would, in the course of a very short time, spread throughout the mass. Exactly as in the case of alcoholic fermentation under the influence of the yeast-plant would the fermentation spread.

Putrefaction, then, is a fermentation, a true fermentation, characterised by the power of self-multiplication of the ferment. Then, gentlemen, if we examine microscopically, we find in the putrefying blood, as we found in the fermenting grape-juice, microscopic organisms, termed bacteria from their rod-shape, which we have represented in this diagram on the same scale as we had the yeast-plant; of different sizes, but all very much more minute than the yeast-plant, and commonly endowed with a remarkable power of locomotion. I say that, in the putrefying blood, we find these organisms developing *pari passu* with the fermentation.

Now, the question is, Are these bacteria the cause of the putrefactive fermentation, or are they merely accidental concomitants? These are two views which are entertained at the present day by men of high eminence. It may be said, "Why should there be any doubt that the bacteria are the cause of the putrefactive fermentation, any more than there is a doubt that the *Torula cerevisiæ* is the cause of the alcoholic?" Well, one reason I believe to be that the bacteria are so exceedingly small. They are not so easily defined as the yeast-plant. We cannot get them in a mass as we can get a mass of yeast; at least without a great deal of trouble; and, besides that, they occur very similar in appearance in a great number of different fermentations. There is, therefore, so far some colour for doubting whether bacteria are the cause of a special fermentation, like this putrefaction. Then there is another ground justifying such a view; for certain it is that organic substances are liable to extremely remarkable alterations, decompositions, under the influence of agents which are endowed with no life at all. As good an example of this as we can take is what occurs in the bitter almond when it is bruised with water. You all know what takes place under those circum-

stances; that there is prussic acid developed, and essential oil of almonds, and other materials. Now, these did not exist beforehand in the bitter almond, but they are the result of the mutual action upon each other of two constituents of the bitter almond, neither of which was hydrocyanic acid, nor oil of bitter almonds, &c. These two constituents are termed emulsin and amygdalin. Amygdalin is a crystallizable substance, and can be obtained separate. Emulsin, though not obtained in a state of crystallization, can also be obtained separately. Till these two materials are in a state of solution in water, they do not act upon each other at all; but, as soon as they are in watery solution, the emulsin so acts upon the amygdalin that the amygdalin becomes broken up into the constituents to which I have referred. This is an exceedingly remarkable fact. Undoubtedly, the emulsin is dead; there is nothing living about it. It is not an organism. It is obtained by a process of alcoholic extraction, and so forth. It is thoroughly a chemical substance, a merely dead substance, if we may so speak, and yet it does produce this remarkable effect upon the amygdalin. But, when we come to consider this case, we find that this process, remarkable as it is, lacks the true character of genuine fermentation, that of the faculty of self-propagation of the ferment. Liebig himself, who was the great advocate of the doctrine of so-called chemical ferments, and who, along with Wöhler, discovered this action of emulsin on amygdalin, pointed out, and showed by irrefragable evidence, that the emulsin does not undergo any multiplication; not only so, but that, after a while, the emulsin loses the property of acting on the amygdalin; but, for a considerable time, it continues to act upon it without undergoing apparently either increase or diminution of its bulk. It may be called a resolvent, the amygdalin being the resolved material.

There are other cases equally striking that might be mentioned, not only in the chemistry of vegetables, but in the chemistry of our own bodies. There exists, for instance, in the saliva a material called ptyalin, which has a remarkable power of acting upon starch, so as to convert it into soluble compounds. In the gastric juice there is a material called pepsin, which has an equally remarkable property of acting on albuminous materials, fitting them for solution in digestion. But here again we find, when we come to consider the matter, that there is no evidence whatever that either pepsin or ptyalin is capable of self-multiplication. Each is secreted for the purpose and in the quantity in which it is required, but it has no faculty of self-propagation; and I believe, if you search through the whole range of organic chemistry, you will not find a single recorded instance where any ferment, so-called, destitute of life has

been proved to have the power of self-multiplication. At the same time, gentlemen, it may be admitted that the thing might be theoretically possible. It is conceivable, for instance, that a resolvent, if we may so speak, of comparatively simple constitution might, by its action upon a resolvable compound, resolve it into substances, one of which should itself be the resolvent, and, if that were so, the process might go on *ad infinitum*. That is conceivable; and although we have no instance of the kind on record, yet we have persons in high authority, as teachers both of physiology and of pathology, maintaining the view that putrefactive fermentation, for instance, in the bacteria are probably mere accidental concomitants; that the real essential agent in the putrefaction is not an organism at all, but some so-called chemical ferment destitute of life. And so long as we have authorities maintaining such a view, it is necessary to test its truth or falsehood by searching inquiry; and such has been the object with which my investigations of the last two months have been conducted.

As regards the putrefactive fermentation, we have already evidence in the flask and in the glass that I have shown you (the flask also has no putrefactive odour emanating from it), that blood has in itself no inherent tendency to putrefy. It must receive something from without, and that something is not mere oxygen or any other atmospheric gas. I have now to point out to you that the addition of water is not of itself sufficient to induce this fermentation. Blood and water constitute a mixture highly putrescible, very much more so than blood itself. But in this flask we have had mixed with water the contents of one of the liqueur glasses of unputrefied blood like that before shown to you. The water, however, had been previously boiled, so as to kill any organisms in it; boiled and cooled under the protection of a cotton cap, and then, the cotton cap being raised, careful provisions (into which I must not enter) against the entrance of dust being taken, the clot was spooned into the water; a fresh cotton cap, perfectly pure, was put on, and so we got, I believe for the first time, a permanent cold watery extract of blood, and here it retains the same brilliant clearness that it had in the first instance, more than a month ago. Mere water, therefore, is as inadequate to induce the putrefactive fermentation of blood as are the gases of the air.

But the fermentation which I have been especially investigating has not been the putrefactive, but one which seemed to me more convenient for the purpose, the lactic fermentation, by means of which milk sours and curdles, through conversion of the sugar of milk into lactic acid. This is a curious instance of a chemical transformation. The composition, as regards the pro-

portions of the three elements, carbon, hydrogen, and oxygen, remains identically the same; but those of you who are chemists understand what I mean when I say the atomic weight of the lactic acid is one fourth of the atomic weight of the sugar of milk. Each atom of milk sugar is resolved into four simpler atoms of lactic acid. Now, it may be naturally supposed, if you observe what happens in a portion of milk obtained from a dairy, that there is an inherent tendency in the milk to this souring and curdling. If you get milk from a dairy and keep it long enough, it is certain to turn sour and curdle, then, after a while, there comes a certain mould upon the surface, the *Oïdium lactis*, which constitutes the sort of bloom there is upon a cream cheese; then comes on, often simultaneously with the growth of this mould, the butyric fermentation, in which butyric acid is produced; and afterwards, if you keep the milk long enough, it will probably putrefy. When you see, time after time, specimens of milk, taken from various dairies, undergo this succession of alterations, you may be tempted to suppose that these were changes to which the milk was disposed from its own inherent properties as it comes from the cow's udder. The late eminent Professor of Chemistry in this College, Professor Miller, in his excellent work on Chemistry, states that the ferment of the lactic acid fermentation is the caseine of the milk. I am bound to say, however, in justice to Professor Miller, that he also adds that M. Pasteur has expressed his belief that there exists an organic living ferment which produces this fermentation; but Professor Miller does not profess to decide between these two opinions. On the contrary, his first statement, that the caseine is the ferment, might lead you to suppose that he is inclined to the former view.[1] If this were the case, as there is caseine always in the milk, there should always be the lactic acid fermentation. But it was pointed out long ago by M. Pasteur that, if you examine any specimens of souring milk with the microscope, you find little organisms.[2] These, when you come to look at them carefully, you see to be obviously of the nature of bacteria. Bacteria may either have the faculty of motion or they may not. This particular bacterium is a motionless bacterium, so far as I know; still it has the essential nature of a bacterium: a microscopic fungus, multiplied by fissiparous generation, the lines of segmentation being transverse to the longitudinal axis of the organism. I have ventured to give to this little organism the name *Bacterium lactis;* for, gentlemen, no doubt there are different kinds of

[1] *Vide* Miller's 'Elements of Chemistry,' third edition, vol. iii.

[2] Vide "Mémoire sur la Fermentation appelée Lactique," 'Annales de Chimie et de Physique,' 3me série, tome lii, 1858.

bacteria. The circumstance that they are minute must not make us shut our eyes to this truth. You sometimes hear bacteria spoken of as if they were all alike. The fact that some do not move and others do, is one indication of a difference between them. Another indication of a difference is, that some bacteria will thrive in a medium in which others cannot live. For instance, the *Bacterium lactis* refuses to live at all, according to the more careful experiments I have been lately making, in Pasteur's solution; the very fluid provided by Pasteur for bacteria, torulæ, and other fungi to live in, is a medium in which the *Bacterium lactis* refuses to grow at all, although many bacteria grow in it with rapidity. That is clear evidence that this is a different kind of bacterium from those which both thrive and move in Pasteur's solution. You will observe, also, it is somewhat peculiar in the form of the segments; they are oval, and not so rod-shaped as bacteria generally. These you will always find in milk when it is souring.

But, gentlemen, neither the souring of milk nor the organism which is found associated with that change is the result of any inherent tendency in the fluid. This is a flask of boiled milk prepared on August 27th, with the same arrangements for ensuring purity of the vessel and excluding dust that we had in the flask of Pasteur's solution. It has not coagulated; it has undergone none of the changes to which I have alluded. There has been no butyric fermentation, no *Oïdium lactis* has formed upon it, no putrefaction has occurred. This milk is as sweet as when it was first prepared; and if you were to examine it with the microscope, you would find in it no organism of any kind. From this same flask, with precautions with which I will not detain you, I have charged various purified liqueur glasses. This one has been charged for more than four weeks, yet the milk remains fluid, you observe, although there is abundantly free access of air to it. The oxygen of the air and the caseine which still exist in the boiled milk have together been unable to bring about the lactic fermentation. As regards boiled milk, this is sufficient evidence that the lactic fermentation is not something to which the liquid is spontaneously prone; it requires something to be introduced into it from without. For you must not suppose that the boiling has rendered the milk incapable of souring. All that it requires is the introduction of the appropriate ferment. If you were to touch the edge of the milk in this glass with the point of a needle dipped in souring milk from a dairy, within two or three days the whole would be a sour clot, showing both the proneness of boiled milk to souring and also the genuine fermentative character of that change as indicated by the faculty of self-multiplication of the

ferment. And on microscopic examination you would be sure to find the *Bacterium lactis* present throughout the mass.

But though the ferment which occasions the souring of milk is present in the milk obtained from any dairy, it appears to be by no means common in the world in general. Suppose you take a series of glasses of boiled milk like these, and introduce into them a series of drops of ordinary unboiled water, you will get fermentation in them. If you put into each, for instance, a drop as large as a quarter of a minim, you will have a fermentation in every one, and an organism in every one; but you will neither have, according to my experience, the lactic acid fermentation nor the *Bacterium lactis*. You will have bacteria of other sorts; fermentations of other kinds. Again, suppose you take a series of such glasses, take off the glass shades and the glass caps, in different apartments or at different times, and expose the milk to the air-dust for half an hour; you will get fungi and bacteria of various sorts, but, according to my experience, you will not get the *Bacterium lactis;* nor will you get the lactic fermentation. And thus it turns out, so far as boiled milk is concerned at all events, that the ferment that brings about this particular fermentation is a rare ferment. So far from boiled milk being spontaneously prone to the change, it requires something to be introduced from without, which is a rarity both in ordinary water and in ordinary air.

But then, it may be urged, indeed such arguments have been used, this may be very true for boiled milk, but how about unboiled? "May it not be that, by boiling the milk, you have destroyed certain chemical ferments, purely hypothetical we must admit, but which we think likely to exist?" For, according to the views of some persons, it may be that in the unboiled milk there may exist certain chemical substances prone to evolve into organisms by spontaneous generation, and prone to produce these and other fermentations, but which, by the act of boiling, we deprive of this tendency. Therefore, with a view to meeting this objection, the first part of my investigation was devoted to endeavouring to see whether or not milk, as it comes from the cow, really does or does not contain materials tending to the development of organisms or to fermentation of any kind.

An exceedingly simple experiment will probably serve to convince you to a considerable extent with regard to this matter. If you go to a dairy where there is also a cow-house, take a couple of clean bottles, and fill one with milk from the dairy and the other with milk direct from the cow in the cow-house, the milk obtained from the dairy will be certain to sour, but the milk that you get direct from the cow will very probably never sour at all. It will probably acquire a nasty bitter taste, and will not have

the *Bacterium lactis* or the *Oïdium lactis*, but some other kinds of fungi. That very simple experiment is enough to show that the lactic acid fermentation is not a change to which unboiled milk is spontaneously prone. And it occurred to me that, if all organisms and fermentations which occur in milk really depended on accidental introduction from without, by performing the experiment with a number of purified glasses and taking the milk in small quantities into each, we might by thus subdividing elude the foreign element and get the milk, in some of the glasses at least, not only without the lactic acid fermentation or the *Bacterium lactis*, but without any fermentation or any bacterium, or any sort of organism. Accordingly, I prepared little glasses like these; little test-tubes with test-tube caps, arranged upon a stand made of pieces of glass-tube and silver wire. The stand containing the test-tubes was placed under a glass shade on a plate of glass and purified by exposure to 300 deg. Fahr. in the hot box. Then some milk having been received from the cow into a purified vessel by means of a a syringe attached to this pipette, the pipette having been also purified, milk was drawn up into the pipette, and then, by means of the syringe, each little cap being in succession raised, a few minims of milk were introduced into each of the glasses, the caps being immediately reapplied. The result was, every one of the milks underwent fermentation, and every one of them contained organisms, some of them as many as three different species. The great majority of those twelve glasses presented little orange specks, such as were never seen, I suppose, in any milk before; and, on examining these, I found them to be little organisms belonging to a group to which I have ventured to give the name Granuligera, because they consist of granules, different from bacteria in this respect, that you might suppose them not to be organisms at all till you had the opportunity of seeing them undergoing multiplication by fissiparous development, in a manner, however, differing from the transverse fissiparous multiplication of bacteria, in being crucial.[1] But, besides the Granuligera, there were among the contents of these test-tubes bacteria of different kinds, to judge by form and size, and in one of them was a toruloid organism, and in two others two species of filamentous fungi, one of which was of the most exquisite delicacy, though in general type of the same sort of arrangement as the common blue mould or the *Oïdium lactis*. The size of the filaments was so exceedingly small that twenty of them would lie abreast in a single human red corpuscle; they were smaller in diameter than even the *Bacterium lactis*, smaller than the great majority of bacteria. I doubt if any such exquisitely delicate filamentous fungus has ever been seen before even by a

[1] Vide 'Trans. of the Royal Society of Edinburgh,' vol. xxviii, p. 319.

professed botanist like my colleague Professor Bentley. But there was no *Bacterium lactis*, and there was no lactic acid fermentation.

What inference were we to draw? Was I to suppose that, although the lactic acid ferment had been excluded, it was impossible to exclude others; that others were present in the milk as it existed in the cow's udder; or was it that I had not been sufficiently careful? The latter was the view I was disposed to take. The experiment had been performed in the cow-house, where certainly the air might be supposed to be reeking with organisms. I, therefore, performed the experiment a second time, and this time in the open air. It must be confessed it was not far from the cow-house, and it was a fine day at the very time of the year in which organisms most abound. On this occasion, I used twenty-four of the little covered test-tubes; those which you see before you. The result was that this time, again, every glass had organisms developed in the milk which it contained. At the same time, every glass seems to be different from all the rest. Such fermentations as there are here, I venture to say, were never seen in milk before. I have brought before you a diagram, showing some of them on a large scale. I want particularly to direct your attention to these strange scarlet spots which occurred in almost all of them. They began as tiny scarlet dots, which spread as fermentative changes capable of self-multiplication in the substance of the milk. Here is one glass that is green, and here is another of an orange-yellow colour. Here are two that have two kinds of filamentous fungi. I have not examined them microscopically, but I shall very likely find there are some species that have not been described.

I felt little doubt that these organisms had got in for want of sufficient care on my part. But how are we to explain these unheard-of appearances? Simply this. If the *Bacterium lactis* had been here, it would have taken the precedence of all other organisms in its development, and the changes which it would have induced would have made the milk an unfit soil for these other numerous species. And the novelty of the appearances depended not on the presence of an unusual variety of organisms, but merely on their having enjoyed an unprecedented opportunity for coming forward. Under ordinary circumstances they would have been smothered—killed—by the effects of the *Bacterium lactis* and the other ferments that commonly develop in its wake. Such being my belief, I determined to make one more attempt. This time I used again the original twelve glasses, but charged them with greater care. I mentioned that a large proportion of these glasses of the second experiment had scarlet spots; and in the former experiment in the cow-house the great majority had orange spots, and those, as we have seen, were composed of heaps of

granules. It occurred to me that one cause of failure might be this. Suppose one single group of such granules to exist, and to become disturbed and broken up in the process of transference to the glasses, it might vitiate the whole specimen of milk; therefore, instead of drawing up the milk into the pipette with a syringe and then expelling it, I determined to have it introduced as directly as possible into the little glasses. With this object I employed these two glass tubes, connected together, as you see, with a short piece of india-rubber tubing, the wider tube being for the purpose of receiving the milk, the narrower to conduct it into the glasses. The glass tubes had been purified by a high temperature, and the piece of india rubber connecting them, as it would not bear a very high temperature, had been boiled for half an hour. The same cow was taken out again into the open air, and this day the elements were in my favour. It had been a drizzly morning, and I might fairly hope that some of the multitudes of organisms existing in the little orchard might have been washed down and that the air might thus have been somewhat purified. I was also more careful in this respect. I got the dairywoman to milk the cow without drawing the hand over the teat, performing the operation by an action of the fingers in succession, so that the end of the teat should always be exposed. Her hands were washed with water, and the cow's udder also, and she having squirted out a little milk to wash away any organisms from the orifice of the duct, the glass cap which protected the larger tube from dust was removed and the end of the tube was held in the immediate vicinity of the teat; a few drachms were introduced, then the cap was readjusted, and then these little glasses were filled by the simple expedient of alternately relaxing and compressing with the finger and thumb on the caoutchouc, so that there was as little disturbance as possible of the organisms that might be supposed to be introduced in spite of my care. It is six weeks since this was done. At first sight, you might suppose, contrasting these appearances with those of the other tubes which were charged only three days earlier, that the milks of this last experiment were all pure. The truth is, all but two have organisms in them; but I may mention that all but four had obviously organisms in them before I went for my trip on the Continent three weeks ago. On my return I found that in the course of the three weeks that had elapsed, two others had gone; but they already showed organisms which, though very pale and insignficant, were quite easily seen by a magnifier in such considerable mass that I felt sure they must have already been growing for a considerable time; and, therefore, in all probability those that still seemed to the naked eye and to the magnifier free from organisms were really so. Accordingly, two

days ago I drew out milk from one of those that seemed to be still pure, and I had the great satisfaction of finding it not only perfectly fluid and tasting perfectly sweet, with a perfectly normal reaction, purpling both blue litmus paper and red litmus paper—the norm alreaction of perfectly fresh milk —but under the microscope I could not discover any organism of any kind whatsoever. Therefore, I think we are justified in saying that in unboiled milk as in boiled milk, provided, of course, the cow be healthy, there does not exist any constituent having the power of giving rise to organisms or producing the lactic or any other fermentative change.

This, gentlemen, was the first step of the investigation : to the second I must beg your special attention, because I bel eve you will agree with me that it is the far more important step of the two.

The object of the second part of the investigation was to find absolute evidence, if possible, whether the *Bacterium lactis* was or was not the cause of the lactic fermentation. It occurred to me that, if we could estimate with some degree of accuracy the number of bacteria present in a given quantity of souring milk, and then if we were to dilute the milk with a proportionate quantity of boiled water, we might have the diluted milk so arranged that every drop with which we should inoculate boiled milk might contain, on the average, one bacterium; and if we should do so, as it would be practically certain that the bacteria would not be distributed with absolute uniformity, we should expect that we might have, as the result of these various inoculations, some glasses with the lactic fermentation, some glasses without it, some with the *Bacterium lactis*, and some without it; and, if it should turn out that those glasses which underwent lactic fermentation should all contain the *Bacterium lactis*, and, on the other hand, those glasses which had no fermentation should be free from bacteria, that would prove the point; as, I think, you will agree with me, when we come to discuss the matter at a little more length after we have all our facts before us. Well, how were we to determine the number of bacteria existing in the liquid? This was done in a simple manner. A circular covering glass, just half an inch in diameter, was used. Of course, we know how many square thousandths of an inch there are in the area of this little glass. We also know by the micrometer how many thousandths we have across the field of our microscope, and, therefore, by calculation we know how many square thousandths there are in our field, and thus we can tell how many fields there are in the covering glass. To measure the liquid, I used this little syringe, with the piston rod in the form of a screw, on which resolves a disc, graduated for 100ths of a minim, by which means you can, with perfect precision, emit 1-100th of a minim, or 2-100ths, or any number you choose. I found that

2-100ths, or 1-50th, exactly occupied the covering glass; so that, when it was put down upon a glass plate, with 1-50th of a minim interposed, the rim of fluid round about the covering glass was not one quarter of the diameter of the field, using the highest magnifying power; so that practically the liquid was all under the covering glass. I knew, therefore, that there was 1-50th of a minim under the covering glass. If, then, I counted how many bacteria there were in a field, taking a number of different fields and striking an average, I could ascertain how many bacteria there were on the average in a field; therefore, by calculation, how many there were under the covering glass; or, in other words, how many there were in the 1-50th of a minim; and, consequently, I knew how much boiled water I ought to add in order that the drop, of whatever size I might wish it to be, should contain, on the average, one bacterium, and one only. This being done with a particular specimen of souring milk, I found that it was needful to add no less than one million parts of boiled water to the milk to ensure that there should be rather less than one bacterium, on the average, to every drop. Then with drops of that size I inoculated five glasses of boiled milk, and the result was that out of the five only one curdled; but one did curdle and soured, and that one had the *Bacterium lactis* in abundance; the others did not curdle, underwent no fermentation whatsoever, and had no bacteria in them. You may say, perhaps, "How was it that there were none of these numerous different organisms and fermentations that you have been showing us?" Simply for this reason, that although these existed, and one of them existed probably in every two or three minims of the milk, yet they were in exceedingly small proportion to the *Bacterium lactis*, so that you might have searched, perhaps, for a whole day, with the high power of the microscope which it was necessary to use, and never discovered one. We are apt to forget how difficult it is to find these minute objects with high powers of the microscope, unless they are very numerous indeed. Therefore, when we came to dilute the milk with a million parts of water, the chances of getting anything but the *Bacterium lactis* were exceedingly small. It was with reference to the *Bacterium lactis* that the dilution had been made, and not with reference to these other organisms so exceedingly small in quantity. It so happened that we saw in the souring milk before making that dilution that there was another kind of bacterium present, a moving kind different from the *Bacterium lactis*; it was in every field, but not nearly so numerous as the *Bacterium lactis* and, consequently, it did not occur in the one milk that curdled.

Now, therefore, we had every reason to hope that we had got the ferment pure, and thus we had the opportunity of performing other experiments; and the last experiment that I shall mention

is this. Having induced the lactic fermentation in a glass of pure boiled milk by means of our presumably pure ferment, and estimated the number of bacteria per minim, I diluted with boiled water accordingly and then proceeded as follows:—These five covered test-tubes which you see before you, containing boiled milk in their lower part, were inoculated each with a drop calculated to contain two bacteria; these other five similar test-tubes were inoculated each with a drop calculated to contain one bacterium; these five liqueur glasses were also inoculated with drops each calculated to contain one bacterium; and one other liqueur glass with a drop calculated to contain four bacteria. The result was that the specimen with the drop calculated to contain four bacteria soured and curdled in a few days; and all these five calculated to have two bacteria to a drop curdled also in a few days. The milk, you see, is perfectly solid. You will also observe that no other change has taken place except the lactic fermentation, no *Oïdium lactis* has grown, and no other alteration has taken place; it is as pure in whiteness as when it was first coagulated. I may here mention that, although all these coagulated, they did not all coagulate at the same time. There was a time in the twenty-four hours during which the coagulation went on, in which I hoped that some of them were going to be permanently fluid, implying, as you would expect, that the particles of the ferment were not uniformly distributed; some had more than others, though each happened to have at least one. But, of the five test-tubes calculated to have only one bacterium on the average to each inoculating drop, three have remained fluid, and so have two of the liqueur glasses; so that, of the ten calculated to have on the average one bacterium each, exactly five, it so happens, have remained fluid without any curdling. I may consider myself somewhat fortunate that I have succeeded in bringing these specimens all the way from Edinburgh in this condition. I will now deprive this one of the protection in which it has hitherto lived. [Professor Lister, having removed the glass shade and glass cap from one of the liqueur glasses, proceeded to drink part of its contained milk.] It is perfectly sweet. It has a slight flavour of suet, which M. Pasteur has described as resulting from the oxidation of the oleaginous material of the milk. If any gentleman likes to taste it after the lecture, he can do so.

Let me note this curious circumstance, that, of those specimens which did coagulate, those in the tubes coagulated considerably earlier than those in the more open vessels. At first, it seemed as if, for some strange reason, those in the open vessels were going to remain permanently fluid—even that which had, according to the calculation, four bacteria to the drop. I presume this is to be explained on the same principle as Pasteur has explained a corresponding fact with regard to the yeast-plant. He has

shown that, if a saccharine solution be put in a very thin layer in an open vessel with yeast, the yeast-plant developes very rapidly, but very little fermentation occurs; on the contrary, if it be put into a deep vessel, the development of the yeast-plant does not go on so rapidly, but more fermentation results. He explains the fact in this way: that the yeast-plant requires oxygen for its nutrition; if it get it easily, as it does in a shallow vessel in the air, it produces comparatively little effect in breaking up the sugar into its constituents, and *vice versâ*. So here, in the test-tubes the carbonic acid accumulated, supposing any to exist, as in a well, and the *Bacterium lactis* had but little opportunity for getting oxygen. Accordingly here, just as in M. Pasteur's experiments with a sugary solution with yeast in a deep vessel, the *Bacterium lactis* produced more rapidly its fermentative effect.

But this, you say, is assuming that *Bacterium lactis* is the ferment. Now we are coming to that point. But first I have to mention an additional fact. For the satisfaction of others rather than for my own, I went through the laborious process of investigating portions of the contents of all these vessels; and I found that, in every one in which the lactic acid fermentation had taken place, where there was curdling and souring, the *Bacterium lactis* was present; and in no instance in which there was no lactic fermentation was any bacterium of any sort to be discovered. I believe that fact demonstrates that the *Bacterium lactis* is the cause of this very special lactic fermentation. Let us assume for a moment that there did exist some other material besides the *Bacterium lactis* in the milk capable of causing the fermentation; that the lactic ferment was not the bacterium at all, but some chemical ferment. First of all, you will please to observe that we have from this experiment absolute evidence that the ferment, of whatever nature, is not in solution, but in the form of suspended insoluble particles. If the ferment had been in solution, every equal sized drop of the water of inoculation would have produced the same effect. The fact that some drops were destitute of the ferment proves that that ferment was not in a state of solution. That is absolutely demonstrated. Now, suppose we admit, for the sake of argument, that the lactic acid ferment consisted of particles of some non-living substance, capable of self-multiplication as rapidly as the bacterium, but not living; a strange hypothesis, no doubt—but suppose we assume it. Suppose we admit that the true lactic ferment and the *Bacterium lactis* were merely accidental concomitants of each other, it would be absolutely inconceivable that these two accidentally associated things should be present in exactly the same numbers. And yet, according to the hypothesis, such would be only another mode of stating our observed fact, which

amounts to this, that wherever there was a fermentative particle there was a bacterium, and wherever there was a bacterium there was a fermentative particle. But, suppose you admitted that—that there were exactly as many of the *Bacterium lactis* as there were of the hypothetical true fermentative particles—suppose you admitted that inconceivable thing, I say it would be again inconceivable that, if mutually independent, they should accompany one another in pairs, that invariably where there was *Bacterium lactis* there should be a ferment particle, and where there was no *Bacterium lactis* no ferment particle. That would be a thing as inconceivable as the other. Therefore, we have two inconceivables, one of which would have been sufficient to show that we cannot admit any other hypothesis than that *Bacterium lactis* is the cause of the lactic acid fermentation.

But the experiment tends to even more than this. Where we find the effect so exactly proportioned, as regards the number of glasses affected with fermentation, to the adult bacteria that we count, we are led to infer that this particular bacterium, at all events, has not any spores—that there are no spores existing in addition to the bacteria. People seem often to assume that bacteria must necessarily have spores or germs. It seems to me an unlikely thing that they should. They are, as it were, a generative apparatus *per se*, they are constantly multiplying; why should they have spores? I do not say that bacteria may not have spores. There are very different kinds of bacteria; some may have spores, and some may not; but this sort of result seems to indicate that this particular bacterium has no spores; at least, in the condition in which it exists in souring milk; because, if we had, besides the bacteria that we can count, spores of bacteria disseminated through the liquid also, we should have the effect more than in proportion to the bacteria that we have counted. The only fallacy here is that it may be that the bacterium has not been diffused uniformly through the milk. Therefore, I do not say that in this case it is absolutely proved. But, at all events, this experiment gives us a line of inquiry, by means of which we may probably settle that point with regard to any individual case of bacterium. This, however, is a point I do not desire now to insist on; but, what I do venture to urge upon you, is that you will seriously ponder over the facts which I have had the honour of bringing before you to-day; and, if you do so, I believe you will agree with me that we have absolute evidence that the *Bacterium lactis* is the cause of the lactic acid fermentation. And thus I venture to believe that we have taken one sure step in the way of removing this important but most difficult question from the region of vague speculation and loose statement into the domain of precise and definite knowledge.

NOTES AND MEMORANDA.

On the Concentric Bodies of the Thymus.[1] By Dr. B. Afanassiew, of St. Petersburg. Anatomical Institute at Strassburg. (With Plate.)—The concentric bodies of the thymus, known as Hassall's corpuscles, represent, as is well known, multinuclear elements of a more or less concentric arrangement in the interior. Their development and nature has been variously described by different observers. Afanassiew shows that they owe their origin to a retrogressive metamorphosis of blood-vessels from whose endothelium they are directly derived. Preparations obtained from glands which, while fresh, were hardened in monochromate of ammonia, then washed in distilled water and alcohol, pencilled and stained in haematoxylin and ammoniacal eosin solution, show all intermediary forms, viz. vascular tubes whose endothelial cells are enlarged, further tubes, whose lumina appear entirely plugged with these enlarged cells, and finally, vessels whose endothelial cells possess a concentric arrangement, and partially or entirely occupy the lumen.

The presence of the blood-corpuscles within the concentric bodies, already noticed by Tendrassik, is now easily explained. In injected preparations of human thymus Afanassiew traced the injection up to the concentric bodies and several times also into them.

The vessels whose endothelium degenerates into the concentric bodies show a thickened wall; this probably represents the capsule which is formed round many of the concentric corpuscles.

In the first stages of development of the gland neither Afanassiew nor Professor Waldeyer could detect any concentric bodies—against the assertion of Berlin, His, and Friedleben. They are found most numerously during the involution of the organ. It thus appears probable that the involution of the gland and the development of the concentric bodies—or the retrogressive change of blood-vessels—stand in an intimate relation to one another.—E. K.

[1] 'Archiv f. Mikroskopische Anatomie,' Bd. xiv, Heft. i, p. 1—6, v. la Valette St. George und W. Waldeyer.

The Iodine Reaction of the Cells of Cartilage and Notochord.[1] —By Professor E. Neumann, of Konigsberg, in Prussia. (With Plate.) In a previous paper Professor Neumann ("Observations on the Cartilage-tissue and the Process of Ossification," in 'Archiv. d. Heilkunde,' xi, p. 414, 1870) stated that cartilage-cells under the action of solutions of iodine assume a red-brown or even dark-brown colour. Ranvier ('Traité Technique d'Histologie') supposes this colour to be due to the presence of glycogen in the cartilage-cells. "To obtain the reaction it is best to use weak solutions of iodine, which give to the other tissue-elements only a pale yellow tint. The 'iodine-red' cartilage-cells appear then in just the same colour as the tissue of amyloid degeneration." The protoplasm of the cartilage-cells that shows the above iodine-red colouration either represents only part of the cell-substance, or this latter appears wholly in that tint. In the first instance there is generally a marked boundary between the red and yellow part of the cell-substance. The nucleus does not participate in the red reaction.

The iodine reaction was very beautifully shown in the cells of the gelatinous particles forming the contents of the cavity of an enchondroma. The portions of the cell-substance which assume the iodine-red colouration presented everywhere "the character of a homogeneous, somewhat glistening substance," probably of viscous consistency.

Already in the embryo the cartilage cells show the above iodine reaction, and they retain it all through their life. The small, flat cartilage-cells situated in the periphery underneath the perichondrium form an exception, as they never show the reaction.

Not only the cartilage cells of hyaline, but also those of fibrous, as well as elastic cartilage, show the iodine-reaction.

In precisely the same manner comport themselves the cells of the notochord of Petromyzon, Rana, and human embryos.

The chemical examination of the notochord of Petromyzon, carried out by Professor Jaffe, *proved most conclusively the presence of glycogen.*—E. K.

The Juice-Passages in Hyaline Cartilage.[2] By Dr. Albrecht Budge. (With Plate.) By means of injections of soluble Berlin-blue, asphalt dissolved in chloroform or in benzol or turpentine, Budge was able to convince himself of the following points :—(1) From the periosteal (resp. perichondial) lymphatics it is possible to inject cartilage capsules near the ossification-zone, so that there exists a free communication between the two.

[1] 'Archiv f. Mikroskop. Anatomie,' Bd. xiv, Heft. i, p. 54—59.
[2] Idem.

(2) In the articulation-cartilage, close under the synovial membrane, it is possible to inject large districts of cartilage capsules; thus fine passages connect the capsules.

(3) By direct pressure of a column of Berlin-blue it is possible to demonstrate directly the fine passages uniting the cartilage capsules.

As objects of his researches, Budge used feet of calf and sheep, frogs and tortoises.—E. K.

Further Communication on the Structure of the Cell-Nucleus, with Observations on Ciliate Epithelium.[1] By Professor H. Eimer, in Tubingen. (With Plate.)—Continuing his researches on the structure of nuclei of cells (Archiv fur Mikroskop. Anatom. Bd. vii and viii), in which he demonstrated within the membrane of the nucleus of various kinds of cells the presence of a hyaline zone round the nucleolus, which he called "Hyaloid;" Professor Eimer found this surrounded by a zone of minute granules, which he called "Körnchenkreis" (granular sphere); between this and the membrane lies the peripheral opaque part of the nuclear contents. His more recent observations enable the author to say that the above elements of the cell-nucleus are not the result of reagents (Auerbach), but are present in the living unaltered nucleus of various cell-species.

Professor Eimer finds from numerous observations (ciliated epithelial cells of palate of *Salamandra maculata*, the large nuclei of cells lining the inner surface of the tentacular wall of *Aegineta*, the sensory cells and the cells of the ectoderm of *Carmarina hastata*, the branchial epithelium of Axolotl) that the "granules" of the "Körnchenzone" are only the optical transverse sections of numerous protoplasmic filaments, which permeate the interior of the nucleus in all directions and anastomose with each other so as to form a very minute network. This network of filaments extends up to the membrane of the nucleus. But also the above-named "Hyaloid" is penetrated by numerous fine threads, which radiate from the "granules" of the "Körnchenzone" and terminate in the nucleolus. The peripheral part of the nucleus, *i.e.* the part between "Körnchenzone" and membrane, contains likewise a dense network of filaments. Under a low power it presents an opaque granular aspect, owing to the numerous fibrils being viewed in optical transverse section.

From this type there are numerous variations as regards the nucleolus and the distribution of the filamentous networks within the nucleus. The filaments of the intranuclear networks are in

[1] 'Archiv f. Mikroskop. Anatomie,' Bd. xiv, Heft. i, p. 97—117.

connection with extranuclear minute filaments, which permeate as networks the substance of the cell itself. In the ciliated cells above named the cilia extend into the cell-substance and appear to identify themselves with those filaments.

[Heitzmann described a similar network of minute fibrils within the substance of the cell-protoplasm].—E. K.

On the Postembryonal Growth of Bone.[1] — By Professor Schwalbe. Professor Schwalbe finds that in man, beginning with the ninth month, or end of first year of life till the fourth or fifth year, the periosteum deposits only minimal quantities of bone substance, so that in tubular bones (femur, tibia, humerus) no appreciable growth in thickness takes place. As the medullary cavity continues to expand during the just named period it follows *that the compact substance is thinner at a later* (four years) *than at a younger stage* (three years). Calculation on the volume of the compact substance showed that the total volume of the cortex of diaphysis (formed from the periosteum) is smaller in individuals of four years than in those of three, *a physiological absorption of bone* is therefore proved beyond doubt. As regards the microscopic appearance of growing bone, Professor Schwalbe finds the following: *in the first period,* after birth till about the sixth month, the tubular bone grows from the periosteum after the embryonal mode, and possesses the peculiar structure common to embryonal bone—viz. that of a trellis-work (v. Ebner). Beginning with the sixth month a distension takes place in all vascular spaces, which become converted into wide Haversian spaces, owing to an absorption of bone-substance. *This second period may be called the stage of osteoporosis.* The osteoporosis extends as a rule only on the inner half or the inner two thirds of the shaft. Before this, however, is completed, *the first formation of lamellar bone takes place in the innermost parts that had been subjected to the osteoporosis.* This formation is due to the medulla. Gradually all parts of the shaft (including the peripheral portion in which meanwhile Haversian spaces have been formed) become provided with lamellar, *i.e.* Haversian systems of bone-substance. This represents *a third stage,* viz. *the stage of formation of the Haversian lamellæ.* Through all this time, however, the tubular bone has not increased in thickness, and it follows, therefore, that up to the end of the fourth year the periosteum takes no part in the formation of lamellar bone-substance.

Beginning with the fourth year the periosteum produces ground-lamellæ. In these the Haversian spaces are formed

[1] 'Sitzungsber. der Jenaischen Gesellschaft f. Med. und Naturwiss.,' 6th July, 1877.

round the vessels, and they become finally also lined by lamellar bone-substance.

These observations of Professor Schwalbe are of great interest with reference to the pathology of rickets, inasmuch as there exists a definite relation between this disorder and the stage of osteoporosis above mentioned. The examination of rickety bones proved (1) an imperfect filling out of the osteoporotic shaft with lamellar substance; (2) a new deposit of embryonal bone-substance by the osteogenetic layer of the periosteum on the surface of the cortex. So that in rickets the periost continues to form bone on the embryonal type, while the formation of lamellar substance in the cortex proceeds only slowly or imperfectly.[1] E. Klein.

Salensky on the Polyzoa Entoprocta. — When preparing the abstract of Vogt's paper on *Loxosoma*, which was published in a late number of this Journal, I was unacquainted with Salensky's researches on the *Entoprocta* which have recently appeared in the 'Annales des Sciences Naturelles.'[2] The paper in which they are recorded takes much the same line as that of Vogt, and is in some measure complementary to it, and I propose to give a brief account of its most interesting points.

Salensky's investigations were conducted at Naples, and were undertaken, he tells us, for the purpose of comparing the *gemmation* of the *Entoprocta* with that of the *Ectoprocta*, and so determining the relations which exist between the structure of these two groups. Two species of *Loxosoma* were examined, which he believes to be new; *L. crassicauda*, Salensky, living as a commensal on a species of Annelid, and *L. Tethyæ*, Salensky which occurs in immense quantity on the *Tethyæ*, and on them only. The former belongs to that section of the genus which is destitute in the adult condition of a pedal gland; it possesses eighteen tentacles, and is not attached by an expanded disc. It is also furnished with numerous, strongly developed unicellular glands in the integument, which strike the eye at once. The author believes that the mode in which the peduncle of *L. crassicauda* is attached is a specific distinction, but in this point it agrees with *L. Phascolosomatum*, Vogt, which is also destitute of an adherent disc, and is fixed by a special secretion.

[1] The abstract in the last number of the Journal (January, 1878) of Professor Boll's paper on nerves, as well as that of Professor Bizzozzero and Salvioli, have been ascribed, by error, to me. They were prepared by Dr. John Cavafy.

[2] "Etudes sur les Bryozoaires Entoproctes," par M. Salensky, Professeur á l'Université de Kazan, Annales d. Sc. Nat., sixième série. Zool. T. V., Nos. 3 à 5, June, 1877. 60 pp., plates xii—xv.

L. Tethyæ is furnished with a very long cylindrical stem, terminating below in an enlarged, foot-like extremity, in which a pedal gland is lodged. It is said to be nearly related to *L. Raja*, Schmidt.

We require a much more minute and precise description of these species.

The interest of the paper, however, lies in its studies of structure and development.

In the first place, the author has determined the nervous system of *Loxosoma* which had escaped both Nitsche and Vogt, and traced its connections with the organs of sense described by the latter author. The only representative of a central nervous system is a small ganglion placed in the middle of the body above the stomach, between the extremity of the œsophagus and the commencement of the intestine, and more on the dorsal than the ventral side. In the adult it is extremely difficult to make it out, as it is concealed by the reproductive organs, but it is easily detected in the young polypide. The ganglion is a small ovoid body, which gives off nerves in many directions. The smaller nerves the author was unable to follow; the largest pass off on both sides of the ganglion towards the dorsal portion of the body. Each of them gives off many lateral branches, and in the middle of its course presents a small thickening composed entirely of nervous cellules. As they approach the surface of the body, the nerves become thinner at first and then swell out into small pyriform knots, which are enclosed in tubercular elevations of the integument. These tubercles, placed on the dorsal surface, on each side of the longitudinal axis of the body, are the *organs of sense*. Their structure is identical with that of the "antennæ" of the *Rotifera*. They consist of small tegumentary swellings the cavity of which is filled by the nervous matter, bearing on the summit a cluster of fixed setæ, which are united at their base to the nerve-knot.

This description of the structure of the *tactile organ* differs from that of Vogt, who represents the *papillæ* as occupied by a number of conical cells.

The plan of the nervous system as just described is similar to that which has been demonstrated by Nitsche for *Pedicellina*.

Another organ is described by Salensky, which has escaped the notice of previous observers. It is composed of a pair of multicellular glands, having the form of two bunches of grapes. They are lodged in the parenchyma of the body on each side of the intestine. Each gland consists of eight cells borne or pedicles; the cells are ovoid and composed of a delicate membrane and transparent protoplasm. All the pedicles of the cluster

unite in a common stem, which opens on the side of the body by an extremely minute orifice. The author conjectures thae these structures may be *excretory organs*—renal glands. Ht then proceeds to give an elaborate account of the *gemmation*, as observed in *L. crassicauda*.

He first notes the fact that buds are formed upon the primary buds before their separation from the parent, and points out that in these secondary buds the earliest stages of the development are most readily studied. I shall give a very brief outline of the process of development as he has reported it.

The buds in this species are produced on the ventral surface of the body, and are placed symmetrically on each side of the longitudinal axis of the cup.

First rudiment of the bud, a circle composed of a group of cells in which two cellular layers are distinguishable, one peripheric, the other central; the first made up of many cells and equivalent to the *ectoderm;* the second of a single cell, afterwards multiplying by division, and constituting the *endoderm*. These layers are the representatives of the germinal leaves of other animals.

In this account of the *primitive contents* of the bud the author agrees with Nitsche, and differs from Vogt, who holds that they are not cellular, but consist of an "undivided sarcodic mass."

I shall notice the principal divergences between these observers, in order to direct attention to points requiring further investigation.

Stage 2.[1] Endoderm composed of two cells resulting from division of the primitive cell.

Stage 3. Development of a third layer, the *mesoderm*, and of the rudiments of certain organs. The form of the whole bud is modified. It now appears as an oval body borne on a short peduncle, a direct continuation of the ectoderm of the parent. Two regions are distinguishable, an anterior and a posterior, the first corresponding to the part on which the crown of tentacles is developed, the second to the stem. A slight longitudinal fissure in the ectoderm on the anterior part, the rudiment of the orifice leading into the intra-tentacular space (the *Vestibule*). The endoderm and ectoderm are also modified. The cells of the former multiply and it increases in bulk. It is now situated in the anterior portion of the bud, and adheres firmly to the ectoderm, a significant change with reference to the formation of the hood and the digestive tube. The *mesoderm*, between the two

[1] The stages correspond to the figures by which the course of development is illustrated.

primitive layers, consists of oval cells much crowded together. In the peduncle two nucleated cells make their appearance, the rudiments of the *pedal gland*.

Stage 4. Very slight changes except in size. The three layers are enlarged. The two cells representing the pedal gland become pyriform, and the longitudinal fissure is elongated.

The author here notes that the ectoderm and endoderm of the bud are *the product of the ectoderm or integument of the parent*, a point upon which Vogt also insists.

He then proceeds to follow the development of the different internal organs, the further modification of the form of the bud itself being unimportant.

Stage 5. According to Nitsche and Oscar Schmidt, the cavity of the digestive tube is formed early. In *L. crassicauda* the rudiment of this organ is a completely solid body, viz. the compact mass of endodermal cells already mentioned. Vogt also describes the stomach as at first perfectly solid.

The differentiation of the rudimentary digestive canal commences by the formation of a cavity at the top of the bud, immediately below the longitudinal fissure in the ectoderm previously noted. This primitive hollow is due, the author thinks, to the atrophy of some of the endodermal cells. It gives origin to the cavity of the digestive canal and to the intratentacular space (the *Vestibule*).

I am quite unable to harmonise the account given by the author of this portion of the developmental history with that which we have from Vogt.[1]

Stage 6. The rudimentary digestive canal takes on the form of a cul-de-sac, in which two regions are distinguishable—the upper, which is broad and furnished with thick walls and represents the *Vestibule*, and the lower, which is bent backwards, and is the rudiment of the *digestive tube* and the *rectum*. The summit of the bud is already obliquely truncated, a character which is distinctive of the genus.

A small oval body is now visible, placed in the fold or bend of the digestive tube, and probably derived from the endoderm. The author considers it to be the rudiment of the nervous ganglion. At this stage, too, some cells are distinguishable on the surface of the stomach, which from analogy he thinks may be the rudiments of the sexual organs. Their origin he was unable to determine.

Stage 7. The bud has increased considerably in size. The rudiments of the tentacles are apparent as minute prominences on

[1] *Vide* the October No. of this Journal, page 369.

the margin of the longitudinal fissure; or, in other words, on the margin of the aperture of the Vestibule.

The upper portion of the digestive tube[1] undergoes a change; the dorsal wall of the rudimentary intratentacular space is carried backward and forms a cul-de-sac, the future Vestibule. On the ventral wall, close to the integument, is situated an opening which is the buccal orifice.

The *anus* is not yet developed. The *rectum*, at this stage, has the appearance of a recurved cul-de-sac, closely applied to the dorsal wall of the Vestibule. The anal orifice subsequently makes its appearance at the point of union between these two.

Stage 8. Œsophagus, stomach, and rectum are now distinguishable. The tentacles appear as small risings on the margin of the cup, and as the latter consists of ectoderm and endoderm the tentacles also possess the two layers. The former constitutes the inner wall, the latter the outer wall of the tentacle which at a later stage is covered with cilia. The number of tentacles is less in the bud than in the adult, and amounts to ten in the present species.

At this stage the formation of the muscles commences; they are developed from the *inferior portion* of the mesodermic layer, the upper portion giving rise to the parenchyma. The reason of this division is evident; it is the lower portion of the bud, in which the muscular fibres are produced, that becomes the peduncle. Each fibre seems to be formed by a single cell.

The peduncle or stem is formed by the extension of the lower part of the bud in which the pedal gland is lodged, but it is only in the latest stages of the development that it is differentiated.

The author has noticed an interesting peculiarity in the young buds of this species. Their tentacles are furnished with a very long, fixed bristle, placed on the external side and close to the summit. It is not present in the adult, and is therefore a provisional structure like the pedal gland. The author conjectures that it is a tactile organ essential to the bud when seeking a place of attachment, but useless afterwards.

If we compare the developmental history which I have now summarised with that which we have from Vogt we shall find very serious discrepancies between them. According to Salensky and Nitsche the primitive contents of the bud are distinctly cellular; Vogt represents them as consisting of masses of homo-

[1] The author may perhaps mislead by speaking as he constantly does of the uppermost section of the cavity scooped out, as it were, in the endodermic mass, as a portion of the digestive tube. At an early stage it seems to be distinguishable from the rest of the cavity, and subsequently becomes the vestibule, which is in no sense a part of the digestive system.

geneous protoplasm. Salensky describes the principal internal organs (*vestibule, œsophagus, stomach, rectum*) as formed by successive modifications of an elongate cavity hollowed out in the solid, central mass of endodermal cells. According to Vogt the *vestibule* has its origin in a small empty space, present from the first in the midst of the protoplasmic contents of the bud. The other organs are formed by "the differentiation of an undivided sarcodic mass." The *stomach* is, in the first instance, a large ovoid body (accumulation of protoplasm), placed transversely below the primitive cavity, which is subsequently hollowed out in the centre, and ultimately brought into communication with the cavities of the œsophagus and intestine. These are not unimportant differences. Must we suppose that such diverse plans of development occur amongst the species of the same natural group? Or are we to believe that there must be errors of observation on one side or the other?

Our author proceeds to give an account of the gemmation of *Pedicellina*, which he finds to be strictly analogous to that of *Loxosoma*. I do not propose to follow him through this portion of his paper, but may remark that his detailed description of the development of the internal organs runs parallel at almost all points to that which he has given us of the same process in *Loxosoma*.[1]

General deductions. The author fully accepts the group of the *Entoprocta* as constituted by Nitsche.

In opposition to the views of the latter author, who considers that the polypide of *Loxosoma* is homologous with the polypide *only* (apart from the zooœcium) of the *Ectoprocta*, and that in this form there are no parts homologous with the zooœcium or cell, Salensky holds that the ectoderm of *Loxosoma* corresponds to the zooœcium of the *Ectoprocta;* its digestive canal and tentacles to the digestive canal and tentacles of the polypide of the *Ectoprocta;* its parenchyma and muscles to the mesoderm and muscles of the *Ectoprocta*. He bases his view on a careful comparison of the gemmation of the two groups. In the *Ectoprocta* and the *Entoprocta* we may always recognise, he holds, the same parts, the zooœcium and the polypide, which together compose, in one case, the *métamère* (*i.e. zooœcium-cum-polypide*) of the colony of the colonial Bryozoa; in another, an independent individual such as *Loxosoma*.

The distinctive generic characters of *Loxosoma* the author considers to be the absence of the diaphragm separating the

[1] This certainly affords a strong presumption in favour of the accuracy of his observations in the latter case.

body from the stem, the solitary condition and the mode of gemmation.

The specific characters, such as the number of tentacles and the presence or absence of the pedal gland, exhibit many modifications in the different kinds. The modifications sometimes take place in individuals of the same species but of different ages. In the bud the arms are fewer in number than in the adult; sometimes also the pedal gland is present in the bud, but absent in the adult. In fact, some species of *Loxosoma* seem to be, relatively to others, the same animals, but in different phases of development. For example, *L. Neapolitanum*, which has few arms and a pedal gland, bears a striking resemblance to one of the early stages in the development of *L. Kefersteini*, which in the adult state is destitute of the gland, and has a larger number of arms. The author regards *L. Neapolitanum*, as the primitive species ("*espèce originaire*"), and thinks that the divergence of the other species has taken place in two different ways—(1) by the disappearance of the pedal gland, and (2) by the multiplication of the tentacles.—T. Hincks.

Development of Acanthocystis.—In the 'Jena Zeitschrift' Dr. R. Hertwig records that his researches have made known to him three modes of increase in the Heliozoon Acanthocystis. The simplest is, of course, that by subdivision, already noticed by Greeff. To the author it seems to be a pretty common process, at least he so judges from frequently noticing two nuclei (on treating examples with osmic acid, followed by Beale's fluid) both lying in a common endosarc, seeming to shut out the idea of a previous conjugation of two individuals. He has also noticed two nuclei in Schulze's Actinolophus. But as to the mode of origin of the binucleated state, he has no very connected observations to record. He found nuclei the nucleoli of which had become elongate, whilst the nuclear membrane showed an annular constriction, showing that the nucleus increases by self-division, as observed by Schulze in Actinolophus.

A second mode of increase the author had an opportunity of observing in *Acanthocystis aculeata*. He met with a single example, with which a globe was in connection, having all the appearance of a second individual. This consisted of a granular protoplasm, but free from foreign bodies, nor could there be detected in it either a nucleus or a special endosarc. It lay in a diverticulum of the 'skeleton' of the Acanthocystis, which was mainly composed of tangential pieces (in *A. aculeata* the skeleton consists of two elements—tangentially posed elongate spicules, composing a thick investment, also

spines furnished with a basal disc, these seated radially on the superficies) ; it gave off no pseudopodia, although the main body-mass sent them off copiously. The author kept this example several days under notice. For forty-eight hours no change was observable, except that the appended portion, wanting pseudopodia, seemed to increase in size. Presently subdivision abruptly set in, and the protoplasm shortly broke up into six portions. These one by one, in the course of an hour, left the inner space formed by the skeleton, this process being begun by their projecting a protoplasmic process through the latter, and gradually forcing their way out, and this, indeed, always at the same place. The little body when set free, whilst undergoing amœboid changes of form, projected at the same time long pseudopodia, passing thus into an actively moving Actinophrys-like body (0·006 mm. in diameter). The author thought he could perceive contractile vacuoles and a nucleus therein, but was not able quite to satisfy himself on the point, nor did he like to apply reagents to his only specimen, but unfortunately, after all, these daughter-organisms got lost to observation, without his being able to follow out any further alterations. The Acanthocystis itself had, meantime, thrown off the empty "brood-capsule," without the spicules falling asunder. The whole finally died, after eighty hours' observation, during which, however, the author was unfortunately unable to observe the mode of development of the new nuclei or to throw a light on the part played by the nucleus in reproduction.

During these efforts, however, he noticed a third mode of development. In a number of individuals he observed within the skeleton roundish or oval corpuscles of about 0·01 mm. in diameter, lying in depressions of the surface of the body-mass. These consisted of protoplasm, poor in granules, rich in vacuoles, and in them the author could sometimes detect a nucleus with nucleolus. There were in some cases as many as six of these bodies, but mostly only two, these constantly separated by a bridge of protoplasm. These by and by passed out, the opened system of spicules closing behind them. Mostly no further change could be noticed, but sometimes each developed two flagella, but their languid action only sufficed to slightly roll these little bodies about, and was not great enough to cause them to move off. The author could never succeed in following these up to an Actinophryan stage. In the mean time the interpretation of the purport of these bodies must remain an open question—are they parasitic or reproductive? In favour of the latter

interpretation is that always after their exit the mother-organism—which before was poor in pseudopodia—sent out pseudopodia copiously.

To the foregoing notes on Acanthocystis the author adds some observations made on *Actinophrys Sol.* He noticed in a certain large example, that its contents were formed of minute organisms, in extremely vivacious swarming movement, so numerous that the alveolar parenchyma had become almost obliterated, leaving only traces in the form of bridge-like connections, reaching between the outer membrane-like attenuated cortex and the protoplasmic investment of the nucleus. The nucleus was unchanged, the pseudopodia sparing and short, and without their granular investment, that is, naked axile threads. After some time the cortex burst at various places, and there issued forth many swarms of extremely minute flagellate organisms, about 0·004 mm. long, 0·002 mm. broad. Appertaining to each of these bodies, which were capable of amœboid alterations of form, the author was able to perceive two flagella, but could not determine if there were a nucleus and contractile vacuoles present. These soon became scattered about, and the author was unable to follow them any further. The parent Actinophrys, on emptying out the zoospores, contracted in mass, forming at last a considerably smaller but ordinary looking example.

The author compares this observation to that of Greeff on *Actinosphærium Eichhornii* already recapitulated in this Journal.[1] Hertwig seems to be of opinion that both were cases of parasitism, for in Greeff's case the parent Actinosphærium was dead (killed by the parasite ?), and in both instances he supposes the 'amœboid' condition of the zoospores renders it improbable they could truly belong to the development of an organism which in its natural state possesses pointed pseudopodia.

To the two observations alluded to, Hertwig might have added the cursory one by myself, recorded in "Minutes of the Dublin Microscopical Club," of the gradual giving off, from that beautiful and large *green* form, (of *A. Sol ?*) of but a few zoospores at a time, and so slowly that the loss to the mass of the parent Actinophrys was scarcely appreciable. Each of these carried off one or two of the chlorophyll-granules. They did not in the least present the appearance or suggest themselves as having the nature of parasites. Nor would it occur to me to suppose that an 'amœboid' state in youth of the zoospores was incompatible with a form at

[1] 'Quart. Journ. Micr. Sci.,' vol. xvi, p. 301.

maturity possessing filiform and pointed pseudopodia. It is the mature form which we must assume as presenting the essentially characteristic conditions; but I, at the same time, wholly agree with Hertwig in regarding the kind of pseudopodia possessed by the mature sarcodine as a constant and eminently characteristic feature.—Wm. Archer.

Calberla's New Embedding Mixture.—The embedding mixture, to which the following account has reference, gives, so far as my experience goes, most useful results. The objects which I wished to embed were the more delicate portions of the nephridia, or segmental organs of the common Earth worm, which are so extremely fragile that I had found it impossible to obtain adequate sections by the ordinary wax and paraffin methods; indeed, I had almost given up the attempt in despair, when the paper which is the subject of the present notice was put into my hands by Professor Lankester. The method of preparation, although somewhat troublesome, is, I think, capable of simplification; the mode in which this might be done, and the difficulties to be overcome, as well as the special merits of the material will be pointed out below.

The embedding mixture was made by Dr. E. Calberla, of Freiburg, on the principle of a somewhat similar substance proposed by Drs. Bunge and Rosenberg. It is meant for small and delicate objects, such as eggs and embryos, which are embedded as a whole, whilst the sections can be mounted and examined without that picking away or dissolving of the embedding material, which is such a fertile source of annoyance and discomfort in the ordinary methods of paraffin embedding. An account of Dr. Calberla's mode of preparation was published in the 'Morphologisches Jahrbuch,' vol. ii, pt. 3, p. 445, 1876. The following is an abridged account:—Make, in the first place, a 10% solution of calcined sodium carbonate, the sodæ carbonas exsiccata of the British Pharmacopœia: then remove the yolks from the whites of some hens' eggs; the number of eggs used will, of course, depend upon the amount of material required; I find that for ordinary purposes three are amply sufficient; pick away the chalazæ, and cut up the whites with a pair of scissors; to every fifteen parts of the albumen thus prepared add one part of the sodium carbonate solution, and shake the whole mixture vigorously: then add the yolks of the eggs which have been used, and again shake energetically for a minute or two: now, pour the mixture into a deep vessel, allow it to stand for a short time, and then skim off all the bubbles with

strips of paper, picking out at the same time, with a pair of forceps, any membranous fragments. The material is then ready for use as an embedding substance. When first using the material a matrix or core has to be made; for preparing this Dr. Calberla uses the method to be presently described, but inasmuch as this involves a great waste of time, I simply pour some of the fluid material prepared as above into a test tube, which is then corked up and put for five minutes or so into a saucepan of boiling water; on then removing the test tube, the substance is found to have set quite firm; the tube is then broken, and a cylinder of embedding material is obtained, which for all practical purposes is identical with the substance hardened by the more elaborate method. A groove is made in a bit of the cylinder thus obtained, about an inch long, and the object to be embedded is placed in the groove; before doing this, however, the object is put into water to free it from any trace of its preservative fluid, where it is allowed to remain for from three to ten minutes: then, if it is very delicate, it is put into some ordinary white of egg, or into the fluid material itself, for a period varying from five to twenty minutes, according to the thickness of the object; if it is liable to curl up the object is placed between two thin shavings taken from the previously hardened material, and is then laid in the groove cut in the cylinder. After placing the object in the groove which has been cut to receive it, the cylinder is put into a small paper box, such as is used in embedding with paraffin, and some of the liquid material is poured over it till a layer is formed above cylinder: the paper box is then suspended by a piece of the thread in a vessel containing strong alcohol in such a way that the paper box is immersed to at least half its height in the alcohol, the vessel itself being placed in a water bath whose temperature is so regulated that the alcohol does not quite boil; a funnel is inverted over the vessel, and thus the paper box containing the object and embedding mixture is exposed to the full vapour of the alcohol; after remaining in this position for 30-45 minutes the material will be found to have attained a fairly marked consistency. It must now be removed, the paper cut away, and the preparation put into strong cold alcohol, which is changed after 24 hours. It is not necessary to use a paper box if the block of hardened albumen is carefully excavated so as to receive the object which is to be cut and a sufficient quantity of the liquid albumen. It is necessary to expose the whole to the action of hot spirit vapour in order to produce such a pellicle on the surface of the liquid albumen as will allow of the whole mass

being sunk in alcohol without displacement of the liquid portion of the albumen. The mass is ready for cutting 48 hours after preparation. The alcohol should, for ordinary purposes, only be changed once, as otherwise the material becomes too hard; if the alcohol is changed three or four times during the first 24 hours, the mass becomes sooner ready for cutting, and the longer the material remains in the alcohol after the first 48 hours the better does it cut. The sections are made in the ordinary way, with a razor wetted with spirit, and are mounted directly in Canada balsam or damar. The temperature of 70°-75° C, to which tissues are exposed by this method of embedding, causes, according to Dr. Calberla, no marked change in them.

The great objection to this method of embedding, putting aside the amount of trouble involved in it, and the danger of the alcohol vapour taking fire, is the necessity of preparing the fluid material from eggs every time an object has to be embedded, for under ordinary circumstances it rapidly dries up and decomposes. It has been suggested to me that salicylic acid might advantageously be added with a view of keeping the material free from germs, and in a cool place in a stoppered bottle it will keep even without antiseptics for a fortnight. The service which this material is capable of rendering is of the very greatest importance. It will be especially valuable for the cutting of sections of minute and friable embryos, giving absolute certainty and corresponding comfort to the student in the preparation of his sections, in place of the anxiety and annoyance due to friable embryos and oily embedding media. Dr. Calberla has earned the gratitude of all embryologists.—D'Arcy Power.

A Central Agency for the Supply of Microscopic Organisms to Students and Class Teachers.—I have very great pleasure in calling the attention of the readers of this Journal to the living organisms offered for sale and sent out through the post by Mr. Thomas Bolton, Hyde House, Stourbridge. Mr. Bolton has supplied me with several dozen specimens of *Hydra viridis* for the purpose of class-demonstration, and he expects to be able to supply these polyps at all times of the year. During the month of February he sent me, safely through the post, and at the most reasonable charge, a bottle containing a dozen specimens of *Actinosphærium Eichornii*, an object which the student, whether young or old, can hardly examine too carefully or too often. Young specimens of Spongilla, a variety of rare and interesting Rotifera, Infusoria, &c., are also offered from time to time by Mr. Bolton as they appear in the ponds

known to him or are received by him from correspondents. If serious students of our pond-fauna and flora will avail themselves of Mr. Bolton's services, and not only purchase from him examples of the specimens he has on hand, but will also *send to him supplies of such rarities as they may find, for the purpose of distribution among his correspondents and customers*, we shall have started among us an agency which will be of immense service not only to the individual student but also (and perhaps chiefly) to the teacher who requires to be able to obtain supplies of given microscopic organisms for his practical classes and to feel with absolute certainty that the specimens needed will be forthcoming on the appointed day. Mr. Bolton can, at present, be depended on for certain forms; after a little time he will be able, no doubt, to enlarge his list. E. RAY LANKESTER.

Zeiss' Objectives.—We wish to draw attention to some objectives made by Carl Zeiss of Jena. We have carefully examined his D, E, and F, and find that they are remarkably fine glasses for his tological work.

D, $\frac{1}{6}$th, equal to about Hartnack's No. 7 (2 guineas), and E, $\frac{1}{9}$th, equal to about Hartnack's No. 8 (3 guineas), are both glasses of great excellence as regards light, power of definition and penetration. The D and E that we have tested stand above the best glasses that we have seen hitherto.

F, $\frac{1}{14}$th (4 guineas), is a dry glass, and is in magnifying power somewhere between Hartnack's No. 9 (new glass with correction), and his immersion No. 10. The F's that we have examined are unquestionably superior to Hartnack's No. 9.

All these three glasses (D, E, F) have a flat field, so that the peripheral objects appear as sharp as those in the centre of the field.

Immersion J ($\frac{1}{13}$th, £7 4s.), Im. K ($\frac{1}{20}$th, £10), and Im. L ($\frac{1}{25}$th, £13 10s.), which I have examined, appear to me as good as any corresponding immersion lens of English or continental manufacture. E. KLEIN.

Celebration in honour of Theodor Schwann, the founder of the Cell-theory.—Professor Schwann, of Liège, has now reached the fortieth year of his professorship. His past and present pupils are about to celebrate this event by presenting him with a marble portrait-bust of himself and with addresses of congratulation. Professor Edouard van Beneden, of Liege, or the editors of this Journal will be glad to receive addresses from English scientific bodies or from individuals for presentation on the occasion.

PROCEEDINGS OF SOCIETIES.

Dublin Microscopical Club.

November 15th, 1877.

Structure of Spine of Amblypneustes ovum.—Mr. Mackintosh exhibited a cross section of the spine of *Amblypneustes ovum*, Lamk. Notwithstanding the small size of the spines—the section measured only $\frac{1}{50}''$ in diameter—its structure is very substantial. The bars of the central reticulations are remarkably thick; the solid wedges which stretch out from it are very broad in proportion to their length, and are united by numerous strong trabeculæ. The structure bears a remote resemblance to that of *Echinus esculentus*, with a section of which it was contrasted.

Sertularella polyzonias, found at Ballybrack, Co. Dublin, was exhibited by Mr. D. Grant. This species occurs but sparingly on the Dublin coast, though common elsewhere.

Geoglossum glabrum and Comatricha Friesiana, exhibited.—Mr. Pim showed transverse sections of *Geoglossum glabrum*, Persoon; he had found this fungus growing in considerable abundance along the roadside in the upper part of Glencullen, also near Kilternan, in November last. The peculiar paraphyses and long multiseptate spores were well marked.—*Comatricha Friesiana* was also shown by Dr. W. M. A. Wright.

Oikopleura rufescens from Bessell's Bay, exhibited.—Dr. Moss, R.N., late of H.M.S. 'Alert,' exhibited specimens of *Oikopleura rufescens* (Fol), captured by him in Bessell's Bay, an inlet of Hall's Sea, north latitude 81° 7′. He remarked that a single individual of the rarer genus Frillaria was obtained in Smith's Sound, and that the discovery of these creatures in the Arctic regions afforded a striking illustration of the wide distribution of oceanic Tunicata.

New Species of Difflugia.—Mr. Archer exhibited an example of the test—he has not yet seen the living state—of a new Difflugia, on account of its vinous colour, as if stained with claret or port wine, to be called *Difflugia vinosa*. This is a large form, broadly truncato-ovate in contour, its outline and size not unlike those of *Euglypha spinosa*, Carter, though, of course, it would be only meaningless to suggest any further or closer comparison between these two Thalmaphores. The test seemed to be

composed of thin, shapeless laminæ, yet superposed so as to produce a tolerably smoothly outlined margin. The test-opening very large, presenting a broadly-arcuate curve, and forming a rather broad, smoothly margined, band-like border, of a pale yellowish colour, or sometimes nearly colourless, thus of a different texture and appearance from the rest of the test. It occurred to Mr. Archer that probably the actual shape of the test was spoon-shaped, that is to say, convex on one side and concave on the other. But as he had seen only empty tests this might be due to a collapse of one of the front, or broad, surfaces. For the same reason it may just possibly be premature to refer this form to Difflugia at all; but still there could hardly be a doubt but that when specimens with the soft sarcode body may turn up, they will show the characteristics of that genus.

Griffithsia setacea.—Dr. E. Perceval Wright, in continuation of his remarks upon the structure of this Alga, showed a series of preparations exhibiting the formation of its filaments, which were generally described as monosiphonious and articulate. In its young condition, however, the filament did not break up into articulations, and even when it did there was a union through means of a linear series of pores kept up, and only in very advanced joints indeed were these pore-openings closed by 'stoppers.' Carl Nägeli long ago pointed out the existence of these pores in Polysiphonia, but Dr. Wright in addition showed in these specimens the origin of the pore-system, its development and its obliteration, and suggested that it formed a connective tissue, which was found not only in Griffithsia and Polysiphonia, but would be found in other so-called Siphonaceous Algæ.

Monad-form, probably undescribed, exhibited.—Mr. Archer drew attention to a Monad-form, which, occurring as it does habitually in pairs, or rather twins, did not seem to be noted. This character would seemingly distinguish it from *Glenomorum tingens*, which the monads themselves somewhat resembled. The truncato-cylindrical pairs of monads hung together, joined by their posterior end, biciliated at the other extremities; they flitted up and down in a fidgetty manner, now one way, now the other. The eye-speck, so to call it, was here much diffused, that is, the red- or garnet-coloured pigment-granules were scattered, but at same time clustered more at the distal or apical extremities, so as almost to appear as but a single red spot under a low power. This is rare, a denizen of the bottoms of the deep old bog-pools in the County Westmeath.

December 20th, 1877.

Further remarks on Spine of Amblypneustes ovum.—Mr. Mackintosh exhibited further sections of the spines of *Amblypnestes ovum*, Lamk., which showed that, though this species has, for the most part, spines with a central axis of reticular tissue, still they are sometimes hollow and surrounded by a ring of calcareous

material resembling the structures to which he had given the name of 'foraminated ring' ('Trans. R.I.A.,' vol. xxv, Pl. 16), and which is characteristic of the spines of the Diademitidæ to which those of Amblypnestes thus come to bear a strong resemblance.

Schistostega Osmundacea, exhibited.—Dr. Moore showed an example of the pretty "Cavern Moss" *Schistostega Osmundacea*, which he had lately brought from Todmorden. It is well known that this plant has the property of partly illuminating the gloomy recesses where it loves to dwell. It forms a pretty and a botanically interesting object.

Diatoms from Arctic Seas, exhibited.—Rev. E. O'Meara presented for inspection a slide containing *Diatomaceous* forms from a gathering made in the Arctic regions by Dr. Moss of H.M.S. 'Alert.' It was collected from a heap of stuff thrown by the tide upon the beach. Great interest is attached to this collection from the fact of its having been made so far north as lat. 82° 23′. Many species common in our own country were met with, *e.g. Navicula Donkinii, N. cyprinus, N. Cleveana, Surirella striatula, Paralia marina, Actinoptychus senarius*, &c. Several forms occurred which hitherto have been met with only in the Arctic regions, *e.g. Thallassiosira Nordenskioldii* Cleve, *Achnanthes Arctica*, Cleve, *Grammatophora Arctica*, Cleve, *Amphora lanceolata*, Cleve, *Synedra Kamskatica*, O'M.

Arctic Dust, which contained a Nostochaceous Organism.—Dr. Moss showed some Arctic Dust containing some organic traces, but the only thing abundant was a Nostoc-like organism, generally of a brownish-red colour, yet few, if any, of the examples showed the cells in chains or chaplets, nor any heterocysts; they were rather clustered without any order; Mr. Archer felt, therefore, rather disposed to refer the form to Glœocapsa.

New Species of Closterium, shown.—Mr. Archer showed examples of what would seem to be a new species of Closterium, coming nearest to *Cl. costatum*, in that it was of a similar contour and curvature, but it was larger, and, whilst the striæ were very like near the similar-looking apices, they speedily got lost, and for from four fifths to three fourths of the extent of the cell they were absent. Unlike other Closteria, then, which are all either equally striate throughout or smooth, we have here to do with a form only partially striate. Mr. Archer would call this form *Closterium mediolære;* he exhibited examples of *Closterium costatum* side by side for sake of comparison.

MEMOIRS.

The Embryology of Clepsine.[1] By Charles Otis Whitman, of Boston.

Contents.

History.

No less than five memoirs have already been written on the embryology of *Clepsine complanata*, Sav. The earliest of these

[1] 'Inaugural Dissertation to obtain the Degree of Doctor of Philosophy submitted to the University of Leipzig.'

—that of F. de Fillippi (No. 36, 1839)—contains but little information in regard to the earlier stages. In this direction the work of Ed. Grube (No. 59, 1844) is a marked improvement. The two most important and most extensive memoirs are those of Heinrich Rathke, revised and published by Professor Leuckart (No. 136,1862), and Charles Robin (No. 143,1875). The most recent paper is that of C. K. Hoffmann (No. 77,1877). Each of these works will be duly noticed in the course of this paper.[1]

The method of making sections for microscopic study—not in vogue at the time of Rathke's investigations—was entirely neglected by Robin, and too exclusively relied upon by Hoffman. This will account for the fact that neither of these authors was able to understand the germ-lamellæ.

Section-cutting has become an indispensable aid in embryological researches, an aid which no embryologist can neglect with impunity, but it is by no means a substitute for former methods of investigation. Section-observation and surface-observation go hand in hand.

My studies, which have been carried on in the laboratory of Professor Leuckart, began with *Clepsine marginata* in the spring of 1876, and in 1877 were renewed and extended to three other species, viz., *C. complanata* (sexoculata), *C. bioculata*, and *C. heteroclita*. I have made *C. marginata* the principal object of study, as the eggs of this species offer special advantages for cutting.

For whatever success has attended these investigations I am deeply indebted to my highly esteemed teacher, Professor Leuckart, whose invaluable aid, experienced counsel, and cordial encouragement I shall always hold in the most grateful remembrance. A complete list of the works referred to in the text, by means of numbers placed above a line, below which the page is given, will be found at the end of this paper.

Methods.

1. *For fresh examination.* I have used a simple microscope, with a magnifying power of 30 diameters. A little higher power is required for observing the formation of the polar globules.

2. *For sections.* *a. Hardened* in osmic acid ($\frac{1}{10}$ per cent. 15—30 minutes; weak alcohol 2—3 hours; strong alcohol 2—3 hours; absolute alcohol 12—24 hours.

b. Stained in toto with Beale's carmine. This method has given the best preparations for the karyolytic figures of Auerbach. For later stages I have sometimes used osmic acid

[1] The Arabic numerals after the word "No." and the numerators in the fractions throughout this paper refer to the list of authors at the end. The denominators indicate the page.

and sometimes chromic acid ($\frac{1}{3}$ per cent.), followed in each case by weak, strong, and absolute alcohol.

c. Imbedding. In clove oil till thoroughly permeated. Imbedded in paraffin to which a little pig's lard has been added.

d. Cutting. Leyser's microtome.

e. Mounting. Sections freed from paraffin by means of benzine. Mounted in balsam.

3. *Surface views of the germ-bands.* Eggs treated with chromic acid 5—10 hours show well the linear arrangement of the nerve-cells. For views of the inner surface it is necessary to free the germ-bands from the yolk. This is done in the following manner :—The fresh embryo is placed in a drop of water upon an object-slide ; a little acetic acid (as much as will adhere to a needle) is added, and all placed under the dissecting lens. With a pair of needles a rupture is made along the dorsal side. By careful manipulation of the needles the embryo, in most cases, can be led away from the yolk and stretched out on the slide.

After partially removing the water by means of a bit of blotting paper, a few drops of osmic acid are added, with care not to disturb the object. At the end of an hour the embryo is washed and stained with Beale's carmine. It is again washed and treated with weak, strong, and absolute alcohol. Mounted in balsam or glycerine. During all this the object is not once removed from the slide.

I. ORIGIN AND GROWTH OF THE EGG.

a. Formation of Primitive Egg-cell.

According to Leydig ($\frac{1}{1}\frac{0}{2}\frac{9}{7}\frac{-}{8}$), who was the first to give attention to this matter, the egg-string is a nucleated protoplasmic mass. Around these free nuclei no cell-limits are visible. The formation of the egg-cell is compared to the cleavage process. "Die Bildung der Eier in ihm (egg-string) findet statt nach Art der Furchungskugeln, d. h. man sieht freie bläschenförmige Kerne, dann um diese einzelne Elementarkörperchen unregelmässig gelagert ; mit Zunahme derselben bilden die Häufchen der Elementarkörner mit dem eingeschlossenen Kern eine länglich-kugelige Form, es tritt eine membran auf."

Ludwig ($\frac{1}{6}\frac{1}{0}\frac{1}{2}$) accepts this view and extends the same to other Hirudinea. Leuckart ($\frac{1}{6}\frac{0}{7}\frac{6}{8}$), on the other hand, speaks of the egg as arising from a ready formed cell. Robin ($^{1}\frac{4}{5}^{2}$) has maintained a singular theory of the egg-formation in the Hirudinea. According to this theory the egg forms, after copulation, within a spermatophore. The spermatophore with its inclosed ovules is called an "*ovo-spermatophore.*" Although Leukart ($\frac{1}{6}\frac{0}{8}\frac{6}{0}$) pointed out Robin's error as long ago as 1863, the latter still holds fast to the same theory in his last great

work ($^{1}\frac{4}{5}^{3}$). In the case of Clepsine, Robin ($\frac{1}{1}\frac{4}{3}$) admits that no proper ovo-spermatophores exist, and claims only to have found something analogous. As the ovo-spermatophore-theory was corrected by Leuckart, refuted by O. Hertwig ($\frac{7}{0}\frac{1}{1}$) in the case of Nephelis, and practically abandoned by Robin himself in Clepsine, it will be unnecessary to devote further attention to it.

There are then two views with reference to the formation of the egg:—

1. (Leydig).—It arises from a *free nucleus*, which, with other nuclei, lies imbedded in a common protoplasmic mass.

2. (Leuckart).—It arises from a *ready formed cell.* As neither of these investigators made use of sections in the study of the egg-string, it is evident that their statements have reference to the peripheral part alone.

Reproductive Organs.—The external orifices of both kinds of sexual organs are found in the median ventral line of the seventh body-segment, the male in the first and the female in the last (3rd) annulus of this segment. The ovaries are two elongated, tubular sacs, lying on each side of the nerve-chain, between this and the testicular sacs, and extending backwards from the vaginal aperture through two or three body-segments. They show no differentiation into ovarium, oviduct, and uterus, but are of nearly uniform size, form and structure, from the vaginal to the cæcal ends. Just behind their common orifice they stand in open communication with each other, so that the contents of the one may be, and often are, partially at least, driven into the other.

The Egg-string.—In each sac there is a single, much-twisted egg-string. No connection between this string and the wall of the sac exists in the mature worm, although such a connection *may* have existed at an early stage in the development of the ovaries. I have succeeded in isolating this string several times without breaking it, and found that the thin membrane covering it is closed at both ends. The strings lie bathed in a nourishing (?) fluid, in which float some cells, which, as Leydig remarks, have probably arisen from the epithelium of the sacs. One of these strings, measured at a time when the largest eggs were only about ·1 mm. in diam., had a diameter of ·3—·4 mm. at the hind end. From this point it tapered gradually to the fore end, where the diameter was ·1 mm. The larger eggs were found near the hind end and the smaller at the fore hind, while between these points were all intermediate sizes. The string is composed of two well-defined parts, a *central* and a *peripheral.*

1. *The Rhachis.*—The central part (Pl. XIII, fig. 57), which I will henceforth designate as *rhachis*, attains its maximum diameter by the time the eggs measure between ·3 and ·4 mm.

(diam.). Examined in a fresh condition and by reflected light, it can hardly be distinguished from the peripheral part; but, by transmitted light, it appears as a milky white, indistinctly outlined, central stripe. Along the centre of this stripe is seen, by reflected light, a white, opaque, irregularly outlined substance, which becomes less and less compact from behind forwards. This axial substance, which appears like a white thread under a low objective, consists of *yolk-granules* ("Elementarkörner," Leydig). Before the eggs begin to grow, these granules are comparatively few, and scattered uniformly through all parts of the egg-string; but with the growth of the young eggs they multiply much more rapidly in the axis of the rhachis than elsewhere, and soon render the larger part of the rhachis opaque. The ground substance of the rhachis is a fine granular protoplasm, which contains besides the yolk-granules (Deutoplasm, van Beneden) some free nuclei, around which I have never been able to discover the least trace of cell-limits. Under slight pressure the contents of the rhachis will flow out at any point where the egg-string is broken, showing that it is much less coherent than the—

2. *Peripheral Part.*—The peripheral part treated with osmic acid and carmine appears to be composed of deeply coloured crowded oval nuclei (·01 mm. diam.)., each of which incloses a more deeply coloured nucleolus (·0025 mm diam.). Besides the nuclei are seen small groups of deutoplasmic granules (fig. 58), where in earlier stages only single granules were seen. These groups diminish in frequency and in size as we approach the anterior end where they are reduced to single granules. We thus have a picture of their different stages of development on one and the same preparation. The nuclei are imbedded in what seems to be a common ground-substance, no cell-outlines being recognisable. The same is true of sections hardened in alcohol. All this would seem to confirm the statements of Leydig and Ludwig. That these nuclei are not *free*, but the centres of well-defined cells, is proved beyond a doubt by sections of an egg-string treated first with osmic acid ($\frac{1}{10}$ per cent. 10—20 minutes), and then with alcohol, and stained with Beale's carmine. Fig. 57 represents one of these sections drawn with the camera lucida. The cells were on every section remarkably well defined. It can be said, therefore, with certainty, that the peripheral part of the egg-string is composed of ready-formed cells: this accords fully with the statements of Leuckart. Whence come these cells? Do they exist first as free nuclei, suspended in the protoplasm of the rhachis, and, after assuming the cell-form, pass into the peripheral part?

Unfortunately my investigations do not furnish sufficient data for

deciding this question. I am unable, however, to explain the presence of free nuclei in the rhachis, on any other hypothesis than that here is the real place of cell-formation. In accordance with this hypothesis, the egg-cell arises from a nucleated protoplasm, as is the case in most Worms, especially the unsegmented, and in many Arthropods (Crustacea). The structure of the central part of the egg-string bears a striking resemblance to the rhachis of the Nematoids, and for this reason I have given it that name. We have seen (1st) that the rhachis, at the time the young eggs begin to appear, contains yolk-granules tolerably equally distributed; (2nd) that the median part of the rhachis, a little later, becomes charged with these granules; (3rd) that, while the axis distinguishes itself as the place of most energetic formation of such granules, this function is by no means localised here; (4th) that in the rhachis free nuclei are suspended, around which (hypothetically) the protoplasm differentiates into the cell-form, thus giving rise to the peripheral cellular part of the string. According to the researches of Claparède (31), Leuckart (107), and van Beneden (13), all these relations are repeated in the Nematoids. All this, together with the important fact that in Nematoids the entire egg is produced by a single organ instead of two as in the Trematoda, points to a nearer relationship between the Nematoidea and the Hirudinea than exists between the latter and the Trematoda. Opposed to this stands the less important fact that hermaphrodism is the rule among Trematodes and the exception among Nematoids.

b. Growth of the Primitive Egg-cell to the Mature Egg.

The peripheral part of the egg-string, as before stated, is a compact cellular layer four to five cells deep (fig. 57).

These cells are the *primary egg-cells*, of which only a comparatively few at any one time develop into eggs. The first step in this development is signalised by the accumulation of yolk-granules around the nucleus (fig. 58, *a*). At this time the nucleus and nucleolus of the young egg do not differ, to any appreciable extent, in size and general appearance from those of the surrounding cells. In little later stages (*b*, *c*, *d*) the granules have increased, and lie, for the most part, in the periphery of the egg. The germinal vesicle and germinal dot have increased in size, but not in the same proportion as the body of the egg, as appears from the following measurements of *a* and *d* in fig. 58:—

a. Egg = ·02 mm. (longest diam.); germ. ves. = ·01 mm.; germ. dot. = ·0025 mm.

d. Egg = ·10 mm. (longest diam.); germ. ves. = ·03 mm.; germ. dot. = ·005 mm.

While the diameter of the egg has increased five-fold, that of

the germ. vesicle has trebled, and that of the germ. dot doubled. As the egg increases in bulk, becoming more and more opaque from the accumulation of granules, it begins to project from the egg-string, and soon, driving the membrane of the string before it, comes to occupy a position quite external to the neighbouring cells (fig. 59). At this stage the egg measures about ·40 mm., and the vesicle, which can no longer be seen, but the presence of which is indicated by a light spot (by transmitted light), measures ·05 mm. At this time I have generally found two *germ. spots.*

In one case three were found, measuring respectively ·005 mm., ·0037 mm., and ·0025 mm. The membrane of the egg-string, which is pushed before the egg in its outward growth, forms a constriction (fig. 59), which becomes progressively smaller, till the egg is merely pendent from the string. By the time the egg has attained a diam. of ·55 mm. to ·60 mm. (germ. ves. = ·06 mm., germ. dot = ·008 mm.) it bursts the membrane and falls into the ovary. This event generally takes place at the end of eight to twelve days from the time the growth of the primary egg-cell began. The time varies much according to the temperature. In three to four days more the egg attains its full size.

The full-grown egg varies much in size, not only in different species, but also in different individuals of the same species.

The average for *C. marginata* is :

Egg = ·80 mm. × ·75 mm.; germ. ves. = ·08 to ·09 mm.; germ. dot = ·012 mm.

For *C. complanata* about the same.

The egg of *C. bioculata* and *C. heteroclita* measures about ·55 × ·50 mm.

The germinal vesicle of the mature egg lies excentrically, sometimes near the surface, and possesses a distinct membrane. Its transparent fluid contents, after treatment with osmic acid and alcohol, appear to be very finely granular (fig. 60, *b*), and slightly blackened by the acid. The germinal dot is composed of several highly refractive pieces.

Formation of the Yolk-spheres.—According to Leydig ($\frac{1}{1}\frac{0}{2}\frac{2}{8}$), the large yolk-spheres result from the coalescence of the minute yolk-granules. I have never been able to discover any indications of such a consolidation. The fact that the refractive power of these spheres diminishes as the size increase is the reverse of what we should expect as a result of simple consolidation.

At the time the egg falls from the egg-string the largest spheres measure no more than ·0075 mm. In the full-grown egg all sizes are found from ·02 mm. down to ·0005 mm.—the size of the yolk-granules. The spheres have become very numerous, forming the larger part of the egg-contents. They

are in general round, homogeneous, without the slightest trace of nuclear formations. The transparency of these spheres varies directly as their size. The minute granules are quite opaque. From all this I conclude that the spheres arise from the granules by a simple process of growth. This agrees in a striking manner with the results reached by Gegenbaur in his investigations of the vertebrate egg ($\frac{5}{5}\frac{5}{0}\frac{5}{5}$). The chief difference is, that in the vertebrates (birds, reptiles) the yolk develops farther than in Clepsine, differentiating into white and yellow yolk.

Summary.—1. The egg-string consists of a central, nucleated protoplasm (rhachis), and a peripheral cellular layer.

2. The primary egg-cell, the development of which into the mature egg requires about two weeks, arises (probably) from the rhachis.

3. The precipitation of yolk-granules takes place with the greatest energy in the axis of the rhachis, but is at no time localised here.

4. The yolk-spheres arise by a simple growth of the yolk-granules.

II. Copulation, Deposit of Eggs, &c.

Copulation.—As is well known, Clepsine, like all the Hirudinea, is hermaphrodite. Copulation has never been observed. Whether the worm can fructify itself, or whether a union of two individuals is necessary to this end; whether in the latter case the fructification is reciprocal; whether the impregnation takes place within or without the body; all these are questions with respect to which there has been the greatest diversity of opinion.

I have kept large numbers of Clepsine in small glass-aquaria during two summers and a few over winter. When properly cared for, they thrive and produce eggs in abundance. I have generally examined the glasses four or five times a day, besides keeping a few in a glass before me while at work. I have often seen several individuals lying side by side, or across one another for hours at a time; but I have never seen them in a position that would admit of sexual union.

Filippi ($\frac{3}{2}\frac{6}{0}$) and some other naturalists mentioned by Moquin Tandon ($\frac{1}{1}\frac{1}{0}\frac{9}{6}$), regarded it as highly probable that Clepsine was capable of self-fructification, this act being performed at the time the eggs were laid ("emettendo ad un tempo le uova ed il liquor seminale.")

Grube (59) inferred from the fact that he never found spermatozoa on fresh laid eggs that impregnation took place within the body. Rathke makes no mention of the matter. Leuckart ($\frac{1}{0}\frac{9}{7}\frac{6}{3}$) thinks it probable that copulation and reciprocal fecundation take place. According to Robin ($\frac{1}{1}\frac{1}{1}^{3}$) the eggs first come

in contact with the spermatozoa after falling from the egg-string.

I have found that eggs taken from the ovary at the time they are about to be laid develop in the normal manner, and have taken advantage of this to watch the earliest changes in the ripe egg. I have done this many times, and always with success. I regard this as very strong evidence that impregnation takes place while the eggs are in the ovary. This is in harmony with the fact that I have found spermatozoa in the ovary two or three days before the time for depositing the eggs. It is barely possible that these spermatozoa found their way into the ovary accidentally during the dissecting. I can only say that no testicular sacs were ruptured during the process; but the vasa deferentia may have been severed, as they are so minute that one cannot easily see them. The unchanged condition of the germinal vesicle at the time the eggs have attained their full size renders it probable that fecundation does not take place more than four or five days at the longest before the deposit; but this does not prove that copulation may not have taken place at a much earlier date. I isolated a worm which had just sucked itself full of blood, and which showed no signs of eggs through the body-wall, and after fifteen days obtained eggs that developed in the usual manner. Recalling the fact that the growth of the egg from the primary egg-cell requires only twelve to fifteen days, it appears that this specimen was isolated about or just before the time when the egg-cell began to grow. In another case eggs were obtained at the end of twelve days which developed in the normal way.

These facts have only a negative bearing, but they raise a suspicion that Clepsine is capable of self-fecundation. The question as to whether a copulation occurs will be most satisfactorily settled by isolating young individuals and keeping them till they produce eggs.[1]

[1] *May 2nd*, 1878.—Five individuals were isolated in the summer of 1877, at the time of hatching. Each has been kept in a separate vessel from that time to the present. Eggs were laid by one April 24th (this year), and hatched May 1st; by two others, April 29th. The latter are now in the germ-band stage. The water in the vessels was changed in November, March 1, and April 1. The water was taken from a small pond in which these worms are not numerous, and at a time before eggs begin to be laid by either species. The eggs had in each case passed the pronuclear stage, at the time they were first noticed, so that I was unable to demonstrate by section the existence of a male pronucleus. As the eggs developed in the normal manner, it is very probable that they were fecundated. Here is an unquestionable case of self-fructification, or of parthenogenesis—more probably the former. V. Baer ('Müll. Arch,' 1835, p. 224) saw strong indications of self-fructification among "hermaphrodite snails."

Food.—Clepsine marginata is a fish-parasite. It is commonly stated in text-books that Clepsine is a sponger upon snails, which may be true of some other species. They require no food during the late autumn and winter months. If fed at the end of March they will produce eggs without further feeding, *i.e.* one set of eggs. When hungry they attach themselves to some object by the posterior sucker, and swing at full length in all directions from the point of attachment awaiting the approach of a fish.

Time of Deposit.—Clepsine sexoculata begins to lay eggs about the first of April; sometimes if the season be colder than usual, not before the middle of this month. The period of depositing is much shorter than with other species, not extending much over four weeks, as Hoffmann has correctly stated. Only one set of eggs is produced yearly. *Clepsine marginata* begins to produce eggs by the first of May. I have found eggs as late as the middle of August. As the time required for the development of the primary egg-cell into the young worm, which can dispense with the protection of its parent, is in this species about six weeks, it is possible that two or three sets of eggs are produced annually. The season of egg-laying extends in *C. bioculata* from the first of April till September. As this species is often ready to lay eggs as soon as the young are ready to abandon the parent, we have here the possibility of a new generation every month—five yearly. I have not been able to ascertain precisely the period during which *C. heteroclita* produces eggs, but I think it is nearly the same as with *C. marginata*.

Habitat.—I have obtained the greater part of my material from a small brook in the vicinity of Leipsic, in places where the water was about half a yard deep, and the current imperceptible.

I have found the eggs of *C. sexoculata* on stones, bricks, reeds, and fallen branches. The other species deposit their eggs on various reeds, preferably where the bed of the brook is very soft.

Act of Laying.—This process, so far as the behaviour of the worm is concerned, has been correctly described by Grube ($\frac{5}{9}$), for those species which lay their eggs in sacs. Leuckart ($\frac{10}{8}\frac{6}{9}$) has given an excellent description of the same in the case of Hirudo. Grube entertained an erroneous opinion in regard to the source of the material of which the sacs are formed. He supposed that it had its origin in the ovary itself, from which it was expelled just before the eggs. According to Leuckart (loc. cit., p. 685), the sac is a product of the skin glands (Hautdrüsen.) The behaviour of *C. marginata* during the extrusion of the eggs differs from that of the other species known to me,

and has not hitherto been described. The process can be easily watched if the worm be placed in a small glass containing water, but no plants. The worm is thus compelled to lay its eggs on the glass. The animal takes its ordinary position, attached to the glass by its two suckers, and carries on the usual undulations of the body. While these movements are quietly continued, without any of those violent contractions and twistings seen in *C. sexoculata*, the eggs are driven forward in the ovary by contractions of its walls, aided perhaps by a slight contraction of the hind body. Suddenly the undulations cease; the vaginal orifice is directed backwards in consequence of a slight elevation of the segments (somites) that follow the genital segment. The wall of the aperture protrudes; a single egg appears and is pushed backwards by the protruding orifice as far towards the terminal sucker as possible without moving the head. The eggs follow in tolerably rapid succession, each being placed as far back as possible. After fifteen to thirty eggs are laid, a pause of one or two minutes occurs, during which the undulations are again continued; and then the eggs are again extruded as before, each time the eggs previously laid being pushed further back by those last deposited. If there are more eggs than can conveniently lie under the expanded body in a single layer, they are placed in a double, and not seldom in a triple, layer. At the end of the act which may last from ten to forty minutes, according to the number of eggs, the eggs lie in quite regular rows, and are held in place by the edges of the body, which are pressed against the object on which the eggs rest. That the eggs come to lie in rows is not due to any skill of the worm in placing them, but to the fact that they are confined between the two nearly parallel edges of the body. The two outer rows are generally shorter than the central ones, as the body tapers somewhat towards either end.

The eggs are not here enclosed in a sac, but are covered with a transparent fluid substance (probably a secretion of the "Hautdrüsen") which hardens in the course of a few minutes, and thus binds the eggs together and to the object on which they are placed. The worm remains over the eggs for the purpose of protection only, till they hatch. The young, soon after exclusion, become fixed to the ventral side of the parent, and are thus borne about till they are fully developed and able to provide for themselves.

Number and Colour of the Eggs.—There is great variety in the number, colour, and arrangement of the eggs, as will be seen from the following:—

C. marginata.	*No. of eggs.*	*No. of rows.*	*No. of layers.*	*Colour.*
1	19	3	1	white.
2	28	3	1	green.
3	50	3	1	yellow.
4	50	4	1	white.
5	50	5	1	yellow.
6	70	5	1	green.
7	103	6	1	yellow.
8	110	6	2	white.
9	200	7	3	yellow.

C. sexoculata.	*No. of sacs.*	*No. in each sac.*	
1	3	15 to 20	flesh-colour.
2	4	40 „ 50	ditto and white.
3	4	20 „ 25	flesh-colour.
4	5	28, 38, 43, 40, 45 = 194	„
5	8	14, 11, 20, 17, / 18, 23, 20, 16, = 139	„

In No. 5 the numbers representing the number of eggs in each sac are arranged as were the corresponding sacs under the worm.

C. bioculata.	*No. of sacs.*	*No. of eggs.*	*Colour.*
1	1	21	greyish-white.
2	1	25	„
C. heteroclita.			
1	1	29	brownish-white.
2	1	30	„

III. Changes Preliminary to Cleavage.

The history of the changes which transpire between the stage of maturity and that of cleavage—"the prelude to the cleavage-drama," as Auerbach terms it—forms one of the most interesting chapters in the biology of the egg.

So far as yet known, these changes in the egg of Clepsine are unsurpassed in variety by those of any other egg.

Some of them take place on the surface and can be easily followed on the living egg, by the aid of a simple lens; while others are accomplished in the interior, and, owing to the opacity of the yolk, can only be traced by means of sections. In order to connect these two series of phenomena, in such a manner that the events of one series may be placed in chronological relation with those of the other, it is necessary, first of all, to know the sequence of the surface changes. This known, it becomes possible to describe intelligibly the eggs that are to be hardened for sections.

With a view to making sections I have adopted the following course:—The time is divided into three periods, the first extending from the time of deposit to the appearance of the first polar globule; the second, from the first polar globule to the first polar ring; and the third, from the first polar ring to the cleavage. Using the time of deposit, of the first polar globule, and of the first polar ring as three points of departure, eggs

were placed in osmic acid at intervals of fifteen minutes. The selection of three well-marked events as starting-points serves to eliminate, to a certain extent, the error in time which would otherwise be sure to occur, as a consequence of the varying rapidity of the changes under different temperatures.

Before passing to the history of these three periods, I will call attention to the composition of the mature egg, and to an important change in the germinal vesicle.

(*a*) **Composition of the Egg.**—The ripe egg consists of three parts, viz. membrane, yolk, and germinal vesicle. The yolk is composed of two distinct parts—(1) protoplasm and (2) deutoplasm. The transparent protoplasm is the ground-substance of the egg, in which the deutoplasmic elements are imbedded. The deutoplasm is the yolk-granules and yolk-spheres before mentioned. These nutritive elements, the smallest of which exhibit a most lively Brownian movement when brought in contact with water, are perfectly passive with respect to all the movements which characterise the egg in this and the following periods of its evolution. They are simply surplus food material, the most of which serves the wants of a late period in the development. A detailed and accurate description of these elements has been given by Rathke ($\frac{1}{7}\frac{3}{6}\frac{6}{8}$).

The Germinal Vesicle.—The germinal vesicle lies excentrically. Treated with osmic acid and carmine, it assumes a lead-grey shade, slightly stained with the carmine. The germinal dot ("macula germinativa") is sometimes wanting (fig. 60, *c*), sometimes present as a mere heap of fragments (fig. 60, *b*). The contents of the germinal vesicle in fig. 60, *c*, seem to have retreated from one side, leaving vacuole-like spaces, separated from one another by very attenuated walls of the very fine granular substance ("nucleo-plasm," van Beneden.) I am uncertain whether this condition is an artificial production, or an incipient stage in the formation of the *reticulum*, said to be characteristic of the ripe ovum. Though doubtful in this particular instance, I am convinced that nuclei do pass through the reticular condition.[1] I have often met with the same in certain stages of the cleavage (fig. 61). Hoffman ($\frac{7}{3}\frac{7}{3}$) has noted this stage in the ovarian egg of Clepsine. The investigations of Heitzmann (69, various tissues), Bütschli ($\frac{2}{6}\frac{7}{7}$) Nephelis,) Frommann (49, blood-cells), Schwalbe (149, ganglionic cells), Flemming (38, Unio and Anodonta; 39, connective tissue, endothelium, muscle, nerve, cartilage, and epithelium), Flemming ($\frac{3}{7}\frac{9}{0}\frac{9}{9}$) and Giard ($\frac{5}{4}\frac{9}{3}\frac{2}{4}$, Echinus miliaris), van Beneden ($\frac{1}{1}\frac{6}{7}\frac{6}{0}$), Asteracanthion and rabbit), O. Hertwig ($\frac{7}{3}\frac{7}{5}\frac{0}{1}\frac{}{2}$) Toxo-

[1] This reticulum was, so far as I am aware, first described by Kleinenberg in his well-known work on 'Hydra' ($\frac{8}{4}\frac{1}{1}$).

pneustes and mouse), $\frac{7}{7}\frac{3}{7}$ Hertwig ($\frac{7}{7}\frac{3}{7}$, Echinus and frog), Strasburger ($1\frac{5}{9}^{4}$, Phaseolus multiflorus), and Fol ($\frac{4}{8}\frac{6}{8}$), Asterias), leave little room to doubt that this reticulum is characteristic of old nuclei in general. The nucleus represented in fig. 61 is a stage reached after a comparatively long period of rest in the cleavage-activity—a stage which precedes only by a short time, as we shall see hereafter, the process of division. This nucleus shows no trace of a membrane, and the anastomosing rays of the nucleo-plasm are continued directly into the protoplasm of the surrounding yolk. That this is a veritable condition of the living nucleus, and not a deceptive appearance produced by reagents, as Bischoff ($\frac{1}{3}\frac{9}{7}$) is inclined to believe, is evident from the fact that it is to be seen in living nuclei, according to the testimony of Hertwig ($\frac{7}{3}\frac{0}{5}\frac{0}{1}$), Kleinenberg, and others.

(*b*) **Archiamphiaster.**—The next stage in the history of the germinal vesicle, of which I have any accurate knowledge, is that of the bi-stellate figure (karyolytic figure, Auerbach; "Amphiaster de rebut," Fol) which I will designate as archiamphiaster, to distinguish it from the later amphiasters directly concerned in the cleavage. I have succeeded in obtaining only eight or ten sections which show both poles of this amphiaster —most sections cutting the figure obliquely. The axis of the archiamphiaster is generally inclined 20° to 45° to that radius of the egg which passes through its centre (fig. 62). In later stages it is much less, or not at all inclined (fig. 63). The most conspicuous parts of this figure are the two poles encircled with well-defined radial lines. These lines can be traced to a considerable distance beyond the polar areas out into the densely packed yolk-spheres. Their point of convergence is the centre of the polar areas. This central part of the area (strongly shaded in the figure) is deeply coloured with carmine, and contrasts strongly with the rest of the area which is much less deeply stained. Fig. 64 represents one of these polar areas with its radial lines more highly magnified. Between the two poles is a more or less spindle-shaped area free from the yolk-spheres. This area corresponds very nearly in size with the germinal vesicle of the previous stage. Within this space the radial lines of the two stars (=polar area plus radial lines) meet, thus becoming continuous from pole to pole. These inter-stellate lines ("Kernfasern," or "Spindlefasern," Strasburger and Bütschli; "filaments bipolaires" or "intranucléaires," Fol) do not present themselves here in so conspicuous a form as they seem to in the eggs of plants and many animals. They appear to differ in no essential way from the other radial lines. In only two preparations have I found anything in the equatorial zone of these lines at all comparable with the thickened portions

termed *Kernplatte* by Strasburger; and in these cases the appearance is of so doubtful a character that I have omitted it in my drawings. Some authors have laid great stress on the interstellate lines, especially Bütschli. In many of Strasburger's figures also the two poles with their radial lines are entirely wanting, while the spindle-fibres with their equatorial nuclear plate is very prominent. On the other hand, Fol, who was the first to describe these phenomena with any degree of accuracy, in his well-known paper on the development of Geryonia (476), lays particular stress on the star-shaped poles. He maintains (866 472 7–8) that the spindle-fibres are identical with the stellate rays and that their difference in appearance is due to the fact that they are in different media.

Bobretzky (218), who studied these phenomena in the egg of Nassa came to the same conclusion as Fol. My preparations seem to confirm this view. Whether this interpretation can be reconciled with the investigations of Butschli, O. Hertwig, and Strasburger, remains to be seen.

The entire amphiaster reminds one, as Fol (476) and Strasburger (154 200) have already observed, of the picture of iron-dust arranged about the poles of a magnet. This resemblance was at once remarked by Leuckart and others who have seen my preparations. The interstellate lines often appear curved, but no one has yet observed any curves in the radial lines not included within the spindle, which we should expect to see if this radial phenomenon were of a magnetic nature. It is not impossible that such curves do exist, and that they are so inconspicuous that they have been overlooked. Thus far these figures have been studied for the most part in microscopic preparations. If the stellate lines are curved in the living condition, this feature might be obscured or entirely obliterated by reagents. No satisfactory explanation of these radial appearances have yet been given. According to the karyolytic interpretation of Auerbach (220 3–222), they are produced by innumerable fine streams of nuclear fluid from the ends of the spindle. Butschli (27 192202), refers them to a reciprocal action between the fluid of the polar areas ("Centralhof") and the surrounding protoplasm—"optischer Ausdruck einer von dem Centralhof ausgehende physikalisch-chemischen Anderung des Plasmas."

Götte (58–95) maintains that a process of endosmosis begins as soon as the egg comes in contact with water (Unke), and that the radial arrangement is only the optical expression of the process of diffusion. These explanations, as will be seen later, do not account for some very important features of nucleus-action.

Flemming (38–207) refers them to a *structural* relation of

the protoplasm," and thinks they arise independently of the nucleus, although he does not deny that some sort of relation may exist between their appearance and the destruction of the nucleus.

Fol ($\frac{4\,2}{6\,6\,7}$), on the other hand, refers the origin of these two radial systems to two *centres of attraction* which arise in two opposite poles of the nucleus.

Strasburger ($\frac{1}{2}\frac{5}{5}^{4}$) also assumes two centres of attraction, which he erroneously, as Bütschli has shown, supposed to be the poles of the spindle. The reciprocal influence manifested by nuclei, as well as the magnetic-like pictures presented by the radial systems, favor the opinion that the poles of the amphiaster are centres of attraction.

Brandt ($\frac{3\,2}{3\,6\,8}\,\frac{2\,3}{5\,9\,2}$) maintains that these appearances are called forth by amœba-like pseudopodia of the nucleus itself.

Villot ($\frac{1\,5\,6}{1\,0}$) advances the same theory, and asserts that the nucleus ("Protoblast") receives its nourishment through these pseudopodia, which actually drag into it masses of yolk which are assimilated as in an amœba.

Schultz ($\frac{1\,4\,5}{4\,6\,6}$) attributes certain radial arrangements seen in the egg of Torpedo, to a *vital property* of the protoplasm.

These various opinions agree in this,—that there is a radial phenomenon of the nucleus to be explained; but no one of them, if we except, perhaps, that of Götte and Bütschli, get further than a statement of the problem to be solved.

The discovery of these phenomena does not appear to be of so modern a date as some authors have supposed who have attributed it to Kowalevsky. The first, so far as I have been able to ascertain, to mention such stellate figures, was Derbès ($\frac{3\,4}{9\,0}$) in 1847. He described each nucleus as a *centre d'une radiation une peu confuse.* Krohn ($\frac{9}{3\,1}\,{}^{1}_{4}$, Ascidia, 1852) described *Irradiations centren* around which the yolk was arranged in *radienförmigen Streifen;* Remak ($\frac{1\,4\,1}{1\,3\,4}$) (Rana esculenta 1855) found *radiale Striefen* in one of the upper cleavage-spheres; Gegenbaur (54 Sagitta, 1857); Leuckart ($\frac{1\,0\,7}{9\,0}$, Nematoidea, 1867—1876); Kowalevsky ($\frac{8\,3}{4}$, 85, $\frac{8\,6}{6\,0\,9}$, Ascidia, 1866; Euaxes and Lumbricus, 1871; Pyrosoma, 1875); Knpffer ($\frac{6\,2}{1\,2\,3}$, Ascidia, 1870); Bütschli (24, Nematoidea, 1873); Fol ($\frac{4\,0}{4\,7\,6}$, Geryonia, 1873; Pteropod, 1875; Echinoderms, 1877); Metschnikoff ($\frac{1\,4\,7}{1\,0}$, Geryonia, Polyxenia, 1874); Auerbach ($\frac{1\,3}{2\,2\,1}$ Nematoidea, 1874); van Beneden ($\frac{1\,5}{4\,8}$, egg and blastoderm-cells of rabbit, 1875), have seen and described more or less fully the same phenomena.

The Polar Figure.—It is impossible to say with certainty how long before the egg is laid the archiamphiaster is formed. That it is formed before deposit is proved conclusively, not only by sections of eggs taken from the ovary, but also by the examina-

tion of such eggs in a living condition. If a worm, about to deposit, be cut transversely through the middle, the eggs thus liberated will, in some cases, show a white spot on one pole. This spot, examined more closely, shows a distinct radial structure (fig. 1). Sections of such eggs prove that this polar figure marks the place of the external pole of the amphiaster. Sections of those eggs in which the polar figure is not yet visible show the amphiaster lying somewhat deeper in the yolk than is represented in fig 62.

This polar figure is visible on most eggs examined immediately after extrusion, and after a few minutes on all. I have, at least twice, seen both poles of the amphiaster on fresh-laid eggs of C. complanata; but usually the inner pole lies too deep to be seen from the surface. This figure was seen by Grube ($\frac{5}{4}\frac{9}{}$ "Polfleck," Kreidweisserpunkt"), by Leuckart and Rathke ($\frac{1}{8}\frac{3}{3}\frac{6}{}$, "weisse Scheibe"), and by Robin ("zone foncée," $\frac{1}{3}\frac{4}{4}\frac{3}{}$), and is perhaps the same as the "clear spot" seen before the polar globules appear on the egg of Lamellibranchs ($\frac{3}{2}\frac{7}{7}\frac{7}{6}$) and other Mollusca, and on the egg of Euaxes ($\frac{8}{1}\frac{5}{2}$). May not the "Faltenstern" (Geryonia, $\frac{4}{4}\frac{0}{7}\frac{}{4}\frac{}{5}$) be referred to the same category?

Quiescent State.—I once disturbed a worm as she was laying the last eggs, and in consequence of the interruption three eggs were retained in the ovary.

The eggs that were laid were examined, and the polar figure was found as usual. The worm manifested no desire afterwards to part with the remaining three eggs. At the end of 48 hours I resorted to the method of cutting before mentioned, to get the eggs. To my surprise, I found them in the same condition in which I had found the others two days before. The eggs in both cases were kept, and they developed in the normal manner. Here was clear evidence that eggs, after the formation of the archiamphiaster, remain in a comparatively quiescent condition until, at the time of extrusion, they are brought into contact with the water; and that this quiescent period could be maintained at least two days without injury to the egg. I have often observed cases where the eggs were retained in the ovary four or five days after the time when they were fully ripe for deposit. I am the more certain, as, during the second summer of my investigations, I was always able to fix that period with sufficient accuracy to exclude failure, in every case where I made the experiment of cutting the worm to obtain eggs. In some cases, where the worm has not had sufficient food, or has been too often disturbed, the eggs are never laid, but retained in the ovary, where they gradually dissolve, and finally disappear. Grube ($\frac{5}{1}\frac{9}{1}$) observed one such case and inferred, erroneously as

I think, that this course of events was the result of non-fecundation.[1]

The Pellucid Spot.—The polar figure, the outward expression of the radial lines of one pole of the amphiaster, becomes larger and more sharply defined; and at the end of 10—25 minutes after deposit, a minute pellucid spot appears exactly in its centre. This spot is entirely free from yolk-spheres and granules, but appears dark on account of the opaque background. It increases in size and at the end of about 30 minutes after deposit, measures 0·03 mm. to 0·04 mm. (diam.). This spot is the central part of the polar area of the outer star, which is so deeply coloured with carmine in my preparations (figs. 62, 63, *cp*).

This was found by Robin ("espace clair circulaire") in the egg of Nephelis, but overlooked in the case of Clepsine ($\frac{1+3}{4\ 9}$).

(*c*) **Polar Globules** (figs. 1—7).—An interesting phenomenon, overlooked by all my predecessors, accompanies the appearance of the polar globules ("Richtungsbläschen"). Robin gives a very detailed account of the appearance of these globules, and it is therefore all the more surprising to find that he failed to see the most conspicuous part of the whole process.

Thirty minutes[1] after deposit the egg passes, from the oval-elliptical form of fig. 1, Pl. XII, into the biscuit form of fig. 2.

The first time I saw this, I supposed the egg was in process of cleavage. But the constriction in this case does not extend much deeper than in fig. 2, and passes gradually from the middle towards the end which shows the pellucid spot and the polar figure. The constriction is perfectly regular and continuous in its movement towards one pole of the egg.

In 10—15 (45 min.) it is completed, leaving only a nipple-like protuberance, from which the first polar globule begins to project. That part of the polar globule first to appear is perfectly transparent, but the half last eliminated is filled with minute, highly refractive granules, the outer border of which forms a straight line at first. At the completion of the elimination (50 min.) the egg is flattened at this end, and slightly depressed just under the polar globule. The yolk at this time is removed from the vitelline membrane by considerably more than the diameter of the polar globule. This space, filled with a fluid (perivitelline fluid) which is a little less transparent than

[1] Colasanti (127) has just published some interesting observations on the duration of the *quiescent state* ("Lebensdauer") of the unincubated hen's egg. The average time during which this condition may be maintained, without fatal injury, is estimated at three weeks, in very rare cases at four weeks.

[2] The time will be stated according to the average of a few accurately noted cases. Different cases vary much. Time elapsed since deposit will hereafter be placed in parenthesis.

water, begins to diminish, and soon the yolk and membrane are in contact, the polar globule being pushed so far back into the yolk that it is seen with difficulty. The membrane now is almost in contact with the yolk all around, and the egg has again the form of fig. 1. After fifteen minutes the yolk begins to recede again from the membrane, initiatory to the expulsion of the second polar globule. The exit of this globule is not attended with so marked and regular constriction as the first. The expulsion of the two globules is completed in 45—55 min. (1 h. 15 min.).

In C. complanata this process is accomplished in the same manner as already described for C. marginata. The constriction often appears (C. complanata) raised in the middle, giving it the appearance of being double. In the egg of C. heteroclita the constriction is less conspicuous, but is, nevertheless, unmistakeable. I have not been able to see the stages preceding the cleavage in C. bioculata.

The process just described cannot be compared with the irregular movements of an Amœba. They begin at a definite time, proceed in the same regular manner, and are accomplished in about the same time on each egg. Thus far no such constriction has been observed on the egg of any other animal. The "slow periodical changes in form" observed by Flemming ($\frac{3\,8}{1\,0\,9}$, Anodonta), "the attempts to divide" reported by Brandt ($\frac{2\,2}{3\,7\,5}$, Ascaris), the "form-changes" noted by Schultz ($\frac{1\,4\,5}{4\,6}$, Torpedo), the "contractions" and so-called "amœba-like movements" described, from time to time by other authors, as preceding or accompanying the expulsion of the polar globules, may be more or less modified forms of the same phenomenon.

No polar globules were recognized by Grube, nor by Leuckart and Rathke. It is evident, however, that the polar globules were seen by all these investigators; for they saw one or more small "balls" between the first two cleavage-spheres, *i.e.*, before the production of ectoblasts. Bütschli ($\frac{2\,7}{9}$), in his excellent work on "Cell-division and Conjugation of the Infusoria," has interpreted Grube's polar ring as a polar globule; but this is certainly incorrect, as an examination of the ring will show. That the polar globules are produced by the archiamphiaster,[1] and that they are not therefore mere "buds" of yolk, having no genetic connection with the germinal vesicle, as supposed by Robin ($\frac{1\,4\,3}{8\,8}$), is proved by such sections as that given in fig. 63, Plate XIII. This section shows the second polar globule in

[1] Fol ($\frac{4\,4}{2}$, $\frac{4\,6}{1\,1}$), who has followed this process in the living egg (Asterias), thinks that the Archiamphiaster does not produce directly the polar globule, but that it gives rise to a second amphiaster, and that the latter produces the first polar globule.

the moment of its liberation, and it is plain to see what part the amphiaster takes in its formation (*cp*).

Polar Activity.—While the phenomena thus far described—polar figure, pellucid spot, and polar globules—have been confined to one pole, those which are to follow are repeated, with some differences, on both poles. A short period of unipolar activity is succeeded by a long period of bipolar activity which extends through the cleavage stages. In the latter period the contrast between the two poles is still maintained: for the pole thus far active, still asserts its pre-eminence by taking the lead in actions that repeat themselves later and more sluggishly on the opposite pole.

It is as if one pole was trying to mimic the performances of the other. The more active pole is further distinguished by being specifically lighter than the opposite pole, so that, with the exception of the short time during which the first polar globule is being eliminated, this pole is always uppermost. As this pole corresponds to the anterior end of the future embryo—the pellucid spot marking very nearly the position of the future mouth—it may be called the *oral pole*, and the opposite, *the aboral pole*. Thus, the main axis of the egg corresponds to the longitudinal axis of the embryo.

d. **Formation of Polar Rings and Pronuclei.**—The ring-phenomenon, like the constriction accompanying the exit of the polar globules, is peculiar to the egg of Clepsine, nothing of the kind having as yet been found on the egg of any other animal.

The first to describe the polar rings was Grube ($\frac{59}{15-16}$). The manner of their formation, however, was entirely misunderstood. He supposed that the white polar figure ("Polfleck") enlarged and became the polar ring. This mistake was corrected by Robin ($\frac{143}{97-105}$), who has followed the ring-phenomenon from beginning to end, and described its minutest details with great accuracy. So far as an outward description goes, I have but little to add to the observations of Robin; but I shall be under the necessity of giving a brief account of the external appearance in order to bring them into relation with internal changes, of which Robin was, of course, not cognizant, as he made no use of sections.

Immediately after the appearance of the second polar globule, the pellucid spot, which marks the place of its exit, is still visible. A section of the egg at this time (Pl. XIII, fig. 65, 1 h. 45 min.) shows the polar globules (p.g.) lying in a slight depression, caused by the action of the acid. Beneath the globules there is a circular space, free from deutoplasm. This space, open towards the globules, is filled with a very fine granular substance, which has the lead-grey tinge, characteristic of the germinal vesicle which has been treated with osmic acid. The

effect of carmine is alike in both cases very weak. This body, which appears as a pellucid spot on fresh eggs, and which, according to the terminology of van Beneden and Fol, may be designated as *female pronucleus*, is the remnant of the Archiamphiaster. It is without a membrane, perfectly homogeneous, and forms the centre of a radial system. Not far from the opposite pole is another similar body—also the centre of a radial system. The latter body is the *male pronucleus*[1] (Spermakern, Hertwig). There appear to be three polar globules in this case (fig. 65, B), two of which are about the same size (circa .03 mm.), and contain nuclear bodies. Opaque granules are quite numerous except in one which is quite transparent.

Ten minutes later (1 h. 25 min.) I have seen a circular area at the oral pole assume a somewhat darker shade than the rest of the egg (Pl. XII., fig. 8). I have recognised this change but once with certainty (by very favourable light), and can give no explanation of its origin or signification.

Five minutes later (1 h. 30 min.) a transparent fluid substance begins to collect in a shallow groove which encircles the oral pole, thus forming the *first polar ring* (Pl. XII, fig. 9). This ring, at first feebly expressed, soon becomes well defined, and is bordered both on the polar and also on the equatorial side with yolk which is quite free from yolk spheres, but densely packed with fine granules. The borders appear whitish by reflected light.

As the ring begins to advance towards the pole, at the same time deepening, the inclosed polar yolk, on which the polar globules rest, assumes the form of a calotte (fig. 10). About this time (1 h. 40 min.) a similar ring appears around the aboral pole, and the equatorial edge of the first ring (*p.r.*) becomes denticulate, the substance of the ring stretching out towards the equator of the egg in the form of rays. Just before these ring-rays have reached their maximum in extent and clearness on the oral pole, they begin to form in the same manner on the aboral pole (fig. 11). The first ring continues to advance towards the pole, reducing the base of the calotte to a slender column (*b*, cal.).

The second ring (*p.r'.*) advances towards the aboral pole, but not striking deep enough to form a calotte, drives the inclosed yolk in towards the centre of the egg, and collects in the form of a disc (fig. 11).

The calotte (cal.) is often reduced to a much smaller extent than is represented in figs. 12, 13, and 14; but it does not wholly disappear. As the time approaches for the beginning of

[1] June 15th, 1878. I have found the male pronucleus *before* the appearance of the first polar globule. Just after deposit the archiamphiaster is found at one pole of the egg and the male pronucleus, which at this time resembled in size and general appearance one of the amphiastral poles, near the opposite pole.

the cleavage, the ring-rays of each pole become more and more feeble. The calotte approaches that side of the ring which lies nearest the plane of the coming cleavage (fig. 14), giving the ring the form of a semilunar spot. The calotte generally remains circular; but when very small, sometimes stretches and forms a mere line at right angles to the first cleavage-plane. The approach of the calotte to one side of the ring does not interrupt the continuity of the latter as a profile view (fig. 13) proves. At this time the aboral ring-disc is reduced to a mere point, with scarcely perceptible rays. Figs. 10—13 show that although the ring and rays begin later on the aboral pole, they pass through their different phases more rapidly than those of the oral pole. The rays are well seen on eggs hardened in chromic acid.

I will now pass to the consideration of the changes taking place within the egg during the ring-period.

Fig. 66 (Pl. XIII) represents the incipient formation of the first ring (*p.r.*), and corresponds nearly with fig. 9, Pl. XII (1 h. 30 min.)

The female pronucleus has advanced a little towards the centre of the egg. The space between it and the polar globules is still free from large yolk-spheres. In this case the action of the acid was weak, and that of the carmine correspondingly strong, the entire pronucleus being deeply coloured.

Pronucleoli.—On the inner side of the pronucleus are seen two small, highly refractive corpuscles, in close apposition, which together measure 01 mm. dm. These small bodies, which I shall call *female pronucleoli* (f. *pnl*) are sharply defined, homogeneous, and more deeply coloured than the nucleoplasm (fig. 66, D).

The male pronucleus (m. pn) is now near the centre of the egg, and shows in its centre a single oval-elliptical body, of the same nature as the female pronucleoli.

This body is the male *pronucleolus* (*m. pnl.*). Both pronuclei are surrounded with radial lines, and their longest axes lie in the main axis of the egg.

In some eggs of the same date, I found two pronucleolar bodies in the central nucleus (fig. 66, F), lying at some distance from each other. The one lying nearest the oral pole is composed of two parts (*f. pnl*), and corresponds in size to the two female pronucleoli (D). The axes of such nuclei lie sometimes parallel with, sometimes inclined to the main axis of, the egg (*f*), and the radial lines are very faintly, or not at all, expressed. In these eggs only one nucleus was found. They represent probably a stage intermediate between fig. 66, C, and fig. 67 G.

Fifteen minutes after the appearance of the first ring (1 h. 45 min.), the egg reaches the condition represented in fig. 67. The upper ring (*p r*) is here very distinct, but I am unable to distinguish the lower ring, although it is generally present at

this time. The ring-substance is coloured brown by the osmic acid and carmine. The unshaded portion lying below and at both sides of the ring is a zone of protoplasm, which contains yolk-granules, but no large yolk-spheres. It is this zone which forms the two white ring-borders seen on living eggs.

e. **Primary Cleavage-nucleus.**—Lying a little excentrically towards the oral pole is the primary cleavage-nucleus. The nucleoplasm is more strongly colored in the centre around the pronucleolar bodies than at the edges. These nucleolar corpuscles are several times larger than in the preceding fig. In fig. 67, J., two of these bodies (= 0·35 mm. diam.) are seen with their concave sides applied to the third nearly round body (=.03 mm. diam.). They are sharply outlined, but only slightly stained with carmine. Between the ring and the cleavage nucleus (G) a line, more highly colored than the rest of the yolk, is sometimes seen. This line, judging from its position and direction, I interpret as the path[1] taken by the female pronucleus towards the male pronucleus. The three corpuscles in the centre of the nucleus are undoubtedly the pronucleoli described in fig. 66, the two uppermost being the female pronucleoli, and the lower one the male pronucleolus. The longer axis of the nucleus in this stage is in every instance at right angles to the axis of the egg, whereas at the moment of union of the pronuclei, the longer axis was found parallel with that of the egg, and a little later (fig. 66, *f*) inclined about 45°. Whether anything occurs here comparable with the rotation described by Auerbach ($\frac{3}{2\,1\,2}$) for Ascaris, I am unable to say.

The advancement of the rings will be easily understood by referring to figs. 68—71, Pl. XIV. The calotte (cal.) reaches its mimimum dimensions about one hour after the first ring appears (fig. 71, 2 h. 30 min.). As the calotte diminishes, the oral ring concentrates and deepens until it arrives at the cordate form seen in fig. 71. The lower ring, as it concentrates covers the aboral pole more and more, forms a shallow disc (fig. 69), and at length presents the oblong oval form of fig. 71. It is quite certain that both rings are composed of essentially the same substance. It is impossible to distinguish on the living egg the substance of one ring from that of the other; and both, when treated with osmic acid, alcohol, and carmine, present the same characteristic shades of brown, varying according to the intensity of the acid action, between a dark brown and the lead-grey first spoken of in connection with the germinal vesicle.

When hardened in alcohol, and coloured with carmine, both the rings and the nucleus are deep red. It is therefore probable that the ring-substance is nuclear material, or something very

[1] Auerbach saw such a *Strasse* in the egg of Ascaris nigrovenosa ($\frac{3}{2\,0\,6}$).

analogous. By the time the rings have reached the stage of fig. 28, the zone of protoplasm (*p z*) underlying the oral ring begins to plunge into the yolk, and a little later often presents the forms seen in figs. 70 and 71, which remind one of the "sichelförmige Ausstrahlungen" seen by Schultz ($\frac{1}{4}\frac{4}{6}\frac{5}{7}$) under the germinal disc (Torpedo).

The nucleus presents essentially the same appearance in all the stages from fig. 67 to fig. 70; but in fig. 71 P., it has already stretched considerably in a direction perpendicular to the axis of the egg. The nucleolar bodies are of about the same size and maintain about the same position in all these stages, (figs. 67—71).

I will now state my reasons for regarding these three small bodies as nucleoli rather than nuclei. Bütschli and Hertwig have seen in the egg of Nephelis two radial systems ("Strahlensysteme"), and in each system one or more minute corpuscles, which grow at the expense of these systems. The "strahlensysteme" with their central corpuscles correspond without doubt to the bodies which I have called pronuclei and pronucleoli. Hertwig and Bütschli both agree in interpreting these small bodies as nuclei, ($\frac{2}{5}\frac{7}{7}$, $\frac{7}{2}\frac{1}{2}$). Hertwig has studied the same phenomena in the egg of Toxopneustes, Asteracanthion and Sphaerechinus, and has here described as "Spermakern" ($\frac{7}{3}\frac{0}{8}\frac{0}{0}$, $\frac{7}{2}\frac{2}{7}\frac{2}{4}$) and "Eikern" ($\frac{7}{3}\frac{0}{5}\frac{0}{7}$, $\frac{7}{2}\frac{2}{7}\frac{2}{4}$) bodies comparable with the male and female pronucleoli found in the pronuclei of stage 66, Pl. XIII. Bütschli's interpretation of these corpuscles as nuclei is maintained on the ground, that when treated with ascetic acid ($1\frac{0}{0}$) they exhibit a thick dark membrane which incloses a fluid with a few dark granules; and further, that they increase in size at the expense of the central area ("Centralhof") in which they arise. All these points except the last, have already been disposed of by the observations of Hertwig ($\frac{7}{2}\frac{1}{4}$). Hertwig found that, when treated with osmic acid, these corpuscles appear thoroughly homogeneous, presenting no thick membrane ("Hülle"), as described by Bütschli, but only a somewhat thicker peripheral layer ("Rindenschicht"); further, that *they do not coalesce until the first cleavage-spindle begins to form.* This is in harmony with what happens in the egg of Clepsine, and corroborates the view I have taken. Hertwig's remarks awaken the suspicion that Bütschli was misled by artificial appearances in regard to the non-persistence of the central area.

The enormous size of these corpuscles in the figures of Bütschli is possibly only a swollen condition produced by his re-agents. Such artificial pictures might easily mislead one into the belief that the central area was disappearing.

That this central area, which arises by a fusion of the two pronuclei, does not disappear, nor even diminish in size, is certain, at least for the egg of Clepsine (figs. 66—72). The three questionable corpuscles increase a little in size, but are at every step small in comparison with the central area of very fine granular substance in which they are imbedded. Their fusion does not take place till after the central area assumes the spindle form.

In their first and last stage (figs. 66, and 71) they are more deeply stained with carmine than the nuclear substance of the central area; and although well defined, possess no veritable membrane. They are altogether similar to the nucleoli of the germinal vesicle. In some of the intermediate stages, particularly in that of fig. 67, *f*, they are exceptionally paler than the nucleoplasm, and larger than in the following stages. Their peripheral part is here quite highly coloured, and shades off gradually into the pale central part. It is this stage, or condition, more than any other, that bears a slight resemblance to Bütschli's figures. I believe the exceptional size and paleness are here due to a variation in the action of the re-agents.

The size, structure, chemical behaviour, and destiny of these bodies favour the interpretation I have given them. On the supposition that they are nuclei, what name should we give to the substance which holds them in suspension, and which takes the lead in the formation of the first cleavage-amphiaster? That this substance is nuclear is evident from reasons already given; and since it maintains its individuality from beginning to end, and always sustains the same relations to the small bodies in its centre that are generally sustained between nucleus and nucleoli, there seems to be no reason why it should not be regarded as a nucleus. This view seems to me not only most in harmony with the above facts, but also most consistent from a theoretical standpoint. The uninuclear condition is the prime characteristic of the cell. The pronucleus stage presents no difficulty. The egg in this condition is not to be regarded as a single cell with two nuclei, but as *a pair of copulating cells* in which like parts are in process of union. Two individualities are blended in one, and the result is a single cell with a single nucleus and one or more nucleoli. This view of the process of fecundation has recently been emphasised by Strasburger ($\frac{1}{4}\frac{5}{8}\frac{5}{3}\frac{5}{5}\frac{5}{0}\frac{5}{9}$).

The formation of free nuclei, as in the eggs of some insects, creates no real exception to the uninuclear character of the cell. As soon as the germinal vesicle has divided into two parts, the egg is no longer a single cell, but two cells, although their

limits are not yet visible. That each part into which the germinal vesicle divides represents the centre of a cell, receives an ocular demonstration by the formation of the blastoderm. As cleavage is only the outward expression of a change originating in the nucleus itself, it is all the same whether it appears a little sooner or a little later. But according to the view taken by Bütschli the cell may pass during the period of proliferation, from the uninuclear to the multinuclear condition, and from the latter back again to the former condition, without once losing its character as a single cell.

What then is a cell? It is no longer a body of protoplasm with a single nucleus, or with any *definite* number of nuclei, but one in which the number of nuclei may vary without specified limits. According to this, any tissue in the state of syncytium (Häckel), whether produced by the formation of free nuclei, or by a simple concrescence of originally distinct cells, might be called a cell.

It is obvious that any definition of the morphological unit we call a cell, capable of general application, must be based on some constant element of the same. The nucleus is a constant, and in the vast majority of cases at least a single element of the cell.

Bütschli ($\frac{2}{2}\frac{5}{1}\frac{}{3}$), in harmony with his theory that the egg and cleavage-spheres pass through the multinuclear to the uninuclear condition, is inclined to regard the former as the more primitive, and to see in its recurrence after each cleavage, a repetition of the ancestral form of the cell. This view is supposed to be supported by another fact, viz. that in some multinuclear Infusoria the several nuclei coalesce before the division. In reply to this, it may be said that the multinuclear condition is not the earliest condition of the egg-cell. The investigations of Engelmann ($\frac{3}{3}\frac{5}{7}\frac{}{7}$) and Zeller ($\frac{1}{3}\frac{5}{0}\frac{8}{0}$, Opalina) make it certain that in one Infusorian at least the multinuclear is preceded by the uninuclear condition.

Opposed to the interpretation of these corpuscles as nucleoli is the colossal size attained by them in the egg of Rana ($\frac{7}{4}\frac{1}{8}$). And yet the absence of anything in them that could be called nucleoli, and the fact that the fine granular substance surrounding them takes the lead in the process of division, as Götte has shown ($\frac{5}{5}\frac{8}{5}\frac{}{6}\frac{}{1}$), are opposed to the view that they are nuclei. Oellacher ($\frac{}{4}\frac{}{1}\frac{1}{0}\frac{2}{5}\frac{4}{4}\frac{}{1}\frac{}{6}$) has given an interesting account of similar bodies which he found in the early cleavage-stages of the egg of Salmo fario. These "clusters of nuclei" were supposed to arise by repeated division of the first cleavage-nucleus, and each was regarded as a veritable nucleus, destined to become the centre of a future cell. No coalescing of these bodies before, and re-formation after cleavage was observed. The whole

process, as represented by Oellacher was therefore only a precocious division of nuclei, to be followed sooner or later in each case by a corresponding cleavage of the germ-substance, analogous to what happens in the eggs of insects.

While this view is entirely consistent with the uninuclear character of the cell, it cannot be accepted as Bütschli has already shown ($\frac{2}{1}\frac{7}{9}\frac{}{8}$). The peculiar clusters of nuclei found by Balfour ($\frac{1}{3}\frac{1}{2}\frac{}{6}$) in the floor of the cleavage-cavity (Elasmobranch Fishes) are not to be confounded with the small bodies under consideration. According to Balfour these nuclei possess distinct nucleoli, and become the nuclei of blastoderm cells. The idea expressed by Bütschli ($\frac{2}{1}\frac{7}{9}\frac{}{8}$) that these clusters are nuclei in process of coalescing, has not the slightest shade of probability in its favour.

Van Beneden ($\frac{1}{1}\frac{6}{5}\frac{}{6}$) regards the Spermakern of Hertwig as a nucleolus, and the clear spot in which it arises as a nucleus. He maintains the same in respect to the two pronuclei seen in the egg of the rabbit, in both of which one or more nucleoli arise. Van Beneden calls attention also to the characteristic lead-grey or blackish colour, given to the pronucleus by osmic acid—precisely what I have above mentioned as an evidence of the nuclear nature of these bodies.

Auerbach ($\frac{3}{2}\frac{}{0}\frac{}{3}$), to whom we are indebted for the first accurate knowledge of the origin of the cleavage nucleus, recognized the two "clear spots" which he saw arise in the two poles of the egg (Ascaris) as nuclei, and the two or three corpuscles that arose in the centre of each as nucleoli ($\frac{3}{2}\frac{}{0}\frac{}{4}$) Besides, he represented these nucleoli as persisting until the transformation of the nucleus into the first cleavage spindle.

In the same way Strasburger (24, Pl. XXVIII, figs. 69—71) represents the cleavage nucleus as arising by the union of two pronuclei, in each of which small nucleoli are figured.

In both these cases the nucleoplasm persists, as in the case of Clepsine. Hertwig's investigations upon Toxopneustes (21, Taf XII, figs. 15-20) prove conclusively that here also the nucleoplasm of the "central area" persists and stretches to form the bistellate figure before the so-called nucleus disappears.

The cases here referred to will suffice to show that evidence is not wanting in favour of the interpretation of the three corpuscles as nucleoli.

f. **Primary Cleavage-amphiaster.**—Fig. 72, Pl. XIV, represents a stage of the same date as fig. 71 (2 h. 30 min.), but it is plainly more advanced. The nucleus has passed from the spindle-form of stage 71 to a biscuit form. The nucleoli are no longer visible, but stretching through the centre of the biscuit-shaped figure, which is somewhat inclined to the main

axis of the egg, are seen some fine granular lines which together form a sort of spindle, the two poles of which appear to be near the centres of the polar areas These lines, which are the same as the inter-stellate lines before mentioned, are well defined, and, in this case, more strongly expressed than the radial lines of the two polar areas. The oral ring has lost the distinct outline of the previous stage (fig. 71), and plunged deeper towards the centre of the egg. The calotte (cal.), though small, is still plain to be seen, supported by a slender column of deutoplasm. The aboral ring-disc has also lost its sharp outline, and advanced towards the centre of the egg. At the surface of this pole are seen several highly-coloured spots, probably remains of the disc.

Fig. 77 represents a section in the plane of the ring, taken about 20 mm. under the upper pole. The ring-substance is here plainly more deeply coloured around the base of the calotte. I have recognised this but once in transverse sections, and twice in horizontal sections. I am unable to give any farther account of it.

Stage 73 also bears the same date as the two preceding, but is evidently farther advanced than either of them. One pole of the amphiaster is seen more highly magnified in fig. 78. In the centre of the polar area is a clear circular space (ca), around the edge of which the nucleoplasm is a little more deeply coloured, giving the appearance of a more or less well-marked ring around the same. The strongly expressed radial lines end in this ring, and are not to be distinguished from the spindle-fibres.

The ring substance has plunged a little deeper, and is directed towards a point to the right of the centre of the spindle. The zones of protoplasm seen in previous stages about the ring-substance are no longer recognizable. Stage 74 is about 15 minutes later than stage 73 (2 h. 45 min.). The poles of the amphiaster are farther apart than in the previous stage, but are still connected by a band of nucleoplasm in which no lines or fibres are visible. They have become somewhat lens-shaped, with their longest diameters at right angles to the axis of the amphiaster. The clear central spaces (ca) seen in each polar area of stage 73 are replaced by central stripes. The substance of the stripes appears a little coarser than the nucleoplasm, and imperfectly distributed in two to four parallel lines. The radial lines are very strongly expressed, extending to the periphery of the egg. The ring disc of the aboral pole has taken a sagittal form. Ten to fifteen minutes later (3h.) the first cleavage is already in progress (fig. 75). The remnants of the two rings are found a little to the right of the plane of division. The

poles of the amphiaster, still connected by a slender band of nucleoplasm, have passed from a biconvex into a meniscal form, with their concavities facing each other.

Fig. 76 (3 h. 30 min) represents a stage about fifteen minutes in advance of fig. 75, although, according to date, it should be 30 minutes in advance.

The first cleavage is seen at the moment of completion. The larger of the two cleavage segments contains nearly all of what is still visible of the two rings. The poles of the amphiaster, between which only an attenuated thread of nuclear substance is still seen, have returned from the meniscal to the biconvex form, and are much nearer to each other than during the process of cleavage. In each of these poles, which now form independent centres, or nuclei, is a cluster of small bodies, which have the microchemical aspect of the three pronucleoli before described, and which I therefore regard as nucleoli.

The nucleoplasm of each nucleus is indistinctly divided into two areas. The central area is not quite so highly coloured as the peripheral, and corresponds in general appearance to the stripe-areas seen in figs. 74 and 75. I think these stripe-areas correspond to the clear central spaces seen in fig. 73, which become flattened shortly before the cleavage. The rays, which reach their greatest intensity in stage 74, have nearly or wholly disappeared

Fig. 76 reminds one strongly of corresponding stages described by Götte (Bombinator igneus) and Hertwig (Rana temporaria).

The five to six clustered nucleoli evidently correspond to Hertwig's nuclei and to Götte's "Kernkeime" ($\frac{5\ 8}{6\ 1}$).

The clear central spaces (ca) that arise in the poles of the amphiaster (fig. 73) probably correspond to Götte's "Lebenskeim" ($\frac{5\ 8}{5\ 1}$).

Summary.

1. The germinal vesicle of the ovarian egg gives rise to a bi-stellate figure (archiamphiaster), which gradually approaches the surface of the egg, where, at the time of deposit, one pole of the same becomes visible as a *white stellate figure*.

2. The egg may be retained two days, possibly four or five, after the transformation of the germinal vesicle, during which time the archiamphiaster appears to remain in a queiscent condition.

3. The white stellate figure marks the place where the mouth later forms, and hence this pole may be designated as *oral*, and the opposite as *aboral*. The line passing through the centre of the egg, and joining these two poles, is the axis of the egg, and corresponds to the longitudinal axis of the future embryo.

4. The oral pole is specifically lighter than the aboral pole (hence always uppermost), is the seat of the unipolar phenomena, and takes the lead in all the bi-polar phenomena.

5. Thirty to forty minutes after deposit a well-marked constriction passes like a peristaltic movement from the middle of the egg towards the oral pole, at the conclusion of which the first polar globule arises from a pellucid spot in the centre of the stellate figure. Thirty minutes later the second polar globule takes its exit from the same place, attended by a much weaker constriction than the first.

6. After the production of the two nearly equal polar globules, the remains of the archiamphiaster are converted into the *female pronucleus*, in which a pair of nucleoli appear a little later; and at about the same time the *male pronucleus*,[1] containing one nucleolus, appears not far from the opposite pole.

7. Fifteen minutes after the elimination of the polar globules a ring-like depression, or constriction, appears in the yolk around the oral pole, and in this depression a transparent, liquid substance (nuclear ?) is collected, forming the first *polar ring*.

8. Five or ten minutes later pseudopode-like extensions of the ring-substance (ring-rays) are formed on the equatorial side of the ring. The same phenomena repeat themselves a little later on the aboral pole.

9. The ring-rays, having attained a maximum intensity, gradually disappear as the rings concentrate to form two discs, one of which covers the aboral pole, while the other is pierced through the centre by a slender column of yolk, which has a calotte-like summit.

10. Before the first cleavage both discs plunge deep into the egg, and possibly contribute some elements to the nucleus, which may either induce or stimulate the molecular changes, which result in the formation of the *primary cleavage amphiaster*.

11. At the approach of the cleavage, the aboral disc is visible only as a mere point if seen from the surface, while the oral disc has taken the form of a semi-lunar spot; and at the completion of the same, the remnant of both discs is found in the larger of the two cleavage-spheres.

12. The two pronuclei, whose longest diameters are parallel to the axis of the egg, approach and coalesce to form the primary cleavage nucleus, the longer axis of which is soon found at right angles to the axis of the egg.

13. The pronucleoli meet in the centre of the so-formed nucleus, increase a little in size, and maintain their individuality till the first cleavage-amphiaster begins to form, then dissolve.

14. In each pole of this amphiaster a *central area* arises, which colors less with carmine than the surrounding nucleoplasm, and in the edge of which the converging rays end. These areas take the form of striated stripes after the disappearance of

[1] See note, p. 235.

the spindle-fibers, and in the centre of each is found at the completion of the first cleavage, a cluster of four to six refractive corpuscles (nucleoli).

15. That the bodies called pronuclei are composed of nuclear substance is attested by their origin, general appearance, entire history, and by the characteristic lead-grey, or blackish tinge imparted to them by osmic acid.

16. As soon as the cleavage-amphiaster is formed, the two poles begin to move in opposite directions as if repelling each other, and during this recession, which reaches its maximum in the moment of cleavage, they pass from the *round* to the biconvex, and lastly to the *meniscus* form; and at the completion of the cleavage they begin to approach each other again, passing through these forms in inverse order.

Their recession proceeds as if the force driving them asunder was applied to their inner face.

General Considerations

(*Relative to the phenomena above described*).

a. **Germinal vesicle** ("vesicula germinativa," Purkinje).— What is the destiny of the germinal vesicle? Does its history as a nuclear element end with the maturity of the egg? or does it persist in whole or in part, and supplemented by the male element, become the progenitor of the subsequent generations of nuclei? Opinions have been, and still are divided. For twenty-five years after the discovery of this body by Purkinje (1825), it was almost universally believed that it became morphologically extinct soon after the maturity of the egg. Within the last twenty-five years this opinion has been contradicted, in a more or less positive manner, by a considerable number of very eminent biologists, among whom we may mention Joh. Müller ($\frac{1}{1}\frac{2}{7}\frac{2}{}$, 1852), Leydig ($\frac{1}{2}\frac{1}{8}$, $\frac{1}{1}\frac{0}{0}\frac{1}{3}$, 1854), Gegenbaur ($\frac{5}{2}\frac{3}{9}$, 1854), Leuckart ($\frac{1}{6}\frac{0}{7}$, 1858, $\frac{1}{3}\frac{0}{2}\frac{7}{2}$), Keferstein ($\frac{8}{1}\frac{0}{}$, 1868), Häckel ($\frac{8}{2}\frac{5}{1}$, 1869), van Beneden ($\frac{1}{3}\frac{3}{0}$, 1870), Kowalevsky ($\frac{8}{2}\frac{5}{1}$, 1871), Frey ($\frac{4}{9}\frac{8}{3}$, 1873), and v. Baer ($\frac{7}{2}\frac{7}{0}\frac{7}{2}$, 1876). On the other hand many distinguished naturalists, among whom are found some of those just mentioned, have maintained the non-persistence of the germinal vesicle. Of these we give the following: Baer ($\frac{6}{3}\frac{6}{8}$, 1846), Reichert ($\frac{1}{2}\frac{3}{0}\frac{8}{1}\frac{8}{3}$, 1846), Leuckart ($\frac{1}{9}\frac{0}{2}\frac{4}{7}$, 1853), Weismann ($\frac{1}{1}\frac{5}{7}$, 1863), Kupffer ($\frac{0}{1}\frac{3}{6}\frac{3}{9}$, 1870), Kleinenberg ($\frac{8}{4}\frac{1}{2}$, 1872), Oellacher ($\frac{1}{1}\frac{2}{2}\frac{4}{3}$, 1872), Auerbach ($\frac{3}{7}\frac{3}{9}\frac{9}{}$, 1874), Robin ($\frac{1}{8}\frac{4}{7}\frac{3}{}$, 1875), Flemming ($\frac{3}{1}\frac{8}{8}\frac{8}{2}$, 1875), Götte ($\frac{3}{1}\frac{8}{8}\frac{8}{}$, 1875), Balfour (Monogr. Develop. Elasmobranch Fishes, 1876), Kölliker ($\frac{0}{3}\frac{0}{3}$, 1876), and Bischoff ($\frac{1}{3}\frac{9}{4}$, 1877). To these may be added Häckel (62) and van Beneden (16), who have both abandoned the belief in the persistence of the germinal vesicle.

The retrograde metamorphosis of this vesicle bridges over the gap between the cytode and the cell, and thus enables Häckel to begin his ontogenetic recapitulation with the lowest form of organic life—the structureless Moner. Beautiful as this theory may seem to be (phylogenetically speaking), it certainly has some *a-priori* as well as *a-posteriori* objections. The idea (facts for the moment waived) that a cell loses its nucleus and sinks to the cytode-condition, for no conceivable purpose except to establish its phylogenetic lineage with "organisms without organs," is plainly in contradiction with the ordinary course of nature. Ontogeny furnishes numerous examples of reversion, but I believe no case in which the reversion is followed by a progression to the same point again. There is of course no objection to the theory that cytodes, sometime in the history of the organic kingdom differentiated into cells, nor can we deny that such a differentiation is possible at the present time; but such a possibility is quite insufficient to sanction the belief that an organism begins its evolution by making a phylogenetic excursion to its ancestral cytode-condition. Besides such an excursion, viewed in the light of facts now well ascertained, loses the last vestige of its supposed significance.

If the egg, after maturity, sinks to the cytode-condition, then it is certain that it reverts to this primordial state, not only before the first cleavage, but also before each subsequent one. As a result of a perfectly regular cleavage, we should pass through a series of reiterated reversions to the primary condition of life, to the archimorula-stage (according to Häckel), which in its turn, before advancing, would become a conglomeration of undifferentiated cytodes! But recent investigations upon the process of cell division demonstrate clearly that Häckel's "Monerula" does not belong to the ontogenetic series. It is no presumption now to say that those who have supposed that the egg passes through an enuclear condition, have drawn this conclusion from the negative fact, that a condition occurs in which the existence of the nucleus can no longer be demonstrated *by the methods employed;* while those who have maintained its persistence, have confounded the primary cleavage-nucleus with the germinal vesicle. Bütschli ($\frac{1}{2}\frac{0}{1}\frac{9}{8}$) has also rejected the Monerula-stage on the ground that, in many cases, the germinal vesicle is not eliminated till after fecundation.

Van Beneden (16) has published an interesting and able defence of his views in regard to the dissolution of the germinal vesicle, which appeared in the 'Quart. Journ, Mic. Sci. 1876.' The results of Van Beneden's researches on the ovum of the rabbit and of the starfish (Asteracanthion rubens), may be most concisely given in his own words ($\frac{1}{1}\frac{0}{5}\frac{6}{4}$). "My researches on the

ovum of the rabbit have proved to me that no morphological part of the germinal vesicle is found in the yolk at the moment of fecundation. The nucleolus united with the substance, which constituted the membrane of the vesicle, is eliminated to form one of the directive bodies; the nucleoplasma with the pseudo-nucleoli are thrown off into the perivitelline liquid, to form there the second polar globule. The liquid of the vesicle remains in the yolk, and becomes confounded with the cortical substance of the ovum, which from this moment is no longer distinguishable from the medullary substance. There can not then be, in the rabbit, any genetic connection between the germinal vesicle or one of its parts, and the embryonic nucleus, which appears in the egg after fecundation ($\frac{1}{17}\frac{6}{5}$), starfish). The successive phenomena which precede the complete disappearance of the germinal vesicle are these:—1. The solution of the nucleoplasmic mass and of the pseudo-nucleoli in the nuclear juice; 2. The breaking up of the germinal spot into fragments, and the progressive solution of these fragments in the nuclear substance; 3. The perforation of the membrane, followed by the partial expulsion of the contents of the nucleus; 4. The complete solution of the membrane in the juice of the germinal vesicle; 5, lastly, the solution of the nuclear substance in the vitelline protoplasm." Are the results arrived at by van Beneden, accurate as they undoubtedly are in most respects, *decisive* on the point in question?

It is asserted with positiveness that the germinal vesicle disappears, and that polar globules arise in both cases, but there is a striking difference in the manner of disappearance.

In the rabbit, the nucleolus, nucleoplasm, and membrane are eliminated as two polar globules, the nuclear fluid alone remaining and mixing with the vitelline mass; while, in the starfish, *all these elements are dissolved and confounded* with the protoplasm of the egg, no genetic relation being found between them, or any part of them, and the polar globules. It is this failure to bring the polar globules into connection with the germinal vesicle, that shows conclusively that van Beneden's results are, in this case at least, *undecisive*. The egg of the starfish is by far more favourable for the study of these phenomena than that of the rabbit, for the successive phases can here be brought under direct and continued observation, while in the rabbit this is impossible. We cannot, then, accept the results in either case as decisive. Had van Beneden supplemented his observations on the *living egg*, by the use of re-agents, at the moment of the supposed disappearance of the germinal vesicle, as Hertwig and Fol have recently done, he would undoubtedly have arrived at a very different conclusion.

It would be difficult to prove that the germinal vesicle

becomes neither physiologically nor morphologically extinct, more conclusively than has been done by the last two-mentioned authors. Both have followed these changes repeatedly in the living egg, and both have confirmed what they saw in this way by the examination of corresponding stages treated with re-agents. Both have arrived at essentially the same conclusions, differing only in regard to the rôle played by the nucleolus. The conclusion reached in each case is, that the polar globules and the female pronucleus are products of the germinal vesicle.

The investigations of Bütschli ($\frac{3}{2}\frac{0}{3}\frac{}{4}$, Neritina fluviatilis) corroborate all this, and are all the more convincing as they compel Bütschli to abandon the opinion previously expressed ($\frac{1}{2}\frac{0}{4}$), that the entire germinal vesicle is expelled in the form of polar globules, and to accept the view maintained by Hertwig—namely, that the polar globules are composed not only of nuclear substance, but also of protoplasm, and that a part of the germinal vesicle remains in the egg, as a nuclear element, after producing the polar globules.

A similar case of confirmation is found in Strasburger's experience with Phallusia mamillata ($\frac{1}{3}\frac{5}{0}\frac{4}{4}$). Strasburger found, upon a second examination, by using osmic acid and carmine, that the germinal vesicle had not wholly disappeared, as he at first supposed, and sums up his conclusion thus: "Aus allen diesen Betrachtungen scheint mir hervorzugehen, *dass ein Theil des alten Keimbläschens stets im thierischen Ei verbleibt.*" The results attained by these authors appear to me to be decisive against the opinion that every part of the germinal vesicle is extruded in the form of polar globules, or completely confounded with the vitelline protoplasm.

So far as Clepsine is concerned, it is not of course possible to prove by direct observation that the archiamphiaster is a product of the germinal vesicle; but if this point be admitted—and there seems no longer any room for doubting it—then it is perfectly clear that a part of the germinal vesicle remains permanently in the egg. Plate III, fig. 63, proves that half of the amphiaster which produced the second polar globule, remains in the egg. This remaining half is seen as a pellucid spot in the living egg, and on sections as a clear, round body, composed of nuclear substance, and containing two nucleoli. I consider therefore that in Clepsine the proof is as complete as it can well be for opaque eggs, that *a part of the germinal vesicle persists as a nuclear element.*

(*b*) **Pronuclei** (Van Beneden) and **Cleavage-nucleus** (Hertwig).

The cleavage-nucleus, one or the other pronucleus, and pronucleoli, have been seen at different times in the past; but their

origin and relation to each other have remained unknown up to the most recent times. It would hardly be profitable to give here the long list of cases which I have catalogued, in which some of these bodies have, possibly, probably, or certainly, been observed, but entirely misapprehended; I shall therefore refer only to the more important of those bearing a recent date.

The first accurate account of the origin of the cleavage-nucleus was given in 1874 by Auerbach ($\frac{3}{202 - 214}$). This author saw two nuclear bodies arise at opposite poles of the egg (Ascaris and Strongylus), in each of which two or three nucleoli soon appeared. These two bodies approached, and, coming in contact with each other near the centre of the ovum, performed a rotation of 90°, on an axis passing through the median point of contact, perpendicular to the longitudinal axis of the egg. A complete fusion of these two pronuclei followed this rotary movement—thus giving rise to the cleavage-nucleus ("central nucleus"). Bütschli ($\frac{24}{101\ 4}$) published as early as 1873 an account of the same bodies, seen in the egg of Rhabditis dolichura; but he was uncertain whether the pronuclei arose independently of each other, or by division of a single nucleus, and left it equally undecided in regard to their complete union. Early in 1875 appeared a preliminary communication from Bütschli ($\frac{25}{201}$), containing results obtained in the study of several genera of nematoids (Tylenchus, Cephalobus, Rhabditis, Diplogaster, Cucullanus) and two mollusca (Lymnaeus, Succinea). This communication confirmed the statements of Auerbach in regard to the coalescence of the pronuclei, but raised a doubt in regard to their normal number, which according to Auerbach is two, an opinion confirmed by the latest papers of Fol and Hertwig.

In the same year the beautiful work of Strasburger was published, in which the cleavage-nucleus ("Keimkern") is represented as arising from two pronuclei ($\frac{154}{212}$, Phallusia mamillata). Shortly after (1875) came the very important investigations of Oscar Hertwig on *Toxopneustes lividus*. Auerbach ($\frac{3}{248}$) had already compared the union of the pronuclei with the "*Copulation zweier Individiduen, oder wenigsteus zweier Zellen,*" for the purpose of propagation; but it was reserved for Hertwig to show, with little less than positive evidence, that the two pronuclei ("Spermakern," and "Eikern") represent *male* and *emale* elements, and the fact was thus distinctly formulated ($\frac{706}{383}$): "Der unmittelbar vor der Furchung in der Eizelle vorhandene einfache Kern, um welchen die Dotterkörnchen in Radien angeordnet sind, ist aus *der Copulation zweier Kerne* hervogegangen."

Almost simultaneously with Hertwig's paper, appeared the not less important preliminary communication from van Beneden (16), on the maturation, fecundation, and development of the

mammalian ovum. The object of study (egg of rabbit) was much more difficult to follow than those selected by Hertwig, but the same conclusion was reached in regard to the *sexual character* of the pronuclei, although not in regard to the exact origin of the same. These points of difference have been discussed at length by van Beneden (16, 1876), and so far as they concern the structure of the male pronucleus, have already been noticed.

According to the latest paper on this subject by Hertwig ($\frac{7}{274}$-$\frac{2}{276}$) the female pronucleus represents that half of the amphiaster that remains after the formation of the last polar globule.

On the other hand, van Beneden ($\frac{16}{154}$) believes that there is no "genetic connection between the germinal vesicle, or one of its parts, and the embryonic nucleus" (cleavage-nucleus). I have already shown that this opinion is entirely incompatible with what takes place in the egg of Clepsine, and it is evidently untenable in view of the researches of Fol. I shall venture to point out what seems to me an inconsequence in van Beneden's statements, which, as I think, makes it quite unnecessary to accept his conclusion on this point. He affirms ($\frac{16}{154}$) that in the rabbit a part of the germinal vesicle, the nuclear fluid ("liquid clair") remains in the egg *after* the expulsion of the polar globules, and becomes "confounded with the cortical substance of the ovum, which from this moment is no longer distinguishable from the medullary substance." All this happens before fecundation ($\frac{5}{9}$). Soon after fecundation two pronuclei are found, one of which ("peripheral pronucleus") arises in the superficial layer, and the other ("central pronucleus") in the central layer of the vitellus.

How then is it possible to know that neither of these pronuclear bodies contains any of the "liquid clair" that was confounded with the vitellus? If this is not known, then the possibility of a genetic relation between the germinal vesicle and the cleavage-nucleus, which is the product of a coalescence of the two pronuclei, still remains. Is this possibility denied because the nuclear fluid mixed with the protoplasm of the ovum? How then in the case of the starfish ($\frac{16}{178}$) can Van Beneden believe that the polar globules have the same origin as in the rabbit? It is asserted positively that *every* part of the germinal vesicle (starfish) dissolves and becomes diffused through the vitellus; and yet van Beneden does not hesitate to say—"Since in the starfish directive bodies are eliminated by the yolk, it is probable that in the Echinodermata, as in Mammalia, these bodies are formed by the nucleoplastic substance on the one hand, and by the nucleolar matter, joined to the substance of

the membrane, on the other hand" ($\frac{16}{178}$). Notwithstanding then the complete dissolution of the entire germinal vesicle, it is regarded as "probable" that between it and the polar globules a genetic connection exists, precisely as in the rabbit. How van Beneden can believe that such a connection is *probable* in the one case (polar globules) and *impossible* in the other (embryonic nucleus), is quite incomprehensible.

I have succeeded, I believe, in making evident a direct histological continuity, in Clepsine, between the germinal vesicle and the cleavage-nucleus. Bütschli and Hertwig have done the same for Nephelis. The same genetic bond has been traced by Bütschli in Nematoids and Molluscs, and by Hertwig and Fol for Echinoderms. I have not been able to determine the origin of the male pronucleus in Clepsine, and Hertwig failed to produce the positive proof for his theory of its origin in Toxopneustes.

This positive proof, however, is no longer wanting, thanks to the successful researches of the distinguished naturalist of Geneva. Fol's discoveries confirm in the most positive and decisive manner the opinion of Hertwig; and, as they fill up the gap in my own observations, deserve special notice in this connection. A brief account of his study in Echinus and Asterias, which will appear in a large memoir before this paper goes to press, has been given in several papers, published in the first half of 1877, in the 'Comptes rendus,' and in 'Arch. des Sci. de la Bibliothèque universelle' (32, 33, 34, 35, 80). In order to follow the process of fecundation from the moment when the spermatozoa first come in contact with the eggs, Dr. Fol placed a drop of water containing spermatozoa on the object-glass of his compressorium, and another drop containing the eggs on the under surface of the cover of the same. The two parts of the compressorium were adjusted under the microscope, so that the moment of contact was under perfect control. These precautions remove many doubts that might otherwise arise in regard to the value and importance of these investigations.

As the cover of the instrument is pressed down, the two drops of water are brought together. The spermatozoa approach the egg and apply their heads to its gelatinous envelope. Soon one of them plunges deeper, and by the time it has passed through half the the thickness of the envelope, a small disc of transparent substance forms in the surface of the vitellus. The centre of this disc soon shows a rounded protuberance ("bosse hyalin"), which, rising higher, assumes the form of a cone, from the apex of which a filament of protoplasm rises to meet the spermatozoon. As the spermatozoon advances it becomes more and more indistinct, apparently fusing with the substance of the cone which sinks into the surface of the vitellus. At

this moment the tail of the spermatozoon is seen projecting from the yolk, and the point of penetration has become a clear spot ("tache clair") which soon forms the centre of a radial system. This body is the *male pronucleus*. As soon as the spermatozoon has entered the yolk, the clear disc, from the centre of which the cone arose, begins to extend in all directions from the point of penetration, and ends by enveloping the entire yolk. This envelope is the vitelline membrane. In normal cases this membrane forms very rapidly, thus preventing the admission of more than one spermatozoon into the yolk. In abnormal cases, when it forms more tardily, several spermatozoa reach the yolk, and the membrane forms from as many centres. In such cases the several male pronuclei unite one after the other with the female pronucleus, but the cleavage is always abnormal.[1]

Strasburger (155) has studied the same phenomena in the vegetable kingdom with no less success than Fol in the animal kingdom. From these interesting researches of Strasburger it is evident that the male pronucleus in Phanerogams (Picia and Orchis) forms at the expense of the contents of the pollensack, just as in animals it arises at the expense of the spermatozoon.

Hoffmann $\frac{7\,7}{3\,4}$ was the first to report pronuclei for Clepsine, but he was able to give no account of the origin of either of these bodies, nor of their destination, and failed to find the pronucleoli.

The investigations thus far made, justify, I believe, the following general conclusion: *fecundation, throughout the organic kingdom, consists in the coalescence of corresponding parts of a pair of sexually-differentiated cells, to form a unicellular asexual individual. It is a re-union, not of exhausted, but of complementary energies.*

c. **Polarity.** Hatschek ($\frac{6\,8}{5\,2\,4}$, Pedicellina) has called attention to the universality and early appearance of polar differentiation in the egg; and Ray Lankester ($\frac{1\,0}{3\,8}$) has pointed out the importance of the same in the evolution of multicellular organisms. Its universality is attested by the production of polar globules, by discoidal cleavage (Aves, Reptilia, Pisces, Cephalopoda), and by unequal cleavage (Amphibia, Petromyzon, Mollusca, Vermes). Even in cases of superficial or peripheral cleavage (Insecta) such a differentiation is evinced both by the shape of the egg and by the formation of *pole-cells*. The manifestation of this polarity in the egg always follows the transformation of the germinal

[1] June 19, 1878. Selenka ('Befruchtung des Eies von *Toxopneustes variegatus*,' Engelmann, 1878) and Calberla ("Der Befruchtungsvorgang beim Ei von Petromyzon," 'Zeitschr. f. wiss. Zool.,' B. xxx, p. 437) have both traced the male pronucleus to a single spermatozoon.

vesicle into the archiamphiaster, which event marks the beginning of the period of proliferation. It is therefore initiated and, in all probability, sustained throughout its various phases by chemico-physical changes originating in the nucleus itself.

The most remarkable example of this polarity hitherto described, is furnished by the egg of Clepsine. The period of unipolar activity is introduced and maintained throughout at the expense of nuclear changes; and the period of bipolar activity, beginning with the formation of the pronuclei, reaches its climax soon after their union, and then gradually subsides. The scene of action is now transferred from the poles of the egg to the poles of the cleavage-amphiaster. Here as before, the energy displayed is at first weak, but rises gradually and culminates in sundering the hemispheres of the egg. A repetition of the same process divides the hemispheres into four quadrants. It is interesting to note that these phenomena are displayed successively at the poles of different axes, each of which cuts the preceding at right angles.

(1.) *Polar Rings.*—It is very remarkable that nothing has yet been observed by embryologists which can with any degree of certainty be compared with the polar rings before described. It is hardly possible to believe that these rings and rays, in which at one epoch the vital energies of the egg seem concentrated, have no parallel in the eggs of other Metozoa. They would be easily overlooked in transparent eggs and recognized with difficulty in opaque eggs, if faintly expressed. The fact that they form so conspicuous a feature of egg-life in Clepsine, makes it extremely probable that they occur in others, at least in closely allied animals, such as Nephelis, Hirudo, &c. Kowalesky ($\frac{8.5}{1\cdot2\cdot\cdot1\cdot3}$) has described a clear elliptical spot, found on one pole of the egg of Euaxes, which I am inclined to believe represents the remains of a ring. This body was found on fresh laid eggs, and was supposed to represent the germinal vesicle, which was no longer recognizable in the interior of the egg. This spot marks that side of the egg on which the cleavage-depression first appears, and is always found on the larger part at the close of the cleavage—precisely as in Clepsine. Its disappearance after the first division is thus described:—"Das Verschwinden konnte leicht beobachtet werden; die Ränder des hellen Fleckes wurden immer mehr und mehr unregelmässig, so zu sagen zerfressen, da in das helle sie zusammen-setzende Protoplasma feine Dotterkörnchen eintraten; der ganze Inhalt des Fleckes trübte sich weiter und verschwand endlich vollständig." A small globule was found resting on this body in eggs treated with chromic acid, and was interpreted as an artificial product, as it was not seen on fresh eggs. The entire description corre-

sponds so exactly with the appearance of the oral ring-disc in Clepsine, on which the polar globules rest, that I feel warranted in assuming, in the absence of proof to the contrary, that the "clear spot" in Euaxes is homologous with the ring-disc of Clepsine. If this be the case, then it is more than probable that the globule which Kowalevsky supposed was produced by the action of the acid, was one or both of the polar globules. That this interpretation is correct is all the more probable from the fact that the cleavage and embryo-formation in Euaxes are strikingly similar to the same in Clepsine.

2. *Ring-rays.*—So far as the ring-rays are concerned, very little has been described, which offers more than a distant analogy. The irregular stellate ("dendritisch—sternförmig") figure seen by Brandt ($\frac{2}{5}\frac{3}{9}\frac{}{0}$, Lymnaeus) may be better compared with the radial arrangement around one pole of an amphiaster than with these ring-rays. Some of his figures of cleavage-nuclei present a striking resemblance with the later stages of the ring-discs.

Some very interesting radial arrangements in the eggs of spiders have been observed by H. Ludwig ($\frac{1}{4}\frac{1}{7}\frac{2}{4}$).

These columns of deutoplasm ("Deutoplasmasäulen") arrange themselves in the form of a rosette around the cleavage-nucleus, and therefore seem to bear a close relation to the nuclear ray-systems. Kleinenberg ($\frac{8}{4}\frac{1}{9}$) mentions peculiar pseudopodial processes of the yolk which accompany the early stages of cleavage in Hydra. It is remarkable that these so-called pseudopodes appear almost *simultaneously* with the cleavage, and *only* on that pole of the egg where the cleavage depression first appears. Why do they not accompany the cleavage in its entire circuit? I am inclined to believe that the "folds" observed by Metschnikoff ($\frac{1}{4}\frac{1}{0}\frac{7}{}$) on the walls of the cleavage-groove, in the egg of Siphonophores (Epibulia aurantiaca) are the same thing as the pseudopodes of the hydra-egg, only less intensely expressed.

3. *Faltenkranz.*—The same remark applies to the well-known cleavage-folds (corona plicarum) of the amphibian egg, which were discovered in 1824 by Prévost and Dumas, and afterwards observed by Von Baer, Götte, and others, and made a subject of special study by Reichert and Max Schultze. Three explanations of these folds (Faltenkranz, Reichert) have been given.

1. Reichert ($\frac{1}{3}\frac{3}{3}\frac{7}{4}\frac{}{3}$) in harmony with his theory that cleavage is only a setting free of preformed cells, regarded them as wrinkles produced in the closely adhering membranes of cells in process of separating. "Das Entstehen des Faltenkranzes is nur dadurch zu erklären, dass die beiden ersten, eng an einander gepressten und fest adhärirenden Furchungskugeln bereits vor

dem Auseinanderweichen vollständig von elastischen Hüllen umgeben seien, und dass die letzteren, indem die Kugeln, *wahrscheinlich in Folge der Schwere* (!), mit ihren Randpartieen sich allmälig trennen und abrunden, durch die ungleichmässige and schwierig erfolgende Lösung der Adhärenz ungleichmässig angespannt und zur Faltenbildung veranlasst werden ($\frac{1\,3\,0}{1\,3\,4}$).

2. Max Schultze ($\frac{1\,4\,8}{1\,0}$) maintains that cleavage results from contractions of the yolk, and that these contractions create in the viscid cortical layer of the vitellin the so-called "folds." "Quum vero vitelli substantia imprimis corticalis glutinosam quandam et viscosam praebeat consistentiam, non mirum videri protest, eodem tempore quo sulcus contractilitate vitelli paullatim exoriatur, incisuras apparere minores, plicas seu rugas sulco vicinas et recto angulo in sulcum vergentes."

3. Götte ($\frac{4\,1}{5\,8}$) interprets them as a mere "Ausdruck für die Ausgleichung an der Oberfläche des dickflüssigen Dotters . . . . gleichwie etwa bei einem Stich in eine teigige Masse, oder bei einer Einschnürung derselben, Falten enstehen."

Three facts seem to be irreconcilable with the above interpretations.

1. These "Falten" appear only on one pole of the egg.

2. They are at right angles to the cleavage-groove only in the centre, diverging more and more towards either end of the same. In consequence of this divergence a radial system is formed, or as Reichert ($\frac{1\,3\,7}{5\,3\,4}$) expresses it, a *long star*.

3. The lively play ("lebhaftes Spiel," Götte) of these "Falten."

According to the explanations of Reichert, Schultze and Götte, the "Falten" should accompany the cleavage-furrow entirely around the egg, and should run at right angles to the plane of division. This, however, is not the case. To what class of phenomena then do they belong? Are they not to be classed with those now well-known radial arrangements of the yolk during cleavage, and consequently to be referred to nuclear influence. Their appearance on one side of the egg, their radial arrangement, and their behaviour, all seem to suggest this interpretation. The pseudopodes of the Hydra-egg can be interpreted in the same way. I have already pointed out the fact that the amphiastral radiations reach their maximum intensity at the time the cleavage begins. It is at this time that the "pseudopodes" appear in the egg of Hydra, and the "Falten" in the ovum of the frog. Furthermore, my sections show that the first cleavage-amphiaster lies nearer the oral than the aboral pole, and it is on this account that the cleavage-depression begins sooner on the upper than on the lower pole. The same is probably true in all cases where this manner of cleavage

occurs. The relative distance of the amphiaster from the upper pole of the egg is about the same in Clepsine as in the frog, and the cleavage progresses from the upper (oral) to the lower pole alike in both cases. In the case of Hydra, where this one-sided cleavage is carried to the extreme, it is highly probable that the amphiaster lies much nearer the surface of the egg than in Clepsine or the frog, and the radial influence of the nucleus is accordingly much more strongly manifested, producing the pseudopodes. The nearer the amphiaster lies to the middle of the egg, the less marked will be these peripheral manifestations. If it lie exactly in the centre, then the cleavage will appear in a perfectly regular manner, the meridian depression encircling the egg from the outset and progressing steadily from its periphery to its centre. That no pseudopodes, folds, or wrinkles make their appearance in such cases of uniform cleavage, finds an easy explanation in the statements just made.

d. **Polar Globules** (Robin).—Flemming (38), Fol (41), and Bütschli (27) have given more or less complete bibliographical references on this topic, which renders it unnecessary for me to go beyond the line of my remarks in this direction.

The evidence in favour of the general occurrence of polar globules in the animal kingdom is rapidly accumulating. The earlier observations of O. Hertwig and Fol seemed to raise a doubt in regard to Echinus and Asterias, but the latest investigations of these authors, as well as those of van Beneden and Giard, prove that these globules are of general occurrence among Echinoderms.[1]

Although, according to the statements of different authors, the *number* of polar globules varies from one to four or five, yet it is evident that *two* is by far the more general, and perhaps the normal number. This is the case in some Coelenterata, in Hirudinea (and many other worms), Echinodermata, a large number of Mollusca, Petromyzon, and Mammalia. O. Hertwig ($\frac{7}{2}\frac{7}{4}\frac{1}{3}$) has shown that two directive cells are normally produced in Nephelis—whilst the third, which is sometimes seen, arises by a division of the first. Later these globules were seen to unite into a single discoidal body. This division and coalescence have been observed in other cases, and they furnish an explanation of the variation in number.

[1] I must here call attention to an oversight of O. Hertwig. He has stated in at least two places ($\frac{7}{5}\frac{1}{9}$, $\frac{7}{2}\frac{7}{7}\frac{2}{6}$) that other authors have failed to find polar globules in the egg of Toxopneustes. Hertwig seems to have entirely overlooked a classic memoir on the embryology of Echinoderms by A. Agassiz (1), published as long ago as 1864. Agassiz has not only figured these globules, but distinctly states (p. 7) that he has found them in both Asteracanthion and Toxopneustes.

The occurrence of polar globules is still a matter of doubt in Birds, Reptiles, Amphibians, most Fishes, Tunicates, Arthropods, and Rotifers. The elimination of the entire germinal vesical, as represented by Balfour and Oellacher, for Birds and Fishes, can hardly be compared to the production of polar globules by amphiastral division. The same may be said of the thin veil of substance found on the animal pole of the amphibian egg, after the disappearance of the germinal vesicle.

The "*pole-cells*," which appear on the aboral end of the egg of insects, notwithstanding that their genetic relation with the germinal vesicle, is now an established fact ($\frac{1}{4}\frac{1}{1}\frac{3}{1}$), cannot, in consequence of their forming the basis of the sexual organs (Miastor, Chironomus, $\frac{1}{4}\frac{1}{2}\frac{4}{1}$), be compared with the directive corpuscles.

The so-called "*testa-cells*" ("Testa-tropfen," Semper) of Ascidians arise, according to Kupffer, Metschnikoff, and Semper, from the yolk—according to Stepanoff and Kowalevsky, from the follicle-cells. In neither case case can they be compared, as Semper ($\frac{1}{1}\frac{5}{0}\frac{2}{1}$) has done, with the polar globules, since they arise not only before fecundation ($\frac{0}{1}\frac{2}{2}\frac{2}{4}$), but also before the transformation of the germinal vesicle ($\frac{0}{3}\frac{3}{0}\frac{3}{0}$, $\frac{8}{6}\frac{6}{0}\frac{6}{7}$). Semper is equally unfortunate in this comparison in other respects. He declares that the "testadrops" arise simultaneously with the cleavage; but in no case is this true of polar globules. He states furthermore that neither the "testa-drops" nor polar globules have nuclei, and that they both move freely around the egg, all of which we now know is entirely incorrect so far as it concerns the polar globules.

Various opinions have prevailed in regard to the morphological value of these corpuscles. Older authors (Dumortier, Pouchet, van Beneden, sen., Reichert, Kölliker, Vogt, Bischoff, Lovén, &c.) from the time of their discovery by Carus in 1824 up to 1848, supposed that they represented either the germinal spot or the germinal vesicle. Rathke ($\frac{1}{1}\frac{3}{5}\frac{5}{8}\frac{5}{9}$, 1848), whose views received the assent of most naturalists up to a very recent date, maintained that they were unimportant *drops of liquor vitelli*, expelled by contractions of the yolk during cleavage, precisely such as are seem to come out of the egg if it be artificially pressed. According to Robin ($\frac{1}{2}\frac{1}{0}\frac{4}{3}\frac{3}{4}$) they originate pecisely as the first four ectoderm-cells (Hirudinea) and the blastoderm-cells (Insects), all of which he represents as arising by a process of "budding" from the protoplasm of the egg, without the aid of nuclear elements. "En résumé, c'est par le mode d'individualisation des éléments anatomiques, appelé *gemmation* et s' opérant à l'aide et aux dépens de la substance hyaline du vitellus, que naissent les *globules polaires*" ($\frac{4}{2}\frac{3}{9}$). Fol (41) and Bütschli (27) were the first to show in a decisive manner a genetic relation between the polar globules and the germinal vesicle; and

O. Hertwig ($\frac{7}{2}\frac{1}{7}$) was the first to prove that they are the *morphological equivalents of cells*.[1]

In regard to their physiological signification, little or nothing is known. Friedrich Müller ($^{1}\frac{2}{3}^{1}$) supposed that the direction of the cleavage was determined by the influence of these corpuscles, and therefore named them "*versiculae directrices*." That they play no such important rôle in the developement of the egg, is now generally admitted. That they have no such function appears evident from the fact that in eggs which have no membrane they escape into the surrounding fluid. The fact that the polar globules have no known morphological or physiological relation with the future embryo, has led some authors to interpret them as refuse material, which is thrown off as excrement ("Koth des Eies," Selenka, $\frac{1}{4}\frac{5}{4}\frac{0}{4}$, $\frac{1}{1}\frac{5}{0}\frac{1}{7}$; "Corpuscule excrété," Fol. $\frac{4}{1}\frac{1}{0}\frac{1}{7}$), or ejected by way of defecation (Semper $^{1}\frac{5}{3}\frac{2}{2}$). That no such interpretation is admissible has already been clearly shown by Bütschli ($\frac{2}{1}\frac{7}{7}\frac{7}{3}$).

The question as to the *historic origin* of the polar corpuscles is undoubtedly one of considerable interest, and has already begun to engage the attention of investigators. Owing to the fact that we are but just beginning to see what really takes place during the process of fecundation, few naturalists have ventured to approach this question from a phylogenetic standpoint. Theories however have their value even when based on few and imperfectly understood facts; for we never approach the truth more rapidly than when we are "hunting down" a theory. Rabl, who was the first to attack this problem, assigns to the polar globules a cenogenetic origin. These "*elastic balls*," he says ($\frac{1}{2}\frac{2}{2}\frac{8}{3}$, $\frac{1}{3}\frac{2}{3}\frac{9}{7}$), are "*nothing but protective organs of the embryo, acquired in adaptation to unequal cleavage*." This theory, ingenious as it may be, seems to have no basis whatever in fact. There is not the slightest evidence that the embryonic cells are protected by these globules, nor is there any evidence that they *need* protection against a protective envelope. The egg of Clepsine furnishes a good example of unequal cleavage and is a fair case for studying the point in question.

The formation of the first embryonic cells shows plainly that they are not easily injured by pressure. The first cell is nearly round at the completion of its formation, but, while the second cell is forming, is pushed out of its original place and so pressed into one of the large cleavage-spheres, that it is difficult for a time to recognise its outline. Shortly after the production of

[1] Brandt ($\frac{2}{6}\frac{3}{0}\frac{3}{1}$) recognised the cell-character of the polar globules, but thinking that they were wholly derived from the germinal vesicle, was compelled to regard this vesicle as a cell. This view of the germinal vesicle is also entertained by Bischoff ($\frac{1}{1}\frac{9}{2}$) and Villot ($\frac{1}{3}\frac{5}{-}\frac{9}{4}$).

the first four embryonic (ectodermic) cells, we find them wedged into a somewhat conical space on the oral pole of the egg, having passed from the globular to a pyramidal form. Is it probable that cells can undergo such pressure and still be liable to injury from contact with the membrane? Besides, during the cleavage period, the membrane is removed from the oral pole of the egg by a distance equal to several diameters of the polar globules, and could not therefore be supported by these globules. There is no time in the whole history of these corpuscles when they could be said to contribute to the maintenance of the space between the membrane and egg. That this space increases or diminishes entirely independently of these "elastic balls," is well attested by the fact that during the elimination of the first polar globule it is sometimes present on both poles of the egg, and by the fact that this globule after its expulsion, is thrust back into the yolk till it is quite out of sight. Bütschli (30 37) has called attention to the fact, that in Paludina and Neritina, and in all cases where eggs unprovided with membrane swim about in the fluid contained in the cocoon, the polar corpuscles could afford no protection to the embryo. The same is true of many eggs which are not laid in cocoons (Echinus and some Cœlenterates), and of all eggs where the membrane stands from the outset at a great distance from the yolk (many Molluscs).

Bütschli (2 7 2 7 1 6, 1 9, 30 37) comparing the process of fecundation with the conjugation of Infusoria, claims for the polar globules a *palingenetic origin*. In some cases (Vorticella, Stylonychia) conjugation results in a complete coalescence (copulation, Engelmann) of two unicellular individuals (male and female); but in most cases the conjugating individuals may be regarded as hermaphrodite (O. Hertwig 70 386, Engelmann 35 306, Bütschli 27 216), and during their temporary union a reciprocal fecundation takes place, the "nucleolus-segments" with a little protoplasm being interchanged (according to Engelmann). Bütschli (27 83) has observed this interchange of nucleoli in Paramœcium Bursaria and P. putrinum. Both Hertwig and Engelmann regard the nucleolus as *a male element* and the nucleus as *a female element;* and Hertwig sees in the pronuclear-stage of the egg a repetition of the hermaphrodite condition of the Infusoria. These authors differ widely in regard to the morphological value of the nucleolus. Hertwig compares it with the *Spermakern*, and the nucleus with the *Eikern;* Engelmann, on the other hand, says that "the nucleus *plus* the nucleolus is homologous with the ordinary cell-nucleus." Bütschli (26 31, 27 71), who first advanced the idea of the nuclear character of the "nucleolus," admits (27 31) the plausibility of Hertwig's comparison, provided the exchange of nucleoli be an event of normal occurrence; but

concludes that it is untenable in view of the so-called bud conjugation ("knospenförmige Conjugation") of Vorticella. Here, where a complete and permanent fusion of two individuals occurs, we should expect (according to Hertwig's theory) to find only a nucleolus in one (the male) and *only* a nucleus in the other (the female). This however is not the case, each individual possessing both nucleus and nucleolus. While this fact seems to render the view of Hertwig untenable, it is quite reconcilable with the theory of Engelmann, that nucleolus plus nucleus are equivalent to the ordinary nucleus, the only difference being that in one case both elements remain united, while they differentiate and separate in the other.

It is first of all important to know which of the above modes of conjugation is to form the basis of comparison. Shall it be that of *complete and permanent fusion*, such as has been observed in Stylonychia ($\frac{3\,5}{6\,1\,3}$), and in Vorticella nebulifera ($\frac{2\,7}{2\,3\,9}$), where corresponding parts coalesce? or shall it be that of incomplete and temporary union, which consists in an exchange rather than a fusion of elements? With reference to the latter and more common mode of conjugation, Bütschli has ventured to compare the ejection of the nucleolus ("secondary nucleus," Infusoria) with the production of polar globules by the egg-cell ($\frac{2\,7}{2\,1\,6}$). "Wir sahen bei einer Anzahl Infusorien in Folge der Conjugation, eine völlige Ausstossung des secundären Nucleus stattfinden und haben anderseits beobachtet, dass nach der Befruchtung der Kern der Eizelle eliminirt wird. *Wir würden nicht anstehen, diese beiden Erscheinungen in näheren Zusammenhang zu bringen*, wenn eben bis jetzt eine grössere Uebereinstimmung darüber erreicht wäre, ob die Kernausstossung der Eizelle thatsächlich eine Folge der Befruchtung sei."

The objection to this theory, here anticipated by Bütchli, has recently been placed in a stronger light. The fact that the ejection of nucleolus-segments is a *consequence* of conjugation, while the production of polar corpuscles is, at least in a few well ascertained cases, entirely *independent* of fecundation (this is always the case with the "Canal-cell," the supposed homologue of the polar globule), seems to be quite irreconcilable with the above theory.

Minot (119), who appears to have accepted the view suggested by Bütschli, sees a confirmation of the same in the formation of the *Kernspindle! How* the formation of a nuclear spindle confirms the supposed homology between polar globule and the nucleoli of Infusoria is not explained. It simply proves the fact that we have to do with nuclear substance in both cases, but confirms the homology in question no more than it confirms a homology between ectoderm-cells and entoderm-cells. Bütschli

($\frac{2\,7}{2\,3\,0}$), in the supplement to his work, concedes that the polar globules are in some cases formed parthenogenetically, and finds in this mode of production something analogous to the process of rejuvenescence in Diatoms (Auxosporenbildung), which in certain cases is accomplished without the union of two individuals, by a single Diatom. According to this view of the matter, the production of polar globules is a process by which the nucleus is rejuvenated (Verjüngungs process des Kernes)—a phenomenon, not of the maturation of the egg, but of the earliest phase of its development, which may take place either parthenogenetically, or under the influence of fecundation. Its meaning is therefore to be sought *in der Entfernung eines Theils des Eikerns* ($\frac{3\,0}{2\,3\,7}$).

This may be correct, but it is not the only interpretation, nor is it, as I believe, the one most in harmony with the phenomena of conjugation, the characteristic feature of which is the *addition* rather than the *removal* of substance. This is well illustrated by the first of the above modes of conjugation which, as Bütschli himself claims ($\frac{2\,7}{3\,3\,1}$), comes nearest of all to the process of fecundation in Metazoa. In the case of partial conjugation there is no diminution, but simply an interchange—a replacement of substance. The object in both cases appears to be the reunion of complementary forces, that have been sundered in the course of multiplication by division.

The process is then fundamentally the same in both instances, the second case being, so to speak, an abridgment of the first.

Now, impregnation in both plants and animals consists in a complete and permanent fusion between corresponding parts of two unicellular individuals, fully analogous to what happens in the first mode of conjugation, with this difference, that polar globules and "canal cells" are produced before the fusion begins, or at least before it is completed. In what relation then do polar corpuscles stand to impregnation? That there is no necessary connection between them is in harmony with the absence of such corpuscles in conjugation. I believe that the formation of the "canal cells" (Muscineæ, Cryptogamæ vasculares, Coniferæ), furnishes a clue to the above question. Hertwig, Strasburger, Bütschli, Fol, and others, see in these cells a pendant of the polar corpuscles. The formation of these canal-cells is everywhere essentially the same, and may be briefly stated. The entire archegonium arises from a single peripheral cell. This cell, in the ferns, for instance, divides first into an outer and inner cell, the plane of division being parallel to the surface of the prothallium. The inner cell divides again in the same manner as before, thus giving three cells, an outer, inner, and middle cell (central cell). The

outer cell divides into four, each of which gives rise to a column of cells which together form the neck of the Archegonium. The inner splits into a number of cells which form the body-wall of the archegonium, within which lies the central cell. The latter divides twice, producing the two canal-cells and the egg.

Of all these cells having a common pedigree, only one—the egg—is destined to survive. The canal-cells are the first to suffer disintegration, after which impregnation takes place. Is there anything in all this to justify the assumption that the canal-cells are produced for the purpose of removing a part of the egg-nucleus? Why assign such a function to these cells, to the exclusion of all the others, since they all have the same origin, and are produced in precisely the same manner? The case is plain; the canal-cells stand at the end of a series of asexual generations; the impregnated egg begins a new series which will end like the preceding. It is easy here to find a parallel with the events initiated by conjugation.

Just as in plants, fecundation is followed by cell-proliferation culminating in sexually differentiated cells, destined to copulate and renew the cycle of changes, all other products of the proliferation (canal-cells with the rest) eventually dying out; so in Infusoria conjugation is succeeded by reproduction by fission, the ultimate products of which are sexually differentiated individuals. The chief difference here is, that in one case all (?), in the other only a comparatively few, individuals become capable of gamic reproduction; but this difference, having reference only to a specialisation of function which necessarily accompanies the development of a multicellular organism, authorizes no fundamental distinction. In the Metazoa, likewise, a gamic cell-generation is followed by a line of agamic generations, the last of which are the small cells called by Robin polar globules. With the production of these globules we arrive at the sexually ripe egg. In accordance with all this I interpret the formation of polar globules as *a relic of the primitive mode of asexual reproduction*, which normally precedes fecundation, and is therefore no part of the process of impregnation. This interpretation accounts for the otherwise inexplicable fact that amphiastral divisions of the nucleus introduce the formation of the directive cells, and is in harmony with the absence of such cells among Infusoria, and their general occurrence among plants and animals.

The two poles of a nuclear spindle are the exact counterparts of each other, and the division of the archiamphaster cannot, any more than the division of the primary cleavage-nucleus, be regarded as a *removal* of nuclear substance. The

two processes are identical, and, if in one case the object is reproduction, how can we say that in the other it is simply to get rid of a part of the nucleus? According to the view I have taken, the production of polar globules, or something very analogous, in the formation of spermatozoa, as Strasburger ($\frac{154}{1921933093133}$, $\frac{155}{511-513}$) has shown, is nothing surprising. Such effete formations are the results of abortive efforts to reproduce in the original way. I should be compelled perhaps to abandon this theory if polar globules should be found in eggs that develope, either exceptionally (moths) or regularly (case of drones among bees), without impregnation. In the case of Neritina (Bütschli $\frac{30}{233-5}$) the unfecundated eggs are said to produce polar globules, and then, after performing a number of irregular cleavages, break up and serve as "food-material" for the single developing ovum. There are two unsettled points here. According to Professor Lankester ($\frac{97}{30}$), only one egg is subject to cleavage; and Bütschli admits an uncertainty in regard to whether both sorts of eggs are impregnated or not. Should it turn out, however, that in this case *unfecundated eggs* both produce polar globules and cleave, it would then be possible to explain the anomaly on the supposition that an event palingenetically introduced tends to repeat itself even after the cenogenetic cancellation of the factor by which it was introduced. This is illustrated by the appearance of cleavage in the egg of birds and Echinoderms, even when fecundation is omitted. We should then have to assume that originally all the eggs of a capsule developed embryos.

IV. Cleavage.

The importance of accurate and detailed study of the cleavage process is well illustrated by the brilliant results attained by Kowalevsky (Euaxes), van Beneden (Rabbit), and Rabl (Unio). In the fecundated egg slumbers potentially the future embryo. While we cannot say that the embryo is predelineated, we can say that it is predetermined. The "Histogenetic sundering" of embryonic elements begins with the cleavage, and every step in the process bears a definite and invariable relation to antecedent and subsequent steps; or, as Bergmann and Leuckart have expressed it, "Jeder einzelne Entwicklungsmoment is die nothwendige Folge des vorausgegangenen und die Bedingung des folgenden" ($\frac{10}{19}$) It is, therefore, not surprising to find certain important histological differentiations and fundamental structural relations anticipated in the early phases of cleavage, and foreshadowed even before the cleavage begins.

The egg is, in a certain sense, a quarry out of which, without waste, a complicated structure is to be built up; but more than

this, in so far as it is the architect of its own destiny. The raw material is first split into two, four, or more huge masses, and some or all of these into secondary masses, and some or all of these into tertiary masses, &c., and out of these more or less unlike fragments the embryonal building-stones are cut, and transported to their places of destination. The cleavage in Clepsine has been described by Grube, Rathke, and Robin, all of whom have fallen into some grave errors, in consequence of which the cardinal points of the process were missed. On this head I can speak with the fullest assurance, for I have followed the cleavage in four species, and have seen it many times over in two of those species. What I have seen in living eggs is verified in the most positive manner by my sections.

a. **First two Meridional Divisions** (Pl. XII, figs. 12-19).—At the approach of the first division, as we have before remarked, the egg has a long elliptical form, flattened at the poles. The aboral ring-disc is reduced to a mere point, slightly stellate, and the oral disc has assumed the form of a crescent, the two horns of which point towards the plane of the advancing division.

The egg takes an oblong, slightly biscuit-shaped form as the cleavage depression passes (figs. 13 and 14, 3 h.) gradually from the upper to the lower pole. In rare cases this cleavage encircles the entire egg from the moment of its appearance. By the time the groove has passed about one third of the distance towards the centre of the egg; the plane of division takes the form of a fine line, owing to the fact that the two segments are checked in their movement away from each other by the egg-membrane. Fig. 15 (3 h. 30 min.) represents the egg after the completion of the division. It will be observed that the walls of the groove approach each other sooner at the upper than at the lower pole. A few minutes later the two segments appear to be separated only by a fine but well-defined line, and 30 minutes later (4 h.) the egg presents the oval form of fig. 16. The remnant of the oral ring-disc, seen on the larger segment, has a clouded appearance, and is without the slightest indications of rays. A trace of the aboral disc is sometimes still visible, but in most cases no longer recognisable. Thirty minutes later the remnant of the upper disc is considerably smaller and less distinct, while the white border, which has encircled the ring substance from the beginning, has grown larger, reaching over to the corresponding pole of the smaller segment. At this time the whole oral pole seems mantled with a greyish-white substance, the character of which has before been indicated. The second division begins from 60 to 90 (5 h.) minutes after the completion of the first (fig. 17). As this division begins the

close adherence of the two segments seems to be relaxed. The plane of division passes to the right of the vanishing spot from the centre outwards. By the time it has reached the centre of this segment (fig. 18, 5 h. 10 min.) the two inner angles of the cleavage walls have fallen together, the advancing end of the cleavage groove assuming a rounded form. About this time the cleavage of the smaller segment begins, advancing, as before, from the centre outwards. The second division severs the smaller segment into nearly equal parts (*a* and *c*), but cuts off only a third or a little more of the larger segment (6). At its completion (fig. 19, 5 h. 30 min.) the appearance of the two segments *a* and *c* reminds one of the condition presented by the first two segments in fig. 15. The inner angles are closed, while the outer are still far apart. The same phenomenon recurs as often as the cleavage appears on one side sooner than on the other.

(*b*) **Formation of the first four Ectoblasts.**—Rathke entirely overlooked the *formation* of these ectoblasts. Grube ($\frac{5}{1}\frac{9}{8}\frac{}{2}\frac{}{1}$) speaks of small "parietal spheres" ("Wandungsballen"), which he supposed were formed in the interior of the blastomeres (Huxley),[1] and afterwards ejected from the "active pole" (oral pole). Grube evidently mistook nuclear bodies for these ectoblasts, and probably confounded the latter with polar globules, for he saw one of these "parietal cells" at the close of the first division, lying between the two hemispheres on the oral pole. This body could not have been an ectoblast, since these cells arise *after* the second meridional cleavage.

Robin ($\frac{1}{1}\frac{4}{3}\frac{3}{8}\frac{}{1}\frac{}{4}\frac{}{0}$) asserts that these small cells arise as buds from the three blastomeres *a*, *c*, and *b*, the fourth and larger blastomere (*x*) producing none. The first two are produced simultaneously by the two opposite blastomeres *a* and *b*, the second two simultaneously by *b* and *c*. In the same way Robin derives the four ectoblasts in Nephelis ($\frac{1}{1}\frac{4}{2}\frac{3}{9}\frac{}{1}\frac{}{3}\frac{}{2}$) from three of the primary blastomeres, without the participation of the fourth.

Bütschli ($\frac{2}{9}\frac{7}{1}\frac{}{0}$), on the other hand, supposed that each of the four large spheres produced one of these ectoblasts; but, as he did not follow the process of this development on the living egg, he was inclined ($\frac{3}{2}\frac{0}{4}\frac{}{0}$) later to yield his opinion to that of Robin. As Robin is certainly wrong in respect to Clepsine, I am inclined to accept provisionally Bütschli's earlier opinion, inasmuch as it is in perfect accord with what happens in Clepsine.

About one hour after the completion of the two meridional divisions, the two lateral blastomeres, *a* and *b*, are found to be

[1] This term is used with reference to the four primary cleavage spheres alone.

wedged apart by the dorsal (*x*), and the ventral (*c*) blastomeres above and below, though more below than above. Soon (6 h. 30 min., fig. 20) the upper angles of the large dorsal blastomere (*x*) lengthens towards the ventral blastomere (*c*), and at the same time a constriction begins to separate this bud-like extension from the mother-cell (*x*), ending in the formation of the first ectoblast. This ectoblast during the next thirty minutes is pushed towards the left by a prolongation from the upper angle of the right blastomere (*b*), and finally becomes so imbedded in the left blastomere (*a*) that one would easily mistake it for a prolongation similar to that of the opposite side (fig. 21, 7 h.) had not one followed the process from beginning to end. It is probable that in this way Robin was led into error. I have followed this entire process without interruption many times and always have found the phenomena repeated as given above. I can affirm also that the process is identical in the four species that I have studied. Thirty minutes later (fig. 22, 7 h. 30 min.) the formation of the first two ectoblasts is already completed, and that of the second two is in progress. The latter are formed simultaneously from the lateral blastomere (*a*) and the ventral blastomere (*c*). The result is four ectoblasts lying exactly in the boundary lines of the four large quadrants where these lines cross, thus presenting a cruciform arrangement.

(*c*) **Formation of the two Mesoblasts and the primary Neuroblast.**—The result of the cleavage thus far is represented by four large primary cleavage-spheres (*a, b, c, x*) and four small ectoblasts, one of which was produced by each blastomere. With the production of these eight cells the regularity of the cleavage ends. The next step is the breaking up of the largest blastomere (*x*) into three parts, two of which give rise to the cells of the future mesoderm, and are therefore designated as mesoblasts, while the third, after dividing into a definite number of parts, becomes the source of cells that are to form the nervous system, and will therefore be spoken of as the primary neuroblast. Rathke failed to understand this step, and Robin has fallen into the gravest errors and confusion with reference to the same. First of all he states ($\frac{1}{1}\frac{4}{6}\frac{3}{3}$) that the four ectoblasts multiply to the number of eight by division, *before* the cleavage of the dorsal segment begins. Robin has given essentially correct drawings of the first division of *x* in the figs 249—251, but that he failed to understand it is proved by his letter-designation in fig. 251. The blastomeres *b* and *c* (fig. 251) are not the parts designated by these letters in fig. 250, but the two parts that have arisen by the division of *d*; and the pair of blastomeres marked *d* in fig. 351 correspond to *a* and *c* in fig.

250. In regard to the second division of x (d in Robin's figs.) and the destination of the products, Robin's statements and figures are sadly at variance with fact. The cleavage of the dorsal blastomere furnishes the guiding thread to all that follows, and Robin's failure to follow it threw him into a maze of inextricable difficulties. About one hour (8 h. 30) after the formation of the four ectoblasts, the segment x shows a depression which begins a little to the left of the upper and inner angle (fig. 23), and thirty minutes after its first appearance it has completely encircled x (fig. 24, seen from below). In 15—20 minutes more this cleavage completes itself cutting off about one-third of the original blastomere as the primary neuroblast (x^1) Several hours now intervene before the second division appears, during which three to five small cells are added to the ectoblasts from the lateral blastomeres (a and b). Possibly one or two are added from the neuroblast (x^1), but I am unable to speak with certainty. Figs. 25 and 26 represent a stage reached two hours before stage 27, and correspond with Robin's fig. 251. Four to five hours after the first division of x (14 h.) it begins to divide again in a direction at right angles with the first plane of division (fig. 27).

The products of this cleavage are the *two mesoblasts* (x and xy, figs. 28 and 29), one of which (xy) is on a level above with the primary segments a, b, c, fig. 30), and the other occupies the aboral polar field, around which the remaining segments lie in a circle (fig. 29). Robin has represented this central mesoblast in fig. 257. He ($\frac{1\,4\,3}{1\,6\,0}$) supposed that this cell (g) originally occupied a peripheral position and that it had been driven from this position by movements among the cells. "Pendant la durée de ces glissements l'une (g) d'elles est souvent chassée derrière les autres." My figures account for its position without the aid of such movements. According to Robin the entire dorsal blastomere (x) is converted into the "dorsal ectoderm," from which it is evident that he lost sight of the two mesoblasts. The origin of the central mesoblast (x) is correctly given by Grube ($\frac{5\,9}{9}$), but he knew nothing of its character and subsequent history.

(d) **Formation of the Neuroblasts.**—The neuroblasts are formed by successive divisions of the primary neuroblast (x^1), the first of which begins about 21 hours after the egg is laid (fig. 30), and cuts x^1 into two equal parts. Three hours later (24 h.) these two parts (x^2) occupy the position seen in figs. 31 and 32, at which time x and xy are still in their original position. The four ectoblasts are now quite surrounded by smaller and less opaque cells which have been produced by the

blastomeres (*a*, *b*, and *c*). Fifteen minutes later the neuroblasts (x^2) begin to divide, the cleavage progressing from above downwards and cutting each into two equal parts (fig. 34, x^3, 24 h. 30 min.). Two hours after the formation of these four neuroblasts (x_3), the two inner ones begin to push each other apart by means of nipple-like protrusions of their contiguous faces (fig. 35, x^4, 26 h. 30 min.). This pushing forces at the same time the two outer neuroblasts somewhat farther apart. In the course of ten minutes the two protrusions are completely constricted from the mother-cells. The two small central cells (x^4) thus formed are always very distinct for a short period, but soon break up into four small ectoderm cells (fig. 37). One hour after the production of these two cells (x^4) the mother-cells divide again, producing this time two cells from their anterior faces (fig. 37, x^5). The last two cells are generally more or less covered with the small cells of the blastodisc and therefore difficult to recognise. A little later the two outer anterior neuroblasts (x^3) divide, producing the two cells x^6 which lie between x^3 and x^5 of each side, and which appear at first considerably smaller than the other neuroblasts. There are now eight neuroblasts (fig. 37, 28 h. 30 min.) arranged in two symmetrical groups at the posterior border of the germinal disc. The rôle played by these neuroblasts will become apparent when we come to speak of the formation of the germ-bands.

e. **The four primary Ectoblasts and their relation to the Mouth.** These ectoblasts differ from the blastomeres only in having a little more protoplasm in proportion to the amount of deutoplasm. A transverse section of stage 26 (fig. 79, S) shows that they are not enuclear buds as supposed by Robin. Fig. 79, T.) shows a nucleus of one of the ectoblasts as found in stage 23. This section (S) cuts two of the ectoblasts, between which and the lateral blastomeres (*a* and *b*) two smaller cells of similar composition are wedged. The nuclei of the blastomeres (one is seen in *a*), lie near the upper surface at this time, which is in harmony with the production of small ectodermic cells from their upper faces—a process which continues at least as late as stage 34. After maintaining their individuality for a comparatively long time, the four ectoblasts are found at length (fig. 34, 24 h. 30 min.) to have undergone two or more divisions; but the division-products remain in situ, so that one can easily recognize the limits of the original cells. The same is still true in stage 37. In stage 38 it is plainly to be seen that the daughter-cells of the ectoblasts form the cephalic portion of the embryo. They rise slightly above the niveau of the neighbouring cells. Beginning in their centre (*m*) and running towards the ventral

blastomere (*c*) is a linear depression which widens a little towards *c*. This shallow depression, the anterior end (*m*) of which marks the place of the future pharyngeal orifice (mouth), is destined to be continuous with the primitive groove formed by the conjunction of the two germ-bands.

f. **Movements among the Cleavage-products.**—In passing from stage 32 to stage 38 important changes of position take place on the lower pole of the Blastula (this term will apply to stages 23—28), which have hitherto escaped observation. These changes originate in the cleavage of x^1 (fig. 30). As this cell begins to divide it lengthens transversely and thus disturbs the equilibrium of pressure. The pressure on the lateral walls of the Blastula (figs. 29 and 30) is increased, while the pressure on *x* from above and behind is correspondingly diminished. The consequence is that the mesoblast (*x*) moves backwards and upwards, followed by (*c*) and, to a certain extent by *a* and *b*. The next division of the neuroblasts (fig. 33, x^2) operates in the same way, except that the pressure is exerted still further above the equatorial plane of the Blastula. This gradual elevation of the plane of pressure is still more evident in stage 35 and 36, and must obviously continue as long as the neuroblasts go on dividing in perpendicular planes. The lateral blastomeres (*a* and *b*) are thus pushed not only towards the ventral blastomere (*c*) but also downward towards the mesoblast (*x*). As *x* moves slowly upward and backward its lower face becomes more and more covered by *a*, *b*, and *c*.

Fig. 36 shows how far *x* has travelled from its original central position. It now lies at the right of *x y* and a little below it. In the course of these movements, none of which are active migratory motions, the left mesoblast (*x y*) becomes quite buried in the left blastomere (*a*), but does not usually disappear. The right mesoblast (*x*) however, is soon completely enveloped by what we may now call the posterior ends of *a*, *b*, and *c* (fig. 37). The relative positions of the two mesoblasts at this time (28 h. 30 min.) are seen in fig. 81 (transverse section just behind the blastodisc). The right mesoblast lies under the neuroblasts in the ventral blastomere (*c*), a little to the right of the middle of the Blastula and on a somewhat lower plane than the left mesoblast (*x y*). In the upper pole of each mesoblast fine granular protoplasm is collected around the nucleus. Above each mesoblast—more distinctly over the left—is seen a line of small cells composed of the same kind of protoplasm. That these cells arise from the upper (formative) poles of the mesoblasts is established by serial sections, by dissection, and by examination of the living egg. If eggs at this epoch are heated

in water to near the boiling point and then treated with alcohol, the mesoblasts can easily be removed with needles without breaking. In this way I have generally found a nipple-like protuberance at the upper pole of each, which shows that they are in process of proliferation.

I have not succeeded in obtaining sections in which the full amphiastral division could be seen; but I have preparations which show parts of the amphiasters and the connection of the same with the nipple-like protuberances. This proliferation begins as early as stage 33 (24 h. 15 min.), at least with the left mesoblast. The path of cells leading from the upper pole of this mesoblast may easily be seen on the living egg in this and some of the following stages (figs. 33—38).

(*g*) **Relation of the Mesoblasts and Neuroblasts to the Germ-bands.**—As before remarked, the cells of the blastodisc multiply first, at the expense of the primary segments (*a*, *b*, *c*). In stage 33, and perhaps earlier, the two mesoblasts begin to produce cells that take their places below the lateral edges of the blastodisc (figs. 81, 82, 84, 85, 87). Those produced by *x y*, although at first visible from the surface, are soon covered by the smaller cells of the centrifugally expanding disc. The eight neuroblasts begin as early as stage 37 to take a conspicuous part in the cell-proliferation. From this time (28h. 30m.) the germinal disc receives new material from only two different sources, namely, the neuroblasts and the mesoblasts. It is at this epoch that we begin to see distinctly lines of cells leading away from the inner nuclear poles of the neuroblasts. In stage 38 (36h.) the lateral borders of the disc are plainly thickened and transversely arched. These thickened borders are the germ-bands (notæ primitivæ), Each of these "embryoplastic" bands is composed of four longitudinal lines of cells produced by the neuroblasts, and of larger subjacent cells produced by one of the mesoblasts. The linear arrangement of the neuroblast-products may be seen on the living egg, but is more distinct after treatment with osmic or chromic acid. The two bands taper a little towards the cephalic ends, which terminate near the boundary lines of the ventral blastomere (*c*.)

The signification of the neuroblasts has not been hitherto understood.

Grube ($\frac{5\,9}{5\,1}$) found only two—at most three—at the anal end of each band. He supposed that they contributed elements to these bands, but to what extent and to what purpose was unknown. Rathke ($\frac{1\,3\,6}{9\,8\,9}$) found three joined by as many rows of cells to the posterior end of each band, and was inclined to believe that they entered into the structure of the terminal

sucker. Robin ($^{1}\frac{4}{0}^{3}$) ascribes a very insignificant rôle to these cells. They are called "ectoderme dorsal," in consequence of their having an analogous origin with the cells, which really form the dorsal ectoderm in Nephelis, although here (Clepsine), only the two small median cells (x^4) are said to enter into the ectoderm. "Nous verrons en effet que *ces cellules,* situées comme celles de l'ectoderme dorsal des Nephelis, ne donnent qu'un nombre restreint de cellules à l'arrière de l'ectoderme sur les Clepsines, par *subdivision ultérieure de deux d' entre elles* (x^4), pendant que les six autres restent longtemps sans changes (!), à la place qu' occupera l'anus." They disappear by atrophy some time after the embryo hatches. Leuckart ($\frac{1}{6}\frac{0}{9}\frac{6}{8}$) has expressed the opinion that these cells represent primordial kidneys, comparable with those described by Gegenbaur for Gastropods. He now concurs, however, in the opinion that the segmental organs have no connection whatever with these cells.[1]

Kowalevsky ($\frac{8}{1}\frac{5}{5}$) found two cells at the hind end of the germ-bands ("Keimstreifen") in Euaxes. (See his figs. 11, 12, K, Pl. III.) He regarded them as mesoblasts. In the case of Tubifex ($\frac{8}{2}\frac{5}{0}$), five such cells were found, and the germ-bands were composed of *five longitudinal lines of cells.* As the cleavage and neurulation (Rauber) in Euaxes and Tubifex are closely similar to the same in Clepsine, it seems very probable that these cells are of the same character as the neuroblasts before described. The five longitudinal lines of cells observed in Tubifex would then be nerve-cells, derived from five neuroblasts, just as four such lines are derived from four neuroblasts in Clepsine.

(*h*) **Formation of Entoplasts.**—About the time the germ-bands begin to form, a number of free nuclei appear in the surface of the entodermal blastomeres (*a*, *b*, *c*.) These nuclei (fig. 37) are very distinct in the egg of C. complanata, and it is remarkable that they have so long escaped observation. They appear like dark spots in the opaque yolk, just as the unclei of the neuroblasts or of the blastodisc. They are oval, oblong, or biscuit-shaped, and measure ·02 to ·05 mm. At the time of appearance they number three to four in each blastomere, two or three of which occupy the position seen in the figure, while the others are near the lower pole. They are encircled by white rings, such as are generally seen around the nuclei of the neuroblasts. The substance of these

[1] Ratzel and Warschawsky ($\frac{}{5}\frac{}{5}\frac{1}{6}\frac{6}{6}-\frac{}{5}\frac{}{6}\frac{}{0}$) described two cells at the hind ends of the germ-bands (Lumbricus agricola). The exact relation of the same to the bands was not ascertained.

rings is the same as that of the white borders of the rings and ring-discs.

I have seen these nuclei pass through the successive forms of a dividing amphiaster. They multiply rapidly, and in stage 38 (36h.) are scattered over the whole outer surface of the blastomeres. In stage 40, and following stages, they can also be seen on the upper faces of *a*, *c*, and *b*, through the thin ectodermal layer. By the time the germ-bands are fully united, they are very numerous, and much smaller than at first.

Such nuclei have been observed in the egg of Nephelis according to Balfour ($\frac{1}{3}=\frac{1}{9}$). "Dr. Kleinenberg has followed a single egg through the whole course of its development, and concludes that the nuclei of Nephelis never become the nuclei of new cells." With reference to Clepsine, I have come to a very different conclusion, as will appear when I come to speak of the origin of the entoderm.

Whence come these nuclei? In stage 35 they are not to be seen. A horizontal section of this stage (fig. 80) shows that each blastomere possesses a single nucleus. The nucleoplasm has a somewhat stellate form; the rays vary in length, sometimes reaching to the irregular circular outline of the nucleus. The same condition has been described for Nephelis by Bütschli (30, fig. 5, Pl. XVIII.). Fig 61 represents one of these nuclei in a little earlier phase. The nuclei now lie nearer the inner than the outer faces. Fig. 83 represents a horizontal section of the stage 37 (nearly), which passes beneath the neuroblasts and the blastodisc. Here only two nuclei were hit, but these lie near the outer faces of the blastomeres. The nuclei of the blastomeres then pass from their original central position to the periphery, and can here be seen on the living egg. They are much more distinct in *C. complanata* than in *C. marginata*.

V. Gastrula and Neurula.

The Neurula (Rauber) arises by the concrescence of the thickened rim of the blastopore (Lankester). It follows the Gastrula, but takes its origin with the germ-bands, *i.e.*, long before the Gastrula-phases (invagination, &c.,) are completed. This is a good illustration of ontogenetic concentration—the earlier phases of one stage appearing before the later phases of the previous stage are completed.

(*a*) **Growth of the Germ-bands and concomitant Invagination.**—In stage 38 the blastodisc has a quadrilateral form with rounded corners, and is bounded on either side with thickened margins, the germ-bands (g.b.). Each band results, as before indicated, from the confluence of five streams of cells, and these streams—if the comparison be allowed—all flow from behind

forward along the lateral edges of the germinal disc. The cells composing the central area of the disc are in a process of rapid multiplication by division, resulting in centrifugal expansion. This expansion takes place, to a certain extent, in all directions: but predominantly in a transverse direction, inasmuch as the chief points of resistance are offered by the cephalic and anal regions. One of the important results of this laternal expansion in which the germ-bands of course participate, is seen in the movements of the blastomeres. The lateral blastomeres (*a* and *b*) are pressed outward and downward, and the ventral blastomere (*c*) moves necessarily upward. This movement is followed without difficulty on the living egg, and it was thus that I first became aware of it. Figs. 37, 38, and 39 show the relative positions of the blastomeres at about the time the movement begins. The ventral blastomere (*c*) has still the cuneate form form seen in fig. 79. Twelve hours later (figs. 41, 42, 43, 84, 48 hours) the upper face of this blastomere, already visible from above in stage 40 as a narrow area tapering backwards, has attained a considerable breadth at the expense of the lower. In stage 44, 45, (54 h.) the upper face of *c* is much broader than the lower, as is best seen in section (fig. 86). This movement culminates in stage 46, 47. The full extent of the change in position which takes place between stages 37 and 47 is at once seen by comparing fig. 78 with fig. 86. The wedge (*c*) is inverted. The successive positions of the mesoblasts (figs. 84-86) show how they are involved in the same movement.

In *C. complanata*, the object studied by Robin, this solid form of invagination is quite as marked as in *C. marginata*. Clepsine thus furnishes a beautiful illustration of the fact that epiboly (Selenka) is only a modified form of emboly. Abolish the limits between *a*, *b*, and *c*, or divide them into small spheres, and it is easy to see that the invaginatory movement might still take place although it might be impossible to recognise it.

In order to understand the form and the movement of the germ-bands, it is necessary to bear in mind that their two ends are, approximately speaking, fixed. Their anterior ends abut against the cephalic portion and their posterior ends are supported by the neuroblasts. As the bands lengthen, the central field simultaneously expanding, the slight outward curve, which they exhibit in stage 38, is rapidly increased. In this way the central area of the blastodisc soon takes the dumb-bell form seen in fig. 40 (42 h.). At this time it has become so thin that it is easy to recognise the limits of the large blastomeres.

While the expanding disc contributed strongly at first to the outward bending of the embryonic bands, it is plain that from this time (fig. 40) forward, the form of the band-curves is mainly

controlled by the pressure of the cells constantly being formed by the terminal neuroblasts and mesoblasts. This pressure reacts upon the neuroblasts themselves, especially the two outer ones (x^6 x^3). That these two cells on each side retreat downward and backward is seen by comparing fig. 40 with fig. 38. The same movement is still more apparent in stage 41, where the two cells (x^6) are nearly in contact, the inner cells (x^3 x^5) having meanwhile moved farther apart in consequence of the invaginatory movement of the blastomeres. At this epoch (figs. 41-42) the anterior ends of the bands are already in contact, and between their concrescent edges is seen the primitive groove (p. gr.) which is continuous with the postoral linear depression.

In stage 40 the two lines (*oc*) which show where the bands border upon the cephalic portion, are marked not so much by a depression as by an increasing elevation of the former above the level of the latter. The foremost extremities of the bands being pushed by the cells behind and resisted by the cephalic mass, rise to a height which plainly exceeds that of any other portion. This pressure, furthermore, causes them to expand a little on each side of the head-portion. In stage 41, in consequence of the concrescence of the fore ends of the bands, the lines of abutment (*oc*), instead of lying nearly parallel with the postoral depression as in fig. 40, lie almost at right angles to the same and now form well marked linear depressions, the distal ends of which are rounded. The raised extremities of the bands form the first somatomere of the embryo. At this time the expanded blastodisc, the marginal bands of which alone are seen in my figures, covers about one half of the egg.

As the bands lengthen the concrescence along the median ventral line continues until finally they are united from end to end (fig. 48). The somatomeric division of the embryo follows closely upon the closing of the blastopore, progressing from the cephalic towards the anal end.

The embryo leaves its protective envelope soon after stage 47 (72 h.) and becomes attached to the ventral side of its parent, under whose protection it remains until it is fully developed and able to seek its own food. The point of attachment is a place on the neural side of the embroyo, just *behind* that part destined to form the anterior sucker.[1]

In what manner the attachment is effected I am unable to say. Embryos taken from the parent at the time of exclusion almost invariably unite in pairs, and the place of contact is always that by which they are attached to the parent. In such cases they adhere so strongly, that they are generally injured by

[1] Hoffmann ($\frac{7}{45}$) thinks the embryo attaches itself by the future suctorial surface itself.

separation. As soon as the posterior sucker is developed they attach themselves by this to the mother, the body and head swinging free.

At the time of exclusion the neuroblasts have diminished much in size. They continue the process of proliferation for at least a day or two after hatching. Remains of the neuroblasts are seen three days after they leave the egg-envelope (fig. 50), but they have ceased to make contributions to the embryo, and are soon lost in the yolk. The mesoblasts continue their activity for about the same period, and finally blend with the yolk.

b. **Pharyngeal Clefts.**—The two depressions (*oc*) noted in stage 41, which, starting from the primitive groove, pass right and left between the fore ends of the germ-bands and the cephalic portion, are in stage 46 extended around a circular area destined to become the protrusible pharynx. Stages 48 and 50 show that these depressions are the incipient invagination of the pharyngeal atrium (*o a*).

These clefts are very distinct on specimens hardened in chromic acid, and, after they have been once seen on such preparations, are recognised without difficulty in a fresh condition. A more advanced stage of the invagination is seen in fig. 96, *o a*.

The permanent mouth is the pharyngeal orifice (*p a*). Both atrium and mouth are ectodermal invaginations.

c. **Nerve-chain.**—Although my investigations here suffice only to form a basis for more detailed study, they settle a point of cardinal interest, namely, the precise origin of the neural elements. In addition to what has been said on the origin and composition of the germ-bands, it remains only to consider sections of later stages.

Fig. 84 represents a median transverse section of stage 41. The origin of the large mesoderm cells is here placed beyond all doubt. The same is equally clear in section 85, which shows some of the anterior neuroblasts. On each side is seen a line of small cells leading from a neuroblast. In fig. 84 the superficial part of each germ-band consists of four of these neuroblastic productions. It is these cells that give the germ-bands their fourfold striated appearance. The same cells are seen again in fig. 87, which is a horizontal section of an embryo in stage 47. The section passes just under the neuroblasts and cuts the two unclosed ends of the bands. In the anterior portion of the section, where the germ-bands have united, the nerve-cells form a line of eight cells lying just under the epidermis. The expansion of the ectoderm is more rapid than that of the bands, and hence the epidermis comes to cover the nerve-cells, and even

advances beyond them, as seen in the posterior part of the figure (*e p*). Kowalevsky (§ 5 / 7) has noted the same thing in Euaxes. Whether any of the epidermal cells enter into the nerve-chain in the manner described by Kowalevsky is uncertain. Thus far I have seen no evidence of this.

d. **Segment-cells.**—In fig. 87 are seen two colossal cells (*s*), with plain nuclei and nucleoli, lying just above the two outer rows of nerve-cells. Somewhat later, owing to the concentration of the nerve-cells and the growth of the mesoderm towards the dorsal side, these segment-cells are no longer found above, but to either side of the neural cell-group (figs. 88, 89).

Fig. 88 would seem in this respect to contradict fig. 90; but the seeming inconsistency in the relative positions of the nerve-cells and the segment-cells is at once removed when we remember that the germ-bands close earlier at the fore end than at the hind end, and that consequently the differentiations are further advanced in the former region than in the latter. A surface view of the Neurula (fig. 91, 2½ days after exclusion) shows the paired arrangement of these cells in each body-segment. Although these cells are present all through the Neurula period, it is not easy to obtain unbroken preparations much before the conjunction of the germ-bands, on account of the want of coherence among the embryonic elements. Such surface views reveal *four rows of segment-cells*, two on each band. A little later (twelve hours after exclusion) they are found in pairs on each side of the ganglionic chain, so arranged that four cells lie in the same transverse plane—the plane of a septum. Two days after exclusion the cells of the two median rows have both diminished in size and changed their position. They appear to be connected with the groups of cells destined to become the segmental organs, and to follow these in their growth towards the dorsal region. I am unable to give any further account of them. The segment-cells of the two outer rows can be followed for three or four days, but are finally concealed by the tissues forming about them. They can even be seen on the living embryo when viewed in profile (figs. 49, 50). One of these cells is seen in fig. 92 in contact with the cells of the segmental organ, which suggests perhaps that it may become the ciliated mouth of the organ. I am more inclined to think, however, that these cells are the mother-cells of the future testes. Their position in the walls of the septa, which they maintain so far as I have been able to follow their history, favours this hypothesis. The ciliated funnels of the segmental organs are, on the contrary, always found in the middle of each somatomere.

(*e*) **Segmental Organs.**—The segmental organs appear first as

simple groups of mesodermic cells—two in each somatomere, to the right and left of each ganglionic centre. Fig. 91 shows the position of these organs two and a half days after exclusion. Fig. 92 represents one of these organs one day later. The cells are now arranged in the form of a double-looped string, and have shifted their position between the segment-cells (fig. 91) to a position outside the same. This change of position is due to the centrifugal growth of the mesodermic elements so characteristic of all bilateral animals.

In fig. 56 I have given one of the segmental organs (*nephridia*, Lankester) in its fully developed form. The string of cells seen in fig. 92 has lengthened immensely, extending from near the median ventral line along the floor of the cœlom, to the margin of the body and mounting from here to the median dorsal line. The main body of the organ lies within a single somatomere, but the external orifice (*ea*) is in the ventral floor of the following somatomere. The internal orifice (*ia*) is formed by a ciliated funnel. A duct of very small calibre accompanies the string of cells from end to end. This duct does not pass through the cells, with the exception of the one at the outer extremity (*ea*), but is apposed to one side of the same. From the duct short lateral branches pass to each cell. Whether these branches have a lumen or not, I have not been able to ascertain.

The duct with its cells passes by a tortuous course from the ciliated funnel towards the margin of the body where it becomes labyrinthiform. Issuing from the labyrinthic coil near the central marginal notch of the gastric diverticulum, it ascends the hæmal side of the same a little obliquely backwards, crosses the space separating this from the following diverticulum, and, after reaching the median dorsal line, bends fowards aronnd the interspace of the diverticula and passes down along the posterior side of the diverticulum entering the labyrinth again. It then makes another excursion to the dorsal region and back, in a course parallel at every step with the preceding, thus completing the 8-shaped figure seen in the drawing. A third shorter loop is then added to the 8-figure, after which it passes obliquely backwards to near the centre of the following somatomere and here ends by a vesicular enlargement of the duct (*ea*). A part of the seventh diverticulum is cut away to show the position of the external orifice. The duct diminishes gradually in calibre from the external ventral aperture to the free ciliated end. There are in the adult worm sixteen pairs of segmental organs, fifteen of which correspond to the fifteen pairs of gastro-pleural cæca. The remaining pair is in the somatomere preceding that which contains the most anterior pair of diverticula.

(*f*) **Number of Somatomeres.**—The original number of body-

segments, corresponding with the number of postoral ganglia, is *thirty-three*. *Eight* are converted into the suctorial disc (*d*).

Some of the posterior ganglia are always rudimentary, and it is rather difficult to obtain preparations which show all the ganglia of the disc region; hence the general opinion that only seven segments enter into this part. The divisions of the nerve-chain are at first quite alike from end to end.

The definitive differentiation into *four regions* is already beginning to show itself, two and a half days after the exclusion (fig. 91). The first four divisions (1—4) are a little broader than the following, and are destined to coalesce more or less to form the sub-œsophageal ganglia.[1] The next region includes seventeen divisions (5—21), which later stand at considerable intervals from one another, connected by the double longitudinal commissures.

The third region embraces the last four divisions (22—25) of the body proper; they concentrate to a continuous tract of ganglia. The fourth and last region includes the eight divisions (26—33) found in the terminal sucker. The concentration in this region will later obliterate the limits of the original ganglia. The dotted transverse lines in this figure mark the position of the septa. The dots represent yolk-granules contained in the embryonic cells. The preparation is seen from the hæmal side. In the mesial line of each ganglionic mass are two pairs of small cells, the signification of which is unknown.

VI. Alimentary canal.

This consists of four parts (fig. 56): (1) The protrusible pharynx (p), which ordinarily lies enshcathed in the pharyngeal atrium; (2) a short œsophagus; (3) a sacculated stomach (st.) or crop (Gratiolet), which stretches through the greater part of the body; (4) a narrow intestine (int.) ("gostroileal" intestine, Gratiolet).

The formation of the pharynx and the pharyngeal cavity has already been considered. The histogenetic origin and structure of the remaining parts will be better understood after a description of surface changes.

The adult form of the digestive tract is much like that given in fig. 56. The stomach is divided into three well-marked regions. The small anterior and posterior regions are almost the exact counterparts of each other, each having four pairs of diminutive lateral sacculations. Those of the anterior part point obliquely forward, and those of the posterior part obliquely backward (excepting the foremost pair, which point forward). The main central region has seven pairs of large lateral diverti-

[1] Hoffmann ($\frac{77}{40}$) states that only three so coalesce.

cula, the seventh and largest one of which lies behind and stretches through five somatomeric chambers.

The diverticula of the anterior and posterior regions are entire, while those of the central region are lobed. The lobes repeat themselves with considerable regularity on each pair of diverticula. The seventh pair show five subdivisions, corresponding to the number of body-chambers, and each subdivision presents the principal marginal notches seen on the anterior diverticula of this region. The first pair is an imperfect counterpart of the seventh; it reaches forward through two chambers instead of five, and has a corresponding number of subdivisions. The seventh pair of diverticula and that part of the alimentary canal included between them (intestine and posterior region of stomach) recall the picture of the iliac bones and coccygeal style of the frog.

The intestine is a narrow tube, which ends in the dorsally placed anus. Diaphragmatic septa are interposed between the walls of the diverticula, and through the central and ventral emargination of these septa passes the trunk of the stomach. How do these diverticula arise? Figs. 49 to 55 are supposed to answer this question in part.

The somatomeric division begins soon after the conjunction of the germ-bonds, and progresses from the neural, outward and upward, towards the hæmal side, and at the same time centripetally. The centripetal growths of the mesoderm are the septa above described. In stage 50 (three days after exclusion) the septa (marked by transverse lines) have already reached the median lateral line of the embryo, but their centripetal growth has not yet made any marked changes in the form of the yolk. On the sixth day after exclusion all intermediate forms are found between figs. 50 and 52. The neural side of the embryo is still much longer than the hæmal, and the latter is, therefore, concave, and the face of the terminal disc turned upward. The septa have already cut sufficiently deep into the yolk to mark off the cæcal divisions and the primary regions of the future stomach. The seventh diverticulum (c. 7), which was at first simple, now has three of its five subdivisions. As this diverticulum lengthens backward, the yolk in the intestinal region diminishes (fig. 53). In fig. 54 the intestinal part has become still more reduced, and the seventh pair of cæca correspondingly larger. At this time the dorsal side is nearly as long as the ventral, and the face of the sucker is at right angles to the longitudinal axis of the embryo. In stage 55 (six days after exclusion) all the principal form-differentiations of the alimentary tract are to be seen.

At about this time the eyes become visible as two pairs

of crescent-shaped orange-coloured spots on the dorsal side of the lanceolate head. The concave side of each looks forward and outward. The posterior eyes are three to four times as large as the anterior ones. A few days later the eye-pigment has become dark brown. These eyes are sack-like involutions of the epidemis (fig. 93, eight days after exclusion). Some of the cells of the inner walls become very large and glassy, and are connected, according to Leydig, with nerve-filaments.

a. **The Entoderm.**—Whence arises the entoderm? Thus far we have found only two germ-lamellæ—ectoderm and mesoderm. Fig. 93 represents a sagittal section of an embryo eight days after exclusion, but in about the same condition as stage 55 (7 ds.). This section is constructed from two successive sections, on one of which appeared the pharyngeal atrium (*oa*) and on the other the anal aperture. All the diverticula are cut in a plane a little one side from the middle. Only two of the four diverticula of the posterior region of the stomach appear (*i.d.*). Fig. 95 is a part of fig. 93 more highly magnified. The cæcal cavities are still filled with the deutoplasm, or "residual yolk" (Lankester). The septa are composed of mesoderm-cells, the nuclei of which appear as mere dots. These walls are lined by a loose layer of oval-elliptical cells (circa ·01 mm.). In preparations treated with osmic acid and hæmatoxylin these cells are very clear, and the deeply-coloured nucleus is very distinct. The cells lie partially *in* the periphery of the yolk, the large yolk-spheres being sparsely scattered through the cell-area. The cells lie at some distance from one another (dorsal portion of fig. 95), or in contact, with their longest diameter for the most part parallel to the walls of the septa. Under a low objective they appear as a light border around the central field of yellow yolk. At a little earlier date these cells (ent.) are even more loosely arranged and intermixed with the peripheral yolk spheres. Earlier still they are not to be recognised at all. One or two days later (fig. 94, 9 ds. after exclusion) they are smaller, more numerous, and compactly arranged in a single layer, with their longer axes perpendicular to the septa-walls (fig 94 = horizontal section). A longitudinal perpendicular section like that of fig. 93 proves that the formation of the entoderm progresses more rapidly in the anterior and posterior than in the median region. The intestine is still closed, and reaches to the anal aperture. A little later this blind end becomes perforated, and thus the alimentary canal is complete.

b. **Origin of the Entoderm.**—What is the origin of these entoderm-cells? Do they arise de novo, or have they a genetic relation with the nuclei of the three primary blastomeres, *a*, *b*

and *c*? In stage 37 free nuclei were found which were regarded as descendants of the original nuclei of the blastomeres.

These superficial nuclei go on multiplying by division during the whole period of the epiboly. Finally they are seen as mere white dots scattered over the entire surface of the yolk. Six to seven days after exclusion the entoderm-cells make their appearance as *clear cells* with small nuclei, in the periphery of the yolk already cut up into compartments by the septa. What hypothesis is more probable than that these cells originate from the free nuclei? My sections have convinced me that these entoderm cells arise in the surface of the yolk, and that they do not originate in the products of the blastodisc. To account for their origin on the hypothesis of generatio equivoca is quite as unnecessary as unsatisfactory. The view I have offered above seems to be the only way to account for all I have seen. The positive proof, however, is wanting, and may be difficult to obtain; but I hope, as soon as fresh material can be had, to trace the history of the superficial nuclei farther. The muscular walls of the alimentary canal are probably derived from the same mesoderm-cells which build the septa.

c. **Final position of the "residual yolk."**—From what has been said it will be seen that the "*residual yolk*" (Lankester) becomes inclosed by the permanent entoderm. Here it is gradually dissolved and assimilated. It is the only food the young worm has before it abandons its parent. This is proved by the fact that the development completes itself in the same manner and in the same time when the embryo is removed from the parent. According to Bütchsli ($\frac{3}{5}\frac{0}{4}$) and Robin, the three large blastomeres in Nephelis lie *outside* of the entoderm, and play a very subordinate roll, if any at all, as "food material." It is almost, if not quite, certain that these large cells correspond to those designated as *a*, *b*, and *c* in Clepsine. It is quite remarkable that the remains of these cells should be found in one case (Clepsine) in the entoderm, and in the other (Nephelis) in the body-cavity. Furthermore the early appearance of the entoderm in Nephelis is in marked contrast with what happens in Clepsine; but the contrast is somewhat diminished on the supposition that the *entoplasts*, which I have described in the latter, represent the nuclei of the future entoderm cells. Such a formation of the entoderm is easy to explain from a comparative standpoint. The less deutoplasm an egg contains, the longer the total and regular cleavage continues. With the accumulation of the same the more sluggish becomes the cleavage, until a point is reached where the dividing nucleus has no longer sufficient power to sever the entire mass. Thus we arrive at the discoidal and peripheral cleavage. In Clepsine

we have a combination of all kinds of cleavage. At first it is total and quite regular; then it becomes unequal, and then discoidal, and, so far as the blastomeres, *a*, *b* and *c*, are concerned, peripheral. The cleavage power of the nuclei in *a*, *b* and *c* is no longer sufficient to overcome the resistance of the masses of yolk, and hence they begin a process of free division, precisely as in the eggs of insects, where this sort of division prevails from the outset. That these three segments (*a*, *b*, and *c*) should furnish the entoderm, is in harmony with what Bütschli has observed in Nephelis, and also with other cases of unequal cleavage, where the entoderm arises from the larger of the cleavage products.

The formation of the entoderm in Euaxes ($\frac{8}{18}\frac{5}{19}$) is essentially the same as in Clepsine, the only difference being that in the former the yolk is broken up into a larger number of primary spheres. The nuclei pass from the centre of these spheres to the outer surfaces, precisely as in Clepsine, and here finally become the centres of the entoderm-cells, leaving the residual yolk in the aliment cavity. The same result is accomplished in *Astacus fluviatilis* ($\frac{1}{15}\frac{4}{3}\frac{0}{7}$) in a little different way. Here the entoderm-cells are at first *within* the yolk, but ultimately *outside* of the same. The passage from one condition to the other is a curious process, which, according to Dr. Reichenbach, is accomplished in the following manner: The interiorly placed entoderm-cells *devour* the yolk by means of amœba-like pseudopodia, which they throw out around the yolk-elements. At length the entire yolk becomes included *within* the entoderm-cells, which now have a long pyramidal form, the bases of which lie in the outer surface of the yolk, and the apices form the boundary of the gastrula-cavity (Archenteron). During this process of lengthening outwards at the expense of the yolk the nuclei shift their position, passing from the apical to the basal ends of the pyramidal cells. In this position the cell-protoplasm gathers around them, and finally splits off from the deutoplasmic portions. Thus the yolk is finally inclosed within the entoderm, as in Clepsine and Euaxes.

According to Rabl ($\frac{1}{2}\frac{2}{0}\frac{8}{3}\frac{8}{7}$) a similar splitting of the entoderm takes place in the fresh-water Pulmonates, in consequence of which the vitellus nutritivus is enclosed in the cœlom.[1]

The same position of the residual yolk occurs in many other Mollusca (Nassa and Fusus, Bobretzky ($\frac{2}{11}\frac{1}{9}$), and Ptero-

[1] According to Lankester ("On the Development of the Pond-Snail," 'Quart. Journ. Mic. Sci.,' vol. xxii (n. s. xiv), p. 384—5) this is incorrect. Primitively (Lymnæus) the *whole* entoderm forms the wall of a bilobed cavity—the archenteron. Later the metamorphosed "gastrula-endoderm-cells" lie on each side of the "stomach," where they are "eventually absorbed as nutritive matter by diverticula of the alimentary canal, which give rise to the liver."

poda, Fol (41)). In Phascolosoma ($\frac{1}{4}\frac{5}{4}\frac{0}{5}$), and Vermetus, and Natica ($\frac{2}{1\,3}\frac{1}{1,1\,6\,0}$), it remains always *in* the entoderm-cells themselves. Lankester, in his valuable "Contributions to the Developmental History of the Mollusca," p. 18 and 25, has shown the same contrast between Aplysia (major) and Pleurobranchidium, in respect to the position of the food-material, as exists between Clepsine and Nephelis, or between Euaxes and Lumbricus (Kowalevsky). In Aplysia the food-material lies *in* the endodermal sac, in Pleurobranchidium it lies *outside* the same, in the form of two big cells which remain persistently with their large pellucid nuclei and give rise to no progeny. From these few examples it is apparent that the ultimate place of the deutoplasm may be either (1) *in*, (2) *outside*, or (3) *inside* the entoderm. The first position is undoubtedly the original one, and the other two may be regarded as departures from this, resulting from the increase of the passive food-yolk.

This is in harmony with the fact that in some cases of unequal cleavage the first position is *followed* by the third (Clepsine) or the second (Lymnæus); while in other cases (Natica, &c.) the first position is maintained throughout.

(*d*) **Free Nuclei.**—As Leuckart ($\frac{1}{6}\frac{0}{7}$) long ago pointed out in his paper on Melophagus, there is no essential difference between ordinary segmentation and the formation of cells from free-formed nuclei. In one case the cleavage is simultaneous with the division of the nucleus; in the other it follows after a shorter or longer interval. Passive yolk obstructs and, if increased beyond certain limits, prevents cleavage. It is not, therefore, surprising to find in eggs loaded with nutritive material the cleavage retarded or even interrupted for a time while the nuclear activity is continuous. The polar or peripheral segregation of the proper cleavage-material, which becomes more and more marked with the accumulation of deutoplasm, accounts for the simultaneous occurrence of both modes of cell-formation, as in Clepsine, and numerous other cases.

The wide distribution of free nuclei formation in two of the three secondary modes of cleavage (*unequal* and *discoidal*) is a fact of late discovery. So far as I am aware Ray Lankester (1872) was the first to recognise such phenomena in the eggs of Mollusca (Loligo, Octopus, and Sepia). His account of these bodies which he termed "*autoplasts*," supposing them to originate as independent segregations of the "formative material," leaves no doubt that they correspond to what I have called "*entoplasts*" ($\frac{9}{3\,9}\frac{8}{4\,7}$). "Before the superficial extension of the cap of klastoplasts (blastodisk) has commenced there appear in a deeper stratum of yelk *pellucid nuclei*, at first arranged in a circle around the cap of klastoplasts as I have figured them in

'Annals and Mag. Nat. Hist.,' April, 1873. . . . . The feature in which they differ from the nuclei of cleavage-segments is this, that no area becomes segmented around them." These nuclei multiplied *not by division* of pre-existing nuclei, but by independent segregration. Towards the close of the epiboly they were very numerous and scattered over the entire surface of the egg. They were found to form "*a large portion of the deeper substance of the embryo.*"

In another place ($\frac{100}{03}$) Lankester expresses the opinion that the cells of the perimorula (Gammarus fluviatilis) arise as "isolated cells," in the same manner as the "autoplasts" in the Cephalopods. Kowalevsky ($\frac{861}{009}$) reports similar formations found in the yolk under the edge of the blastodisc, in the egg of Pyrosoma. Their origin was unknown.

The phenomenon of "free nuclei" has long been known among Arthropods. The opinion formerly entertained (Weismann, $\frac{157}{006}$) that these nuclei were "new formations," having no genetic relation with pre-existing nuclei, has not been corroborated by the later and more trustworthy investigations on this point.

Metschnikoff ($\frac{115}{006}$, $\frac{114}{411}$) and Mayer (113) have traced the nuclei of the blastoderm directly to the primary egg-nucleus in Aphis, Cecidomyia, Miaster, &c. Besides, Ganin (50), van Beneden and Bessels (14), Häckel (65, Peneus), Mayer (113, Eupagurus), and others have shown that in many Crustacea a genuine cleavage takes place. Ludwig (112) has established the same fact in reference to spiders. The studies of Kowalevsky (85), taken in connection with the supplementary observations of Dohrn (33), show clearly that the larger part of the yolk in the eggs of many insects is subject to cleavage. *Apis mellifica* furnishes a good illustration. Kowalevsky's statements is ($\frac{855}{455}$) as follows:—One finds on sections clearly outlined nuclei, like those seen in the cells of the blastoderm. They are found in all parts of the yolk, but are most numerous near the surface, just under the blastoderm. The maximum number of the nuclei, which at first are few, is reached about the time of exclusion. These nuclei were supposed to disappear with the yolk, taking no part in the embryonic tissues, and consequently having no other physiological function than that of hastening the dissolution of the yolk. Like the nuclei of the blastoderm, they were supposed to be derived from the egg-nucleus. In the case of Lepidoptera also ($\frac{855}{774}$) the entire yolk, beginning at the periphery and progressing towards the centre, breaks up into "Dotterballen," in each of which a *clear spot* is seen. Dohrn ($\frac{3}{1}\frac{31}{18}$) goes farther, recognising the "clear spot" as a nucleus and the "Dotterballen" as cells. In conclusion Dohrn remarks, p. 122: "From all this it is certain that the yolk in the egg of

insects, at a time when the embryo is almost fully developed, is full of *cellular elements*." In regard to the fate of these elements, Dohrn states, in opposition to Kowalevsky, that they enter into the composition of the embryo as cells (*middle intestine, blood, &c.*)

The occurrence of free nuclei in the egg of fishes is established by a large amount of concurrent testimony. Kupffer (2 1 7, Gasterosteus, Spinachia), Bambecke (8, Cyprinoids), Götte (5 6 4), Oellacher (1 2 5), His (7 4), and Klein (82, Salmo; Balfour (3 2 6–3 2 9, Mustelus) and Schultz (4 7 1 0–4 7 1, Torpedo), among others, have reported such nuclei.

There is a difference of opinion in regard to the origin of these elements. Klein maintains that they are formed *de novo*. Balfour leaves the question open as to whether they spring from pre-existing nuclei, or have an independent origin. Oellacher and Schultz derive them from the cells of the blastodisc. In regard to the rôle they play Balfour, Schultz, and Klein believe they enter into the entoderm and mesoderm. The following remark concerning the significance of these phenomena is from Balfour (3 1 2 7): "We are, therefore, forced to believe that the fine granular, and probably the coarsely granular, yolk of this meroblastic egg (Mustelus), consists of *an active organised basis with passive yolk-spheres embeded in it*."

For the occurrence of such nuclei in the egg of *birds*, we have the testimony of Götte (1 1 5 8–9), Rauber (1 3 1 0, 1 3 4 6, 1 3 2 9–5 7 0), and Balfour (3 0 2). Götte saw what he interpreted as a process of segmentation about these nuclei. His (1 6 7 1) and Disse (1 6 0 8) think, on the other hand, that Götte was misled by artificial productions. Whether these formative cells enter into the entoderm is not yet determined.

From the references here given it will be seen, not only that free nuclei are of very general occurrence, but also that they play no unimportant part in the formation of the embryo in bilateral animals. It is also plain, as Balfour and Lankester have stated, that the yolk is interfused with active protoplasm and consequently more or less subject to cleavage in one or another form. The production of free nuclei is only an abridged form of cleavage, and these nuclei undoubtedly have the same genetic relation with the egg-nucleus as have the nuclei of the blastoderm. That they form entoderm-cells in the case of Euaxes is quite certain, and in Clepsine the evidence all points in the same direction. This conclusion is, however, in plain contradiction with the opinion of Grube, Rathke, Robin, and Hoffmann, all of whom, however, overlooked the formation of entoplasts, and consequently were, as I believe, misled. Grube (5 0 3 6), Rathke (1 3 6 7), and Hoffman (7 7 4 9) all derive the entoderm by delamination from the blastoderm. According to Robin (2 9 7 1–2 3 9 8) the entoderm

(epithelium) arises from a solid cylindrical mass of cells which lies in the axis of the embryo, *between* the large blastomeres (*a*, *b*, and *c*). These cells are regarded as a prolongation of the "cord of cells" from which the walls of the pharyngeal atrium and the pharynx are formed. The lumen of the œsophagus gradually extends backwards through the cellular cylinder, and thus a digestive cavity arises lined with the axially placed cells, external to which are the segments, *a*, *b*, and *c*. The same mode of formation is maintained for Nephelis, but incorrectly according to Bütschli (30).

In regard to the role performed by the primary blastomeres (*a*, *b*, and *c*), Robin has arrived at conclusions utterly at variance with what is taught by figs. 93—95. Soon after exclusion segmentation sets in, beginning with *c*, which according to Robin occupies at this time the posterior end of the embryo, and extending to *a* and *b*. The result is that these blastomeres are broken up into a large number of cells lying externally to the epithelium. The part they take in the composition of the alimentary canal is stated by Robin himself thus:—"Ce n'est pas par atrophie qu'ils disparaissent, mais en se segmentant en grosses cellules qui forment *la couche moyenne de* l'intestin, et particulièrement *la couche hepatique*." On the other hand, I have found that these blastomeres preserve their individuality during the entire period of invagination and *neurulation*, and that no cells, save those before mentioned, are ever found in their interior. Furthermore, that the entoderm *incloses* these large yolk-spheres, instead of developing by an axial extension of the "œsophageal cord" through the *centre* of these.

VII. Circulatory Apparatus.

That we do not to-day possess a complete knowledge of the circulation in the Hirudinea is not the fault of neglect nor of unskilful hands; for among those whose patience and ingenuity have been taxed by this problem are such men as Cuvier, Moquin-Tandon, Siebold, Joh. Müller, Leydig, Wagner, Gratiolet, and Leuckart. Clepsine, Nephelis, and Hirudo medicinalis have been the principal objects of study. Fillippi, O. F. Müller, Grube, Leydig, Bidder, and others have made Clepsine an object of study in this particular. Filippi (36) found only the two lateral lacunæ and supposed that these were in direct communication with the digestive cavity. O. F. Müller (120) saw all the main channels except the median sinus.

Grube (59, 60) makes no mention of the lateral lacunæ, but says the dorsal vessel gives off to either side as many branches as there are lateral diverticula in the stomach. Leydig (108), whose account is the most accurate of any that has yet been given, saw all five longitudinal channels and gave a correct

account of the connection existing between the lateral and the median sinus. In one important respect Leydig's statements cannot be accepted—namely, that the dorsal trunk stands in free and open communication posteriorly with the median sinus. This error which was not corrected by Bidder, has been accepted by most authors and has found its way into our best text-books. Leydig (109) claims to have found the same free communication between dorsal trunk and median sinus in Piscicola. Here he describes six pairs of loops at the posterior end of the ventral trunk, each of which begins and ends at nearly the same point of the same vessel! Bidder ($\frac{1}{3}\frac{8}{9}$) did not find the ventral vessel, and of course could not determine how the dorsal vessel ended.

My own observations are confined to *Clepsine marginata*. Specimens from 10—15 days old have been found the most favorable for study. The entire circulatory apparatus is at this time fully formed, and the pigment has not developed to such an extent as to render it very difficult to trace the main channels and branches with a low magnifying power.

I have found the ordinary live box an indispensable instrument in this part of my work. The pressure applied was generally sufficient to *check* the flow of the blood, but not to stop the pulsation. The entire circulation, I hardly need to say, cannot be determined by the examination of a single animal. For tracing different parts, different degrees of pressure are necessary, and the best views of some parts are only seen when the pressure is so severe as to result in the almost immediate death of the worm. It is only after one has succeeded in tracing all the parts individually that one, in rare cases, is able to follow all in one individual. A constant supply of fresh material is of course indispensable to success in such a study. To this end eggs were collected in different stages, and by the time older specimens were exhausted, new ones were ready for use.

Fig. 56 represents the circulatory apparatus of a worm fourteen days old. It embraces two distinct systems:—(1) a *closed vascular system*, consisting, as in the Annelids, of a *dorsal* and *ventral trunk*, connected by lateral and terminal *branches*; (2) a *lacunar system*, consisting of a *marginal sinus* and a *median sinus*, which communicates with the marginal sinus by means of lateral branches, of which a single pair (right and left) is found between each somatomere, The first system is coloured red, and the second green. The median dorsal trunk, which alone is contractile, takes a zigzag or meandering course just above the alimentary tract. In the anterior third of the body it gives off three pairs of lateral branches and one odd pharyngeal branch, and just behind the eyes bifurcates, thus producing two cephalic branches. The posterior pair pass outward and backward over four pairs of diverticula (I think they extend still farther back-

wards later), and then bend forward along the margin of the body, and enter the ventral trunk near the fore end of the body. The next pair pass outward over the first pair of diverticula to the margin of the body, and then forward, entering the ventral trunk just a little in advance of the posterior pair. The next pair leave the dorsal trunk asymetrically, the left taking its departure at a point farther forward than the right. Both pass forward to the lateral lobes or angles of the head, and then backward, entering the ventral trunk just behind the cephalic pair. The odd pharyngeal branch (*p. b*) leaves the dorsal trunk just behind the cerebral ganglia (*c. g*), then passes forward or backward, according to the position of the pharynx (backward in the figure), to the hind end of the pharynx (*p.*), then forward along its median dorsal line; near the fore end it splits into two branches which, diverging, encircle the pharynx and unite again on its ventral surface; the course is then backward to the base of the pharynx; from this point it passes forward again, and finally enters the ventral trunk between the cephalic branches (*c. b.*), thus forming the anterior end of this trunk. The ventral trunk lies just above and in close apposition to the ganglionic chain. When the pharynx is protruded it passes through the œsophageal nerve-ring, and with it the pharyngeal branch (*p b*); the main ventral trunk (*v. t.*) remains in the position given in the figure. The dorsal trunk (*d. t.*) splits into two branches posteriorly, which unite again just behind the anus, thus producing an anal ring. Into this anal ring seven pairs of branches enter, which come from the posterior end of the ventral trunk. All the branches above given I have traced many times with great care, and I believe they represent all the connections between the dorsal and ventral trunk. Leydig and Bidder found only two of the four pairs of anterior branches which I have described. Leydig supposed that they terminated in the posterior disc, but Bidder was undecided about it. In the dorsal trunk are found, as Bidder has already stated, several "valves." These consist of cell clusters, which have but one point of attachment to the wall of the vessel. During the diastole the valves are thrown forward and to one side, and during the systole they lie transversely, closing the lumen of the vessel. The nearly colourless fluid of the system just described contains a few corpuscles, which are easily seen when the blood is made to flow slowly by pressure.

The marginal sinus, usually spoken of as two lateral sinuses, is a continuous channel, passing entirely around the animal and returning into itself. The median sinus, in which the ganglionic chain and ventral trunk appear to lie, can be traced through the entire length of the body proper. The transverse channels, joining the median with the marginal sinus, often anastomose

with each other and with the marginal sinus, as seen on the right side of the figure. Between some of these channels other transverse but smaller channels are often seen. The circulating fluid is almost colourless, and contains numerous corpuscles.

Bidder ($\frac{1}{4}\frac{8}{2}$) states that both on the dorsal and the ventral surface of each somatomere are found branches coming from the marginal sinus. I have never observed such branches. Although I have never been able to find any connection between the two circulatory systems, I cannot of course say positively that there is none. That there is no such communication as Leydig supposed is perfectly certain. In regard to the nature of the lacunar cavities, I fully adopt the opinion maintained by Leuckart ($\frac{1}{6}\frac{0}{7}$), that they are parts of the body-cavity. I am unable to state anything definite in regard to the way in which the blood-vessels originate. Fig. 96 represents a horizontal section, in which are seen parts of the subœsophageal ganglionic mass (*s g.*) and two body-ganglia. The intermediate ganglia lay beneath the plane of this section. Just behind the subœsophageal ganglia is seen a longitudinal collection of mesoderm cells, from which four pairs of lateral branches take their departure. Judging from the position of these cells and the number of the branches, it seems quite possible that they represent the anterior end of the ventral trunk, with its four pairs of tributary branches. Another interpretation is, however, possible, namely, that these branches are sections of the body-septa.

Summary (Sections IV—VII).—1. The first meridional cleavage, passing from the oral to the aboral pole, divides the egg into two unequal segments, the larger of which contains the remains of the ring-discs. This segment includes the *greater part of the future ectoderm*, the *entire mesoderm*, and about *one third of the entoderm;* the smaller segment contains *two thirds of the entoderm* and a *little ectoderm*.

2. The second meridional cleavage, passing from the centre outwards, cuts off about one third of the larger segment, and divides the smaller into nearly equal parts. Thus four large blastomeres are produced, three of which (*a, c, b*) are nearly of the same size, and contain *the entire entoderm;* the fourth and larger blastomere (*x*) contains *the entire mesoderm* and *a large* share *of the ectoderm*.

3. The oral pole of each blastomere is split off as an ectoblast. The first ectoblast is produced by *x*, the second by *b*, and the third and fourth simultaneously by *a* and *c*. The four ectoblasts lie in cruciform order in the boundary-lines of *a, c, b*, and *x*. During the divisions of the primary neuroblast, other ectoblasts are added to the four original ones from *a, b*, and *c*.

4. The dorsal blastomere (*x*) (so called because it is opposed

to the blastomere *c*, which later is ventrally placed) divides into the *primary neuroblast* (x^1) and *two mesoblasts* (x and $x\ y$).

5. The primary neuroblast divides into *ten cells*, the two smaller of which (x^4) are soon broken up into ectodermic cells, while the remaining *eight neuroblasts* are arranged in *two symmetrical groups* at the posterior border of the blastodisc.

6. In consequence of movements originating in the successive meridional divisions of the primary neuroblast, the mesoblast (x) moves upward and backward, and so takes a position at the right and a little below the left mesoblast (xy), where it soon becomes buried in the posterior ends of *c* and *b*, just beneath the right group of neuroblasts. The left mesoblast ($x\ y$) lies likewise just under the left group of neuroblasts. *The bilateral symmetry* thus reached in the arrangement of the neuroblasts and mesoblasts is a little imperfect in respect to the mesoblasts, inasmuch as they lie nearer the left than the right side.

7. The eight neuroblasts and two mesoblasts are the *builders of the germ-bands*, each of which has accordingly five builders, four neuroblasts, and one mesoblast. These bands appear as thickened lateral margins of the blastodisc, each of which is composed of *four parallel lines of cells* (produced by the neuroblasts) and of larger subjacent mesoblastic productions.

8. The *form* taken by the bands during the epibolic expansion is determined by *pressure in two principal directions* at right angles to each other. The *transverse pressure* is due to the expansion of the central field of the blastodisc, which is stronger in a lateral direction, in consequence of the obstructions at the anterior (cephalic mass) and the posterior border (neuro-blasts).

The longitudinal pressure arises from the proliferation of the neuroblasts and mesoblasts, and its direction is therefore forward. The *predominant direction* of the circumcrescent expansion is then in the *diagonal direction*, or *obliquely forwards*. It is on this account that the bands which, approximately speaking, are fixed against the cephalic mass, exhibit the strongest curve in their anterior halves.

9. A *solid invagination* of the ventral (*c*) and lateral blastomeres (*a* and *b*) accompanies the epibolic extension of the blastodisc, and is caused by this extension (reckoning from the moment the primary neuroblast begins to divide). This invagination consists mainly in forcing *c* towards the dorsal side, in such a manner that the broad ventral side becomes narrow and the narrow dorsal side becomes broad. The original wedge-shape is thus inverted.

10. About the time the germ-bands begin to form, the nuclei of *a*, *b*, and *c*, abandon their central position and pass to the periphery, where they multiply by free division during the whole period of epibolic invagination. The *entoplasts* thus

formed are numerous and scattered over the whole surface of the blastomeres at the close of the neurulation, and later appear as *entoderm-cells.*

11. Owing to the forward pressure of the germ-bands, a *pair of depressions* (pharyngeal clefts) form at their junctions with the cephalic mass, which deepen into an invagination that finally encircles the pharyngeal portion and forms the *pharyngeal atrium.*

12. The *mouth,* or *pharyngeal orifice* (*p a*), like the pharyngeal cavity, is an ectodermic invagination, which begins as a slight depression in the centre of the cephalic area, at a point corresponding nearly, if not exactly, with the *centre of the four original ectoblasts.*

The mouth invagination is at first continuous, by a linear depression, with the *primitive groove,* which is formed by the junction of the two germ-bands.

13. The *conjunction of the germ-bands,* in harmony with the oblique forward direction of predominant growth, is accomplished first at the cephalic ends, and from here progresses gradually towards the anal end, which is reached about the time of exclusion. The *somatomeric segmentation* follows closely upon the union of the bands, and progresses in the same direction. The final somatomere is completed at the end of one or two days after exclusion, after which the remnants of the neuroblasts and mesoblasts and the primary blastomeres (*a, b, c*) soon lose their individuality, and form only a common mass of yolk, which is driven back and forth by the contractions of the embryo.

14. The *ganglionic chain* is formed from the *eight rows of cells* produced by the neuroblasts, and does not probably include a other elements. The precise origin of the cerebral ganglia is unknown.

15. The *number of pairs of ganglia* corresponding to that of the somatomeres is *thirty-three. Four* of these are consolidated in the *subœsophageal ganglia, eight* in the *ganglia of the disc,* and *four* in the *terminal ganglia* of the body.

16. At the time of exclusion *two rows of colossal cells* (segment-cells), products of the mesoblast, are found on each side of the median ventral line of the neurula beneath the neural elements. The two median rows appear to be connected with the cells of the segmental organs, but in what way is unknown.

The two outer rows maintain nearly their original position in the walls of the septa, but are finally lost sight of in the growing tissues. The position of these cells and their prominence suggest that they are the mother-cells of the male sexual organs.

17. There are *sixteen pairs of permanent segmental organs,* fifteen of which correspond to the fifteen pairs of enteric diverticula, and the sixteenth lies before the anterior pair of diverticula. They arise from mesodermic cell-groups, of which two are

originally found on the floor of each somatomere, to the right and left of the nerve-chain. The direction of the growth is transverse along the inner wall of the body, from the median ventral to the median dorsal line.

18. The closing of the germ-bands is followed by a *bilateral growth of the mesoderm* in two directions:—(1) around the alimentary tract, meeting in the median dorsal line; and (2) around the nerve-chain, meeting in the median ventral line (*evolutio bigemina*, Baer).

19. The *septa* are diaphragmatic growths of the mesoderm between the somatomeres.

20. The *entoderm* (epithelium), which has its origin in the peripheral entoplasts, *encloses the yolk remains as food.*

21. The *diverticulate form* of the digestive tracts is produced by the *antecedent* growth of the mesoderm (septa, muscles, &c.).

22. The *circulatory apparatus* consists of two systems:—(1) a *closed vascular system*, and (2) a *lacunar system*. The first has a *dorsal* and a *ventral trunk*, communicating anteriorly by four pairs of branches and an odd pharyngeal branch, and posteriorly by seven branches. The second has a *median* and a *marginal sinus*, the former communicating with the latter by a pair of transverse channels in each body-segment.

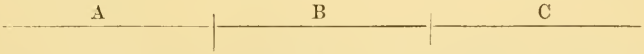
General Considerations.

a. Axial Differentation.

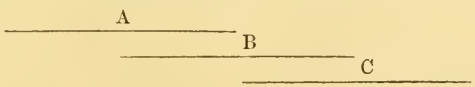
The early appearance of structural axes in the developing egg is a very significant fact, and deserves special mention. Among those authors who have called attention to this point may be mentioned Auerbach ($\frac{3}{1\,0\,6}$, Ascaris and Strongylus), Hatschek ($\frac{1\,6\,8}{5\,0\,8}$, Pedicellina), Selenka ($\frac{1\,5\,1}{1\,7\,4}$, Holothuria), A. Agassiz ($\frac{2}{3\,6\,4}$, Ctenophora), His ($\frac{7\,4}{6}$, Salmo), and Rauber ($\frac{1\,3\,3}{1\,8\,0}$, bilateral animals). The unripe ovum is characterised by a *spherical symmetry*. At maturity, or immediately after, a *main axis of symmetry* appears (*radial symmtry*), and with, or during, the cleavage, the *lateral axes* (*bilateral symmetery*). This is the order of axial differentiation in bilateral animals. The first indications of a main structural axis appear with the passage of the germinal vesicle to the periphery of the egg. The pole of the axis thus localised marks, in some cases, the oral end of the future embryo. The lateral axes, which in most cases becomes recognisable during the cleavage, can be located in Clepsine before the cleavage begins by means of the crescent shape assumed by the upper ring-disc. At the completion of the second meridional division, the right and left sides are given in the lateral blastomeres (*a*, *b*). A complete bilateral symmetry is established with the appearance of the neuroblasts and mesoblasts.

Thus the order of axial differentiation is in harmony with the supposed phylogenetic order of development. A point of considerable theoretical interest demands attention in this connection. In Clepsine, as in numerous other forms, a well-marked bilateral arrangement appears *before* the definite gastrula. This is only one of the many instances of what Häckel ($\frac{6}{4}\frac{5}{1}\frac{}{2}$) has called *ontogenetic acceleration* (heterochrony), and it finds an explanation in the principle of *precocious segregation* (Lankester ($\frac{0}{1}\frac{1}{3}$)). Every definite differentiation of material presupposes a *preliminary segregation*. Clepsine furnishes some striking illustrations of this fact. Nerve-cells are preceded by neuroblasts, these by a primary neuroblast, and the latter by antecedent stages of segregation. The same is true of the mesodermic and entodermic elements. Precisely when this segregation begins it is impossible to say; but it is certain that it begins in the great majority of cases long before cleavage. So likewise every ontogenetic *form* presupposes a *preliminary arrangement*. A *radiate* arrangement precedes the gastrula; a bilateral arrangement the definitive bilateral form. Thus it happens that, before a given ontogenetic stage is completed, the preliminary segregations and arrangements for the following stage are already more or less advanced. Thus the gastrula —and more rarely the blastula—is pre-stamped with the antimeric character of the ultimate bilateral form. Such antecedent segregations and arrangements illustrate the tendency to concentration in ontogenetic recapitulation. This concentration does not, however, essentially disturb the palingenetic order of events.

Let A, B, and C represent three successive phylogenetic forms; thus:



The ontogenetic concentration would then be represented by the same forms progressing side by side; thus:



If only the extremities of the lines are kept in view, it will be seen that the palingenetic order is preserved.

b. Cleavage-cavity (Blastocel, Huxley.)

A morula, as defined by Häckel ($\frac{6}{4}\frac{5}{2}\frac{}{1}$), does not occur in the ontogeny of Clepsine. Before subscribing to the opinion that such a stage really belongs to the ontogenetic series, some more convincing proof than mere surface views must be adduced in its favour. In most cases where cleavage has been subjected to

detailed and accurate study it has been found to end in a blastula and not in a morula. "A solid sphere of *indifferent* cells" is, to say the least, a very improbable form, so improbable that its existence may be held questionable until established by positive evidence. The doubt is all the more justifiable, as more careful investigation has, in many cases, already shown, that the so-called *mulberry-stage* is not a morula, but a blastula or even a gastrula.

What is the origin and signification of the blastocoel? Baer ($\frac{5}{4}\frac{5}{5}$) supposed this cavity to be the place originally filled by the germinal vesicle. According to Häckel ($\frac{6}{3}\frac{2}{7}$, $\frac{6}{4}\frac{5}{2}$), it is a cavity formed by the collection of a fluid in the centre of the so-called morula. What this fluid is, where it comes from, and why it appropriates a central position, forcing the cells into the periphery, we are not informed. I will give some reasons for the opinion that the *blastocoel, whenever it appears, forms as a necessary result of the cleavage process.* As is well known, a dividing cell lengthens in a direction at right angles to the plane of cleavage. An interesting phenomenon follows the close of the cleavage, viz. the approach of the two cleavage products, in consequence of which a sphere, composed of two distinct hemispheres, is formed (fig. 16). This phenomenon is familiar to all who have followed the cleavage-process. Some authors have contented themselves with a simple report of the fact, while others have attempted to find an explanation in the confinement of the egg in a membrane. This explanation, however, cannot apply in cases where the membrane stands at so great a distance from the egg that it offers no resistance to the complete separation of the division products.

I once, accidentally, while endeavouring to separate the eggs with a dissecting needle, freed an egg from its membrane. The egg divided, and the two spherical parts, at the close of the division, touched each other at a single point. Immediately after this they began to approach, flattening against each other, and finally they formed a single sphere, as perfect as that in fig. 16.[1] This convinced me that the membrane had nothing whatever to do with the phenomenon. I am unable to give anything more than a hypothetical explanation. The cause of the separation and of the subsequent approach is undoubtedly the nucleus. If we suppose that the two poles of the amphiaster are similar poles, they will of course repel each other. To account for the approach, it is necessary to assume that subsequent to the division one nucleus becomes positive and the other negative. The proof that this is an electrical phenomenon

[1] The first cleavage of the egg of Lymnæus, or of Planorbis, is performed in the same manner.

is at present wanting, but the facts seem to point in this direction very strongly. The explanation of the cleavage-cavity, however, does not depend upon the decision of this question, but upon the fact that cells lengthen and push each other apart in cleaving. This cavity arises very early in Clepsine, and at the place where the first three plains of division cross one another (fig. 79, seg. *c*). According to Götte ($\frac{5\,8}{1\,2\,9}$), it forms in precisely the same manner in the amphibian egg. Here, as in Petromyzon ($\frac{1\,4\,7}{8\,-\,9}$), the wall of the Blastula is at first one cell thick, but soon becomes several cells thick. A transitory cavity appears in the eight-cell stage, in the egg of osseous fishes, according to His ($\frac{7\,4}{6}$). The same was observed by Bambeke (9) and Lereboullet (102). Balfour and Schultz did not find it. Oellacher (124) doubts the existence of such a cavity, but has certainly indicated something of that nature in his fig. 24, Pl. XXXIII. Kowalevsky, Owsjannikow, and N. Wagner ($\frac{8\,9}{1\,7\,3}$) testify to the occurrence of the same in an early stage (6—8 segments) in the egg of the Sturgeon. Rauber ($\frac{1\,3\,1}{9}$) alleges that a segmentation cavity, entirely distinct from the later embryonic cavity ("Keimhöhle"), occurs in the egg of the bird at a time when only four segments have been formed. According to Kowalevsky ($\frac{8\,3}{4}$), a small hole appears between the first four blastomeres in Ascidia, which increases in size during the cleavage, and becomes the blastocœl. The Blastula form is reached in the seven-cell stage in Pedicellina (Hatschek, $\frac{6\,8\,5}{5\,0}$), and at about the same time in Holothuria (Selenka, $\frac{1\,5\,1}{1\,5\,0}$).

In Geryonia (Fol. $\frac{4\,0}{4\,7\,8}$) it arises between the 8-cell and 32-cell stages. Metschnikoff ($\frac{1\,1\,7}{1\,1\,8\,-\,2\,3}$) says that no blastocœl occurs in certain Aeginidæ. Rabl ($\frac{1\,2\,8}{1\,9\,8}$), who seems to be very successful in confirming some of Häckel's most doubtful views (*e. g.* origin of mesoderm, monerula, and morula), asserts that in fresh water Pulmonata (Lymnæus, &c.) the cleavage ends in a morula, in the centre of which a blastocœl subsequently forms. In Euaxes (Kowalevsky) the blastocœl appears in the 8-cell stage, just as in Clepsine. In Lumbricus ($\frac{8\,5}{2\,1}$) it is never much more than a simple fissure. In *Cucullanus elegans* (Bütschli, $\frac{2\,8}{1\,0\,4}$) the cleavage ends in producing two cell-plates, between which there is no open space. Only a narrow fissure is found between the cleavage elements in Paludina ($\frac{3\,0}{2\,1\,8}$).

F. E. Schultz ($\frac{1\,4\,6}{2\,6\,9}$) describes an interesting segmentation-hole in the egg of Sycandra. The cleavage cells, sixteen in number, form two rings of eight each (apical and basal). The cleavage hole passes through the centre of each ring, and is a little smaller in the apical than in the basal ring. Both ends of the hole finally close, but the apical first. Thus a simple cavity is formed. According to Flemming ($\frac{3\,8}{1\,2\,4}$), a lenticular cavity

("Binnenhöhle") arises between the first two blastomeres (Anodonta and Unio).[1] This space (p. 126) disappears during the second division, but reappears after it is completed. The same phenomenon is repeated in the subsequent divisions. "Die Blasenform des Keimes, schon von der ersten Theilung angedeutet . . . . ist bisher allgemein verkannt worden" (p. 163). From the cases here cited, it is evident that the blastocœl begins very early either as a cavity or a simple hole. The case of Cucullanus is that of Lumbricus and Paludina carried to the extreme. Here there is no proper cavity—not even a fissure—but the two cell-plates must nevertheless be regarded as a Blastula, and not as a Morula.

It is worthy of remark that the blastocœl, wherever it is present, is at first bounded by a single cell-layer. The case of Ascidia (Kowalevsky), of Sycandra (Schultz), of Anodonta and Unio (Flemming), of Clepsine and Euaxes, and numerous cases like the latter, show that the blastocœl arises by the cells being pushed asunder in the process of cleavage. The case of Cucullanus shows that the cleavage may proceed in such a manner as to avoid any such cavity. The fluid which collects in the hole or cavity is the perivitelline liquid.

(*c*) Mesoderm.

The orgin of the mesoderm is a time-honoured problem, the solution of which is still obscure. Some investigators derive it from both ectoderm and entoderm; others assert that it arises independently of either. One claims that it owes its origin to the ectoderm alone; and still another to the entoderm plus certain migratory elements. Many believe that it arises by delamination, a few by a process of infolding; others by migration, and others still, by growths of one of the primary lamellæ into the blastocœl. In short, almost every possible theory has been suggested, and advocated, at one time or another, so that nothing remains but to test opinions already stated. Whoever is now ambitious to launch a new theory, must approach the subject from a phylogenetic stand-point; here one can venture without much danger, provided only he is content to set up one of those theories which can never be proved or disproved. Of such theories we have already at least two good examples. The most plausible view of the genealogical origin of the mesoderm is that first suggested by Lankester, which will be considered farther on. The question, whence comes the mesoderm, is by no means easy to answer, as might be inferred from the fact that so many different opinions exist in regard to it. In many cases, all we know of its origin is, that it arises *between* the two primary lamellæ just where these are continuous (Properistoma, Häckel.) As the edge of the blastopore is, so to speak, a neutral zone, how

[1] I have observed the same in the egg of Planorbis.

can the origin of the mesoderm in this place be referred to one of the primary germ-layers, to the exclusion of the other? In such cases the resemblance of the cells, to those of the ectoderm or entoderm is usually regarded as evidence of relationship; but such a test is by no means decisive. The difficulty in the case of most fishes, birds, and mammals, is even greater. In a very few instances the mesoderm has been traced to distinct cleavage-cells, and in no case has this been done more completely than in Clepsine. Yet the question, which lamella gives it origin? admits of a difference of opinion. One might be inclined to think that the two mesoblasts arise from the entoderm, judging from their size, position, and composition. Like the entodermic blastomeres (*a*, *b*, *c*,) they contain a large amount of yolk, which later serves as food for the embryo. But this is no criterion, as the neuroblasts are also loaded with the same food-material.

On the other hand, the origin of the mesoblasts from that one of the four original blastomeres (*x*) which is preëminently ectodermic, would seem to favour the opinion that it is derived from the ectoderm. The question is therefore debatable. I incline to the former opinion for this reason, that the mesoblasts represent the lower pole (entodermic pole) of the blastomere (*x*) while the neuroblasts represent the upper pole (ectodermic pole). This view, it seems to me, is most in harmony with known facts. The first two meridional divisions do not completely separate the elements of the future lamellæ in Clepsine; for the upper pole (oral pole) of each is ectodermic, and the lower, if we except the fourth (*x*) is entodermic. The first sharp separation of ectoderm from entoderm begins with the parallel division, which produces the four original ectoblasts at the oral pole.

I shall enter into the historical part of this subject only in so far as it, in conjunction with my own observations, bears directly upon a few important points, the most significant of which is *the bilateral origin of the mesoderm*.

The first, so far as I am aware, to trace the mesoderm to to a single pair of mesoblasts was Kowalevsky (1871). He stated ($\frac{M.5}{2.3}$) that during the invagination (Lumbricus) a single cell (*m*), on each side the median line, steps out of the entoderm into the blastocœl. His figures (10-16, Pl. VI.) show that these two cells appear *behind* the blastopore, and that they produce *forward* two longitudinal masses of mesoderm-cells. The next case of this kind was established by Rabl (1876). Rabl ($\frac{9.7}{3.2.4}$) traced the origin of two mesoblasts, *situated at* ONE END *of the blastopore*, to a large entoderm-cell, and was the first to emphasise the general importance of the double origin. Hatschek (1877, $\frac{6.8}{5.6.7}$) found also two mesoblasts, derived from entoderm, at the *hind end* of the blastopore in Pedicellina. In addition to these four cases (Clepsine included) where it is certain that the

mesoderm arises from a single pair of cells, placed at the posterior end of the blastopore, there are others which are more or less doubtful. Bütschli ($\frac{30}{24\ 3-4}$) who was the first to devote special attention to the germ-lamellæ in Nephelis, found the mesoderm at first as two lateral lines of cells. Whether they were produced by two primary mesoblasts, was not ascertained. Kowalevsky ($\frac{5}{2}$) did not succeed in tracing the mesoderm in the Hirudinea. In Euaxes ($\frac{8\ 5}{1\ 3}$) the mesoderm was referred, in part at least, to two cells. Whether these two cells were really mesoblasts or neuroblasts, admits of doubt. It seems probable, though not certain, that the mesoderm has a similar double origin in Nassa (Bobretzky, $\frac{21}{1\ 1\ 0}$). Bobretzky derived the mesoderm from the ectoderm. Rabl ($\frac{1\ 2\ 8}{2\ 0\ 2-4}$) found a double symmetrical arrangement of the mesoderm in Lymnæus, and derived it from entoderm and ectoderm. According to Bütschli ($\frac{2\ 0}{5\ 2\ 0}$, $\frac{3\ 0}{2\ 2\ 0}$) the mesoderm appears as a few cells (probably bilaterally symmetrical), at the hind end of the blastopore in Paludina. Bütschli thinks they are of entodermic origin, while Lankester ($\frac{2\ 0}{1\ 6\ 0}$) refers the origin to both ectoderm and entoderm. Langerhans ($\frac{9,5}{1\ 7\ 2,1\ 7\ 5\ 4\ 8}$) found in several Gasteropods (Acera, Doris, Aeolis) *two large cells* at the hind end of the blastoporic cleft, but was able to give no explanation of the same.

Bütschli ($\frac{3\ 0}{2\ 3\ 8}$, Neretina) mentions a pair of cells (x) as arising from the large blastomeres (see fig. 46, Pl. XVII), the fate of which was unknown. The same may be said of a pair of cells found in the egg of Helix (78, fig. 5). These doubtful cases are mentioned only in the hope of turning the attention of embryologists more to this point.

Indications of this double origin are not wanting in other classes of animals. In Sagitta (Kowalevsky, $\frac{8\ 5}{8\ 7}$) the mesoderm arises as lateral diverticula of the entoderm.[1] Kowalevsky, in a very valuable paper ($\frac{8\ 7}{1\ 8\ 0-7}$), has demonstrated the same thing for Amphioxus, and at the same time proved the correctness of Balfour's explanation ($\frac{1\ 2}{2\ 2\ 4}$) of a fact noted in the development of the Selachians ($\frac{1\ 1}{3\ 4\ 7}$)—namely, that the body-cavity extends originally to the top of the protovertebræ. The first to suggest that the mesoderm, viewed phylogenetically, arose as paired outgrowths of the entoderm, was Lankester. Commenting on Professor Huxley's view of the body-cavity (this Journal, January, 1875), Lankester says: "I wish now very briefly to point out that viewing the matter genealogically it is quite possible that by the obliteration of the lumen of gastro-vascular outgrowths of the primitive alimentary canal a large bulk of cellular elements should be furnished to the so-called 'mesoblast' from the hypoblast, and that subsequently this solid mass of cellular elements

[1] In Pleurobranchidium, according to Lankester, a part of the entoderm lies outside the alimentary cavity, and suggests a comparison with the development of Sagitta.

should by splitting develop a cœlom. In this way it is conceivable that the schizocœlous condition might develop from the entero-cœlous and gradually lose all trace of its ancestral origin further than is afforded by the derivation of some mesoblastic cells from hypoblast." Balfour ($\frac{1}{2}\frac{2}{2}\frac{2}{3}$), pursuing the same line of thought a little later, remarks: "It might then be supposed that the muscular system of part of the alimentary canal took the place of the primitive muscular system of the body; so that the whole muscular system of higher animals would be primitively part of the muscular system of the digestive tract." The origin of the mesoderm (Selachii) as "two lateral masses" (Balfour, $\frac{1}{3}\frac{1}{3}\frac{1}{5}\frac{1}{6}$) has been confirmed by His ($\frac{7}{1}\frac{5}{1}\frac{1}{8}$). The investigations of Kowalevsky upon Sagitta and Amphioxus, above referred to, furnish indubitable evidence of the origin of the mesoderm in two cases. It furnishes such a complete explanation also of the formation of the body-cavity in the Selachians, that one can scarcely doubt that the mesoderm and body-cavity arise in essentially the same manner in both cases. Kowalevsky ($\frac{8}{1}\frac{7}{9}\frac{7}{0}$) furthermore states that the origin of mesoderm in the Brachiopods is similar to that of Sagitta and Amphioxus. Metschnikoff ($\frac{1}{1}\frac{2}{4}\frac{3}{7}$) and A. Agassiz (1) have shown that in Echinoderms the body-cavity arises as lateral diverticula of the entoderm. Putting all these facts together the conclusion first drawn by Lankester appears exceedingly plausible. If this view be correct, it is easy to account for the early appearance of the mesoderm as two mesoblasts, as in Lumbricus, Unio, Pedicellina, Clepsine, and perhaps many other worms and molluscs. It is simply an early expression of its primitive bilateral origin. Before leaving this subject I will call attention to some facts which seem to lend a certain degree of plausibility to the opinion that the mesoderm may at one time arise with the entoderm and at another with the ectoderm.

In the case of Unio (Rabl) the first division splits the egg into two unequal segments. The larger segment contains all the entoderm, all the mesoderm, and some ectoderm. In Clepsine the larger segment contains one third of the entoderm, all of the mesoderm, and the larger part of the ectoderm. The difference in these two cases, so far as the mesoderm is concerned, is that in Clepsine the mesoderm goes with the segment that is preëminently ectodermic, while in Unio it goes with the segment that is preëminently entodermic. Van Beneden ($\frac{1}{2}\frac{5}{3},\frac{1}{4}\frac{5}{1}$) has found also that the mesoderm goes with the entodermic segment in the rabbit. Selenka has observed the same (Holothuria, $\frac{1}{1}\frac{5}{5}\frac{1}{9}$). If the mesoderm in one case is cut off with the ectoderm, and in another with the entoderm, it seems not improbable that by a species of cenogenetic heterotopy (Häckel) the mesoderm should

sometimes arise *with* the ectoderm, and so give the appearance of arising *from* the ectoderm (Bobretzky, $\frac{2}{1}\frac{1}{0}\frac{1}{9}$).

(*d*) The Gastrula.

Supported by the investigations of the celebrated Russian embryologist, Kowalevsky, as well as by their own observations, Häckel and Lankester arrived, independently of each other, at fundimentally similar views in regard to the importance and universality among Metazoa of the Gastrula or Planula-phase of development. Both these investigators published sketches of their views at about the same time (61, 96), Häckel's appearing but a little earlier than that of Lankester.[1] The latest form of Häckel's theory appeared in the 'Jenaische Zeitschrift,' vols. viii. and ix. (64, 65). A complete view of Lankester's Planular theory, and its points of divergence from the Gastrula theory was published in this journal, Oct. 1877.

The chief points of difference between the two theories in their latest forms concern the interpretation of the Gastrula orifice (blastopore, Lankester), and the genealogical relationship between the delaminate and invaginate forms. Häckel has from the outset adhered to the opinion that the "gastrula invaginata" (Lankester) is the primitive form, and the "gastrula delaminata," if such exist, a secondary form which has arisen by cenogenetic changes from the former ($\frac{6}{5}\frac{5}{4}\frac{-}{5}$). What these changes are, or in what conceivable way the one form could pass into the other, Häckel does not attempt to say. Manifestly there is some difficulty here.

Lankester, who at first entertained the same opinion, has in his last paper (101) strongly advocated another view, viz., that the delaminate Planula is the primordial form. Lankester has undertaken to account for the substitution of invagination for delamination on the hypothesis of "precocious segregation." This principle, which he has recognised in former papers, but which is here for the first time clearly formulated, and its application to the question under consideration, will be best understood if stated in the author's own lucid words. "Though the substance of a cell may appear homogeneous under the most powerful microscope, excepting for the fine granular matter suspended in it, it is quite possible, indeed certain, that it may contain, *already formed and individualised*, various kinds of physiological molecules. The visible process of segregation is only the sequel of a differentiation already established, and not visible. The descendants of the Diblastula (diploblastic Planula),

[1] See also "Development of the Pond-Snail," this Journal, vol. xxii (n.s. 14), pp. 365—367

which had gradually acquired a separate *deric* and *enteric* cell-layer in place of one cell-layer with an external deric moiety and an internal enteric moiety to each cell, must have tended in their individual development from the egg-cells of parent Diblastulæ to have established more and more early, in the course of their growth, the important separation of deric and enteric cells, of ectodermic and endodermic elements. In so far as the differentiation of the two kinds of factors or molecules, the deric and the enteric became dependent on heredity, and less dependent on the direct adaptative causes which first brought about the differentiation, in so far would it be possible for the differentiation, the segregation of deric molecules from enteric molecules, to take place at an earlier point in the embryonic development than that (namely, the blastula stage), at which the direct adaptative causes could come into operation. Thus, since the fertilised egg already contained hereditarily-acquired molecules, both deric and enteric, invisible though differentiated, there would be a possibility that these two kinds of molecules should part company, *not* after the egg-cell had broken up into many cells as a morula, but at the very first step in the multiplication of the egg-cell. In fact, some or all of the deric molecules might remain in one of the two first cleavage-cells, and all of the enteric molecules, with or without some of the deric molecules, might remain in the other. We should not be able to recognise these molecules by sight; the two cleavage-cells would present an identical appearance, and yet the segregation of deric and enteric factors had already taken place. This hypothesis may be called that of Precocious Segregation, "precocious," since it is the acquirement of a condition in the developing organism, in virtue of heredity, at an earlier period of development than that at which such acquirement was attained by its forefathers through adaptation."

The principle of segregation here so clearly enunciated can hardly be doubted.

The question on the answer to which everything turns is this: Is the segregation which leads to invagination more precocious than that which terminates in delamination? A negative answer to this question would be inconsistent with the above explanation of the transition from delamination to invagination.

Van Beneden has reported an exceptionally sharp differentiation of ectoplasm from endoplasm, which appears with the first cleavage (rabbit). So far as yet known this case is without a parallel.

In the vast majority of cases where invagination occurs each of the two first segments contain both ectoplasm and endoplasm. In the case of Unio one blastomere is wholly ectodermic, but the other contains both elements, and the separation of the two

factors is only completed after the so-called morula stage is reached.

In Clepsine ectoplasm and endoplasm are not fully segregated till about the time the germ-bands begin to form.

Without citing further examples it may be stated, as a general fact, that the segregation of ectoplasm and endoplasm goes on *during* cleavage as well as previous to cleavage. In the more typical forms of invagination this differentiation manifests itself later than in the modified forms of epibolic invagination. Indeed, in some cases there is no appreciable differentiation till after the invagination begins.

But how is it in the case of delamination? According to Fol (40. Geryonia) the ectoplasm is distinct from the endoplasm before the cleavage begins, and remains distinct during the whole period of cleavage. To be sure, the *definitive* separation of one from the other is accomplished with the formation of the Planula. In what case of typical invagination do we find so complete precocious segregation? A similar precocious differentiation is manifested in some other Cœlenterata (*e. g. Escholtzia cordata*, Kowalevsky, 84), where a more or less modified form of invagination takes place. Such cases do not, however, lessen the force of the above objection. The *qualitative* and *topical* differentiation of elements which characterises the egg of Geryonia appears to me irreconcilably opposed to the hypothesis of primary delamination.

Another objection is the fact that the delaminate Planula is reached by a more direct route than the invaginate planula, and hence bears the mark of ontogenetic abridgement.

Still another objection is the occurrence of the invaginate development in the very lowest Metazoa (Dicyemida, $\frac{17}{101}$; Gastrophysema, $\frac{16}{40}$; Sycandra raphanus, $\frac{14}{71}$).

A fourth objection is found in the *progressive differentiation* of the primary lamellæ during cleavage, and especially during the invagination. The theory of primary invagination disposes of these objections and furnishes an easy explanation of the primary differentiation into ectoderm and entoderm.

The primary cause of invagination is undoubtedly the cause which operates to-day. This cause, so plainly seen in epibolic invagination, is *the unequal growth of the two poles* (hemispheres) *of the Blastula*. This is well illustrated in that rare but instructive form of the Blastula described by Bütschli ($\frac{28}{104-108}$, Cucullanus).

Before the invagination as Bütschli remarks (p. 106), the character of the two cell-layers is the same. The process of invagination is thus described:—"As the outer cell-layer (ectoderm) enters upon a rapid growth in which the future inner layer (entoderm) does not participate, the cell-plate begins to bend, becomes hollow, and finally the edges close over the cavity."

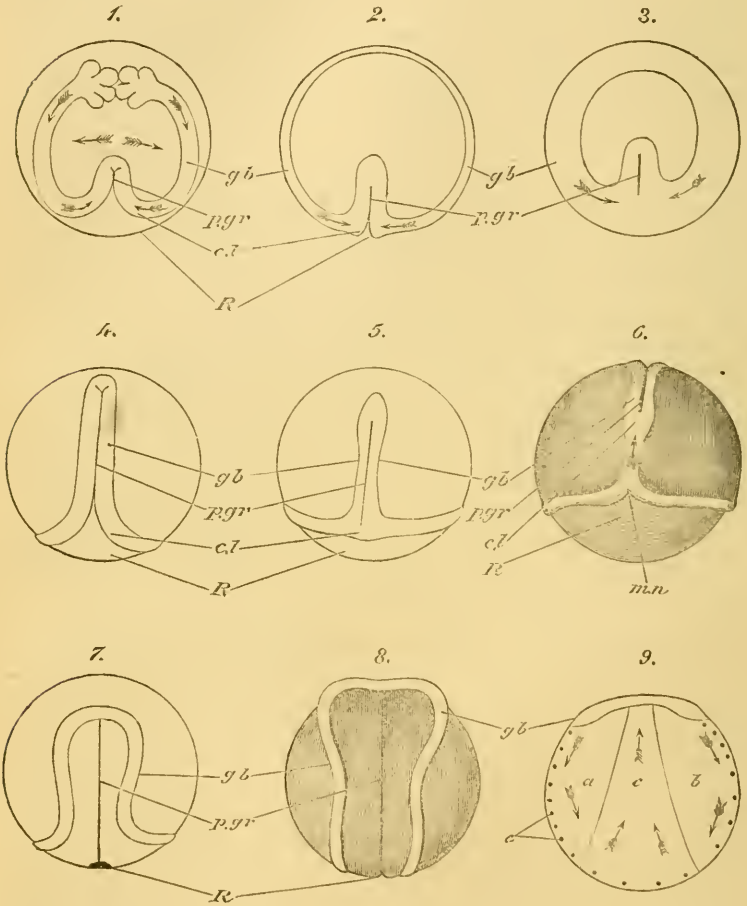
With this process "a change in the character of the two cell-layers goes hand in hand. The cells of the outer layer become larger . . . quite clear and transparent. Those of the inner layer, on the other hand, do not grow, but become darker, yellowish, and finely granular." All this is in harmony with the opinion that the differentiation of entoderm and ectoderm was originally the *result* rather than the cause of invagination. That indiscernible differences may have existed in the character of the two cell-layers before invagination is not at all improbable, and this would present no difficulty.

I have already called attention to the fact that cleavage may proceed in such a manner as to produce no proper blastocœl; but in most cases such a cavity arises as a necessary accompaniment of the cleavage. Starting with a typical Blastula it is not necessary to assume that unequal growth would always result in invagination; but it is easy to see that the more rapid growth of one hemisphere, accompanied perhaps by an absorption of the blastocœlic fluid, *might* lead to a double-walled Gastrula. The unlike conditions into which the two hemispheres would thus be brought would necessarily result in a physiological as well as morphological differentiation. Such an invaginatory mode of growth would act as an economy of space, and the advantage thus offered would afford an opportunity for the operation of Natural Selection. According to this view the blastopore, as Lankester has stated, would be "simply the necessary accompaniment of the invagination." Whether it remained open and functioned as a mouth and anus, or closed up, need not here be discussed.

The transition to the delaminate development is not of course, on one hypothesis more than the other, effected by intermediate *forms*. The invagination once established, the consequent differentiations would become hereditary and thus render it possible for "the segregation of deric molecules from enteric molecules to take place at an earlier point in the embryonic development than that (namely, the blastula-stage), at which the direct adaptative causes could come into operation." The hereditary differentiation carried to the extreme would manifest itself in the egg even before cleavage, and we should thus arrive at conditions represented in the egg of Geryonia. The cleavage would result as before in a Blastula, and the first tangential cleavage (the direction of the cleavage probably being determined by the heriditarily acquired concentric arrangement of the ectoplasm and endoplasm) would result in the delaminate Gastrula.

According to this view the phenomenon of polarity, so universally exhibited in eggs, may with some plausibility be regarded

as a precocious appearance of a character which originated in the differentiation of the two hemispheres of the primordial Blastula, and the concentric segregation as a peculiarity inherited from the Archigastrula.



Figs. 1, 4, and 9 =	Clepsine.	*g. b.* = Germ-band.
Fig. 2 =	Pristiurus (after His).	*p. gr.* = Primitive groove.
Figs. 3 and 6 =	Chick (after Rauber).	*c. l.* = Caudal lobes.
Fig. 5 =	Salmo (after His).	*R.* = Anus of Rusconi.
Fig. 7 =	Accipenser (after Kowalevsky).	*m. n.* = Marginal notch.
Fig. 8 =	Frog (after Rusconi).	*e.* = Entoplasts.

(*e*) **The Neurula of Clepsine compared with that of Vertebrates.**

The germ-bands in Clepsine, their epibolic growth, and final conjunction at the median neural line, are so remarkably similar to the embryonic rim and the process of neurulation in Vertebrates, as to indicate a fundamental relationship. This similarity has already been noticed by Semper (153) and Hatschek (67), and adduced as an argument in favour of a geneological relationship between the vertebrates and the invertebrates. Of the justice of the comparison I am thoroughly convinced, and I propose here to add some considerations in its favour which have hitherto passed unnoticed. I believe I have already made clear the manner in which the ectoderm with its marginal bands incloses the blastomeres. If my account of the præcession (Rauber) of the germ-bands is correct, it is evident that Hatschek($\frac{6}{7}$) has a very incorrect notion of the cause for their closing first at the cephalic ends. Hatschek attributes this to a more rapid development of the mesoderm at this end of the bands. I have shown that the mesoderm develops from the posterior ends of the bands. The multiplication of mesodermic elements in the fore ends has nothing whatever to do with the early conjunction at this point.

In the case of Lumbricus, as Kowalevsky and Hatschek have stated, the præcession is more rapid at the hind end, just the inverse of what takes place in Clepsine. The same is true in the frog, as Rusconi (144) long ago pointed out, and in Accipenser ($\frac{8}{17}\frac{9}{0}$). In both these cases, however, the approach of the two halves of the embryonic band is at first more rapid near the middle (figs. 7 and 8, *g b*). Whether the union of the germ-bands, or two halves of the embryonic rim, takes place earlier at the fore end than the hind end, or the inverse, is a matter of secondary importance. The fact that such a union does take place, and that the leading features of the præcession in each case are fundamentally alike, is the point of central interest.

Professor His has given an excellent account of the manner in which the fish-embryo lengthens backward by the apposition of the two halves of the embryonic rim. That his words give a complete picture of what happens in Clepsine is good evidence that the process described is one and the same in both cases.

"Man kann den Vorgang veranschaulichen, wenn man einen zum Ring geschlossenen Gummischlauch an einer Stelle so einbiegt, dass er eine dem Centrum zustrebende Schleife bildet. Bringt man beide Schleifen-schenkel zur Berührung und verlängert sie mehr und mehr, so wird der Ring immer kleiner und schliesslich geht er in der Bildung des zwei-theiligen

Stranges auf" (7 5 / 1 0 9). Compare also (7 4 / 2 1). This description is in harmony with the investigations of Kupffer (94), Kowalevsky (88), Balfour (11), and Schultz (145). Oellacher (124) on the other hand regards the hind end of the embryo, instead of the fore end, as fixed, according to which, as His has remarked, the embryo must lengthen forward by intussusception.

A comparison of figs. 1, 2, and 3 with somewhat later stages, figs. 4, 5, and 6, will show that the neurula of the chick, or of the fish, belongs to the same type as that of Clepsine. The embryonic rim in the Selachian egg appears first in the form of a ring; but this ring is composed of two homotypical parts, as evinced by their progressive concrescence which begins at the fore end and advances towards the hind end, precisely as in Clepsine. In Clepsine there is a cephalic portion in front of the primitive groove (*p. gr*). The same condition is seen also in the shark (fig. 2) and in the chick (fig. 3). The primitive groove in Clepsine is continuous with the blastopore, or the anus of Rusconi (R.). The same is true of the sharks, but not of the chick. This discontinuity in the case of the chick is, however, made easy to understand by what happens in osseous fishes. Bring the two marginal lobes (*c. l.*) in the Selachian egg (fig. 2) into close apposition, and a single lobe, like what we see in Salmo (fig. 5, *c. l.*), is formed. This closing up of the two lobes would obscure or interrupt the continuity between the primitive groove and the blastopore. In the chick (figs. 3 and 6) this modification of the typical condition is carried still farther, as Rauber, in his excellent paper, "Primitivrinne und Urmund," has made clear. Here the homotypical halves of the embryo are not only blended into a single lobe at their posterior point of junction, but this lobe (*c.l.*, fig. 6) has lost its marginal position. This latter fact is in harmony with the fact that only a small part of the blastoporic rim is used in the formation of the chick-embryo. Evidence of the original connection between the primitive groove and the blastopore is seen in the marginal notch ("Randkerbe," Rauber) which sometimes makes its appearance in the edge of the blastopore, just behind the primitive groove (fig. 6, *m. n.*).

This interesting remnant of the ancestral condition was first seen by Pander (126, Pl. I, fig. 4). His (7 5 / 1 2 4, 7 6 / 1 1 8) has observed the same in several cases, and so has Rauber who was the first to interpret it as "the hind end of the primitive groove" (1 3 2 / 6 4). This interpretation is indirectly supported by the typical relation between the digestive tract and the neural canal, first made known by Kowalevsky. The direct continuity between these two tubes found in Mustelus and Acanthias (8 3 / 7 3) by Kowalevsky, according to a citation by Rauber (1 3 / 7 5), has been confirmed by Balfour

($\frac{1\,1}{3\,3\,8}$) and by His ($\frac{7\,5}{1\,1\,3}$). Kowalevsky ($\frac{3\,0}{1\,7\,5}$) has found the same connection in Accipenser and the frog, Bobretzky ($\frac{2\,0}{1\,1\,4}$) the same in Axolotl, Max Schultze ($\frac{1\,4\,7}{1\,3}$) and Owsjannikow the same in Petromyzon, Kupffer and Kowalevsky the same in Ascidia and Amphioxus ($\frac{8\,3}{3\,3\,9}$). Recently (Oct. 26, '78) the discovery of the same connection in the bird has been reported by Gasser (51). He says, "Bei Gänse-Embroyonen von ungefähr 17-20 Urwirbeln besteht an einer bestimmten Stelle der Schwanzanssschwellung *eine offene Communication des Centralnervenrohres mit dem Lumen der Chorda und dem Entoderm, also das Entoderm setzt sich direct in das Ectoderm, Centralnervenrohres fort.*" Should this be confirmed it will be only a new and convincing evidence of the relationship between the neurula of the chick and that of the fish, and at the same time of the correctness of Rauber's interpretation of the marginal notch (*m.n.*)

In comparing the neurula of Clepsine with that of Vertebrates, an interesting question arises in regard to the cause of the central thinning of the blastodisc and the concomitant formation of a marginal rim or band. Does the embryonic rim thicken only relatively? or does it thicken absolutely? Intimately connected with this question is that in regard to the origin of the primitive streak. These questions have long engaged the attention of embryologists, but have not yet been fully answered, especially in the case of discoidal development.[1] As a contribution to the solution of these problems, Professor His (76) has given a large number of embryometrical tables from which it is plain that the embryonic rim (chick) not only increases in surface but in depth, and that it is thicker in the posterior ("retrocentral") than in the anterior ("præ-embryonal") region. The latter fact indicates that the posterior region is the place of most energetic growth or concentration; and this is what we should expect if the development here is comparable with the concrescence of germ-bands.

According to Rauber ($\frac{1\,3\,4}{4\,2\,4\,3}$) the primitive streak results from a concentration of the "entodermic lunula" to both sides of the longitudinal axis of the future embroyo—in other words—it is a "phenomenon of conjunction." The forward growth of the streak, both in duration and extent, is unimportant as compared with the growth in the opposite direction, which takes place, in the main, by a conjunction ("association") of the two lateral halves of the embryonic rim. Disse ($\frac{1\,6\,9}{8\,6}$) puts the matter in the same light when he says. "diese Verdickung (primitive streak) entsteht durch centripetale Zellverschiebung in der unteren Keimschicht aus dem Randwulster." Thus the embryo chick

[1] Balfour (12) was the first to suggest the identity of the primitive streak with a part of the blastopore.

lengthens backwards, like the embryo of the fish or of Clepsine. This is also in harmony with the latest investigations of His, who admits that the bird passes through a stage comparable with the Gastrula of other animals ($\frac{7}{1}\frac{6}{5}\frac{}{3}$).

The cause of the central thinning of the blastodisc, the direction of growth, and the shape of the band-curves assumed at successive stages, are all quite clear in the case of Clepsine. Is the thinning of the central field to be explained in the same way in the case of the bird and the fish? Since the process in both cases leads to similar results, it is natural to infer that it is controlled by the same general laws. Fig. 9 represents a diagrammatic section of Clepsine at a little earlier stage than that of fig. 1.

The cells of the blastodisc are rapidly multiplying by division and lengthening as they divide. The consequent expansion, due not only, as before explained, to the multiplication of the cells of the central field, but also to the addition of cells to the germ-bands from behind, disturbs the equilibrium of pressure. The effect of the increased pressure at the margin of the disc on the underlying yolk manifests itself in the downward movement of the lateral blastomeres (*a* and *b*) and the upward movement of the central blastomere (*c*). Suppose the yolk, instead of being divided into three segments, to be a single mass as in the egg of the fish or the bird; the pressure exerted would still be in the same direction and would generate movements in the yolk similar to those represented in the figure. If the resistance offered by the yolk were great it might cause the disc to arch against the vitelline membrane and thus produce an embryonic cavity (Keimhöhle). The arch would, however, be opposed by the membrane and thus the pressure at the margin would overcome the resistance of the yolk. The equilibrium of pressure would tend to re-establish itself by movements equivalent to those indicated by the arrows. The downward pressure at the periphery of the yolk would cause the central yolk to take the direction of least resistance—upward.

The comparison above sketched may, I believe, be extended to the formation of the entoderm.

In Clepsine the ectoderm develops from one pole of the egg; the primary entoderm consists of three large blastomeres (*a*, *b*, *c*) on which the ectoderm rests. The entodermic spheres are so loaded with nutritive material that a regular cleavage cannot take place. The consequence is a free formation of nuclei by the repeated division of the original nuclei. These nuclei, surrounded by a little protoplasm, take a peripheral position (fig. 9, *n*), and later, by a sort of superficial cleavage, appear as cells of the secondary (permanent) entoderm. The epibolic expansion of the ectoderm with the mesodermic elements causes a solid invagination

of the primary entoderm. I have before called attention to the general occurrence of free nuclei, especially in the case of discoidal development. In the egg of the fish and the bird the ectoderm develops from the upper pole. The lower pole is primary entoderm, so enormously distended with nutritive yolk (deutoplasm), that no regular cleavage takes place. But here, as in Clepsine, free nuclei form in the periphery of the yolk, and later become the centres of cells. That these cells form the entoderm in the fish and the bird, as in Clepsine, is highly probable as appears from what was said under the head of free nuclei and entoderm. I may here call attention to a significant remark by Rauber ($\frac{1}{5}\frac{3}{0}\frac{2}{0}$). Speaking of free nuclei which are formed even *outside* the embryonic rim in the bird's egg, he says: It looks as if we had to do with a superficial cleavage, the products of which are added to those of the blastodisc. . . . Future investigation will have to teach us whether *the entoderm is partly formed in this way.*" It was with no small degree of pleasure that I read the following remarks by van Beneden.[1] Comparing the development of the Dicyemida with the early phases of evolution in the fish, van Beneden says: " Dans le manteau protoplasmique du globe vitellin (couche intermédiaire de van Bambeke) se développe á la fois, par voie endogene, toute une génération de noyaux. Autour de chacun de ces noyaux se délimite un corps cellulaire; *il en résulte la formation d'une couche distincte de cellules; c'est l'endoderme.* C'est de cette manière que j'interprète les recherches de Kupffer, de van Bambeke, de Balfour, et de Klein " ($\frac{1}{1}\frac{7}{2}\frac{}{0}\frac{}{4}$).

The same view is more fully given in an article published in this Journal January, 1878. (161).

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Observations *on the* Structure *of* Cells *and* Nuclei.

By E. Klein, M.D., F.R.S. (With Plate XVI.)

I.

The knowledge of the structure of cells and cell-nuclei has of late years been greatly extended by the observations of Kleinenberg, Eimer, Heitzmann, Auerbach, Strassburger, Frommann, Schwalbe, Bütschli, O. Hertwig, R. Hertwig, Kupffer, van Beneden, W. Flemming, Eberth and others. It is shown by the work of these observers that the substance of cells, as well as that of their nuclei, is of a far more complex nature than is indicated by the term 'granular' or 'hyaline'—usually applied to it.

Heitzmann[1] asserts that the substance of various cells—amœbæ, blood-corpuscles, cartilage cells, bone cells, epithelial cells, &c.—contain networks of minute fibrils, into which pass fibrils radiating from the interior of the nuclei of those cells. Kleinenberg, W. Flemming, O. Hertwig, and E. van Beneden,[2] observed a network of fibrils in the nucleus of the ovum of various invertebrate and vertebrate animals.

[1] "Untersuchungen über das Protoplasma," 'Sitzungsber. d. k. Akad. d. Wiss. Vienna,' Bd. lxvii and lxviii, Abth. iii, 1873.

[2] For the detailed references see W. Flemming's paper in 'Archiv f. Mikrosk. Anatom.,' Bd. xiii, p. 715.

Frommann[1] describes, in accordance with Heitzmann, a minute network of fibrils in the nuclei of blood-corpuscles of *Astacus fluviatilis;* this network passes through the nuclear membrane into a similar network of the substance of the blood-corpuscles. From a reference quoted in a note ("Zur Kenntniss des Zellkerns"), by Professor W. Flemming, in 'Centralblatt f. med. Wissen.,' 1877, No. 20, I learn that C. Frommann had observed and described already in 1867, *i.e.* before Heitzmann, a network of fibrils in the nuclei of many kinds of cells.

Bütschli[2] observed in the neuclei of coloured blood-corpuscles of frog and newt minute fibrils with granular thickenings, but no nucleolus, as asserted by Ranvier.

Schwalbe[3] made extensive studies on the nuclei of ganglion-cells of the retina and found their nucleoli often possessed of minute filamentous prolongations; the nuclear membrane shows prominences on its inner surface. Nucleolus and filaments, nuclear membrane and its prominences represent what Schwalbe designates as 'Nucleolarsubstanz,' and he distinguishes it from the rest of the nuclear matter, which he calls 'Kernsaft' (nuclear juice). Schwalbe thinks that this arrangement of the 'Nucleolarsubstanz' is brought about by a process of vacuolation, which in young individuals is of much greater extent than in older ones. According to Schwalbe between the bulky mass of the nucleolar substance in small young nuclei and the small rudiments of this same substance—limited entirely to the membrane of the adult nuclei, in which the nucleolus is not present any more, there are intermediate stages brought about by vacuolation, one of which is the above-mentioned condition, viz. nucleolus with processes, smaller or larger prominences on the inner surface of the nuclear membrane.

Kupffer[4] maintains of the liver cells of frog, of the odontoblasts, of the epithelial cells of the urinary tubules, and of the cells of salivary gland of *Periplaneta orientalis,* that their substance is composed of a hyaline (non-fluid) ground substance, 'Paraplasma,' and of a granular-fibrillar contractile 'Protoplasma' embedded in the former. The relation

[1] "Zur Lehre von der Structur der Zellen," 'Jenaische Zeitschrift f. Naturw.,' Bd. ix, p. 280.

[2] "Studien über die ersten Entw," &c., 'Abhandl. d. Senkenbergischen Naturf. Gesellsch.,' Bd. x.

[3] "Bem. über d. Kerne d. Ganglienzellen," 'Jenaische Zeitschrift f. Naturw.,' Bd. x, p. 25.

[4] "Über Differenzirung d. Protoplasma an den Zellen thierischer Gew.," 'Schriften des Naturw. Vereins f. Schleswig-Holstein.,' Heft iii, und 'Beitr. z. Anat. u. Physiol. Festshr. f. Carl Ludwig,' 1875.

and distribution of the protoplasmic fibrillar substance varies in the cells of different kinds.

R. Hertwig[1] and Bütschli[2] distinguish, like Schwalbe, the former a 'nuclear substance' from a 'nuclear juice,' the latter a 'nuclear matter' from a 'nuclear fluid.' The 'nuclear matter' of Bütschli comprises the nuclear membrane, the nucleolus and a fibrillar stroma; the latter in some instances extends and a radial manner from the nucleolus.

E. van Beneden[3] saw a fine protoplasmic reticulum in the large axial entoderm cell of Dicyema, which (reticulum) exhibited slow spontaneous movements. In the nucleus of the ripe ovum of *Asterocanthion rubens*, v. Beneden observed within the nuclear membrane and besides the nucleolus a delicate network of a finely granular substance, 'Nucleoplasma,' including several 'Pseudo-nucleoli.' Also the germinal vesicle of the ripe ovum of rabbit contains a minute network.

Arndt ('Uber den Zellkern. Sitzung. d. medicin. Vereins zu Greifswald,' Nov., 1876), distinguishes in the nucleus a homogeneous ground substance and elementary globules; the former possesses a reticular structure and encloses in its meshes the latter.

W. Flemming[4] made very important observations on the structure of the nuclei found in the membrane of the urinary bladder of *Salamandra maculata*. This author, to whose paper I shall have to refer more minutely hereafter, saw a very delicate and dense network of fibres uniformly pervading the interior of the nucleus and attached to the nuclear membrane. This network—'Gerüstformige Structur'—was seen by Flemming in the nucleus of all cellular elements of the bladder of Salamandra—epithelial cells, connective-tissue cells, migratory cells, unstriped muscle-cells, nerve-cells, endothelial cells and blood-corpuscles.

The clearness and extent of the observations of this author leave no doubt that the network in the nucleus represents a definite and pre-existing structure. Although this is to a certain extent questioned by Langhans[5] as regards the fresh cells of the human decidua serotina, Flemming's assertions cannot be, I think, in the least shaken, considering that he observed the above structure, not only after the use of

[1] 'Morpholog, Jahrbücher,' vol. ii.

[2] L. c., vol. x.

[3] 'Bulletins de l'Académie roy. de Belg.,' 2 sér., t. 41, No. 1 and No. 6, 1876.

[4] "Beobachtungen über die Beschaffenheit des Zellkernes," 'Archiv f. Mikrosk. Anatomie,' Bd. xiii, p. 693 and following.

[5] Langhans, 'Centralbl. f. Medic. Wissensch.,' 1876, N. 50.

reagents, *e.g.* acetic acid, chromate of potash, alcohol, chromic acid with or without subsequent staining in carmine or hæmatoxylin, but in the absolutely uninjured bladder, *i.e.*, while this organ was being observed in the living (curarised) animal. In a subsequent note ("Zur Kenntnis des Zellkerns") in 'Centralblatt f. medic. Wiss.,' 1877, No. 20, Flemming states that he observed the same network in the nuclei of various cells also *in the living and perfectly uninjured larva of Salamandra.*

In Strassburger's[1] great work ('Uber Zellbildung und Zelltheilung,' Jena 1875), we notice in developing cells of *Phaseolus multiflorus* a network of fibrils radiating from the nucleolus, permeating the interior of the nucleus in connection with a similar network of the cell-substance.

Mayzel[2] met in the epithelium of the cornea of frog, rabbit and cat, during regeneration, with large round nuclei, in which he observed filamentous masses either in a convoluted manner or radiating from a central point. Mayzel regarded these forms as due to a particular stage of division, as described by Bütschli and Strassburger.

Eberth[3] noticed similar nuclei in the epithelium of the cornea and the endothelium of the membrana Descemeti under normal and abnormal conditions, *i.e.* nuclei containing anastomosing filaments in their interior. Eberth also regards them as peculiar forms in the development and division of nuclei.

Eimer[4] found in numerous nuclei that the granules of the 'granular zone' surrounding his 'hyaloid' are due to protoplasmic filaments, which permeate the interior of the nucleus and anastomose with each other so as to form a network. This network extends to the membrane of the nucleus, and also sends radiating fibrils through the hyaloid into the nucleolus. Such or similar is the condition in the nucleus of ciliated epithelial cells of the palate of *Salamandra maculata*, in those of cells lining the inner surface of the tentacular wall of Aegineta, the sensory cells and the ectoderm-cells of *Carmarina hastata* and others. The network of fibrils of the nucleus is in some instances also in connection with fibrils and networks of such, belonging to the cell-substance itself.

[1] For an excellent abstract see Mr. J. Priestley in this Journal, Vol. XVI, p. 138, and Plate XII, figs. 1—6.

[2] 'Centralbl. f. Medic. Wiss.,' N. 50, 1875.

[3] "Uber Kern- und Zelltheilung," 'Virchow's Archiv,' Bd. 67.

[4] "Weitere Nachrichten über den Bau des Zelkernes," &c., 'Archiv f. Mikrosk. Anatomie,' Bd. xiv, p. 94. See also "Notes and Memoranda" of April Number of this Journal, 1878.

In the present paper I propose to show by a simple method and on an object easily attainable at all seasons of the year that the statements of Professor Flemming, of Kiel, above quoted, as regards the delicate network of fibrils more or less uniformly pervading the interior of the nucleus of various cells is in all respects perfectly correct. In addition to this I shall have to notice several observations I made with reference to the structure of the cell-substance itself and the relation of it to the intranuclear network.

A *The stomach* of a freshly killed newt (*Triton cristatus*) is cut open and placed into a 5 per cent. solution of chromate of ammonia in a closed vessel—(a reagent now well known through the investigations of Heidenhain[1] on the rod-like structures in the epithelium of some of the urinary tubules, and afterwards used by MacCarthy[2] for the demonstration of the rods in the medullary sheath of nerve-fibrils)—where it is kept for about twenty-four hours. It is then washed in water for about half an hour and placed after this in a dilute solution of picro-carmine, where it is left till it assumes a deep pinkish-yellow tint. It is now washed in water, and microscopic specimens are prepared in this manner:—The mucous surface of the organ is scraped with a small scalpel, whereby smaller or larger flakes may be easily removed; they are placed in a very tiny droplet of glycerine on a glass slide; by slight knocking with the rounded or flat top of any thin rod or needle holder these flakes are broken up into microscopic fragments; a drop of glycerine is placed on a covering-glass, and this is inverted over the above specimen.

Examined under a moderately high power—say Hartnack's 7 or 8, or Zeiss's D or E—we recognise easily innumerable isolated or groups of epithelial cells, and a great many isolated nuclei or fragments of nuclei. If the scalpel has been drawn over the surface of the mucous membrane with a little energy, the preparation contains great numbers of gland-cells, isolated and in continuous masses, and also other elements belonging to the tissue of the mucosa.

1. What arrests the attention of the observer at once is the striking appearance presented by *all nuclei, every one of them showing an extremely beautiful network of fibrils*, which permeates its interior uniformly. In some instances this network, which we will designate '*intranuclear network*,' does not extend quite up to the nuclear membrane, which in all instances is well defined, but leaves a narrower or broader

[1] 'Archiv f. Mikrosk. Anatom.,' Bd. x, 1873.
[2] This Journal, 1875, p. 377.

zone next this membrane unoccupied. But in all cases the network is in connection with what is known as the limiting membrane by numerous fibrils. These connecting fibrils appear naturally longer in those instances where the intranuclear network does not reach up to the nuclear membrane. The network stains a pink colour; the colour is more pronounced, *cæteris paribus*, the more shrunk the network is, but apart from this some portions of the network are stained deeper than others.

The fibrils of this network are highly refractive, and vary in their thickness, course and arrangement. In some nuclei they are delicate, fine, cylindrical and smooth, or stiff and short, and so densely arranged as to leave more or less uniform small spaces between them; in others they are coarse, membranous, and possessed of an irregular outline, are more or less convoluted, and so arranged that the spaces formed by their anastomoses are not uniform, being sometimes larger in the central parts of the network than in the periphery, at other times the reverse is the case. There are nuclei in which the fibres appear to possess a spiral or circular arrangement in the periphery of the network. Seen from the narrower side, some nuclei—especially those of the surface-epithelium—show a chiefly longitudinal arrangement of the fibres of the network. We find all forms between a network of fibrils as represented, *e.g.* in a net, and a honeycomb of membranous structures, as represented in a sponge. In almost all instances however, we observe a greater or smaller number of minute bright spots, which as careful focussing proves are fibrils of the network seen in optical transverse section or at the point of anastomosis. But it seems also that some fibrils are possessed of irregular thickenings. This is shown with remarkable clearness in those instances in which the nuclear membrane has been broken at one or the other point and the fibrils of the intranuclear network protrude through this opening. In fig. 8, of Plate XVI, such protruding fibrils are shown. Comparing the different nuclei one cannot help noticing that the number of the above-named bright dots is greater, the denser the network, the more convoluted its fibrils are, or the more shrunk the network is as a whole.

Numerous fragments of nuclei are met with in our preparations; these consist of greater or smaller portions of the intranuclear network isolated from the nuclear membrane; in fig. 3, Plate XVI (*a*, *b*, *c*), may be seen such isolated networks.

The nuclei of the epithelial cells are slightly flattened, and when, therefore, seen in profile, they do not show the net-

work so clearly as when seen from the surface. The network appears more delicate in the nuclei of the gland-cells and endothelial plates of the mucosa than in those of the epithelial cells of the surface.

In some nuclei the ground substance, in which is embedded the intranuclear network, appears slightly tinged red, in others it is perfectly colourless.

Such are the appearances presented by the nuclei of the surface-epithelium, of the gland-cells, and of the endothelial plates of the mucosa of the stomach, prepared simply in 5 per cent. solution of chromate of ammonia. Of the greatest clearness and distinction, and of greater preference as regards preservation in microscopic specimens that are permanently to be sealed up, are the nuclei of the above cellular elements, if, after having been kept for about twenty-four hours in 5 per cent. solution of chromate of ammonia, the stomach is placed for abont ½—1 hour in a mixture of 2 parts of 1-6th per cent. solution of chromic acid and 1 part of methylated alcohol—a mixture which I am in the habit of using with great advantage for hardening glandular organs, and such as contain connective- and muscular tissues—then washed and treated as above.

But also by keeping the fresh stomach for twenty-four hours in Müller's fluid, washing and then staining it in picro-carmine, the intranuclear network may be perceived in some nuclei, although with far less distinctness than after the above methods. In many nuclei the network is too much shrunk and presents, therefore, the well-known 'granular' appearance. The appearance is somewhat, though not very much, improved by placing the stomach, after Müller's fluid and before washing it, in the above mixture of chromic acid and spirit for about ½—1 hour.

The nuclei examined in the perfectly fresh condition show distinctly, although faintly, part of the intranuclear network, a good magnifying power, as Zeiss's F or Hartnack's Imm. 10, and good light, as that reflected by white clouds, being indispensable. Other reagents, as osmic acid, alcohol, chromic acid, bichromate of potash, give not very satisfactory results, although osmic acid and bichromate of potash show the intranuclear network in some instances more or less distinctly.

Measurements which I made of the nuclei in specimens prepared after the 5 per cent. chromate of ammonia method and mounted in glycerine, give the following numbers as the mean:

(*a.*) For the nuclei of surface epithelial cells 0·017 by 0·007 millimètres.

(*b.*) Nuclei of gland-cells 0·017 by 0·014 mm.

(*c.*) And the same numbers for those of endothelial plates.

There are several questions which suggest themselves in connection with the observations just detailed: First, as regards the 'granules' or bright dots that present themselves in the intranuclear network, we have to ask what their exact nature is? As has been mentioned already previously, many of the bright dots or granules can be distinctly recognised as due to optical transverse sections of fibrils, these being either twisted or bent, or being altogether placed vertically; this is the view also expressed by Flemming (l. c., p. 698). But I have no doubt that in some nuclei the more irregularly shaped dots are due to a thickening of fibrils from place to place, the fibrils not being always cylindrical, but some showing a perfectly irregular outline.

It is clear from this that the more shrunk the intranuclear network or the more twisted and convoluted the fibrils the more does the nucleus present the appearance of being 'granular.' And it is, no doubt, owing to this condition that in hardened specimens we are able to distinguish sometimes only a granular condition of the nucleus. Thus, for instance, the 'granular' nuclei of epithelial cells, lymph-cells, unstriped muscle-cells, muscle-corpuscles, &c., of different organs in many animals, including man and mammals, are due merely to a shrunken or convoluted condition of the intranuclear network, or to the fibrils having a dense arrangement, as I shall have occasion to show on a future occasion.

The few 'granules' that one also observes occasionally in nuclei in a fresh condition are due to some of the fibrils being seen in optical transverse section, the fibrils themselves being not easily differentiable. A careful examination with a good lens and a good light shows the correctness of this in most cases. Thus, for instance, the examination of the nuclei of fresh epithelium of frog, toad, or newt, the nuclei of fresh coloured blood-corpuscles of these animals, especially of toad, with a Zeiss's F Lens or a Hartnack's Immersion No. 10, reveals fibrils in the nucleus, and also shows that the 'granules' are due to the twisted or bent condition of them.

Another point which deserves consideration is the so-called nucleolus. It is well known that many nuclei of the most different kinds of cells contain a very conspicuous, highly re-

fractive, large dot, called nucleolus; the best known examples are the nucleus of ganlion-cells and the germinal vesicle or nucleus of ovum. Many nuclei contain more than one nucleolus, which are described as of the same, or as is more commonly the case, of different sizes. Now, to every experienced student of histology it must have become apparent that if there is one thing unsatisfactory, unreliable, puzzling and inconstant about the nucleus of vast numbers of cells, it is this very nucleolus. Flemming (l. c., p. 702) maintains that in the case of the nuclei of the bladder of Salamandra 'nucleoli' are present and may be demonstrated by anilin staining. But looking at his drawings (figs. 9, *a*, *b*, *c*, and *d*) I fail to see the justice of this conclusion. What is here shown in the nucleus are *numbers* (a dozen and more) of more deeply stained, irregularly-shaped masses placed in the fibrils of the network.

Thus, for instance, in *d* of his fig. 9, Flemming shows us two nuclei of unstriped muscle fibres, each of which contains in the network of fibrils, only faintly seen, an uncountable number of irregularly shaped particles. Why he should regard all these particles as definite anatomical structures identical with the spherical bright large spot, which is generally characterised as nucleolus, I fail to comprehend. Arguing against Schwalbe, Flemming (l. c. p. 713) thinks that "the nucleoli at all events do not represent merely local accumulations of the network, but are or may be in many cases something different."

In the nuclei of the cells of the stomach of newt, prepared in the above manner, I do not find in the great majority of instances any signs of what is usually accepted as a nucleolus; only in a few instances I saw one or two particles (larger than the ordinary bright dots above explained) which correspond to Flemming's 'nucleoli.' Now, I have after a very prolonged examination arrived at the conclusion that these large particles are due to one of two things; in some instances they are distinctly thickenings of the network, in others they appear to be merely due to the shrivelling up and intimate fusion of a part of the network. And this seems to me to be borne out also by the examination of nuclei of the stomach or palate of newt in the fresh condition. I have seen distinctly that when the neucleoli are present—the instances are fewer than is generally supposed—they are accumulations of the fibrils of the network. The inconstancy as regards size, shape and number of the so-called nucleoli seems to me to point very strongly in the above direction, viz. that they are due merely to a local thickening (natural

or artificial) of the fibres of the intranuclear network. The observations of Van Beneden, O. Hertwig, Schwalbe, Langhans, Flemming himself, and last, not least, Auerbach and Eimer, appear to me to lend support to the view that in most cells the so-called nucleoli are local accumulations of the intranuclear network, that they are inconstant in size and number, and that they are only transitory appearances. As regards this last point, the observations of Strassburger, Schwalbe and Langhans, may be here referred to, especially Langhans (l. c.), according to whom the single nucleolus or multiple nucleoli met with in the nuclei of cells of the human decidua serotina, are the result of the shrinking of the network.

The assertions that have been made as regards spontaneous movements of nucleoli (Auerbach,[1] Brandt,[2] Eimer,[3] Kidd,[4] and others) are quite compatible with the above view, for E. van Beneden, as previously mentioned, has observed movements in the intranuclear network, and it is quite possible that the above assertions might refer to contractions of part of the network.

A last point to be considered is the nuclear membrane in it relation to the intranuclear network. I shall have to mention this more fully hereafter in connection with the cell-substance itself, but will limit myself here only to say that the examination of the nuclei in my specimens, especially of nuclei that are isolated, and whose limiting membrane has become broken at one place or another (see fig. 8, Pl. XVI), shows that what usually appears as nuclear membrane is composed of an outer thicker portion, which is the limiting membrane proper, and—closely connected with it—of an inner, more or less incomplete—probably because reticular—delicate layer, which is, properly speaking, a peripheral condensation of the intranuclear network, with which it is, of course, connected by longer or shorter threads. The clear space which may be observed in some instances between the 'membrane' of the nucleus and the intranuclear network is due, as mentioned on a former page, to a retraction of the latter from the former, and is a space, not between the two layers of the limiting membrane, but between the inner layer of this and the bulk of the intranuclear network.

2. The following forms of cells present themselves in our

[1] 'Organologische Studien,' &c., Abth. 3, Breslau, 1874.
[2] 'Archiv. f. Mikr. Anatom.,' Bd. x.
[3] 'Archiv. f. Mikr. Anat.,' Bd. xi.
[4] This Journal, 1875.

specimens :—*a*. Columnar epithelial cells varying somewhat in length and thickness ; they are goblet-cells of the same or a similar character as those represented in figs. 1 and 2. They consist of an upper swollen transparent part, and a lower part more opaque and including the nucleus. The first has lost its cover, the second terminates in a more or less indentated, serrated, or truncated manner. In some cells the nucleus lies close to the boundary line of the two parts, in others there is a longer or shorter mass of protoplasm interposed between the two. In the latter case the cell has a much more graceful form, being relatively less plump in both parts. If the preparation be made by scraping the foregut, we meet also with a few ciliated cells, conical or cylindrical in shape. Most of the ciliated epithelial cells of the foregut, however, present themselves likewise as goblet-cells, the cover with the cilia having been removed.[1]

The columnar goblet-cells mentioned before may be grouped, according to size, in two distinct categories—one comprising the larger, the other the smaller forms. The larger cells belong to the surface, the smaller ones to the ducts of the gland. (Besides cells, which, to all appearances are perfect, there are a good many, which present themselves only as larger or smaller fragments.)

b. Flat transparent cells with nuclei somewhat larger than the nuclei of those mentioned above. These cells are met with in groups forming the wall of larger or smaller portions of gland-tubes ; when in a group they are imbricated, and being flattened they do not appear conspicuous when thus seen in profile ; their bulky nucleus seems to be the chief thing that attracts attention. It is the nuclei of these cells which show the intranuclear network most splendidly. When these cells are met with isolated and looked at in profile they are seen to be composed of a bulky oval nucleus, to one pole of which is attached a short plump process, to the opposite pole one that is many times longer. Some of these cells are seen only as fragments.

c. Oval, biconvex, more or less polyhedral, or irregularly shaped cells, consisting of a relatively opaque, or what appears as a granular cell-substance, and including an excentric large oval nucleus of the same aspect and size as that of

[1] I may here mention that I know of no better reagent for the demonstration of goblet-cells than 5 per cent. chromate of ammonia. A piece of trachea of a mammalian animal placed fresh in 5 per cent. chromate of ammonia, kept there for twenty-four hours, and treated in the manner above detailed, yields excellent specimens for the study and demonstration of goblet-cells in all different stages between ordinary normal epithelial cells and bulky goblet-shaped mucus-containing cells.

the preceding cells. They are met with isolated or in groups; in the latter case they are likewise imbricated and form the wall of gland-tubes, somewhat narrower than those mentioned *sub b*. Both are sections of the same gland-tube; the broader, lined by the transparent flat cells, appear to be the more superficial, the narrower lined by non-granular cells, the deeper portion of the stomach glands.

d. Exceedingly large transparent placoids, with an oval nucleus in the centre; they are met with relatively rarely, and only in preparations which were made with a view of including in the scraped flakes the tissue of the deeper parts of the mucosa. When looked at in profile they appear more or less spindle-shaped, the cell-substance appearing at each pole of the nucleus as a long filamentous prolongation. The nucleus of these endothelial plates—for such they are in all probability—resembles in aspect and size perfectly that of the flattened transparent gland-cells; the intranuclear network presents itself in great clearness. Fragments of the endothelial plates cannot be, therefore, distinguished from those gland-cells.

As regards the epithelial cells mentioned *sub a*, it is a fact too conspicuous to be overlooked, even under a moderately high power (*e. g.* about 300), *that the substance of the upper transparent, as well as the lower or opaque part of the goblet-cells contains a great number of delicate fibrils*, more or less distinctly stained, many parallel to the longitudinal axis of the cell. They are especially distinct in the upper or transparent part of the goblet-cell. These fibrils can, in many cells, be traced up to the free margin of the goblet, *i. e.* the margin of the cell which, as has been mentioned above, has lost its cover; they anastomose with each other by lateral branchlets. If such a cell is looked at along its longitudinal axis these lateral branchlets are not very conspicuous, but in an oblique, or, still better, in a bird's-eye view, we obtain a clear insight into their arrangement, and we thus convince ourselves *that the longitudinal fibrils and their lateral branchlets form a very delicate and more or less dense network*. This network we shall designate the *intracellular network*; it is represented in figs. 1, 2, 4, 5. That this *network* of the intracellular fibrils comes out with such distinctness in these goblet-cells is no doubt due to the *interfibrillar* or *ground-substance* having swollen up very much; by doing this the meshes of the network become of course distended, and, therefore, distinctly perceptible.

The ground-substance or interfibrillar substance of these goblet-cells is mucin, and it stains in a characteristic

manner deeply blue with hæmatoxylin. This is the reason why hæmatoxylin-staining does not yield such clear views of the intranuclear, and especially the intracellular network of fibrils, as carmine or picrocarmine, viz. because hæmatoxylin stains the ground-substance of the cell too deeply, on account of its containing mucin, and thereby these networks become more or less obscured, whereas carmine or picrocarmine leaves the ground-substance transparent and unstained, but stains both the intracellular and intranuclear network.

The intracellular fibrils and their network is best seen in the slender goblet-cells of stomach (of newt) kept for twenty-four hours in Müller's fluid, placed then for about half an hour in a mixture of two parts of chromic acid ($\frac{1}{6}$ per cent.) and one part methylated alcohol, washed after this in water, and stained in picrocarmine. The fibrils running in the long axis of the cell come out with remarkable clearness; if the upper part of the cell is inspected in an oblique manner it is seen that the fibrils form a dense network. In fig. 12 I have faithfully represented two such cells.

In the epithelial cells of the foregut that have retained their cilia we recognise in the last-named specimens with great clearness *that the cilia pass into the cell-substance through the cell-cover*—hence the striated or granular appearance of this latter—*and indentify themselves with the intracellular network of fibrils*. Eberth, Marchi,[1] and especially Eimer[2] have noticed a similar condition.

Another point not less conspicuous, is *the direct connection of the fibrils of the intracellular with those of the intranuclear network*. In our preparations with 5 per cent. chromate of ammonia this connection of the intranuclear network, both with the fibrils of the upper and lower portion of the cell, is much more distinct than in specimens prepared with Müller's fluid, on account of the intranuclear network being more clearly perceptible in the former than in the latter.

What I have stated with reference to the structure of the epithelial cells mentioned *sub a*, applies likewise to those mentioned *sub b*, *c*, and *d*, viz. that we have to distinguish in the cell-substance two parts, one the homogeneous ground-substance and the other the intracellular network of fibrils. This latter is in direct anatomical continuity with the intranuclear network. But there exists a difference in the density of the intracellular network between the different

[1] 'Archiv. f. Mikr. Anat.,' Bd. iv.

[2] L. c., p. 115, figs. 3, 11, 20, and 21.

cells; for those *sub c* mentioned, viz. the opaque or 'distinctly granular' gland-cells situated in the deeper parts of the gland-tubes contain a very dense network of fibrils, and of course little of the interfibrillar ground-substance is seen, hence the 'granular' aspect of these cells. I have represented such a cell in fig. 7. In the flattened gland-cells described *sub b* and the endothelial plates, *sub d*, the hyaline ground-substance forms a formidable portion, the intracellular fibrils forming a network with relatively wide meshes; looked at from the broad surface the cell-plate presents longitudinal fibrils, in some places placed more closely than in others, and anastomosing by lateral branchlets. When seen in profile the continuity between the intracellalur and intranuclear network is very distinct (figs. 9, 10, and 11).

B *The mesentery* of a freshly killed newt (the black species) is cut out together with the intestines and placed in a solution of 5 per cent. chromate of ammonia, where it remains for twenty-four hours; it is then carefully cut into several portions while under water, then cut off the intestine and left in water for about $\frac{1}{2}$—1 hour; after being stained in carmine, or picrocarmine, or hæmatoxylin, it is ready for microscopic examination. For this purpose one or the other portion is floated on an object-glass, and after having being spread out it is covered with a covering-glass, a drop of glycerine having previously been placed on the latter. It is necessary that the preparation should be well spread out, but should not be subjected to any undue pulling or other mechanical injury, for this does invariably annihilate many of those exquisitely delicate structural peculiarities that I am going to describe below. But I must add that it requires not much more than ordinary delicacy of handling in order to obtain success, and I will likewise add that I know of no more beautiful object in histology than a preparation of the mesentery of newt prepared in the above manner, well stained with hæmaxotylin or doubly stained with picrocarmine first and hæmatoxylin afterwards and well spread out. Mounted in glycerine the preparations preserve all their fine qualities.

In our specimens we have to notice the following structures:—(1) the endothelium of the surface, (2) the ground substance, (3) unstriped muscle-fibres (4) connective-tissue corpuscles, (5) blood-vessels, and (6) nerve-fibres.

I. *Endothelium of the surface.*

In our specimens we find the endothelium of the surface, when not detached from the subjacent membrane, represented as a transparent hyaline layer containing large

oval nuclei at more or less regular intervals. The nuclei measure in the mean 0·018 by 0·014 mm., and show in all instances an exquisitely pretty network uniformly pervading their interior. This *intranuclear network* possesses the same characters as that described of the cells of the stomach. The network varies in form and arrangement between that of a delicate net and that of a honeycomb of a sponge; the bright dots to be observed in it correspond mostly to fibrils seen in optical section. There are no structures comparable to the nucleolus. In some parts of the specimen we find the endothelial membrane of the surface broken up into isolated plates corresponding to the individual endothelial cells, or into smaller groups—two or three—of them. The isolated endothelial cells show generally a single excentric nucleus—in exceptional instances the nucleus is double—and are in many instances more or less folded or rolled up. If we get the endothelial plates in our specimen clear of the membrane of the mesentery and unfolded we notice that *their substance shows a beautiful network of fibrils*, such as I attempted but only imperfectly succeeded to delineate in figs. 13, 14, and 15; in the drawings the network is composed of far too coarse fibrils. The fibrils are exceedingly delicate, run in bundles which anastomose and thus form a fenestration with large or smaller fenestræ. At first sight, and looked at with only a moderately high power they (fibrils) seem to be only streaks of a 'granular substance.' That this latter is composed of fibrils becomes clear when examined more carefully and with a higher power, such as Zeiss F, or Hartnack Imm. 10. Thus we have to distinguish also in the endothelial plates a hyaline ground-substance, which I wish to call *ground-plate*, and in it a network of fibrils—the *intracellular network*. Thus network is in all endothelial cells of our specimens in direct communication with the intranuclear network (see figs. 13, 14, and 15.) In groups of endothelial plates not showing the outlines of the individual cell-plates we notice that the intracellular fibrils of one plate merge into those of its neighbours, apparently without any distinct interruption.

Schwalbe[1] describes each of the endothelial cells lining the canal of Schlemm as consisting of a hyaline, delicate plate, with an oval nucleus and 'reticular thickenings;' in figs. 30 and 31 accompanying his paper this arrangement is well shown on isolated endothelial plates prepared in Müller's fluid. I have no doubt that these 'reticular thick-

[1] "Unters. über d. Lymphbahnen d. Auges," &c., 'Archiv. f. Mikr. Anatom.,' Bd. vi, p. 305.

enings' are not in reality thickenings of the endothelial cells, but correspond to our intracellular network of fibrils.

Tourneux[1] asserts that the endothelium lining the peritoneal surface of the septum cysternæ lymphaticæ magnæ of batrachian animals consists of two superimposed strata of cells, a superficial one composed of a single layer of hyaline, thin, non-protoplasmic plates, with or without a nucleus, and a deep layer of protoplasmic cells. Tourneux thinks that the superficial cells become detached after they have lost their nucleus, and that the lower protoplasmic cells by division produce their substitutes.

v. Ewetsky[2] maintains of the endothelium of the membrana Descemeti of frog, pigeon, cat and calf, that its cells consist of a hyaline membrane and a subjacent nucleated, branched, protoplasmic corpuscle.

I have little hesitation in saying that the hyaline plate of Tourneux and Ewetsky correspond to our *ground-plate*, and that their subjacent protoplasmic corpuscle is identical with our *intracellular network* in connection with the nucleus. Contrary to these observers I regard both the *ground-plate* and *intracellular network* as forming one individual endothelial plate, that is to say, *the intracellular network* connected with the intranuclear network lies *embedded* in the hyaline *ground-plate*.

II. The ground-substance of the mesentery is a delicate connective-tissue membrane, being composed of a feltwork of very fine fibrous bundles, to which are added the ordinary fine elastic fibrils branching and anastomosing. But this ground-substance is in our specimens, in which the other elements are shown with all clearness of detail, only very faint.

III. *The unstriped muscle-fibres.*

The mesentery of the newt contains a very beautiful plexus of unstriped muscle-fibres. They are arranged[3] in flat bundles, crossing each other, but chiefly interchanging its fibres. The bundles are made up of a limited number of fibrils, ranging between ten or more abreast down to slender bundles of three or two fibrils. Most of the unstriped muscle-fibres are included in this plexus, but there are individual and couples of fibres leaving this plexus and

[1] "Recherches sur l'épith. des Sereuses," 'Journ. de l'anatomie et de la physiologie,' Jan. et Fév., 1874.

[2] "Uber d. Endothel. d. Membr. Descemeti," 'Untersuch. aus d. path. Inst. Zürich.,' iii, 1875.

[3] The description given here refers to specimens that have been well spread out. The arrangement naturally differs if the specimens are in a more or less shrunken condition.

terminating freely in the ground-substance of the mesentery. Whether the bundles be large or small, the individual fibres are sufficiently separate from each other as to allow their examination in all parts. Of great beauty are those parts of our specimens in which the minute bundles are composed of a limited number of fibres (two—five), and when they freely intercommunicate with each other.

Most of the muscle-fibres remain undivided, and terminate at each extremity in one single pointed end; there are, however, such as divide close at the nucleus or at a part not distant from it into two, seldom into three, branches.

The nucleus of the muscle-fibres is oval and slightly flattened; when seen in profile it measures in the mean 0·027 mm. in the long, 0·008 mm. in the transverse, diameter. The nucleus lies not exactly in the middle of the long diameter of the muscle-fibre, but not very far from it, as the following measurements show:

In muscle-fibre (*a*), 0·306 mm. from middle of nucleus to the corresponding extremity of the muscle-fibre.
0·294 mm. the rest.
In muscle-fibre (*b*), 0·282 mm. from middle of nucleus to the corresponding extremity of the muscle-fibre.
0·354 mm. the rest.

The length of the thin examples is about 0·61 mm.; but there are others which are shorter, being much thicker.

As I have mentioned above, the muscle-fibres are, in specimens that have been well spread out, so much separated from each other that they can be easily followed from one end to the other, and it is thus a matter of great facility in seeing that most fibres are more or less wavy and curved, and that *some possess from place to place varicose swellings*. Examining these varicosities with a high power we observe that they are composed of two substances:—(1) a sheath containing transversely arranged thickenings in the form of annular markings, such as represented in fig. 17 *e*, and (2) a central bundle of fibrils;[1] the thickness of this bundle varies naturally according to the thickness of the point examined, *i.e.* whether a part near the extremity or the nucleus (*i.e.* near the thick part) of the muscle-fibre, but is always slightly thicker corresponding to a varicosity than in the parts between.

[1] That the substance of unstriped muscle-fibres in general is longitudinally striated, *i.e.* probably composed of longitudinal fibrils, has been known to histologists for some time (*Arnold*, 'Stricker's Manual of Histology,' chapter on unstriped muscle; *E. Klein*, 'Handbook of the Physiol. Laboratory,' edited by Dr. Burdon-Sanderson, chapter on unstriped muscle tissue, fig. 67 of plate xxv; *W. Flemming*, l. c., p. 714).

The same distinction into a sheath and a central bundle of fibrils exists also in the part between two varicosities, with this difference, that the sheath is thinner and the annular thickenings are scarcer and not so distinct. Those fibres which in a greater portion, especially next the nucleus, do not possess any varicosities, exhibit the transverse rings of the sheath with great uniformity. When looked at in profile this appearance is in some respects similar to that of medullated nerve fibres (Lantermann, MacCarthy), and especially of preparations of nerves prepared with chromate of ammonia, as described by MacCarthy (l. c.) But in our muscle-fibres the transverse markings being due to rings may be followed over the upper as well as lower surface of the fibre, which of course is not the case in the medullated nerve-fibre where those markings are due to rods placed vertically to the long axis of the fibre.

[In fig. 17, *a* and *b*, the muscle-fibres have not been represented in their whole length, as this would have occupied too great a space, and therefore the varicosities which are especially distinct and sometimes of a very regular distribution near the extremities of the fibre, are not shown sufficiently numerous.]

The sheath with its transverse markings is visible also on the thickest part of the muscle-fibre, *i.e.* the part that includes the oval nucleus. For the demonstration of the transverse markings all specimens are useful, hæmatoxylin specimens, however, being best. The distinction between a sheath with annular thickenings and a central bundle of fibrils, is shown with remarkable clearness in some muscle-fibres in which that bundle had shrunk away from the sheath, and thus presents itself as a wavy or more or less zigzag group of fibrils within the latter. In the accompanying
a woodcut (*b*), a portion of such a muscle-fibre is shown.

Within the limiting membrane of the nucleus of every muscle-fibre we notice *the very distinct intranuclear network of fibrils*. This network is stained in our specimens, whereas the ground-substance is in most instances quite transparent and unstained; in a few instances I have seen it, however, assuming a slight tint. There are muscle-fibres to be met with which, instead of a single oval, possess a constricted nucleus, the constriction being placed either transversely or, more

usually longitudinally. In such a nucleus we notice that this intranuclear network forms two distinct groups—one for each part of the nucleus—connected with each other by parallel threads. There are nuclei possessed of one or two smaller or larger rounded buds; these contain a small network connected with the bulk of the intranuclear network by a few small threads. This is shown in *b*, of the accompanying woodcut.

It is probable, from this, that the division and multiplication of the nucleus of unstriped muscle-fibres is preceded by a process of gemmation. In most muscle-fibres the nucleus is seen in profile, and the bulk of the intranuclear network occupies an axial position; from it pass numerous transverse fibrils towards the sides of the nucleus.

In all muscle-fibres *the intranuclear fibrils may be traced to emerge as a bundle from the pole of the nucleus, and to become identified with the bundle of fibrils representing the core of the muscle-fibre itself.* This point, viz. the connection of the intranuclear network with the bundle of fibrils of the muscular substance is shown well enough in preparations stained with hæmatoxylin, but it is perhaps still better shown in parts of mesentery stained first with picrocarmine and then with hæmatoxylin. In a muscle-fibre which, close to the nucleus, divides into two processes like the one represented in fig. 17, *c*, we observe that the intranuclear network sends forth two bundles of fibrils, one for each division.

A very interesting relation that I have been able to ascertain in some nuclei, is this: the nucleus possesses a small circular opening at each pole, through which the bundle of fibrils emerges. (I have tried to represent this in fig. 17, *d*, but fear not with great success). The same relation probably exists in all fibres.

Thus, we may regard the unstriped muscle-fibre as composed of a sheath with annular thickenings, and a bundle of delicate fibrils which at one more or less central point forms a delicate network; this surrounded by a special membrane—except where the network is in connection with the bundle of fibrils—represent the nucleus.

I am unable to say what the nature of that sheath is, but I think it is probably of the nature of an elastic sheath, and I can well understand its importance in the function of the muscle-fibre. The central bundle of fibrils having its fixed point, as it were, in the intranuclear network, shortens during contraction towards this, and is brought back again into its condition of rest by the elasticity of the sheath.

The above-named varicosities and the different condition of the sheath and central bundle in the varicose and intervaricous portions bear a very strong support to this view; for the varicosities are in all probability portions of the muscle-fibre in a state of contraction, and hence the narrowness of the transverse rings, the thickness of the sheath, and the breadth of the central bundle of fibrils. Another point which can be, I think, only interpreted in the sense of this theory, is the fact that in the thicker muscle-fibres we find the sheath thicker and the annular markings much closer and more numerous than in the thinner examples.

As a last fact of some interest may be mentioned the termination of these muscle-fibres. I have noticed in a few instances that the extreme end does not terminate in a free point, but is either at or near this *in connection with the fine processes of a connective-tissue corpuscle.*

IV. *The connective-tissue corpuscles.*

a. The migratory cells are in some parts more numerous than in others, but they are to be met with in almost all portions of the mesentery. Most of them are possessed of coarse granules and are of various shapes—elongated, constricted, irregular, and possessed of knob-like prominences. Their nucleus is generally single, oval and large, or in some instances constricted, but in all cases it shows a beautiful intranuclear network (see figs. 20 and 21). In some of these cells the nucleus as a whole is more deeply stained (in carmine as well as in hæmatoxylin) than in others, and I conclude that the ground-substance of the nucleus is in these instances of a different nature, the whole cell being younger. A minority of migratory cells possess a pale, slightly and indistinctly fibrillar substance surrounding the relatively large nucleus, and a few knoblike or filamentous processes. The nucleus of these cells shows the network of fibrils with equal distinctness. Also in the nuclei of many of these cells we find the ground-substance of the nucleus staining a deep tint.

b. The connective-tissue corpuscles proper or fixed cells present themselves in great delicacy and beauty, both in specimens stained in picrocarmine as well as hæmatoxylin. What attracts our attention in the first instance is the remarkable distinction between a hyaline, slightly but distinctly tinted *ground-plate* and the *fibrillar substance* or *intracellular network of fibrils,* which forms a collection round the nucleus and is prolonged beyond the *ground-plate* in numerous richly branched processes, which in some instances form a con-

tinuous network with those of neighbouring cells. The nucleus is oval, and measures about 0·013 by 0·011 mm.; it contains a very beautiful and delicate network of fibrils; these pass through the nuclear limiting membrane into the fibrillar substance of the cell, with which they become identified.

(*a*) The *intranuclear network* stains well and contains here as well as in those of the cells of other kinds, previously named, bright dots, owing to the fibrils being looked at in their optical transverse section or at their point of anastomosis, and larger irregular particles—thickened or contracted (shrunk) portions of the network. I have seen nuclei whose network had so much shrunk as to form a central irregular mass, in which the individual fibrils could be distinguished only with difficulty. There could be no doubt as to its nature, although at first it resembled in a high degree what corresponds to the 'nucleolus' of the authors. In these instances, therefore, I see additional evidence for the correctness of the view first expressed by Langhans (l.c.), viz. that the nucleoli owe their origin, probably in most instances, to the shrunk condition of the intranuclear network. The nuclear membrane shows in those instances in which the network is not shrunk too much, besides the faint outline representing the limiting parts of the network, in addition a thicker boundary, such as had been mentioned to exist in the nuclei of the epithelial cells of stomach.

(β) The *fibrillar substance* or *intracellular network* is arranged round the nucleus in a very unequal manner, such as is represented in fig. 23, and it is in this part especially, and also in the thicker prolongations of it, that the character of a fibrillar network can be made out most distinctly. It is possessed of numerous processes unequal in thickness and length; they are all very richly branched. Of characteristic appearance are the irregular thickenings in the course of the processes and the club—or pear-shaped prolongations at the point of branching of these processes.

The branching of the processes is very rich; the anastomosis of processes of neighbouring cells is rarer than it would appear at first sight. Only in rare instances have I seen an anastomosis of two processes coming out of the same cell. In many cases the processes terminate either club-shaped or in the form of an irregular plate. I need hardly say that in this description of connective tissue corpuscles I refer to specimens or parts of them in which the endothelial covering of the surface has become over larger or smaller

areæ loosened and detached, but the elements of the tissue: such as unstriped simple fibres, connective-tissue cells, nerve-fibres and capillary blood-vessels, remained undisturbed, presenting all details of structure. The same details may be of course also seen where the endothelium of the surface has retained its position, only for obvious reasons it requires a more attentive examination.

(γ) The *ground-plate* is faintly stained and therefore not well defined in all parts of its outline. Although the greater number of processes of the *fibrillar substance*—except the thicker branches—pass beyond the limits of the *ground-plate*, still there are some minute processes which remain within its area. I find a good many instances in which I can trace fibrils coming out of the nucleus and running up to the margin of the ground-plate, where they terminate. These fibrils, which appear to connect the nucleus directly to the edge of the ground-plate, are not represented specially in fig. 23, but they are very clear in many connective-tissue corpuscles. An inspection of fig. 23 no doubt suggests to the reader—as a cursory inspection of the specimens would to the observer—that, supposing the ground-plate be not visible, the rest of the connective-tissue corpuscle corresponds to those typical branched connective-tissue cells as they usually present themselves, viz. an oval nucleus unequally surrounded by the 'granular' cell-substance, which is drawn out into more or less branched processes.

I shall have occasion to return to this question of the nature of connective-tissue corpuscles in the second part of this memoir, when I shall be able to discuss it in connection with the cells of tendon, cornea and loose connective tissue, and when I shall be able to examine critically the assertions and observations of various writers, especially *v. Recklinghausen, Stricker, Rollett, Schweigger-Seidel, Schwalbe, Boll, Waldeyer, Ranvier, Grünhagen, Eberth, Ewetsky, Böttcher, Bizzozero, Waller, Lavdovski, Axel Key, Retzius, Spina, Renaut,* but this much I may say here that the two substances, viz, *ground-plate* and *fibrillar substance* connected with the intranuclear network of the oval nucleus form the essential parts of a connective-tissue cell. It is to me clear that in the case of the connective-tissue cells of the mesentery the two parts are not merely superimposed, but form one integral element in the same manner as I stated it to be the case with the endothelial cells of the surface.

V. *The blood-vessels and lymphatics.*

In all blood vessels of our specimens we recognise *the net-*

work in the nuclei. This is of course best shown in capillary blood-vessels, on account of the thinness and transparency of the wall. In some capillary vessels, when looking at the wall from the surface, I have noticed from place to place fragments of a minute network ; it is quite possible that these places correspond to the granular substance observed sometimes in the endothelial plates constituting the wall of the capillary. Some such capillaries contained blood-corpuscles ; and the nuclei of these showed a very distinct network. I have been able to recognise in some specimens apparently *cæcal dilatations of lymphatics, the nuclei of the endothelial wall* of which whether looked at from their broad or narrow side *presented the intranuclear network* with great distinctness, and in all respects similar to that described of the other nuclei. Also the nuclei of lymph-corpuscles present in the lymphatic exhibited a network of fibrils (see fig. 22).

VI. *The nerve-fibres.*

Without wishing to enter here into a description of the distribution of nerve-fibres in the mesentery,[1] I have only to mention the extremely long, more or less wavy and curved course the fine nerve-fibres pursue without giving off any branches. Each such nerve represents a bundle of fine fibrils, *i.e.* an axis cylinder, to which is applied from place to place an oblong constricted or slightly folded nucleus. The number of nuclei along such an axis cylinder depends on the thickness of this latter. I measure, as far as this is possible in a fibre presenting a number of curvatures, three successive segments from nucleus to nucleus, in fibre (*a*): 0·1, 0·16, 0·128 mm. ; in fibre (*b*): 0·16, 0·208, 0·14 mm. But in what appear to be the thinnest fibres the length of a section between two nuclei reaches about 0·75 of a millemètre.

The nuclei are oblong and slightly flattened ; their outline is not smooth, but is wavy or notched in, and therefore looks as if folded. The mean size of such a nucleus is 0·026 by 0·006 or 0·008 mm. ; its outline, however, on careful inspection is not very well defined, especially corresponding to its poles we miss in many instances the limiting membrane. Each nucleus is embedded in a hyaline plate folded over the bundle of fibrils representing the nerve-fibre ; this plate—*ground-plate* we will call it—is very well shown in specimens stained in hæmatoxylin, or doubly

[1] I have described minutely the distribution of fine nerves in the mesentery of frog in this Journal, Vol. XII, 1871 ; the minute nerves belonging to the ground-substance of the mesentery appear to be far less numerous in the newt than in the frog.

stained in picrocarmine and hæmatoxylin, as it assumes a distinct tint. As shown in fig. 23, *b*, it extends a certain distance beyond the poles of the nucleus. In some places the expansion of the ground plate is much greater at one side of the nucleus than at the other. To each nucleus, therefore, corresponds a cell-plate, and consequently we find a greater number of cell-plates, the thicker the nerve-fibres. A microscopic nerve trunk of the mesentery of batrachian and other animals exhibits, by the aid of silver-staining, the presence of a complete envelope of nucleated endothelial plates. (Axel Key and Retzius, Ranvier.)

The nucleus contains in all instances a beautiful network of fibrils, and in this respect it is second to no nucleus of any other kind.

A point that deserves careful consideration is the relation of the fibre-bundle, *i.e.* the axis cylinder, to the intranuclear network. I have examined the nerve fibres in my specimens with great attention, and I have failed to obtain any evidence of a connection of the two; on the contrary, in most instances I am able to follow the axis cylinder *along one or the other side of the nucleus* beyond this latter, and there is every reason to regard this condition as the rule. If in an isolated instance the appearances are against it, I can easily account for it. The reason is this: the nucleated cell-plate, which at intervals ensheathes the axis cylinder, does not consist merely of the hyaline ground-plate and nucleus, as mentioned above, but possesses fibres, which I will call *investing fibres; they are in connection with the intranuclear network and pass beyond the ground-plate, they follow closely the axis cylinder and appear to be twisted round it so as to actasa the sheath for the sections between two neighbouring cell-plates.* If, then, in one or the other instance it would appear as if the axis cylinder were in connection with the intranuclear network, these investing fibres must not be forgotten. I have not represented these fibres in figure 23, because they were not distinctly differentiated in the nerve-fibre delineated in this figure, which is a faithful rendering of the appearances observed in this particular nerve-fibre; but there are others in which I have seen the investing fibres with sufficient distinctness. Thus, there exists a complete analogy between these cell-plates and the connective-tissue corpuscles described in a former paragraph, for in both we distinguish a hyaline ground-plate from the fibres which, on the one hand, are in connection with the intranuclear network, and, on the other hand, pass as the processes beyond the limits of the ground-plate. The difference

between the two kinds of cells lies merely in the distribution and amount of these fibres; in the connective-tissue cells of the mesentery of newt the fibrillar substance forms a considerable portion of the whole cell, being present in a perceptible amount around the nucleus and extending from here in all directions as the branched processes : in the cell-plates of the nerve-fibres, on the other hand, there is no appreciable collection of that substance around the nucleus, and the fibres extend apparently only in the two opposite, or at most three directions. This last fact is easily accounted for, considering that the cell-plate of the nerve-fibre is doubled round this latter, and consequently must be without processes at least on one side, *i.e.*, the margin of the fold.

It is easily understood that the intranuclear network of the cell-plate of a nerve-fibre is in connection with the processes and intranuclear network of a neighbouring connective-tissue corpuscle—a fact which I observed in the instance figured in 23 of Plate XVI.

It is not at all improbable that the assertions of a connection of minute nerve-fibres with the processes of connective-tissue corpuscles—too well known in the literature of the cornea—are to be explained in this manner.

NOTES AND MEMORANDA.

Recent researches on the Nervous System of the Medusæ.—Although the remarkable memoir of Dr. Kleinenberg on Hydra has for some time pointed out to biologists the importance of the study of the Cœlenterate nervous system for the solution of questions on the origin of the nervous system, it is not till within the last two years that the subject has been again taken up. Since then the nervous system of the Medusæ has formed the subject of a series of researches both on the physiological and morphologieal sides. The very brilliant physiological investigations of Mr. Romanes[1] are well known to all naturalists in this country, and we need only call attention in this connection to the investigations carried on simultaneously by Dr. Eimer.[2] From the morphological side there has appeared a memoir by Professor Claus,[3] a very elaborate monograph by the brothers Hertwig,[4] which completely revolutionizes our knowledge on this subject, and a note by Professor Schâfer,[5] which, though unfortunately short and without illustrations, still serves to fill up important lacunæ in the more elaborate memoirs of the German naturalists, and is, we trust, only the prelude to a more complete investigation. We propose giving a short account of the morphological results of the investigations relating to Medusæ, but more especially those of the brothers Hertwig.

The nervous system is differently constituted in the Craspedota and the Acraspeda. We shall commence with the former, for our recent knowledge of which we depend almost entirely on the observations of the brothers Hertwig. The central part of the

[1] 'Phil. Transactions of the Society of London,' 1876 and 1877.

[2] "Ueber kunstliche Theilbarkeit u. uber das Nervensyst. d. Medusen," 'Archiv f. Mikr. Anat.,' Bd. xiv. Dr. Eimer has also published an important memoir on the nervous system of Beroe, 'Zoologische Untersuchungen,' Wurzburg, 1874, of which, however, we do not propose to give an account.

[3] "Studien ueber Polypen u. Quallen d. Ardria." 'Denk. k. Akad.' Wien, 1877.

[4] 'Das Nervensystem u. die Sinnesorgane der Medusen,' by O. Hertwig and R. Hertwig.

[5] 'Proceedings of Royal Society,' Jan., 1878, No. 185.

nervous system is stated by these investigators to be formed by a nerve ring, situated along the line of insertion of the velum, and composed of two bands separated by the structureless lamina interposed between the two epithelial layers of the velum.[1]

The existence of a central nervous system of this type is described in a large variety of forms from all the main groups of the Craspedota, including examples from the Trachymedusæ and the true Gonophores, both Oellata and Vesiculata. The nervous ring reaches its highest development in the Geryonidæ. It is, however, even in its most differentiated form, *not separated from the ectoderm*. The upper band is formed of immeasurably fine fibres with delicate swellings at intervals. Among the fibres a fair number of, for the most part, bipolar nerve cells are scattered. In the lower band the nerve fibres are larger and the ganglion cells more numerous than in the upper. The two bands appear to be connected by delicate fibres passing through the lamina which is interposed between them.

The ectoderm adjoining both the bands is formed of but a single layer of cells which may, however, be divided into two categories. A. Interstitial cells. B. Sense cells. The sense cells are formed of an elongated body with a delicate cilium projecting from the free surface, and prolonged below into fibres which pass into the nervous bands. There is a much larger number of sense-cells connected with the upper than with the lower band.

The peripheral nervous system is formed of multipolar ganglion cells interposed between the superficial epithelium and the layer of circular muscles on the under surface of the disc. Multipolar garglion cells are also present in the tentacles, but, as it would seem, not in the velum. The ganglion cells of the disc form a complete network connected with the lower band of the nerve ring, and probably also with the muscles and sense cells of the tentacles.

The nervous system of the Acraspeda has been investigated by Claus, Schäfer, and the Brothers Hertwig. The fullest description is that by the brothers Hertwig, which on the whole fairly agrees with that by Schäfer.

The central part presents a marked contrast to that of the Craspedota in that it does not consist of a nerve ring round the edge of the disc, but of a series of isolated ganglia usually eight in number, though sometimes more numerous. These ganglia are thickenings of the ectoderm which generally take the form of a ring surrounding the base of a sense organ, and are formed of sense cells continued below into nerve fibres. Nerve cells, similar to those in the central nervous system of the Cras-

[1] Einer gives a similar account of the double nerve-ring.

pedota, are absent, according to the Hertwigs, though stated to be present by Claus. The peripheral nervous system has been especially studied by Schäfer, and its presence is mentioned by Claus. Schäfer describes it as formed of an interlacement of nerve-fibres covering the whole under surface of the umbrella and lying between the ectodermal epithelium and the muscular sheet. Each nerve-fibre presents in the middle of its course a nucleated enlargement in the shape of a bipolar nerve-cell, which is thus interpolated in the course of the fibre. The nerve-fibres are rarely more than four millimetres in length, and do not come into actual continuity with other fibres. They end either by fine tapering extremities, or by dilated expansions enclosing a nucleus. The nucleated expansions are regarded by Schäfer as a form of motorial end plate. It will be seen that that the peripheral nervous system of the Acraspeda appears to differ nearly as much from the same system in Craspedota as does the central system.

In considering the organs of special sense, it will be convenient again to distinguish between the Craspedota and the Acraspeda. In the Craspedota we are entirely indebted to the brothers Hertwig for our facts.

Though both auditory and optical organs are present in this group, they are never associated in a single form. Organs of hearing are formed in the Trachymedusæ and the Vesiculata, and, as the brothers Hertwig clearly prove, are formed on different types in these two groups.

In the Vesiculata the simplest form of auditory organ is that of a series of open pits situated along the attached edge of the velum with the aperture directed downwards. Both epithelial layers of the velum take part in their formation. The epithelium of the upper surface of the velum covers the convex surface of the organs, and its cells are provided with thick membranes, and filled with fluid. The epithelium of the under side of the velum supplies the cells of the under or concave side of the organ, and most of it cells develope a calcarious concretion; but a row of them along the inner edge of each pit, takes the form of sense-cells, provided with auditory hairs, and continuous with the fibres of the lower nerve ring. The above form of auditory organ is found in Mitrotrocha, Tiaropsis and other genera. In Mitrotocha the number of such pits present in a single individual may amount to eighty.

In many Vesiculata (Aequorea Octorchis Eucheilota, etc.) the open pits are replaced by closed sacks. The open and closed forms of the organ appear at first sight rather different, but really stand to each other in the same relation as do the open auditory

pits of an embyro Vertebrate to the closed vesicles into which they become converted.

In the Trachymedusæ the auditory organ appears more in the form of a modified tentacle, and consists, in its simplest condition, of a papilla, formed of a central axis of endoderm and a coating of ectoderm. In the terminal cells of the endodermal axis is situated a concretion, and some of the ectodermal cells are modified so as to form hair cells. In the more complicated types the whole papilla becomes enclosed in a cup, and in the highest forms (*e.g.* Geryonia) by the conversion of the cup into a vesicle, the papilla comes to be situated in a completely closed cavity. In Geryonia the entrance of a nerve into the vesicle, originally described by Hæckel, is fully established by the brothers Hertwig and by Eimer, and the termination of its fibres in the hair cells around the endodermal axis is clearly demonstrated by the former.

An optic organ is confined to the Ocellata. In its lowest conditions it consists of certain areas at the base of the tentacles composed of sense-cells invested by pigment-cells, and in its most differentiated condition a lens formed by a thickening of the external cuticle is added to the structures found in the simpler form of eye.

The sense organs of the Acraspeda have been investigated both by Schäfer, the brothers Hertwig and by Claus. The brothers Hertwig have studied with great detail almost all the main types of the Acraspeda and the observations of Claus have also been made on more than one form, while those of Schäfer are confined to Aurelia. The general type of sense organ appears to be auditory in function and is more or less similar to that of the Trachymedusæ. It consists of a tentacle-like organ situated in a groove on the under surface of one of the lobes of the edge of the disc. The groove is nearly converted into a canal by the presence of a fold or flap closing it over below. The organ itself is somewhat knobbed and consists of an endodermal axis along half the length of which is continued a prolongation of the gastrovascular canal-system, while its terminal portion is solid and contains calcareous concretions. The ectoderm covering the knobbed extremity is flat but around the base its cells become columnar and are provided each with a stiff hair or bristle and prolonged internally into a nerve fibre. They constitute, in fact, what have already been described as the ganglia of the central nervous system. Between the ectodern and entodern is a structureless lamina, which is spoken of as mesoblast by Schäfer though it appears to us hardly to deserve that title. Optic organs of a similar character to those in the Ocellata are added to the sense organs in Nausithöe, Aurelia, and Charybdea.

Claus finds in Aurelia another sense organ present on the dorsal surface of the plate which covers each marginal sense organ, in the form of a pit, lined by an epithelium of sense-cells. He interprets it as olfactory in function.

We may point out in conclusion that the structural features of the nervous system brought to light in the investigations of which we have given an account are exactly such as might be deduced from the physiological investigations of Mr. Romanes.

F. M. Balfour.

PROCEEDINGS OF SOCIETIES.

Dublin Microscopical Club.

17*th January*, 1878.

On the Development of the Siphons in Polysiphonia.—Dr. E. Perceval Wright exhibited some mounted specimens presenting the form of apical growth so characteristically described by Nägeli in the 'Zeitschrift für wiss. Botanik,' 1836, and showing in addition some new facts (as he believed) in the development and growth of the so-called siphons in Polysiphonia. The species selected was one with four siphons, representing the subgenus Oligosiphonia. Such a species would be described as having a "frond consisting of four tubes radiating round a central cell, and generally containing endochrome" (Harvey), or, as by Agardh, in some detail, as "Cellulæ istæ pericentrales, quas siphones vocant, sunt endochromate colorate repletæ, parietibus hyalinis crassis, polystromaticis invicem et a cellulis articulorum proximorum separatæ." But all such-like descriptions appeared to Dr. Wright to be based on a misconception of the structure to be seen when the living plant is carefully studied, and when quite fresh specimens are submitted to the action of re-agents. It then become s pretty evident that the growing filament in Polysiphonia is in reality one continuous cell bounded on all sides by its own well-marked cell-wall, that below its growing point, below the layer of homogeneous protoplasm, and below even that so-called granular protoplasm of Nägeli, there exists a protoplasm, which has, as it were, taken up its maximum quantity of water of organisation, has become a viscid, almost sensitive substance, capable of forming out of itself chlorophyll and starch-granules, and, on occasions, exhibiting, in some allied forms, all the characteristic movements of animal pseudopodia. In this layer the siphons originate. In the species exhibited the process was—(1) a thin lozenge-shaped plate of the protoplasm became detached all but in five parts, where the thread-like continuations kept the little lozenge of protoplasm from falling away from the upper portion; (2) this lozenge increased greatly in thickness, and after a certain thickness had been attained, it divided asunder into a central portion and four equi-dimensioned peripheral portions; each of these latter still attached to the central portion by prolongations from the centre of their long diameter

and each of the free masses, it will be borne in mind, is also attached by similar prolongations to the five masses already below them, and will be equally attached to the five masses that will be formed above them. When these masses, which are the "siphons" of the algologist, reach adult age, a delicate cellulose membrane forms around each, and between these and the common investing cell-wall there is sometimes a well-marked interval, which is in some species filled up by cellules. No true articulation at this stage exists in the stem; it only occurs later in life, and in a manner seemingly as yet undescribed.

Structure of Spines of Toxopneustes variegatus, Lamk.—Mr. Mackintosh exhibited a cross section of the spine of *Toxopneustes variegatus*, Lamk., which showed two cycles of solid wedges, the inner very small, the outer much larger, globular in contour, and connected to each other by single short bars perforated with a few delicate tubes.

Magnetic Particles occurring in Arctic Ice-dust.—Dr. Moss, R.N., exhibited a sample of Arctic Ice-dust, and showed intermingled magnetic particles under the microscope, calling attention to their movement under a magnet caused to move about under the stage.

An Oscillatoria from Australian Seas, occurring in large quantities.—Mr. Archer exhibited examples of an alga forwarded by Professor Thiselton Dyer, an Oscillatoria from the surface of the Australian Seas, often forming large masses and thrown ashore. Locally, this was popularly called Whale-Spawn, and its nature misunderstood, being accounted there as somehow a kind of slough or exuvium from certain polypes. Whether originally colourless or whether by drying it had become bleached was uncertain. The cells were unequal in size. In the mass this production is described as giving off a very foul smell; this however, more than probably, may be only when it is undergoing decomposition in the sun on the beach. Perhaps the most remarkable circumstance connected with it was the vast quantity in which, at certain seasons, it would appear to occur.

Polariscopic aspect of Artificial Gems.—Mr. Grubb showed the polariscopic appearance of certain of the artificial gems (sapphire, &c.) now being manufactured by M. Feble of Paris, in which respect they are not to distinguished from genuine stones.

"*Japanese Isinglass.*"—Mr. Draper showed sections of the so-called Japanese Isinglass, and stated he had arrived at the same conclusion as Professor Mr. Nab, that this was in reality some form of starch.

Navicula Mossiana, n.s., O.'M., *exhibited.*—Rev. E. O'Meara showed a slide from material dredged from a depth of seven fathoms in Discovery Bay 81° 43′ N., for which he was indebted to the kindness of Dr. Moss, R.N., late of H.M.S. "Alert." Amongst the forms contained was one belonging to the genus Navicula, which he considered not hitherto named, and proposed

to designate *N. Mossiana* as follows: Valve broadly elliptical, length ·0027″, breadth ·0018″; median line well-marked, lying in a narrow, unstriate, linear space, with a wide quadrangular expension around the central nodule; striæ distinct, close, punctate, slightly radiate in the middle, more and more decidedly radiate towards the ends; towards the middle of the valve the lines of puncta are interrupted, so as to form on either side of the median line two or three distinct longitudinal bands, nearly parallel to the median free space. Schmidt has figured without names some forms which strongly resemble the present, 'Atlas der Diat.,' t. vi, figg. 35, 36, 39.

27*th February*, 1878.

A New Mineral from Carnmoney Hill, near Belfast, exhibited.—Professor Hull, F.R.S., exhibited a thin section of the Olivine Basalt of Carnmoney Hill, near Belfast, containing a mineral considered by Mr. E. T. Hardman, F.C.S., who has analysed it, to be new. The mineral is black, glossy, and gives an olive-brown streak; hardness 2·5, sp. gr. 1609—1700. It occurs as a material filling cells and cavities in the original rock and often surrounding the crystals of felspar, olivine and augite. It is in a gelatinous, uncrystalline condition, and does not polarize under the microscope; with a low power it is seen to be of a chocolate or yellowish-brown colour, passing into black in the thicker portions of the slice. No special structure is observable, but with a high power faint, wavy lines, like those of stalagmite or chalcedony, were observed, giving evidence of a formation from aqueous solutions. The author had no doubt, both from the characters of this mineral under the microscope and from the chmical analysis of Mr. Hardman, that it was a secondary mineral, derived from aqueous solution after the rock had consolidated from the crystalline state.

The following is the analysis:

Silica . . .	39·352
Alumina . . .	10·452
Peroxide of iron . .	20·769
Protoxide ,, . .	3 699
,, manganese . .	trace.
Calcium oxide . .	4·484
Magnesium ,, . .	7·474
Water (confined) . .	13·618
Zinc oxide . . .	trace.
Organic matter . .	,,
	99·848 per cent.

A New Variety of Stysanus stemonitis, exhibited.—Mr. Pim showed a remarkable mould which grew on a bamboo-stake in his hothouse. It bore no resemblance to any mould figured in

either Cooke or Corda, except *Stysanus stemonitis*, Corda, from which it differed in the fact that the stem, instead of being simple and surmounted by a cylindrical head of concatenated spores, was divided into numerous branches, each terminated by a head of spores, the way in which the threads of the stem passed into the branches strongly reminding one of the divisions of some tendons. A specimen was subsequently shown to Mr. Vize, and by him to Mr. Phillips, of Shrewsbury, and both concur in considering it a branched form of the species named. As its claim to specific rank is doubtful, Mr. Pim would suggest that it be called *Stysanus stemonitis*, Corda, var. *ramosa*, Pim.

A new Micrasterias from Scotland, forwarded by Mr. J. P. Bisset, Banchory.—Mr. Archer exhibited a new Micrasterias, kindly sent to him from Scotland, by Mr. J. P. Bisset of Banchory, near Aberdeen, and collected by that gentleman on the Dee-side. This presented denticulations like those of *M. denticulata*, with some of the contour of *M. angulosa*, but seemed well distinguished by the possession, on each front surface, near the base of each semicell, of a curved series of circular groups of bead-like markings, of which the central group was the largest, the external ones being the smallest. These seemed to Mr. Archer to be composed of leaflike prominences, and the groups he supposed, were it possible to view them laterally, would be seen to stand out as crown-like elevations, somewhat comparable to those, say, of *Xanthidium armatum*, but most likely not by any means so prominently—that is to say, the component bead-like markings did not appear as dots or even granules, but, as he said, thin leaflike projections. Mr. Bisset referred to other "markings," but in the specimen, owing probably to the presence of the cell-contents, no others could be noticed. These examples formed a portion of a set of two or three slides of species carefully selected and exquisitely mounted by Mr. Bisset—one contained six species of Micrasterias, represented by one example of each, *M. rotata*, *M. denticulata*, *M. truncata*, *M. papillifera*, *M. angulosa*, and the new species.

Section of Spine of Mespilia globulus, exhibited.—Mr. Mackintosh exhibited a cross section of the spine of *Mespilia globulus*, which had been kindly sent him by Dr. Günther, F.R.S. The greater portion of the spine was composed of reticulations with thick trabecules, the periphery being formed of a single row of solid wedges, whose form was almost that of an equilateral triangle.

Peculiar condition of Rivularia.—Dr. E. Perceval Wright exhibited sections from examples of *Rivularia*, showing a peculiarity in certain of the component filaments, consisting in the fact that these presented a clavate expansion in place of the ordinary tapering, inside which expansion the cells formed indefinite groups, not linear series. Possibly, in function these might be comparable to the mamillate branches of such forms as *Stigonema mamillosum*, in which, however, the cells remain in single

series, though indefinitely scattered in the stems, and, in Rivularia, like them destined to become removed and serve to the vegetative propagation of the plant. Such a mode of vegetative growth does not appear to have been noticed in Rivularia.

Some Arctic Diatoms, amongst which Biddulphia Balæna, which appears rather to be a Triceratium.—Rev. E. O'Meara showed a slide containing Diatoms, collected in Franklin Bay by Major Fielden, R.A., Naturalist to the Arctic Expedition. The collection is a most valuable one, containing numerous rare species, amongst which are to be found *Coscinodiscus punctatus*, Greg.; *Synedra Kamskatica*, O'M.; *Grammatophora arctica*, Cleve; *Rhabdonema Torellii*, Cleve; *Pleurosigma longum*, Cleve; *Thallasiosira Nordskioldii*, Cleve; *Rhoiconema bollinana*, Grunow; *Triceratium arcticum*, Brightwell; *Biddulphia Balæna*, Brightwell. To this last Mr. O'Meara directed special attention, and expressed his opinion that it would be more properly included in the genus Triceratium. Except as regards its somewhat rhomboid outline the characters closely resembled *Triceratium arcticum*, with which it was not unfrequently associated.

21*st March*, 1878.

Verrick's $\frac{1}{6}$th.—Mr. Richardson drew special attention to the excellence of this objective. It was the power (about $\frac{1}{6}$th) supplied by Verick with his low priced stand, and defined so well that he, Mr. Richardson, preferred it for demonstrating most sections to glasses of some other makers of about the same power in his possession, each of which cost considerably more than Verick's stand and its two objectives. Moreover, the glass without any special arrangement therefor, acted equally well whether as a dry or as an immersion lens.

Structure of Spine of Echinus acutus, Lamk.—Mr. Mackintosh exhibited a cross section of the spine of *Echinus acutus*, Lamk. The specimen from which the spine was taken was dredged off the coast of Youghal by the late Dr. Ball, who described it under the name of *Echinus Flemingii*, but A. Agassiz, ('Review of the Echini,' 1872), has placed it as a variety only of Lamarck's older species. The example was presented by Dr. Ball to the Museum of Trinity College, Dublin, and Mr. Mackintosh was indebted to the kindness of Professor Macalister, the present Director, for the opportunity of examining the structure of the spines. They present in cross-section the usual axis of reticular tissue surrounded by four cycles of solid wedges, separated from each other by spokes of trabecular tissue. The cycles might be described as being produced by the constriction at intervals of a single elongated wedge, such as is seen in the spine of the allied *E. esculentus*, Linn., a section of which was shown for sake of contrast.

Lafoea pocillum, exhibited.—Mr. Grant showed *Lafoea pocillum*, one of the Campanularians, of considerable rarity, Hincks, in his

work on the "Hydroid" Zoophytes, stating that he had met with it only at Oban. Mr. Grant met with it at Howth. It is minute, with short beaded stem and long tubular capsule.

Docidium nodosum, American example, exhibited.—Mr. Archer showed an example of *Docidum nodosum* occurring in a small collection labelled as made at New Jersey, in America, and for some years lying in the Herbarium of Trinity College, and for the examination of which Mr. Archer was indebted to Professor Perceval Wright. For a long time this appeared to be a purely American species, but Mr. Archer had some time ago made its acquaintance at Comemara, but he had seen two examples only. It had since been gathered in Sweden. It is an unmistakable, fine, bold, and handsome species.

Cephalic Armature of Clio borealis, exhibited.—Dr. Moss showed the cephalic armature of *Clio borealis*, and some beautiful original drawings in illustration, showing also the entire animal as it appears in the act of swimming.

New Species of Craspedodiscus, C. Febigeri, exhibited.—Rev. Eugene O'Meara exhibited a new species of Craspedodiscus from the Nottingham deposit. The slide was sent by Mr. Febiger of Wilmington, U.S., through Mr. Habenshaw of New York. Mr. Febiger doubtfully assigns the form to the genus Crasp dodiscus, but close examination removes all doubt as to its proper place. Like *Craspedodiscus elegans*, this species possesses a central rosette of eight elongated areoles. The central part of the disc is not so distant from the border as in some other species, yet sufficiently defined to constitute in form a genuine Craspedodiscus. The areoles are hexagonal, radiately arranged, somewhat smaller in the central portion than in the marginal. The marginal portion is somewhat broader than the radius of the central. The form is large, being in diameter 0·1″ Mr. O'Meara proposed to name this form *Craspedodiscus Febigeri.*

On the Development of the Tetraspores in Polysiphonia.—Dr. E. Perceval Wright exhibited some recent preparations showing the development of the tetraspores in *Polysiphonia nigrescens.* He was not aware of any researches on this subject, but the series of specimens would clearly show that the mass of protoplasm which in time developed into the cell with the tetraspores, took its origin from the base of the central siphon, first presenting the form of an oval mass, then becoming stalked; it next greatly increased in size, and in doing so pressed out the external rows of sephons which surrounded it, giving thus to the tetrasporic ramule its well-known irregular form of outline; after a little the central portion of the cell contents divided into the four portions forming the tetraspores. The stalk, though still plainly visible with a sufficient power, was very slender in comparison with the mass it bore.

MEMOIRS.

On the Oral *and* Apical Systems *of the* Echinoderms. By P. Herbert Carpenter, M.A., Assistant Master at Eton College. (Part I.)

The following essay is an attempt to compare and criticise the various views that have been put forward from time to time, respecting the homologies among the different Echinoderms of the two groups of calcareous plates, which appear at a very early period around the two peritoneal diverticula of the primitive digestive sac of the larva.

To the one group, developed upon the abactinal surface of the body, around the right peritoneal sac from which the aboral division of the cœlom is derived, the name of the Abactinal, Apical, or Dorsocentral System has been applied by the various authors who have studied it. It generally consists of three sets of plates, viz., a single central plate, surrounded by two rings of five plates each. The plates forming the proximal ring are situated *interradially*, as regards the general symmetry of the Echinoderm type, while the plates of the distal ring alternate with them, and are therefore *radial* in position. This compound Apical system forms an essential constituent of the skeleton of all the Urchins, Starfishes, and Crinoids, although the special functions of its various elements differ very considerably in these different groups.

The other group of plates, which I will call the Oral System, is developed upon the actinal surface of the young Echinoderm, around the left peritoneal sac that gives rise to the anterior or oral division of the cœlom. It has hitherto received but little attention from the various naturalists who have studied the comparative morphology of the Echinoderms. It is much simpler in character than the Apical system, as in recent Echinoderms it consists of but one ring of five plates disposed *interradially* around the mouth of the

larva, though in some fossil Crinoids there is a single plate in the centre of this ring, as in the Apical system. Further, it is by no means so constant in its appearance as the Apical system, and often undergoes more or less resorption before maturity is reached. This is the case even in some of the Crinoids, in which group this Oral system seems to reach its fullest development.

It is characteristic of the larval stages of existing Crinoids, and in some cases persists through life, while it is very highly developed in many extinct genera, more particularly those of the earlier geological periods.

The Oral system is consequently of considerable interest from a phylogenetic standpoint, and adds another piece of evidence to the many which we already possess, in support of the view that the Crinoids represent an earlier stage in the phylogeny of the Echinoderms than any other members of the order.

The component elements both of the Apical, and of the Oral systems are better represented in the Pentacrinoid stage of the development of *Antedon*, than in the larval or adult condition of any Echinoderm with which I am acquainted. It will be well, therefore, to take the Pentacrinoid as a starting point, from which we can pursue our study of the

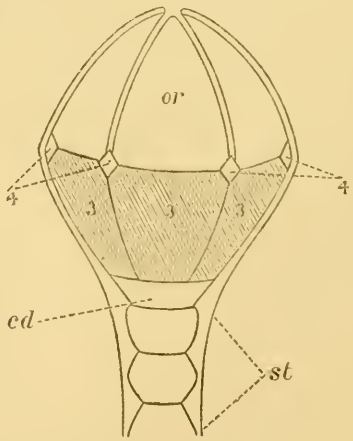

FIG. I.—Calyx of the Pentacrinoid larva of *Antedon rosacea*. (After Allman.) *st*. Upper part of stem. *cd*. Centrodorsal piece = Top stem-segment. *or*. Oral plates = Interradials (Allman). 3. Basal plates. 4. First radials.

N.B.—In this and the following figures, the plates 3, 3, the position of which is *interradial* with regard to the general symmetry of the Echinoderm type, are shaded for the sake of distinctness.

comparative development of these two systems in the other Echinoderms.

The first observer who paid any attention to the homologies of the skeletal plates of the young Crinoid was Professor Allman. He described the body of the larva as consisting of a calyx covered by a pyramidal roof.[1]

"The calyx is composed chiefly of five large plates, very distinct, and united to one another by simple suture (fig. 1, 3, 3). Between the lower edges of these plates and the summit of the stem is a narrow zone (*cd*), in which no distinct indications of a composition out of separate plates can be detected. Between the upper angles of every two contiguous large plates there may, with some care, be made out a minute intercalated plate (4, 4). There would thus be five of these little intercalated plates, which, though by no means so evident as the large plates which alternate with them, are sufficiently so to leave no doubt of their presence. . . . . The pyramidal roof which closes the cup in the contracted state of the animal is composed of five large triangular plates (*or*), each supported by its base upon the upper edge of one of the large plates of the calyx, and with the small intercalated plates encroaching upon its basal angles."

Allman recognised at once that the narrow zone (*cd*), intervening between the five large hexagonal plates and the summit of the stem, represented the centrodorsal piece of the adult *Comatula*, and he regarded it, in complete accordance with Müller's views, as a metamorphosed stem-joint. The five large plates (3, 3) superimposed upon it and constituting almost the whole of the calyx, were regarded by him[2] as corresponding "to the true *Basalia* which immediately surround the stem in such forms as *Platycrinus*, and the great majority of the Crinoidea; while the five small plates (4, 4) intercalated between their upper angles will represent *radialia*."

While believing this view of the homologies of the elements of the calyx in the Pentacrinoid to be the correct one, Allman suggested another as possible, which has not found general acceptance, though it has recently been revived by Agassiz and Lovèn.

It is as follows.—The single centrodorsal piece (*cd*) represents a zone of coalesced *Basalia*, the "Pelvis"

[1] "On a Pre-brachial Stage in the Development of *Comatula*, and its importance in its Relation to certain aberrant forms of Extinct Crinoids," 'Transactions of the Royal Society of Edinburgh,' vol. xxiii, p. 241.

[2] Loc. cit., p. 244.

(Miller) or "Basis" (Müller), while the five large plates (3, 3) above it correspond to the *Parabasalia* of the Palæozoic Crinoids, the intercalated pieces (4, 4) being considered, as before, as *radialia*.

Allman's own view has been accepted by Wyville Thomson, Dr. Carpenter, M. Sars, and (with some modifications) by Götte, all of whom have studied the development of *Comatula* with much care.

Agassiz[1] and Lovèn, however, prefer to regard the centrodorsal piece (*cd*) as a "solidified homologue of the basals of the other Crinoids." There are many and serious objections to this view, which will be discussed at length farther on.

Correct as Allman undoubtedly was in his analysis of the calyx of the Pentacrinoid, he was, nevertheless, misled with respect to the homologies of the five large triangular roof plates (*or*), for he regarded them[2] as "greatly developed interradialia."

Wyville Thomson[3] and Dr. Carpenter[4] have shown, however, that the true interradials[5] of the Pentacrinoid appear between the upper edges of the basals (3, 3), and the lower edges of the roof plates (*or*), to which they have given the name of *Orals* from their position around the mouth. On the other hand, both these observers agree with Allman in regarding these interradially disposed oral plates as "in all probability homologous with valve-like plates surrounding the mouth only, in all Crinoidal genera in which they occur."[6]

In *Haplocrinus* these oral plates remain single, but in *Coccocrinus, Stephanocrinus,* and *Eucalyptocrinus,* they are more or less subdivided. Allman suggests the possibility that the roof of *Lageniocrinus* is homologous with that of *Haplocrinus* and of the young Pentacrinoid.[7] It is difficult to decide whether his, or De Koninck's[8] analysis of this

[1] "North American Starfishes," p. 63. 'Memoirs of the Museum of Comparative Zoology at Harvard College,' vol. v, No. 1.

[2] Loc. cit., p. 245.

[3] "On the Embryogeny of Antedon rosaceus," 'Phil. Trans.,' vol. clv, p. 540.

[4] "Researches on the Structure, Physiology, and Development of Antedon rosaceus," part i, 'Phil. Trans.,' vol. clvi, p. 716.

[5] These are not precisely homologous with the interradials of the *Palæocrinoidea*.

[6] 'Phil. Trans.,' vol. clv, p. 542.

[7] Loc. cit., pp. 250, 251.

[8] 'Recherches sur les Crinoïdes du Terrain Carbonifère de la Belgique,' p. 188.

difficult genus is the correct one, but with regard to Hagenow's *Hertha mystica* I have no hesitation in saying that the five triangular plates forming the so-called pyramidal "roof" are not in any way homologous to the oral valves of the pre-brachial *Comatula*, as suggested by Allman. Müller's[1] determination of them as parts of the (first) radials, which remain attached to the centrodorsal piece is undoubtedly the correct one. In the same way I am convinced that the five pieces forming the pyramidal "roof" of *Eugeniacrinus caryophyllatus* are not in the least to be regarded as an "oral series," but that Quenstedt[2] was perfectly right in describing them as forward processes between the two distal articular surfaces of the third or axillary radials. They are, in fact, homologous with the "clavicular piece" of Schultze,[3] which occurs also, though developed to a far less extent, on the radial and brachial axillaries of *Actinometra polymorpha*.

Among the recent Crinoids the oral plates of the Pentacrinoid larva appear to have a very different fate, according as the adult animal remains throughout life in the pedunculate, and therefore embryonic, condition, or is set free from the stem at the end of its development. In the latter case, as exemplified in *Comatula*, the orals undergo a gradual resorption, which commences before the termination of Pentacrinoid life, and is completed very soon after the young *Comatula* has entered upon the free stage of its existence. On the other hand, in some, though not all, of the recent genera of pedunculate Crinoids, the oral plates of the larva persist through life, supporting the oral valves in the interradial areas of the disc. Sars[4] and Ludwig[5] have met with them in *Rhizocrinus;* and Wyville Thomson[6] finds them in *Hyocrinus*, though not in *Bathycrinus*.

He has also described them in the remarkable genus *Holopus*[7] as five rather large triangular plates which meet in

[1] "Ueber den Bau des *Pentacrinus caput Medusæ*," 'Abhandl. der Berlin Akad. 1843,' p. 28.

[2] "Ueber *Eugeniacrinites caryophyllatus*," 'Neues Jahrbuch für Mineralogie, 1855,' p. 671.

[3] "Monographie der Echinodermen des Eifler Kalkes," p. 5. 'Denkschriften der Wiener Akademie,' Bd. xxvi.

[4] "Mémoires pour servir à la connaissance des Crinoïdes vivants." 'Du Rhizocrinus lofotensis,' p. 17.

[5] "Zur Anatomie des *Rhizocrinus lofotensis*," 'Zeitschr. für Wiss. Zool.,' Band xxix, p. 115.

[6] "Notice of new living Crinoids belonging to the *Apiocrinidæ*," 'Journal of the Linnean Society, Zoology,' vol. xiii, pp. 51—53.

[7] "On the Structure and Relations of the genus *Holopus*," 'Proceedings of the Royal Society of Edinburgh,' vol. ix, p. 409.

the centre of the disc to form a low pyramid covering the mouth, and further states that "these oral plates are *interradial*, and the spaces between them radial corresponding with the arm grooves."

From the descriptions of Duchassaing[1] it is possible that they occur in *Pentacrinus asteria*, though this is doubtful. According to Wyville Thomson[2] there is no trace of them in *P. Mülleri*.

The importance of the Oral system in the morphology of the Crinoids, and of most, if not all the other Echinoderms, has hitherto been but little noticed, and I propose in another paper to attempt to determine its homologies in the *Echini* and Holothurians, though not in the Starfishes, in which it is apparently undeveloped. For the present, however, we will confine our attention to the Abactinal or Apical System.

These names were first used by A. Agassiz[3] for the assemblage of plates at the dorsal pole of the test of the Sea-urchins. It has been called the "dorsocentral system" by Lovèn,[4] and consists of the so-called genital and ocular plates, disposed symmetrically around a central group, the anal system of Agassiz, which is composed of a series of polygonal plates, arranged with more or less irregularity around the anal opening.

The essential element in this anal system is a single plate which is, as a rule, most distinct in the young *Echini*, as it undergoes very important modifications during the passage from the young state to that of the full-grown animal. Lovèn, recognising its morphological importance as an integral part of the dorsocentral system, has named it the *central disc*.

As shown by Agassiz,[5] this central disc appears at a very early period in the development of the young Urchin, before any traces of either the genital or the ocular plates become visible. In its primitive condition it has a fairly regular

[1] Quoted by De Koninck, 'Recherches, &c.,' p. 53. See the 'Canadian Naturalist' for 1868, p. 441. Lütken states that Lovèn, who had examined Duchassaing's original specimen in the Michelin collection at Paris, told him that it did not show any oral valves because it had no peristome at all. This, of course, proves nothing either way.

[2] 'Phil. Trans.,' vol. clv, p. 542.

[3] "Revision of the Echini," p. 635, 'Illustrated Catalogue of the Museum of Comparative Zoology at Harvard College,' No. 7.

[4] "Études sur les Echinoïdées," 'Kongl. Svenska Vetenskaps Akademiens Handlingar," Band ii, No. 7, p. 65.

[5] "Embryology of the Echinoderms," 'Memoirs of the American Academy,' ix, 1864, p. 12, fig. 28. "Contributions to the Fauna of the Gulfstream, &c.," pp. 281, 284, 285. "Revision of the Echini," pp. 280, 286, 300, 683.

pentagonal shape, but as maturity is approached it generally undergoes very considerable modification, being gradually resorbed from one side, simultaneously with the development of the anal opening. This circumstance seems to have led Agassiz[1] to name it the *subanal plate*.

As the test enlarges, other plates are gradually added to the anal system, and the primitive central disc becomes much less conspicuous. But in *Salenia* it is never resorbed to such a considerable extent as in other *Echini*, and retains its original preponderance and pentagonal form throughout life, filling up the central space of the apical system (fig. II, 1). In one species, *S. goésiana*, Lovèn, no secondary anal plates are developed, and even when they do appear, as in the ordinary *Salenidæ*, they remain very small throughout life.

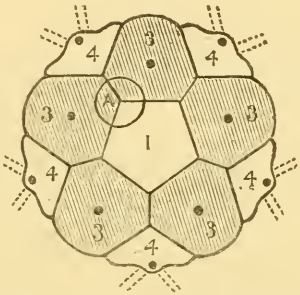

FIG. II.—Apical system of *Salenia* (after Lovèn). A. Anus. 1. Central disc or subanal plate. 3. Genital plates=costals (Lovèn). 4. Ocular plates=radials (Lovèn).

In either case, however, the anal opening is excentric, trenching on one of the posterolateral angles of the partially resorbed central disc (fig. II, A); but in the *Cidaridæ* the anal opening in the adult animal is placed in the central part of the apical system, and, as in most *Salenidæ*, is surrounded by the remains of the central disc together with numerous secondary anal plates.

In young Urchins, and in *Salenia* throughout life, the whole of the apical system is marked externally by a peculiar striation. This is also found on the calyx of the curious fossil Crinoid *Marsupites*, which *seems* to agree so very closely with the primitive condition of the apical system in the *Echinoidea* that Lovèn[2] has been led to institute a comparison between the two. Thus, the large pentagonal plate

[1] 'Lovèn, Études, &c.,' p. 70, figs. 170—176.
[2] Loc. cit., pp. 71, 72.

occupying the centre of the dorsal pole of the calyx of *Marsupites* (fig. III, 1) is regarded by him, as by Müller, as composed of five closely anchylosed basals, and as homologous with the central disc of the apical system in the *Echini* (fig. II, 1).

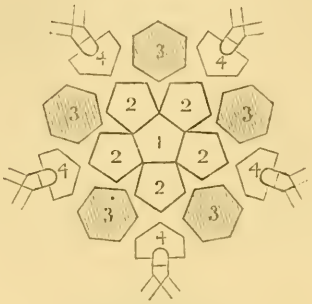

FIG. III.—Apical system of *Marsupites*. 1. Central disc = basis (Lovèn). 2. Under basals = first parabasals (Lovèn). 3. Basals = second parabasals (Lovèn). 4. Radials.

The five genital plates (fig. II, 3, 3) which are in close contact with this subanal plate in the *Echini*, are regarded by Lovèn as homologous with the similarly situated plates forming the first or proximal ring in the *Marsupites* calyx (fig. III, 2, 2). I would, however, venture to suggest that Lovèn is mistaken in this view. The genital plates of the Urchins are *interradial* in position, while the plates of the proximal ring in *Marsupites* are *radially* disposed. This fact seems to me to be a conclusive proof that the two sets of plates, although similarly situated with regard to the central plate of the Apical system, are in no way homologous, as supposed by Lovèn, but merely analogous.

Comparisons such as these, which are made without any regard to the positions of the parts compared, with reference to the general radial symmetry of the Echinoderm type, appear to me to be quite erroneous. Lovèn would, I am sure, be the first to admit this, and I imagine that he has fallen into this error from having inadvertently overlooked the relative positions of the plates in the first and second rings around the central disc of *Marsupites* (fig. III, 2, 2, 3, 3).

The plates of the second ring are situated interradially, and I believe them to be truly homologous with the genital plates of *Echini* (fig. II, 3, 3), which occupy a similar interradial position. Lovèn, however, considers that they have "no analogue" in the Urchins, nor in the other Crinoids, a

point to which I shall shortly return. The ocular plates of the Urchins (fig. II, 4, 4) are indicated by Lovèn as homologous with the third series of plates in the *Marsupites* calyx, namely, the radials (fig. III, 4, 4), a determination in which I entirely concur.

Müller,[1] De Koninck,[2] and Schultze[3] regarded the central disc of *Marsupites* as the basis, and supposed the plates of the second ring (*interradial*) to represent the ordinary "parabasals" or "subradials" of *Cyathocrinus*, *Poteriocrinus*, *Rhodocrinus*, and other *Palæocrinoidea*. De Koninck specially mentioned the fact of their alternating with the radials so as to be *interradial* in position. Following Müller, he spoke of the proximal ring of plates in *Marsupites*, intervening between the true parabasals (subradials) and the central disc, as a second series of parabasals, constituting a new element in the calyx peculiar to this genus. Lovèn, however, for no apparent reason, takes a precisely opposite view, and regards the plates of the first ring as the first or true parabasals, those of the second ring being "parabasals of the second order," which are unrepresented in the Urchins and in the Palæozoic Crinoids.

Having compared the apical system of the Urchins with the calyx of *Marsupites*, Lovèn further proceeds to trace out its homologies with the calyx of the Crinoids generally, and especially of the older and pedunculate forms.

I regret to state that I am unable to agree with him in many of these comparisons, partly because some of them are based upon views which I believe to be erroneous, and partly because he does not appear to have altogether understood some of the eccentricities in Miller's terminology.

In the first place, the *basis* in the nomenclature of J. Müller, d'Orbigny, De Koninck, and Schultze (= pelvis, Miller), or the centrodorsal (and not dorsocentral, as Lovèn quotes it) of De Blainville, is, I am firmly persuaded, in no way comparable to the central disc of the Urchins and of *Marsupites*. These, like Lovèn, I regard as homologous with one another, but I cannot follow Müller and most subsequent writers in considering the central disc of *Marsupites* as representing five closely anchylosed basals. Although Miller,[4] the first naturalist who analysed the calyx of *Marsupites*, spoke of this central disc as the pelvis (= basis), yet he also suggested that it might not be of this nature, and

[1] 'Pentacrinus,' loc. cit., p. 32.
[2] Loc. cit., p. 67.
[3] Loc. cit., p. 4 (116).
[4] 'A Natural History of the Crinoidea,' pp. 137—139.

that it was possibly the top stem-segment, so that the first ring of plates (fig. III, 2, 2) would represent the elements of the pelvis of the other Crinoids. This view was adopted with a slight modification by Pictet,[1] who referred to the absence of a facet on the central disc as indicating the absence of a stem, a view in which, as will be seen later, I entirely concur. I do not, however, agree with Miller and Pictet in regarding the proximal ring of plates (fig. III, 2, 2) in the calyx of *Marsupites* as basals, and the second ring (fig. III, 3, 3) as subradials (= parabasals), for, as shown above, the former are radial in position, while the basals of *Pentacrinus* and the other *Articulata* are situated interradially. But I have referred to their works in order to show that the view here advanced of the essentially simple nature of the central disc of *Marsupites* is not altogether a new one. There are no sutures upon it which would indicate its composition out of five separate elements. Yet this is the true nature of the basis of the other Crinoids, which Lovèn himself admits,[2] though sometimes entire, to be sometimes composed of more or fewer separate pieces, the *basalia*. I have endeavoured to show elsewhere[3] that there is every ground for believing that the basis is primitively a composite structure, and that its occasional apparent simplicity as in *Apiocrinus*, *Rhizocrinus*, *Eugeniacrinus* is only the result of a very close anchylosis of its component elements, and the disappearance of the external markings indicating their faces of lateral union. One important reason why the basis of the Crinoids should be considered as typically consisting of five originally separate plates is its mode of development in *Comatula*, the only Crinoid of which the younger stages are known to us. The ring of five plates (fig. I, 3, 3) which rest upon the top stem-segment [= future centrodorsal piece (fig. I, *cd*)] have been almost universally regarded as representing the basals of the Crinoids generally. This was Allman's intepretation of their nature, and it has been accepted by Wyville Thomson, Dr. Carpenter, Sars, and Götte. They are, however, termed parabasals by Lovèn, who compares them to the so-called first parabasals of *Marsupites* (fig. III, 2, 2) and to the genital plates of the *Echini* (fig. II, 3, 3). In this latter comparison I entirely agree, but I cannot accept the former, for the simple reason that the genital plates of the

[1] 'Traité de Paléontologie,' tome iv, p. 291.

[2] Loc. cit., p. 72.

[3] "On Some Points in the Anatomy of *Pentacrinus* and *Rhizocrinus*," 'Journal of Anatomy and Physiology,' vol. xii, pp. 48, 49.

Echini and the basals of the Pentacrinoid are *interradial* in their position, while, as I have already mentioned, the so-called first parabasals of *Marsupites* are placed *radially* with regard to the general symmetry of the animal (fig. III, *2*, *2*). Consequently, the interradial genital plates of the Urchins (fig. III, *3*, *3*) are compared by Lovèn, on the one hand, to the interradially placed basals of the Pentacrinoid (fig. I, *3*, *3*), and on the other to the radially situated plates in the first ring of the *Marsupites* calyx (fig. III, *2*, *2*). *Both* these comparisons cannot surely be correct. Apart from this question, however, if Lovèn be right, and the proximal ring of plates in the Pentacrinoid (fig. I, *3*, *3*) be parabasals and not basals, why does he give the collective name of basals to the "rosette" which results from their metamorphosis? It is quite impossible to suppose that the homologies of these plates can change during their development, that they can be parabasals in the Pentacrinoid but basals in the adult *Comatula*. He nowhere tells us what he regards as the basis in the calyx of the Pentacrinoid, but in his description of *Phanogenia*,[1] a new *Comatula* from Singapore, some years ago, he used the expression *basals* for the pieces composing the "rosette" both of *Phanogenia* and of *Antedon Eschrichtii*, and this, as shown by Dr. Carpenter,[2] is the result of the metamorphosis of the five interradial abactinal plates (fig. I, *3*, *3*) of the Pentacrinoid, which Allman rightly designated basals, but Lovèn parabasals.

It is not quite clear what has caused Lovèn to give up this view (if indeed he has done so) and adopt another which seems to deny the existence of basals in *Comatula* altogether. For it is impossible that they can be represented by the uppermost stem segment (fig. I, *cd*), although Agassiz appears to think so, since this develops into the centrodorsal piece of the *Comatula*, which becomes covered with cirrhi, and in no known Crinoid do these appendages appear upon the "basis."

Thus, then, the only hypothesis open to Lovèn in this case is that the basals of the Crinoids generally, which constitute one of the most important elements of their skeleton, are unrepresented in the only larval Crinoid about which we know anything. Nevertheless, plates—the so-called parabasals—which are believed by Lovèn to be limited exclusively, or nearly so, to the Palæozoic *Tessellata*, and only to occur in some families of this group, are supposed

[1] "Phanogenia, ett hittills okändt slägte af fria Crinoideer." 'Öfversigt af Kongl. Vetenskaps-Akademiens Forhandlingar,' 1866, No. 9, p. 225.

[2] 'Phil. Trans.,' loc. cit., pp. 744, 745.

by him to reappear in the larval forms of the modern Articulate Crinoids. This hypothesis appears to me to be not only unnecessary but also absolutely incorrect, and for the following reason.

I have already shown[1] that there is every reason to believe in the general existence of a "chambered organ" in most if not in all the Articulate Crinoids. It is contained in a special cavity at the base of the calyx, the exact position of which varies in different genera. But whatever its position, the basals are invariably perforated or grooved for the passage outwards of the five primary fibrillar cords from the interradial angles of the chambered organ (fig. IV, 3, 3).

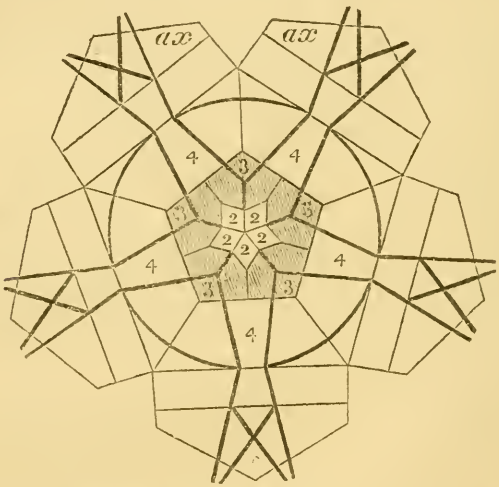

FIG IV.—Calyx of *Encrinus*. (After Beyrich.) 2. Under-basals. 3. Basals. 4. (First) Radials. *ax*. Radial axillaries.

The dark black lines show the arrangement of the canals. which lodged the branches of the five primary cords, proceeding from the interradial angles of the chambered organ. These cords bifurcate within the basals, and the ten secondary cords resulting from this bifurcation proceed onwards to the circular commissure contained within the circlet of radials (fig. IV, 4, 4). This character, the presence or absence of bifurcating grooves or canals, gives us the means of at once determining with accuracy the position of the basals in the calyx of any Crinoid. I have already drawn attention to the presence of a chambered organ in

[1] '*Pentacrinus* and *Rhizocrinus*,' loc. cit., p. 42.

Pentacrinus. Five primary cords proceed from the dorsal angles of its fibrillar envelope, and enter the central canals of the five basals, in which they bifurcate. In the Pentacrinoid larva of *Comatula* there is precisely the same relation between the primary cords proceeding from the chambered organ, and the five plates (figs. I, V, 3, 3) which are placed interradially upon the uppermost stem segment, the future centrodorsal piece (fig. I, *cd*), and have hitherto been regarded as true basals. And yet these plates are called parabasals by Lovèn!

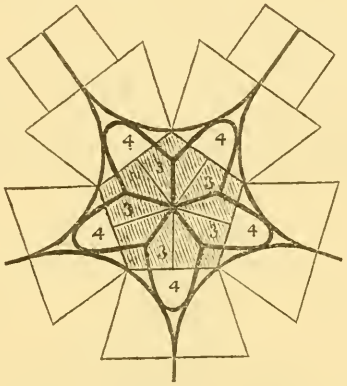

FIG. V.—Calyx of the young *Antedon*. (After Dr. Carpenter.) 3. Basals. 4. (First) Radials. Dark lines as in fig. IV.

This important morphological fact seems to me conclusively to demonstrate the correctness of Allman's interpretation of the proximal ring of plates in the Pentacrinoid (figs. I, V, 3, 3), which, like Lovèn, I regard as homologous with the genital plates of Echini (fig. II, 3, 3). They are true basals, interradial in position, and therefore not homologous with the radially placed "first parabasals" of *Marsupites* (fig. III, 2, 2), as supposed by Lovèn. Miller[1] gave the name "costals" to the last-mentioned plates, and Lovèn has adopted the name as a general one for the plates forming the proximal ring in the apical system of most Echinoderms, some Crinoids excepted, *i.e.* the genitals of *Echinus* (fig. II, 3, 3), and the basals of *Comatula* (figs. I, V, 3, 3). It is extremely unfortunate that he has done so, because Miller was not always consistent in his terminology, and employed this one name in his various generic descriptions to designate plates which are now recognised as belonging to at least three distinct systems. (See the table on page 382.)

[1] Loc. cit., pp. 136, 137.

In the Articulate Crinoids (*Apiocrinus*, *Encrinus*, *Pentacrinus* and *Comatula*), which are supposed not to possess the plates that are now generally known as "Parabasals," and also in *Actinocrinus*, Miller gave the name Costals to the first of the " ray-bearing " plates (figs. I, IV, v, 4, 4), those, namely, which Müller subsequently termed radials. Even in *Rhodocrinus*, in which parabasals are present, Miller spoke of them as intercostals, correctly regarding the radials as costals. In *Poteriocrinus* again, he termed the parabasals intercostals, but as in *Platycrinus*, which has no parabasals, he called the radials not costals, but " Scapulæ " (as in *Marsupites*), a term which he employed in describing the Articulata to designate the radial Axillaries (fig. IV, *ax*). In these two genera he described no costals at all, but in *Cyathocrinus* he gave the name of costals to the plates subsequently termed parabasals by Müller (fig. VI, 3, 3), the position of which is *interradial*, while he called the radials, or true costals (fig. VI, 4, 4), Scapulæ, as in *Poteriocrinus* and *Platycrinus*.

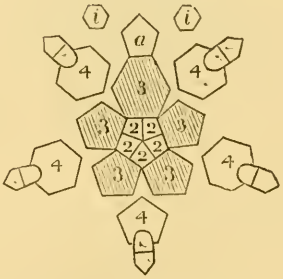

FIG. VI.—Calyx of *Cyathocrinus*. (After Pictet.) 2. Under basals = Pelvis (Miller) = Basis (Lovèn). 3. Basals = Costals (Miller) = Parabasals (Lovèn). 4. Radials = Scapulae (Miller). *a*. Anal plate. *i*. Interradials.

Lastly, in *Marsupites*, he again describes the radials or true costals (fig. III, 4, 4) as scapulæ, and applies the term costals to the so-called first parabasals (fig. III, 2, 2), which though radial in position are not ray-bearing plates at all, but are separated from the bases of the rays by the ring of "second parabasals" (fig. III, 3, 3), termed by him intercostals.

From the radial position of these first parabasals in *Marsupites* it is obvious that they cannot be homologous with the basals of *Comatula* (figs. I, v, 3, 3) nor with the genitals of *Echinus* (fig. II, 3, 3), as supposed by Lovèn, and although Miller called them costals, they are not the homologues of the costals (*i. e.* the radials) of *Pentacrinus*, *En-*

crinus, and *Comatula* (figs. I, IV, V, 4, 4.) These, as pointed out by Lovèn, are homologous with the ocular plates of *Echini* (fig. II, 4, 4),and not with the genitals (fig. II, 3, 3), for the latter represent the basals of *Pentacrinus*, *Encrinus* (fig. IV, 3, 3), and *Comatula* (figs. I, V, 3, 3) which Lovèn, reasoning from the calyx of *Marsupites* (fig. III, 2, 2), has wrongly termed costals.

Consequently, Lovèn's suggestion that the term "Costals" should be used as a general expression to designate the interradial plates of the apical system (figs. I—IX, 3, 3) cannot be regarded as satisfactory. In the genus *Cyathocrinus* only (fig. VI) did Miller use it in this sense, as he generally employed it to designate the true *radial* plates (figs. I—VII, IX, 4, 4), situated upon, and alternating with, those plates which Lovèn would call costals. But in *Marsupites* Miller gave this name to the innermost set of radial plates (fig. III, 2, 2), which are absent from the Apical System of many Crinoids (in the widest sense of the term) and of all the other Echinoderms. In either case, however, Lovèn's use of the term costals is somewhat inappropriate.

It should be noticed here that Lovèn is not the first naturalist who has made use of *Marsupites* in tracing resemblances between the Crinoids and the other Echinoderms. As long ago as 1851 Major Austin[1] suggested that the five genital plates of the Urchins might be regarded as collectively representing the dorsocentral plate of *Marsupites*, and of the Crinoids generally. He used this name to designate the pelvis or basis, and thus compared the genital plates of the Urchins to the basals of the Crinoids, which is precisely the view advanced above. With regard to *Marsupites*, however, I differ from Major Austin, for I have already explained that I cannot agree with the generally accepted view that the central disc of *Marsupites* represents the composite basis of the other Crinoids. Two questions, therefore, have now to be determined, first, What is the true nature of the central disc of *Marsupites*? and second, Which of the plates in its calyx represent the basals of *Pentacrinus* and the other Articulata?

The first question will be considered later, while the second can, I think, be answered in but one way. The basals of *Pentacrinus*, *Encrinus*, and *Comatula* (figs. I, IV, V, 3, 3,) are interradial and lie immediately beneath the radials (figs. I, IV, V, 4, 4), which rest upon them and

[1] "On the connection between the *Crinoideæ* and the *Echinodermata* generally," 'Annals and Magazine of Natural History.' Second series, vol. viii, pp. 285-289.

alternate with them. This is precisely the relation of the "second parabasals" of Marsupites (fig. III, 3, 3) and of the parabasals generally in all those Crinoids (fig. VI, 3, 3) in which they are present. Many reasons which I cannot enter into here, though I shall shortly state them fully elsewhere, have led me to the conclusion that the so-called "parabasals" of the Palæozoic Crinoids are really the basals. According to the present system of nomenclature, two distinct sets of plates are termed basals in this group. In *Platycrinus* and in all the others in which there are only two sets of plates in the calyx, the lower ones resting on the uppermost stem segment, were called basals by Müller.[1] This was perfectly correct, for their position is interradial, and they correspond in every respect to the basals of *Pentacrinus*, the calyx of which genus was taken by Müller as a type, on which he based his analyses of the calyx in all the other Crinoids. On the other hand, the plates which Müller called basals in *Cyathocrinus* (fig. VI, 2, 2), because they rest upon the uppermost stem segment, are radial in position, while the "parabasals" (fig. VI, 3, 3) which intervene between them and the radials (fig. VI, 4, 4) alternate with both series and are therefore interradial. It is these plates which I regard as representing the basals of the other Crinoids. Müller assumed that the basals must always rest upon the uppermost stem segment, and hence fell into an error in which he has since been followed by every one but Beyrich. In the calyx of *Encrinus*, as shown by Beyrich,[2] the true basals (fig. IV, 3, 3) homologous with those of the other Articulata, are separated from the uppermost stem segment by a ring of five small plates (fig. IV, 2, 2) which alternate with them and are therefore radial in position. I shall shortly show that these second or under basals are also present in the calyx of *Pentacrinus briareus* and of *P. subangularis*, though they are absent in the other *Pentacrini*. I believe them to be homologous with the "first parabasals" of *Marsupites* (fig. III, 2, 2), and with the plates which have been hitherto regarded as basals in *Cyathocrinus* (fig. VI, 2, 2), *Poteriocrinus*, *Rhodocrinus*, and the other Palæozoic Crinoids in which the so-called parabasals are present.

Beyrich[3] has already pointed out the resemblance of the two circlets of basals in *Encrinus* to the two series of plates

[1] '*Pentacrinus*,' loc. cit., p. 31.

[2] "Ueber die Crinoideen des Muschelkalks," 'Abhandlungen der Berlin Akademie,' 1857, p. 13.

[3] Loc. cit., p. 13.

below the radials in the Tessellate Crinoids (figs. IV and VI, 2, 2, 3, 3). 'Das Verhältniss des äusseren zum inneren Basalkreis des *Encrinus* entspricht der Unterscheidung von Parabasis und Basis bei den *Crinoidea Tessellata;* jedoch würde die ueberträgung dieser terminologischen Ausdrücke auf die entsprechenden Theile des *Encrinus* vermieden, weil sie dahin führen würde bei nachst verwandten Gattungen, wie *Encrinus* und *Apiocrinus,* Gleichwerthiges mit ungleichen Bennengungen zu belegen.' In this opinion I entirely agree, for I believe that the parabasals of the *Tessellata* (fig. VI, 3, 3) are truly homologous with the basals of *Encrinus* (fig. IV, 3, 3), the second or underbasals of which (fig. IV, 2, 2) represent the plates that have been hitherto called basals (fig. VI, 2, 2) in such Tessellata as possess parabasals (fig. VI, 3, 3).

Lovèn, regarding the radial plates of the first ring in *Marsupites* as the true parabasals (fig. III, 2, 2), states that the "second parabasals" (fig. III, 3, 3), which rest upon and alternate with them, are unrepresented in the Urchins and in most Crinoids. It will be evident from the reasoning given above that this assertion is incorrect. The interradial "second parabasals" of *Marsupites* represent an essential element in the Apical system of the other Echinoderms, namely, the interradial abactinal plates. They are homologous with the genital plates of the Urchins (fig. II, 3, 3), the basals of the Articulate Crinoids (figs. I, IV, V, 3, 3), and the parabasals of most *Tessellata* (fig. VI, 3, 3); while it is the so-called basals of the latter, and the under basals of *Encrinus* (figs. IV, VI, 2, 2), which are homologous with the radial "first parabasals" of *Marsupites* (fig. III, 2, 2), and are unrepresented in the Urchins and in most Articulate Crinoids.

It is now, I think, a view universally accepted that the Starfishes, both Asterids and Ophiurids, have an apical system homologous with that of the *Echini.* L. Agassiz and Müller long ago pointed out, on purely anatomical grounds, the correspondence between the so-called ocular (Intergenital, Müller) plates of the *Echini* (fig. II, 4, 4) and the terminal plates (fig. VII, 4, 4) of the five arms of an Asterid, which support the eyespot and the odd terminal tentacle. The study of the development of the Urchins and Starfishes has proved this correspondence to be a true homology. The beautiful observations of A. Agassiz[1] and Metschnikoff[2] have

[1] 'North American Starfishes,' p. 48, Pl. vi, fig. 10.

[2] "Studien über die Entwickelung der Echinodermen und Nemertinen," 'Mémoires de l'Academie Impériale de St. Petersbourg,' viie série, tome xiv, No. 8, Pl. xii, fig. 1.

shown that in the young *Asteracanthion* there is an apical system developed upon the abactinal surface, which is precisely similar to that of the young Urchin. There is a central plate (fig. VII, 1) as to the ultimate fate of which we are still somewhat in the dark, though it remains distinct up to the age of three years.

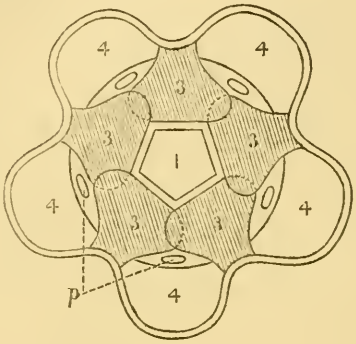

FIG. VII.—Apical System of *Asterias glacialis* (young; after Lovèn). 1. Central disc. 3. Genital plates = Costals (Lovèn). 4. Ocular plates = Radials (Lovèn). *p*. First rudiments of perisomatic system.

Around this are two rings of plates, a proximal series, the interbrachials (fig. VII, 3, 3), and a distal series, the brachial plates (fig. VII, 4, 4). The former, if not resorbed, persist as the genital plates of the adult, while the original radial arm plates are gradually "pushed away from the body, by the addition of new spines formed at the base of the ray," but remain perfectly well defined at the extremity of each ray, in connection with the odd terminal tentacle. The same is the case in the Ophiurids as shown by Müller,[1] and since by Agassiz[2] and Metschnikoff.[3] The terminal plates of the arms are the first to appear, and are carried outwards by the development of new plates between them and the body.

Thus, then, the terminal arm plates of the starfishes (fig. VII, 4, 4) are homologous with the ocular plates of the *Echini* (fig. II, 4, 4), and, therefore, with the (first) radials of the Crinoids, (figs. I, IV, V, 4, 4) which remain fixed, as in the Urchins, in their original positions.

[1] "Ueber die Ophiurenlarven des Adriatischen Meeres," 'Abhandlungen der Berlin Akademie,' 1852, p. 14. "Ueber den Bau der Echinodermen," 'Abhandlungen der Berlin Akademie,' 1854, p. 46.

[2] 'Embryology of Echinoderms,' loc. cit., figs. 29, 32.

[3] Loc. cit., Pl. iv, fig. 17.

In the young of both *Echini* (fig. II) and Asterids (fig. VII) there are two rows of plates surrounding the central disc of the Apical system as in the Crinoids (figs. I, V). It does not appear, however, that the proximal ring of plates, viz., the basals, is always developed in the Ophiurids. Agassiz figures them in *Ophiopholis bellis*, but it is not easy to determine their presence in any of Müller's figures of Ophiurid larvæ, and in Metschnikoff's figure of the young *Amphiura* there is absolutely no trace of them, though the central disc and the radial plates are quite distinct. This gives us a transitional stage from the other Echinoderms to the Holothurians, in which not even a rudiment of an apical system seems ever to make its appearance, though the oral system may occasionally be very well developed (*Psolus*).

Besides pointing out the correspondence between the plates at the apices of Urchins and Starfishes respectively, Prof. Agassiz also instituted a comparison between these plates and those forming the calyx of the Crinoids, which has been extended by A. Agassiz in his memoir on the development of the Starfishes. He says,[1] "Were there a stem on the central plate of its abactinal area, the young Starfish when seen from the abactinal side would have all the appearance of a Crinoid. The central plate corresponds to the basal plate, the set of five plates in the angles of the arms to the interradial plates, and the arm plates themselves to the radial plates of a Crinoid." And again:

"I cannot agree with Professor Allman in considering the central plate otherwise than as a solidified homologue of the basalia of the other Crinoids figured by him; the only difference being that in some cases the plates composing this piece are soldered together as in *Comatula*, while in others they are kept distinct as in *Coccocrinus* and the like."

It will be seen from this last quotation that in Agassiz' opinion the centrodorsal piece, or, as he calls it, the "central plate" of *Comatula* consists of five anchylosed basals, and represents the central plate of the Apical system, *i. e.* the subanal plate of the *Echini*. This opinion involves the anomaly of the basis bearing cirrhi, which has been referred to above in connection with Lovèn's views. Agassiz ignores Dr. Carpenter's[2] proof that the five interradial abactinal

[1] Loc. cit., pp. 62, 63.

[2] It should be mentioned here that Dr. Carpenter's memoir did not appear until after the publication of the first edition of the 'Embryology of the Starfish.' Agassiz, however, has taken no notice of it in the second edition, except by referring to the figures of young *Comatulae* there repre-

plates of the Pentacrinoid larva of *Comatula* are the true basals which, undergoing much metamorphosis to form the "rosette," become entirely concealed in the adult *Comatula* between the radials and the enlarged centrodorsal piece. It is these plates which Lovèn calls parabasals, and compares, perfectly correctly, to the genital plates of the Urchins and Starfishes. Agassiz, however, calls them interradials,[1] thus comparing plates which are essential elements of the Apical systems of both *Echini* and Starfishes to plates which are by no means universally present in the Crinoids, a proceeding in which he is, I think, scarcely justified.

Interradials do not occur in the Articulata at all, nor are they present in all the Tessellata, being absent in *Hexacrinus* (Austin), and in some species of *Platycrinus*. Even when present (fig. VI, *i*) they do not form the proximal ring of plates in the apical system as those plates do in the *Echini*, to which Agassiz compares them [viz., the genitals (fig. II, *3*, *3*)], but they intervene between the plates which correspond to the elements of the distal ring of the Echinoid calyx, namely, the radials [= oculars (fig. VI, 4, 4)].

Further, if the basals of the *Pentacrinidæ*, *Apiocrinidæ*, and the other *Articulata* collectively represent the central plate of the Apical system, as supposed by Agassiz and Lovèn, what are the homologues in these Crinoids of the interbrachials of Asterids and the genitals of the *Echini?* They can have no "interradial" plates (costals of Lovèn), for the only plates in their calyx occupying interradial positions *are the basals*, and these are supposed by Agassiz and Lovèn to represent the central abactinal plate. They are, however, developed from the homologues of the "interradial" plates (parabasals of Lovèn) of the larval *Comatula*, which Agassiz, Lovèn, and myself all regard as homologous with the genital plates of *Echini*. Consequently, the views held by Agassiz and Lovèn involve one of the two following alternatives:—

1. The interradial elements of the Apical system are absent from the calyx of all the Articulate Crinoids except

sented, although he professes to have added notes "on the points where additions have been made by subsequent investigations." On p. 49 he speaks of Lovèn as "having most thoroughly proved the homology of the basal and radial plates of Crinoids with their corresponding plates, still readily to be traced in the young Starfish, and with their homologies in the Apical system of *Echini*," a statement which I cannot endorse. It would seem, therefore, that in spite of Dr. Carpenter's proofs to the contrary, gassiz adheres to his originally-expressed view that the centrodorsal piece *Comatula* represents the basis of the other Crinoids.

[1] Loc. cit., pp. 62, 63.

Comatula, though they are important constituents of the Apical system of the other Echinoderms. This is contrary to fact, for they are present as the basals (Allman, Wyville Thomson, Dr. Carpenter, M. Sars, Götte).

2. The interradial plates of the young *Pentacrinus* represent the genital plates of *Echini*, but the basal circlet of the adult which is developed out of them is homologous with the subanal plate. This means that the homology of these plates changes during their development, which is, of course, impossible.

There is one fact which brings out very clearly the homology of the basals of the young Crinoid (figs. I, V, 3, 3) with the interradial abactinal plates of the young Starfish or Sea Urchin (figs. II, VII, 3, 3).

It is worth mentioning here, because it tells very strongly against the view held by Agassiz and Lovèn, that the basis of the Crinoids represents the central disc in the apical system of the other Echinoderms. Both the basals of the Crinoid (figs. I, IV, V, 3, 3), and the genital plates of the Urchin or Starfish (figs. II, VII, 3, 3), are developed in the form of a spiral around the right peritoneal sac or water tube of the larva. Agassiz[1] first demonstrated this in the *Echini* and Starfishes, and Götte[2] has shown it to be equally true in *Comatula*, pointing out at the same time the homologies of the two sets of plates, and also that the basals appear before the radials in both Asterids and Crinoids.

Apart from this important identity in the mode of development of the two sets of plates, it appears to me that Agassiz and Lovèn are by no means justified in assuming that the single central abactinal plate of the young Starfish or Sea Urchin (figs. II, VII, 1) is in any way homologous to the "basis" of the Crinoids (figs. I, IV, V, 3, 3).

All our knowledge of the development of this element of the Crinoid skeleton goes to show that it primitively consists of five pieces, which first appear quite independently of one another, although they subsequently become firmly united by Synostosis. The mere fact that in some cases the sutures between them become ultimately obliterated does not appear to me to be a sufficient argument for considering the apparently simple piece that is formed by their unusually close union, as homologous with the central abactinal plate of the young Starfish, which is single and undivided from the very first (fig. VII, 1).

1 'North American Starfishes,' pp. 37, 38.

2 "Vergleichende Entwickelungsgeschichte der Comatula Mediterranea," 'Archiv für Mikroscopische Anatomie,' Band xii, pp. 595, 620.

Agassiz, Claus,[1] and apparently also Götte, regard this plate as homologous with the centrodorsal piece of *Comatula* (fig. 1, *cd*).

Götte's statements[2] with regard to the origin of the centrodorsal piece are not in accordance with those of his predecessors. His view of it, as originating in small rods which appear on the lower edges of the primitively independent basal plates, and simultaneously with them, involves the homology of an originally single plate, the central abactinal plate of the other Echinoderms, with another which results from the gradual union of five primitively independent elements. As I have pointed out above, in reference to the views of Agassiz and Lovèn, comparisons such as these do not appear to me to be correct.

I have shown elsewhere[3] that it is very probable that Götte is mistaken upon this point, and that the generally received view that the centrodorsal piece of *Comatula* is simply the enlarged uppermost stem segment is the correct one. From its appearance in the adult *Comatula*, particularly in *Actinometra*, in which it is nearly always a simple flat disc, it would seem quite natural to regard it, as Claus and Agassiz do, as homologous with the central abactinal plate of the young Starfish (fig. VII, 1).

There appears to me, however, to be one serious objection to this view. In the embryos of both Starfishes and Sea Urchins, the central plate of the abactinal system is a simple *plate* from the very first, developed at the centre of the posterior end of the right peritoneal sac. According to Agassiz,[4] it is formed in the Starfish as a small cluster of polygonal limestone cells "round the rod placed in the *very centre* of the abactinal area," and its earliest condition in the young Urchin (*Toxopneustes*)[5] is that of "a single large plate covering the opening of the anus, which leads out on one side of it."

On the other hand, the future centrodorsal piece of the embryo Crinoid is merely the uppermost of a series of calcareous *rings* developed around the elongated hinder portion of the right peritoneal sac (fig. 1, *cd*). Wyville Thomson[6] speaks of an irregular calcareous ring which is early formed immediately beneath the basal plates, and is considerably wider and broader than the ordinary rings of the stem.

[1] Loc. cit., pp. 597, 598.

[2] 'Grundzüge de Zoologie,' Dritte Auflage, p. 289.

[3] See my memoir on *Actinometra*, now in course of publication in the 'Transactions of the Linnean Society.'

[4] 'North American Starfishes,' p. 48.

[5] 'Embryology of Echinoderms,' p. 12.

[6] 'Phil. Trans.,' vol. clv, p. 539.

"This ring, which is subsequently developed into the permanent centrodorsal plate, gradually thickens and becomes more regular in form, maintaining its position at the top of the stem, the lower edges of the basal plates resting on its upper surface. During the earlier stages of the growth of the Pentacrinoid it is simply a circular band of the ordinary calcified areolar tissue enclosing a shaft of the peculiar fasciculated tissue of the stem, gradually enlarging, with a central aperture continuous with the bore of the tube-like stem joints."

According to Dr. Carpenter,[1] the centrodorsal piece "first presents itself in a form which nowise differentiates it from the other joints of the cylindrical stem." The diameter of this original annular disc gradually increases, while the centre becomes filled up by an inward extension of the calcareous trelliswork from the first formed portion. A narrow axial cavity, the remains of the original right peritoneal sac, persists, however, for a long time. This lodges the vascular axis of the stem, and in the young *Comatula* just set free from the stem a minute perforation may be seen in the centre of the lower surface of the centrodorsal piece, which thus preserves its annular character until the commencement of adult life. This perforation thus forms the communication between the cavity of the centrodorsal piece and the central canal that remains in the upper segments, at any rate, of the discarded stem. In the recent *Comatulæ* it is soon closed up by an extension of the calcareous network, so that no trace of it remains visible, either internally or externally. But in the cretaceous *Comatula, Glenotremites*, this was not the case, and the centrodorsal piece seems to have preserved its primitive annular character throughout life.

Sars' researches[2] on the Pentacrinoid stage of *Antedon Sarsii* show in the same way that although the centrodorsal piece of the adult *Comatula* may appear externally to be a solid plate or hemisphere, yet it is really hollow, enclosing a portion of the aboral cœlom, and that its imperforate condition is altogether a secondary character.

This appears to me to be a serious objection to our regarding the centrodorsal piece of *Comatula* as homologous with the central abactinal plate of the other Echinoderms. Similar and similarly placed as they are in the adult animal, the relative positions of the two with regard to the right peritoneal tube of the larva are quite different. The centrodorsal

[1] 'Phil. Trans.,' vol. clvi, p. 706.
[2] 'Crinoïdes Vivants, ii. Du Pentacrinoïde de l'*Antedon Sarsii*,' p. 53.

ring of the embryo Crinoid surrounds this tube near its proximal or anterior end, while the central disc of the embryo Starfish or Sea Urchin lies over the centre of its distal or hinder end, at some little distance from its anterior extremity. The fact that the basals of the Pentacrinoid rest upon the uppermost stem-segment (fig. I) does not appear to me to be a sufficient reason for considering this segment as homologous with the central abactinal plate, with which the homologues of the Crinoidal basals are in contact in the other Echinoderms (figs. II, VII).

Beyrich[1] has shown that in *Encrinus* there is an inner or second circlet of basals intervening between the basals (fig. IV, 3, 3) and the top stem segment, and I have found (as I shall shortly describe elsewhere) that these secondary basals are represented in some species of *Pentacrinus*, but not in others. According to the reasoning followed by Agassiz, tempting as it seems at first sight, we should have to regard these five plates in *Encrinus* and in *P. briareus* as collectively homologous with the central abactinal plate of the other Echinoderms (figs. II, VII, 1), because the true basals = interradials, Ag., rest upon them. But in *P. asteria*, in which they are absent, this plate would be represented by the top stem segment, as in *Comatula*. This, then, is another objection to our considering as Agassiz' view with regard to *Comatula* involves our doing, that the top segment of the Crinoidal stem represents the central abactinal plate of the other Echinoderms, and hence the question arises, What *is* its homologue in the Crinoids?

In the first place, I believe that the second or under basals of *Encrinus* (fig. IV, 2, 2), *Pentacrinus*, and of the Palæozoic Crinoids (fig. VI, 2, 2), and also the interradials of the latter (fig. VI, *i i*) are additional elements which occur in the apical system of some Crinoids, while they are unrepresented in other members of the Order, and in the other Echinoderms. Further, I regard *all* the annular segments of the Crinoidal stem in the same light, and believe that the central plate of the apical system of the other Echinoderms is represented in the Crinoids by the discoidal plate developed close to the posterior extremity of the embryo (fig. VIII, 1), that forms the basis of the expanded disc at the base of the stem by which the larva attaches itself to foreign bodies (fig. IX, 1). In many of the pedunculate Crinoids the stem is attached by a more or less spreading base of this kind. This condition was common in the *Apiocrinidæ* and *Cyathocrinidæ*,

[1] "Ueber die Crinoideen des Muschelkalks," 'Abhandlungen der Berlin Akademie,' 1857, p. 13.

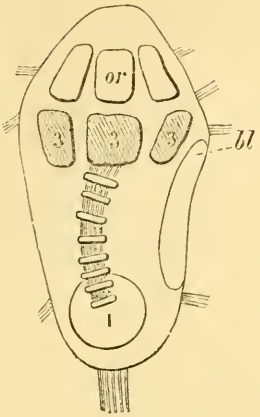

Fig. VIII.—Gastrula of *Comatula*. (After Wyville Thomson.) 1. Terminal plate at the base of the stem. 3. Basal plates. *or*. Oral plates. *bl*. Blastopore.

and Pourtales[1] describes the root portion of the stem of the recent *Rhizocrinus Rawsonii* as appearing to have been "partly attached to a solid body by enlarged surfaces."

On the other hand, in *R. lofotensis* and in *Bathycrinus* the base of the stem is attached by radicular cirrhi, or by strong-jointed branches that descend into the ooze on the surface of which the animal lives, as was the case in some *Cyathocrinidæ*. But it is of course not impossible that in the larval condition, the stem may have had a terminal disc of attachment, which was resorbed when the cirrhi appeared and it became no longer necessary.

The fact that the whole length of the stem intervenes between its disc of attachment (figs. VIII, IX, 1) and the basal plates (figs. VIII and IX, 3) is no argument against my view that the former is homologous with the central abactinal plate of a Starfish or Sea Urchin. A precisely analogous case occurs in the Starfishes, in which the radials do not remain in close contact with the genital plates as in the Echinids, but are carried out of the apical system altogether, to form the so-called ocular plates at the ends of the arms, by the constant formation of new spines at their bases.

[1] "On a New Species of *Rhizocrinus* from Barbadoes," 'Zoological Results of the Hassler Expedition,' p. 27.

There is one Crinoid, however, in which no stem is ever developed, but the elements of the apical system retain their

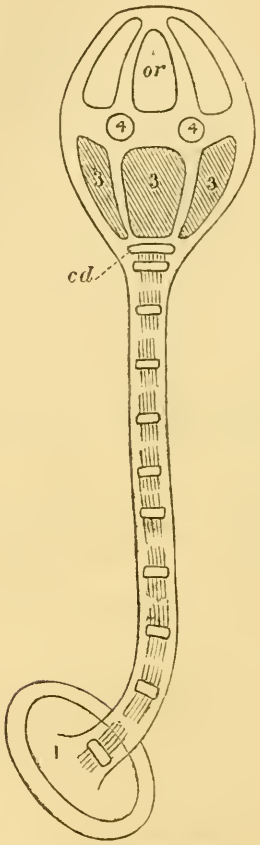

Fig. IX.—Pentacrinoid larva of *Antedon rosacea*. (After Wyville Thomson.) 1. Terminal plate at the base of the stem. 3. Basal plates. 4. Radial plates. *or*. Oral plates.

embryonic relations throughout life. In the remarkable genus *Holopus* the basals and the radials are fused together to form the walls of a tubular chamber, which has an irregularly expanded calcareous base supported by the foreign body to which the animal is attached. Wyville Thomson[1] has pointed out the resemblance of this mode of attachment to that which prevails in the pedunculate *Apiocrinidæ* and *Cyathocrinidæ*, but expressly states that in *Holopus* there is

[1] 'Proceedings R.S.E.,' vol. ix, loc. cit., pp. 407—409.

no trace of anything representing a stem, not even its uppermost segment which indicates its presence in *Comatula*. The problematical Crinoid *Cyathidium* from the chalk of Faxöe seems to have resembled *Holopus* in this respect, and to have had a spreading base of attachment immediately beneath the cup. In these two cases we have, I believe, the nearest approach in the Crinoids to the embryonic condition of the apical system in the other Echinoderms.

I do not think that the extremely irregular shape of the spreading base of attachment of *Holopus*, *Apiocrinus*, and other Crinoids is any serious objection to the view advanced above. In the Pentacrinoid larva of *Comatula* the terminal plate has at first a definite circular form (figs. VIII and IX, 1), but gradually increases both in diameter and thickness, absorbing into itself (as it were) nearly the whole of the organic substance of the basal disk, while its margin usually becomes more or less deeply divided into lobes. We do not know the condition of the original base of the stem in *Pentacrinus*, but in *Apiocrinus* the terminal plate and much of the lower part of the stem are surrounded by a thick extraneous deposit of calcareous matter, forming an irregular cone which would give a very firm support to the animal. I imagine the irregularly expanded character of the base of attachment of *Holopus* to be due to a similar secondary deposit, which has obscured its originally symmetrical shape, in the same way as the partial resorption of the subanal plate in the Echinids causes its primitive pentagonal symmetry to become less marked. In neither case is it fair to regard the condition of the part in the adult animal as an argument against any views of its homology which may be derived from embryological considerations.

In order to make clear the reasons which have led me to the view above suggested, I must go back to a very early period in Echinoderm development, namely, to the Gastrula stage. If Götte's figures of the Gastrula of *Comatula* be compared with those of other Echinoderm Gastrulæ as given by other observers, a very striking difference is apparent between them. The Gastrula of *Asteracanthion*, according to Agassiz,[1] is somewhat "pear-shaped, with rounded extremities, having at one end an opening leading into a pouch which extends half the length of the cylinder" (fig. X, A). The *Toxopneustes* Gastrula closely resembles that of *Asteracanthion*, except that its transverse axis is longer in proportion to the longitudinal one, *i.e.*, to the one occupied

[1] 'North American Starfishes,' loc. cit., p. 9.

by the primitive digestive sac. This is also the condition of the Holothurian Gastrula, as figured by Krohn and Selenka. But the Gastrula of a Crinoid differs extremely

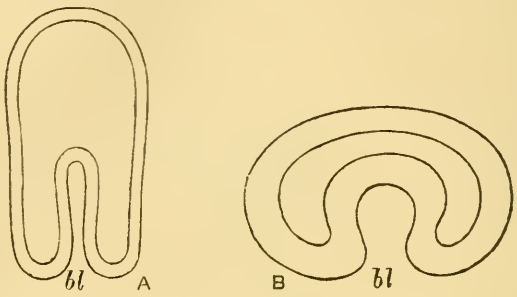

Fig. X.—Echinoderm Gastrulae. A. *Asteracanthion.* (After Agassiz.)
B. *Comatula.* (After Götte.) *bl.* Blastopore.

in its shape from that of any other Echinoderm, for it is very much elongated transversely to the axis of the digestive sac, which is comparatively short (fig. X, B). Consequently, in the Crinoid Gastrula the principal mass of the body lies at the sides of the archenteron, and not in a line with it as in other Echinoderm Gastrulæ. This extreme elongation of the transverse axis provides the space for the development of the future stem, which is so characteristic of the Crinoids. It first appears (fig. VIII) as a series of delicate calcareous rings, that form a curved line passing backwards from beneath the centre of the ring of basals, behind and slightly to the left of the large keyhole-shaped depression of the outer surface of the body, at the narrow anterior end of which is the Gastrula mouth or blastopore (*bl*). As development proceeds this depression becomes obliterated, and the stem lengthens very considerably, so that the part of the embryo body behind[1] the blastopore (which indicates the position of the future anus), becomes very much longer than the corresponding part in front of the blastopore with which, in the Gastrula stage, it was symmetrical. In correspondence with the extreme elongation of that part of the body of the larval *Comatula* which lies behind the blastopore (fig. XI, *st*), the right peritoneal tube (fig. XI, *rp*) sends a backward process into the mass of mesoblast of which it is mainly composed (*rp'*), and it is around this tubular process that the primitive rings representing the future stem segments are developed (fig. XI, *st*).

[1] The term behind is here used to indicate the opposite end of the transverse axis of the Gastrula to that at which the future mouth appears.

This extension of the extreme distal portion of the aboral cœlom throughout the rudimentary stem of the young

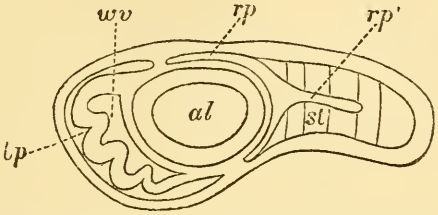

FIG. XI.—Section through a *Comatula* larva. (After Götte.) *al.* Digestive sac. *lp.* Left peritoneal space. *rp.* Right peritoneal space. (*rp'*). Its posterior diverticulum. *st.* Stem. *wv.* Rudiment of water-vascular ring.

Pentacrinoid is of great interest, for it is at the end of the stem, *i.e.*, at the apex of the aboral cœlom, that the terminal discoidal plate is developed; and it therefore appears to me to be strictly homologous to the subanal plate of *Toxopneustes*, which also appears at the centre of the posterior end of the right peritoneal tube.

This terminal plate of the stem of the Pentacrinoid is not developed in the form of a ring like the true joints of the stem, nor does any of that peculiar fasciculated tissue appear in connection with it which forms the principal part of the older stem segments (figs. VIII and IX). But the base of the sheaf of rods passing through the last ring of the series abuts against the centre of its upper surface, and it increases both in diameter and in thickness, to form the skeleton of the round fleshy disc by which the stem adheres to its point of attachment. Hence it is merely a temporary structure, not forming any part of the calyx of the adult *Comatula*. But I do not think that this point can be urged as any serious objection to the view advanced above, that it is homologous with the central plate of the apical system of the other Echinoderms. In most Echinids and Asterids this plate undergoes more or less subdivision and resorption. In *Collyrites* there is scarcely any trace of it, and it is entirely absent in the cretaceous *Ananchytidæ*, in the *Spatangidæ*, and in *Micraster*.[1] Further, in some of the recent *Echini* (*Brissopsis, Meoma*) it moves away from its central position out of the calyx altogether, between the two rows of the last plates of the odd interambulacrum. This is quite analogous to the change of position which I suppose it to undergo in the young Crinoid, though on a smaller scale.

[1] Lovèn, loc. cit., p. 83.

Returning now to *Marsupites* and to the consideration of the nature of the central plate of its apical system (fig. III, 1), we are at once met by the difficulty that we do not know, and are never likely to know, the history of its development. If it passed through a pedunculate stage as *Comatula* does, the central disc of its calyx cannot be homologous with the subanal plate (fig. II, 1) of the Echinids as Lovèn supposes, since the homologue of the latter would, on the view advanced above, have been the terminal plate at the base of the stem. The central disc of *Marsupites* might then be fairly regarded as homologous with the centrodorsal plate (= uppermost stem segment) of *Comatula* (fig. I, *cd*). Except in *Glenotremites*, this piece is imperforate as in *Marsupites*, its primitive annular character becoming obliterated by a secondary extension of its original calcareous reticulation. In most *Comatulæ*, however, it is marked by sockets for the attachment of numerous cirrhi which serve to fix the young animal after it is detached from its stem, and there is no trace of these in *Marsupites*.

Until quite recently I should have considered this as a serious difficulty in the way of our regarding the central disc of *Marsupites* as a centrodorsal piece homologous with that of *Comatula*. But in the examination of the collection of *Comatulæ* brought home by the "Challenger," I have come across many specimens in which there are not only no cirrhi, but no sign of their having once existed. The centrodorsal piece is nearly flat outside, and is not marked with the slightest trace of sockets. This is, however, merely the result of age, the obliteration of the cirrhus socket by a calcareous deposit having been carried to an extreme extent. For in the same as well as in different species, I have met with all kinds of intermediate stages between this and the ordinary condition of two or three rows of cirrhus-sockets which prevails in the younger, and in the commoner *Comatulæ*.

It is not altogether clear to me how these older cirrhusless *Comatulæ* can fix themselves in any one spot, as these animals ordinarily do, and the same difficulty of course presents itself in the case of *Marsupites*, the central disc of which corresponds in many respects with the centrodorsal plate of *Comatula*. On the whole, however, I am inclined in this case to adopt Lovèn's views, and believe that *Marsupites* was an altogether stemless Crinoid like *Holopus*, but that the primitive character of its central abactinal plate never became obscured as it does in *Holopus* by an extraneous calcareous dsposit. The chief reason which has led me to

this conclusion is the great resemblance of the sculpturing on the central disc of *Marsupites* to that on the subanal plate of the young *Salenia*, as pointed out by Lovèn. This sculpturing is found on all the plates of the calyx in many of the Tessellate Crinoids, and appears to be a primary character. I do not think, therefore, that it would occur on the central disc of *Marsupites*, if the imperforate nature of this plate were only due to a secondary calcareous deposit, as must be the case if it be regarded as homologous with the centrodorsal piece of *Comatula*.

The following table has been drawn up to show the various analyses which have been made of the apical system of *Marsupites* and the other Crinoids, and the homologies in the Echini of its various parts, as determined by Lovèn and myself.

There is one feature in the history of the Apical system of the Crinoids which has an important bearing upon Haeckel's celebrated worm theory of the Echinoderms. This theory has been completely adopted by such a distinguished authority on the Echinoderms as G. O. Sars,[1] and is supported by Gegenbaur[2] in the last edition of his 'Comparative Anatomy,' while Haeckel[3] has quite recently spoken of its truth as "durch die Ontogenie und Paläontologie der Echinodermen so schlagend bewiesen, dass jene ältere Auschauung ihr gegenüber unhaltbar geworden ist."

It appears to me, however, that the more we know of the ontogeny and palæontology of the Echinoderms, the greater difficulties do we find in the acceptance of this theory, according to which an Echinoderm is to be regarded as a colony of "persons" which have arranged themselves in a radiate manner around a common centre, somewhat in the same way as the individuals forming a *Botryllus* colony. In the latter case the individvals form for themselves a common egestive aperture, while those forming a Starfish colony have a common ingestive aperture, *i.e.* they are all united together by their heads. For in the Asterids and Ophiurids the first formed arm segment (ocular or radial) (fig. VII, 4) is carried outwards and becomes the terminal plate of the arm, corresponding to the tail end of an Annelid, while new segments are added between it and the disc, according to the usual laws of Annelid segmentation. In the Crinoids,

[1] "Researches on the Structure and Affinity of the genus Brisinga," 'Christiania University Program' for 1875, pp. 75—85.

[2] 'Grundriss der Vergleichenden Anatomie,' ii Auflage, p. 205.

[3] "Ueber die Individualität des Thierkorpers," 'Jenaische Zeitschrift, Band xii, p. 18.

Table showing the Constitution of the Apical System in MARSUPITES *and in the Pedunculate Crinoids, according to different Authors, and its supposed Homologies in the* ECHINOIDEA.

	Elements of the Apical System.	Marsupites.				Pedunculate Crinoids with "Parabasals."						Encrinus.	Pentacrinoid Larva of Comatula.				Echinoidea.	
						Miller.												
		Miller.	Lovèn.	Pictet.	P. H. Carpenter.	Cyathocrinus.	Rhodocrinus.	Müller and De Koninck.	Lovèn.	Agassiz.	P. H. Carpenter.	P. H. Carpenter.	Agassiz.	Claus.	P. H. Carpenter.	Lovèn.	Lovèn.	P. H. Carpenter.
1	Central plate.	Pelvis.	Basis.	Central plate.	Central plate.	?	?	?	Basis.	Basis.	Terminal plate at base of larval stem.	Terminal plate at base of larval stem.	Centrodorsal piece. Basis.	Centrodorsal piece.	Terminal plate at base of larval stem.	?	Subanal plate.	Subanal plate.
2	First ring of plates. *Radial.*	Costals.	First parabasals.	Basals.	Underbasals.	Pelvis.	Pelvis.	Basis.	Parabasals.	?	Underbasals.	Underbasals.	—	—	—	Parabasals.[1]	Genital plates.	—
3	Second ring of plates. *Interradial.*	Intercostals.	Second parabasals.	Parabasals or subradials.	Basals.	Costals.	Intercostals.	Parabasals or subradials.	—	Interradials.	Basals.	Basals.	Interradials.	Basals.	Basals.	?	—	Genital plates.
4	Third ring of plates. *Radial.*	Scapulæ.	Radials.	Radials.	Radials.	Scapulæ.	Costals.	Radials.	Radials.	Radials.	Radials.	Radials.	Radials.	Radials.	Radials.	Radials.	Ocular plates.	Ocular plates.

[1] These plates, the basals of Wyville Thomson and Dr. Carpenter, are *interradial* in position, as indicated in the three preceding columns; but Lovèn disregarding this fact, places them in the same class as the *radially* situated plates of the first ring in *Marsupites*. The same is the case with respect to their homologues, the genital plates of the *Echini*, as will be seen from a comparison of the last two columns.

however, the last tail segment of the worm-like "person," namely, the radial (figs. I, IV, V, IX, 4) remains fixed, and no plates are added between it and the disc. But a large number are added in succession upon its distal side, so as to build up the arm of the Crinoid with its terminal growing point.

Consequently, so far as we are entitled to form an opinion based upon the different modes of growth of the arms of the Starfishes and Crinoids respectively, we must adopt one of the two following hypotheses:—Either, 1st, that the individuals composing a Crinoid colony are united together by their tails, and not, like those of an ordinary Starfish colony, by their heads—a view which no one, I suppose, would entertain for a moment; or, 2nd, that for some reason or other centralisation has proceeded so far, that the principal parts of the bodies of the worm persons which make up a Crinoid have been altogether suppressed, while the tails are developed to a very great extent. The very close correspondence, however, in the general anatomy of the disks of the Crinoids and of the Starfishes lend no support to this latter view. Further, if the arms of an *Ophiura* or *Brisinga* may be considered as representing the body of an Annelid, it is difficult to see why the closely similar arm of a Crinoid should not be regarded in the same light; and although it contains the whole of the genital system, it cannot, according to Haeckel's theory, be anything more than an excessively developed tail.

On the STRUCTURE *and* DEVELOPMENT *of the* VERTEBRATE OVARY.

By F. M. BALFOUR, M.A., Fellow of Trinity College, Cambridge. (With Plates XVII, XVIII, XIX.)

CONTENTS.

The present paper records observations on the ovaries of but two types, viz., Mammalia and Elasmobranchii. The main points dealt with are three :—1. The relation of the germinal epithelium to the stroma. 2. The connection between *primitive ova* in Waldeyer's sense and the permanent ova. 3. The homologies of the egg membranes.

The second of these points seems to call for special attention after Semper's discovery that the primitive ova ought really to be regarded as *primitive sexual cells*, in that they give rise to the generative elements of both sexes.

The Development of the Elasmobranch Ovary.

The development of the Elasmobranch ovary has recently formed the subject of three investigations. The earliest of them, by H. Ludwig, is contained in his important work, on the 'Formation of the Ovum in the Animal Kingdom.'[1] Ludwig arrives at the conclusion that the ovum and the follicular epithelium are both derived from the germinal epithelium, and enters into some detail as to their formation. Schultz,[2] without apparently being acquainted with Ludwig's observations, has come to very similar results for Torpedo.

Semper,[3] in his elaborate memoir on the urogenital system of Elasmobranchs, has added very greatly to our knowledge on this subject. In a general way he confirms Ludwig's statements, though he shows that the formation of the ova is somewhat more complicated than Ludwig had imagined. He more especially lays stress on the existence of nests of ova (Ureierernester), derived from the division of a single primitive ovum, and of certain peculiarly modified nuclei, which he compares to spindle nuclei in the act of division.

My own results agree with those of previous investigators, in attributing to the germinal epithelium the origin both of the follicular epithelium and ova, but include a number of points

[1] Arbeiten, a. d. 'Zool. Zoot. Institut. Würzburg,' Bd. i.
[2] 'Archiv f. mikr. Anat.,' vol. xi.
[3] Arbeiten, a. d. 'Zool. Zoot. Institut. Würzburg,' Bd. ii.

which I believe to be new, and, perhaps, of some little interest; they differ, moreover, in many important particulars, both as to the structure and development of the ovary from the accounts of my predecessors.

The history of the female generative organs may conveniently be treated under two heads, viz. (1) the history of the ovarian ridge itself, and (2) the history of the ova situated in it. I propose dealing in the first place with the ovarian ridge.

The Ovarian ridge in Scyllium.—At the stage spoken of in my monograph on Elasmobranch Fishes as stage L, the ovarian ridge has a very small development, and its maximum height is about 0·1 mm. It exhibits in section a somewhat rounded form, and is slightly constricted along the line of attachment. It presents two surfaces, which are respectively outer and inner, and is formed of a layer of somewhat thickened germinal epithelium separated by a basement membrane from a central core of stroma. The epithelium is far thicker on the outer surface than on the inner, and the primitive ova are entirely confined to the former. The cells of the germinal epithelium are irregularly scattered around the primitive ova, and have not the definite arrangement usually characteristic of epithelial cells. Each of them has a large nucleus, with a deeply staining small nucleolus, and a very scanty protoplasm. In stage N the ovarian ridge has a pointed edge and narrower attachment than in stage L. Its greatest height is about 0·17 mm. There is more stroma, and the basement membrane is more distinct than before; in other respects no changes worth recording have taken place. By stage P a distinction is observable between right and left ovarian ridges; the right one has, in fact, grown more rapidly than the left, and the difference in size between the two ridges becomes more and more conspicuous during the succeeding stages, till the left one ceases to grow any larger, though it remains for a great part of life as a small rudiment.

The right ovarian ridge, which will henceforth alone engage our attention, has grown very considerably. Its height is now about 0·4 mm. It has in section (*vide* Pl. XVII, fig. 1) a triangular form with constricted base, and is covered by a flat epithelium, except for an area on the outer surface, in length co-extensive with the ovarian ridge, and with a maximum breadth of about 0·25 mm. This area will be spoken of as the ovarian area or region, since the primitive ova are confined to it. The epithelium covering it has a maximum thickness of about 0·05 mm., and thins off rather rapidly on both borders, to become continuous with the general epithelium of the ovarian ridge. Its cells have the same character as before, and are several layers deep. Scattered irregularly amongst them are the primitive ova. The germinal

epithelium in the ovarian region is separated by a basement membrane from the adjacent stroma.

In succeeding stages, till the embryo reaches a length of 7 centimetres, no very important changes take place. The ovarian region grows somewhat in breadth, though in this respect different embryos vary considerably. In two embryos of nearly the same age, the breadth of the ovarian epithelium was 0·3 mm. in the one and 0·35 mm. in the other. In the former of these embryos, the thickness of the epithelium was slightly greater than in the latter, viz. 0·09 mm. as compared with 0·08. In both the epithelium was sharply separated from the subjacent stroma. There were relatively more epithelial cells in proportion to primitive ova than at the earlier date, and the individual cells exhibited great variations in shape, some being oval, some angular, others very elongated, and many of them applied to part of an ovum and accommodating themselves to its shape. In some of the more elongated cells very deeply stained nuclei were present, which (in a favorable light and with high powers) exhibited the spindle modification of Strasburger with great clearness, and must therefore be regarded as undergoing division. The ovarian region is at this stage bounded on each side by a groove.

In an embryo of seven centimetres (Pl. XVII, fig. 2) the breadth of the ovarian epithelium was 0·5, but its height only 0·06 mm. It was still sharply separated from the subjacent stroma, though a membrane could only be demonstrated in certain parts. The amount of stroma in the ovarian ridge varies greatly in different individuals, and no reliance can be placed on its amount as a test of the age of the embryo. In the base of the ovarian ridge the cells were closely packed, elsewhere they were still embryonic.

My next stage (Pl. XVII, fig 3, and fig 4), shortly before, the time of the hatching of the embryo, exhibits in many respects an advance on the previous one. It is the stage during which a follicular covering derived from the germinal epithelium is first distinctly formed round the ova, in a manner which will be more particularly spoken of in the section devoted to the development of the ovum itself. The breadth of the ovarian region is 0·56 mm., and its greatest height close to the central border, 0·12 mm. —a great advance on the previous stage, mainly, however, due to the larger size of the ova.

The ovarian epithelium is still in part separated from the subjacent stroma by a membrane close to its dorsal and ventral borders, but elsewhere the separation is not so distinct, it being occasionally difficult within a cell or so, to be sure of the boundary of the epithelium. The want of a clear line between the stroma and the epithelium is rendered more obvious by the fact that the surface of the latter is somewhat irregular, owing to projections formed

by specially large ova, into the bays between which are processes of the stroma. In an ovary about this stage, hardened in osmic acid, the epithelium stains very differently from the subjacent stroma, and the line of separation between the two is quite sharp. A figure of the whole ovarian ridge, showing the relation between the two parts, is represented on Pl. XVII, fig. 5.

The layer of stroma in immediate contact with the epithelium is very different from the remainder, and appears to be destined to accompany the vascular growths into the epithelium, which will appear in the next stage. The protoplasm of the cells composing it forms a loose reticulum with a fair number of oval or rounded nuclei, with their long axis for the most part parallel to the lower surface of the epithelium. It contains, even at this stage, fully developed vascular channels.

The remainder of the stroma of the ovarian ridge has now acquired a definite structure, which remains constant through life, and is eminently characteristic of the genital ridge of both sexes. The bulk of it (Pl. XVII, fig. 3, *str*) consists of closely packed polygonal cells, of about 0·014 mm. with large nuclei of about 0·009. These cells appear to be supported by a delicate reticulum. The whole tissue is highly vascular, with the numerous capillaries; the nuclei in the walls of which stand out in some preparations with great clearness.

In the next oldest ovary, of which I have sections, the breadth of the ovarian epithelium is 0·7 mm. and its thickness 0·096. The ovary of this age was preserved in osmic acid, which is the most favorable reagent, so far as I have seen, for observing the relation of the stroma and epithelium. On Pl. XVII, fig. 6, is represented a transverse section through the whole breadth of the ovary, slightly magnified to show the general relations of the parts, and on Pl. XVII, fig. 7, a small portion of a section more highly magnified. The inner surface of the ovarian epithelium is more irregular than in the previous stage, and it may be observed that the subjacent stroma is growing in amongst the ova. From the relation of the two tissues it is fairly clear that the growth which is taking place is a definite growth of the stroma into the epithelium, and not a mutual intergrowth of the two tissues. The ingrowths of the stroma are, moreover, directed towards individual ova, around which, outside the follicular epithelium, they form a special vascular investment in the succeeding stages. They are formed of a reticular tissue with comparatively few nuclei.

By the next stage, in my series of ovaries of *Scy. canicula*, important changes have taken place in the constitution of ovarian epithelium. Fig. 8, Pl. XVII, represents a portion of the ovarian epithelium, on the same scale as figs. 1, 2, 3, &c., and

fig. 9 a section through the whole ovarian ridge slightly magnified. Its breadth is now 1·3 mm., and its thickness 0·3 mm. The ova have grown very greatly, and it appears to me to be mainly owing to their growth that the greater thickness of the epithelium is due, as well as the irregularity of its inner surface (*vide* fig. 9).

The general relation of the epithelium to the surrounding parts is much the same as in the earlier stage, but two new features have appeared—(1) The outermost cells of the ovarian region have more or less clearly arranged themselves as a kind of epithelial covering for the organ; and (2) the stroma ingrowths of the previous stage have become definitely vascular, and have penetrated through all parts of the epithelium.

The external layer of epithelium is by no means a very marked structure, the character of its cells vary greatly in different regions, and it is very imperfectly separated from the subjacent layer. I shall speak of it for convenience as *pseudo-epithelium*.

The greater part of the germinal epithelium forms anastomosing columns, separated by very thin tracts of stroma. The columns are, in the majority of instances, continuous with the pseudo-epithelium at the surface, and contain ova in all stages of development. Many of the cells composing them naturally form the follicular epithelium for the separate ova; but the majority have no such relation. They have in many instances assumed an appearance somewhat different from that which they presented in the last stage, mainly owing to the individual nuclei being more widely separated. A careful examination with a high power shows that this is owing to an increase in the amount of protoplasm of the individual cells, and it may be noted that a similar increase in the size of the bodies of the cells has taken place in the pseudo-epithelium and in the follicular epithelium of the individual ova.

The stroma ingrowths form the most important feature of the stage. In most instances they are very thin and delicate, and might easily be overlooked, especially as many of the cells in them are hardly to be distinguished, taken separately, from those of the germinal epithelium. These features render the investigation of the exact relation of the stroma and epithelium a matter of some difficulty. I have, however, been greatly assisted by the investigation of the ovary of a young example of *Scyllium stellare*, 16½ centimètres in length, a section of which is represented in Pl. XVIII, fig. 26. In this ovary, although no other abnormalities were observable, the stroma ingrowths were exceptionally wide; indeed, quite without a parallel in my series of ovaries in this respect. The stroma most clearly divides up the epithelium of the ovary into separate masses, or more probably anastomosing columns, the equivalents of the egg-tubes of

Pflüger. These columns are formed of normal cells of the germinal epithelium, which enclose ovarian nests and ova in all stages of development. A comparison of the section I have represented, with those from previous stages, appears to me to demonstrate that the relation of the epithelium and stroma has been caused by an ingrowth or penetration of the stroma into the epithelium, and not by a mutual intergrowth of the two tissues. Although the ovary, of which fig. 26 represents a section was from *Scy. stellare,* and the previous ovaries have been from *Scy. caniculа,* yet the thickness of the epithelium may still be appealed to in confirmation of this view. In the previous stage the thickness was about 0·096 mm., in the present one it is about 0·16 mm., a difference of thickness which can be easily accounted for by the growth of the individual ova and the additional tracts of stroma. A pseudo-epithelium is more or less clearly formed, but it is continuous with the columns of epithelium. In the stroma many isolated cells are present, which appear to me, from a careful comparison of a series of sections, to belong to the germinal epithelium.

The thickness of the follicular epithelium on the inner side of the larger ova deserves to be noted. Its meaning is discussed on page 399.

Quite a different interpretation to that which I have given has been put by Ludwig and Semper upon the parts of the ovary at this stage. My *pseudo-epithelium* is regarded by them as forming, together with the *follicular epithelium* of the ova, the sole remnant of the original germinal epithelium; and the masses of cells below the pseudo-epithelium, which I have attempted to show are derived from the original germinal epithelium, are regarded as parts of the ingrowths of the adjacent stroma.

Ludwig has assumed this interpretation without having had an opportunity of working out the development of the parts, but Semper attempts to bring forward embryological proofs in support of this position.

If the series of ovaries which I have represented be examined, it will not, I think, be denied that the general appearances are very much in favour of my view. The thickened patch of ovarian epithelium can apparently be traced through the whole series of sections, and no indications of its sudden reduction to the thin pseudo-epithelium are apparent. The most careful examination that I have been able to make brings to light nothing tending to show that the general appearances are delusive. The important difference between us refers to *our views of the nature of the tissue subjacent to the pseudo-epithelium.* If my results be accepted, it is clear that the whole ovarian region is an epithelium

interpenetrated by connective tissue ingrowths, so that the region below the pseudo-epithelium is a kind of honeycomb or trabecular net-work of germinal epithelium, developing ova of all stages and sizes, and composed of cells capable of forming follicular epithelium for developing ova. Ludwig figures what he regards as the formation of the follicular epithelium round primitive ova during their passage into the stroma. It is quite clear to me, that his figures of the later stages, 33 and 34, represent fully formed permanent ova surrounded by a follicular epithelium, and that their situation in contact with the pseudo-epithelium is, so to speak, an accident, and it is quite possible that his figures 31 and 32 also represent fully formed ova; but I have little hesitation in asserting that he has not understood the mode of formation of the follicular epithelium, and that, though his statement that it is derived from the germinal epithelium is quite correct, his account of the process is completely misleading. The same criticism does not exactly apply to Semper's statements. Semper has really observed the formation of the follicular epithelium round young ova; but, nevertheless, he appears to me to give an entirely wrong account of the relation of the stroma to the germinal epithelium. The extent of the difference between Semper's and my view may perhaps best be shown by a quotation from Semper, loc. cit., 465:—" In females the nests of primitive ova sink in groups into the stroma. In these groups one cell enlarges till it becomes the ovum, the neigbouring cells increase and arrange themselves around the ova as follicle cells.

Although the histological changes which take place in the succeeding stages are not inconsiderable, they do not involve any fundamental change in the constitution of the ovarian region, and may be described with greater brevity than has been so far possible.

In a half-grown female, with an ovarian region of 3 mm. in breadth, and 0·8 mm. in thickness, the stroma of the ovarian region has assumed a far more formed aspect than before. It consists (Pl. XVII, fig. 10) of a basis in most parts fibrous, but in some nearly homogenous, with a fair number of scattered cells. Immediately below the pseudo-epithelium, there is an imperfectly developed fibrous layer, forming a kind of tunic, in which are imbedded the relatively reduced epithelial trabeculæ of the previous stages. They appear in sections as columns, either continuous with or independent of the pseudo-epithelium, formed of normal cells of the germinal epithelium, nests of ova, and permanent ova in various stages of development. Below this there comes a layer of larger ova which are very closely packed. A not inconsiderable number of the larger ova have, however, a superficial situation, and lie in immediate contact with the pseudo-epithelium. Some of the younger ova, enclosed

amongst epithelial cells continuous with the pseudo-epithelium, are very similar to those figured by Ludwig. It is scarcely necessary to insist that this fact does not afford any argument in favour of his interpretations. The ovarian region is honeycombed by large vascular channels with distinct walls, and other channels which are perhaps lymphatic.

The surface of the ovarian region is somewhat irregular and especially marked by deep oblique transverse furrows. It is covered by a distinct, though still irregular pseudo-epithelium, which is fairly columnar in the furrows but flattened along the ridges. The cells of the pseudo-epithelium have one peculiarity very unlike that of ordinary epithelial cells. Their inner extremities (*vide* fig. 10) are prolonged into fibrous processes which enter the subjacent tissue, and bending nearly parallel to the surface of the ovary, assist in forming the tunic spoken of above. This peculiarity of the pseudo-epithelial cells seems to indicate that they do not essentially differ from cells which have the character of undoubted connective tissue cells, and renders it possible that the greater part of the tunic, which has apparently the structure of ordinary connective tissue, is in reality derived from the original germinal epithelium, a view which tallies with the fact that in some instances the cells of the tunic appear as if about to assist in forming the follicular epithelium of some of the developing ova. In Raja, the similarity of the pseudo-epithelium to the subjacent tissue is very much more marked than in Scyllium. The pseudo-epithelium appears merely as the superficial layer of the ovarian tunic somewhat modified by its position on the surface. It is formed of columnar cells with vertically arranged fibres which pass into the subjacent layers, and chiefly differ from the ordinary fibres in that they still form parts of the cell-protoplasm enclosing the nucleus. In Pl. XIX, fig. 34, an attempt is made to represent the relations of the pseudo-epithelium to the subjacent tissue in Raja. Ludwig's figures of the pseudo-epithelium of the ovary, in the regular form of its constituent cells, and its sharp separation by a basement membrane from the tissue below, are quite unlike anything which I have met with in my sections either of Raja or Scyllium.

Close to the dorsal border of the ovary the epithelial cells of the non-ovarian region have very conspicuous tails, extending into a more or less homogeneous substance below, which constitutes a peculiar form of tunic for this part of the ovarian ridge.

In the full-grown female the stroma of the ovarian region is denser and has a more fibrous aspect than in the younger animal. Below the pseudo-epithelium it is arranged in two or

three more or less definite layers, in which the fibres run at right angles. It forms a definite ovarian tunic. The pseudo-epithelium is much more distinct, and the tails of its cells, so conspicuous in previous stages, can no longer be made out.

Formation of the permanent ova and the follicular epithelium.—In my monograph on the development of Elasmobranch Fishes an account was given of the earliest stages in the development of the primitive ova, and I now take up their development from the point at which it was left off in that work. From their first formation till the stage spoken of in my monograph as P, their size remains fairly constant. The larger examples have a diameter of about 0·035 mm., and the medium-sized examples of about 0·03 mm. The larger nuclei have a diameter of about 0·16 mm., but their variations in size are considerable. If the above figures be compared with those on page 131 of my monograph on Elasmobranch Fishes, it will be seen that the size of the primitive ova during these stages is not greater than it was at the period of their very first appearance.

The ova (Pl. XVII, fig. 1) are usually aggregated in masses, which might have resulted from division of a single ovum. The outlines of the individual ova *are always distinct.* Their protoplasm is clear, and their nuclei, which are somewhat passive towards staining reagents, are granular, with one to three nucleoli. I have noticed, up to stage P, the occasional presence of highly refractive spherules in the protoplasm of the primitive ova already described in my monograph (p. 135, 136, Pl. XI, fig. 15). They seem to occur up to a later period than I at first imagined. Their want of constancy probably indicates that they have no special importance. Professor Semper has described similar appearances in the male primitive ova of a later period.

As to the distribution of the primitive ova in the germinal epithelium, Professor Semper's statement that the larger primitive ova are found in masses in the centre, and that the smaller ova are more peripherally situated is on the whole true, though I do not find this distribution sufficiently constant to lay so much stress on it as he does.

The passive condition of the primitive ova becomes suddenly broken during stage Q, and is succeeded by a period of remarkable changes. It has only been by the expenditure of much care and trouble that I have been able to elucidate to my own satisfaction what takes place, and there are still points which I do not understand.

Very shortly after stage Q, in addition to primitive ova with a perfectly normal nucleus, others may be seen in which the nucleus is

apparently replaced by a deeply stained irregular body, smaller than the ordinary nuclei (Pl. XVIII, fig. 11, *d. n.*). This body, by the use of high objectives, is seen to be composed of a number of deeply stained granules, and around it may be noticed a clear space, bounded by a very delicate membrane. The granular body usually lies close to one side of this membrane, and occasionally sends a few fine processes to the opposite side.

The whole body, *i.e.* all within the delicate membrane is, according to my view, a modified nucleus; as appears to me very clearly to be shown by the fact that it occupies the normal position of a nucleus within a cell body. Semper, on the other hand, regards the contained granular body as the nucleus, which he compares with the spindles of Butschli, Auerbach, &c.,[1] This interpretation appears to me, however, to be negatived by the position of these bodies. The manner in which Semper may, perhaps, have been led to his views will be obvious when the later changes of the primitive ova are described. The formation of these nuclei would seem to be due to a segregation of the constituents of the original nuclei; the solid parts becoming separated from the more fluid. As a rule, the modified nuclei are slightly larger than the original ones. In stage Q the following two tables show the dimension of the parts of three unmodified and of three modified nuclei taken at random.

Primitive ova with unmodified nuclei—

Nuclei.
0·014 mm.
0·012 mm.
0·01 mm.

Primitive ova with modified nuclei—

Nuclei.	Granular Bodies in Nuclei.
0·018 mm.	0·006 mm.
0·018 mm.	0·006 mm.
0·012 mm.	0·009 mm.

For a slightly older stage than Q, the too annexed tables also show the comparative size of the modified and unmodified nuclei.

Unmodified nuclei of normal primitive ova—

0·014 mm.
0·016 mm.
0·014 mm.
0·016 mm.
0·016 mm.

[1] Loc. cit., pp. 361.

Nuclei of primitive ova with modified nuclei—

Nuclei.	Granular Bodies in Nuclei.
0·018 mm.	0·008 mm.
0·016 mm.	0·008 mm.
0·016 mm.	0·01 mm.
0·016 mm.	
0·018 mm.	

These figures bring out with clearness the following points: (1) that the modified nuclei are slightly but decidedly larger on the average than the unmodified nuclei; (2) that the contained granular bodies *are very considerably* smaller than ordinary nuclei.

Soon after the appearance of the modified nuclei, remarkable changes take place in the cells containing them. Up to the time such nuclei first make their appearance the outlines of the individual ova are very clearly defined, but subsequently, although numerous ova with but slightly modified nuclei are still to be seen, yet on the whole the outlines of all the primitive ova are much less distinct than before; and this is especially the case with the primitive ova containing modified nuclei.

From cases in which three or four ova are found in a mass with modified nuclei, but in which the outline of each ovum is fairly distinct, it is possible to pass by insensible gradations to other cases in which two or three or more modified nuclei are found embedded in a mass of protoplasm in which no division into separate cells can be made out (fig. 14). For these masses I propose to employ the term nests. They correspond in part with the *Ureiernester* of Professor Semper.

Frequently they are found in hardened specimens to be enclosed in a membrane-like tunic which appears to be of the nature of coagulated fluid. These membranes closely resemble and sometimes are even continuous with trabeculæ which traverse the germinal epithelium. Ovaries differ considerably as to the time and completeness of the disappearance of the outlines marking the separate cells, and although, so far as can be gathered from my specimens, the rule is that the outlines of the primitive ova with modified nuclei soon become indistinct, yet in one of my best preserved ovaries very large nests with modified nuclei are present in which the outline of each ovum is as distinct as during the period before the nuclei undergo these peculiar changes (Pl. XVIII, fig. 12). In the same ovary other nests are present in which the outlines of the individual are no longer visible. The section represented on Pl. XVII, fig. 2, is fairly average as to the disappearance of the outlines of the individual ova.

It is clear from the above statements, that in the first instance the nests are produced by the coalescence of several pri-

mitive ova into a single mass or syncytium; though of course, the several separate ova of a nest may originally, as Semper believes, have arisen from the division of a single ovum. In any case there can be no doubt that the nests of separate ova increase in size as development proceeds; a phenomenon which is more reasonably explained on the view that the ova divide, than on the view that they continue to be freshly formed. The same holds true for the nests of nuclei and this, as well as other facts, appears to me to render it probable that the nests grow by division of the nuclei without corresponding division of the protoplasmic matrix. I cannot, however, definitely prove this point owing to my having found nests, with distinct outlines to the ova, as large as any without such outlines.

The nests are situated for the most part near the surface of the germinal epithelium. The smaller ones are frequently spherical, but the larger are irregular in form. The former are about 0·05 mm. in diameter; the latter reach 0·1 mm. Scattered generally, and especially in the deeper layers, and at the edges of the germinal epithelium, are still unmodified or only slightly modified primitive ova. These unmodified primitive ova are aggregated in masses, but in these masses the outlines of each ovum, though perhaps less clear than in the earlier period, are still distinct.

When the embryo reaches a length of 7 centimètres, and even in still younger embryos, further changes are observable. In the first place many of the modified nuclei acquire fresh characters, and it becomes necessary to divide the modified nuclei into two categories. In both of these the outer boundary of the nucleus is formed by a very delicate membrane, the space within which is perfectly clear except for the granular body. In the variety which now appears in considerable numbers the granular body has an irregular star-like form. The rays of the star are formed of fibres frequently knobbed at their extremities, and the centre of the star usually occupies an excentric position. Typical examples of this form of modified nucleus, which may be spoken of as the stellate variety, are represented on Plate XVIII, fig. 17; between it and the older granular variety there is an infinite series of gradations, many of which are represented on Pl. XVIII, figs. 12, 14, 15, 16. Certain of the stellate nuclei exhibit two centres instead of one, and in some cases, like that represented on Pl. XVIII, fig. 19, the stellate body of two nuclei is found united. Both of these forms are possibly modifications of the spindle-like form assumed by nuclei in the act of dividing, and may be used in proving that the nests increase in size by the division of the contained nuclei. In addition to the normal primitive ova, a few of which are still present, there are to

be found, chiefly in the deeper layers of the germinal epithelium, larger ova differing considerably from the primitive ova. They form the permanent ova (Pl. XVII, fig. 3, *o.*). Their average diameter is 0·04 mm., compared with 0·03 mm., the diameter of original primitive ova. The protoplasm of which they are composed is granular, but at first a membrane can hardly be distinguished around them; their nucleus is relatively large, 0·02—0·027 mm. in diameter. It presents the characters ascribed by Eimer,[1] and many other recent authors,[2] to typical nuclei (*vide* Pl. XVII, fig. 3, and Pl. XVIII, figs. 13, 14, 15, 16, 17, 18). It is bounded by a distinct membrane, within which is a more or less central nucleolus from which a number of radial fibres which stain very deeply pass to the surface; here they form immediately internal to the membrane a network with granules at the nodal points. In some instances the regularity of the arrangement of these fibres is very great, in other instances two central nucleoli are present, in which case the regularity is considerably interfered with. The points in which the youngest permanent ova differ from the primitive may be summed up as follows:—

(1) The permanent ova are larger, the smallest of them being larger than the average primitive ova in the proportion of four to three. (2) They have less protoplasm as compared to the size of the nucleus. (3) Their protoplasm is granular instead of being clear. (4) Their nucleus is clear with exception of a network of fibres instead of being granular as in the primitive ova. It thus appears that the primitive ova and permanent ova are very different in constitution, though genetically related in a way to be directly narrated.

The formation of permanent ova is at its height in embryo of about 7 centimètres or slightly larger. The nests at this stage are for the most part of a very considerable size and contain a large number of nuclei, which have probably, as before insisted, originated from a division of the smaller number of nuclei present in the nests at an earlier stage. Figs. 14—18 are representations of nests at this period. The diameter of the nuclei is, on the whole, slightly greater than at an earlier stage. A series of measurements gave the following results:—

0·016 mm.
0·016 mm.
0·018 mm.
0·02 mm.
0·02 mm.

Both varieties of modified nuclei are common enough, though

[1] 'Archiv f. micr. Anat.,' vol. xiv.

[2] *Vide* especially Klein, in the last number of this Journal.

the stellate variety prodominates. The nuclei are sometimes in very close contact, and sometimes separated by protoplasm. which in many instances is very slightly granular. In a large number of the nests nothing further is apparent than what has just been described, but in a very considerable number one or more nuclei are present, which exhibit a transitional character between the ordinary stellate nuclei of my second category, and the nuclei of permanent ova as above described; and in these nests the formation of permanent ova is taking place. Permanent ova in the act of development are indicated in my figures by the letters *d o*. Many of the intermediate nuclei are more definitely surrounded by granular protoplasm than the other nuclei of the nests, and accordingly have their outlines more sharply defined. Between nuclei of this kind, and others as large as those of the permanent ova, there are numerous transitional forms. The larger ones frequently lie in a mass of granular protoplasm projecting from the nest, and only united with it by a neck (Pl. XVIII, figs. 14 and 16). For prominences of this kind to become independent ova, it is only necessary for the neck to become broken through. Nests in which such changes are taking place present various characters. In some cases several nuclei belonging to a nest appear to be undergoing conversion into permanent ova at the same time. Such a case is figured on Pl. XVIII, figs. 17 and 18. In these cases the amount of granular protoplasm in the nest and around each freshly formed ovum is small. In the more usual cases only one or two permanent ova at the utmost are formed at the same time, and in these instances a considerable amount of granular protoplasm is present around the nucleus of the developing permanent ovum. In such instances it frequently happens several of the nuclei not undergoing conversion appear to be in the process of absorption, and give to the part of the nest in which they are contained a very hazy and indistinct aspect (Pl. XVIII, fig. 15). Their appearance leads me to adopt the view *that while some of the nuclei of each nest are converted into the nuclei of the permanent ova, others break down and are used as the pabulum, at the expense of which the protoplasm of the young ovum grows.*

It should, however, be stated, that after the outlines of the permanent ova have become definitely established, I have only observed in a single instance the inclusion of a nucleus within an ovum (Pl. XVIII, fig. 24). In many instances normal nuclei of the germinal epithelium may be so observed within the ovum.

The nuclei which are becoming converted into the nuclei of

permanent ova gradually increase in size. The following table gives the diameter of four such nuclei:—

0·022 mm.
0·022 mm.
0·024 mm.
0·032 mm.

These figures should be compared with those of the table on page 396.

The ova when first formed are situated either at the surface or in the deeper layers of the germinal epithelium. Though to a great extent surrounded by the ordinary cells of the germinal epithelium, they are not at first enclosed in a definite follicular epithelium. The follicle is, however, very early formed.

My observations lead me then to the conclusion that in a general way the permanent ova are formed by the increase of protoplasm round some of the nuclei of a nest, and the subsequent separation of the nuclei with their protoplasm from the nest as distinct cells—a mode of formation exactly comparable with that which so often takes place in invertebrate egg tubes.

Besides the mode of formation of permanent ova just described, a second one also seems probably to occur. In ovaries just younger than those in which permanent ova are distinctly formed, there are present primitive ova, with modified nuclei of the stellate variety, or nuclei sometimes even approaching in character those of permanent ova, which are quite isolated and not enclosed in a definite nest. The body of these ova is formed of granular protoplasm, but their outlines are very indistinct. Such ova are considerably larger than the normal primitive ova. They may measure 0·04 mm. In a slightly later stage, when fully formed permanent ova are present, isolated ones are not infrequent, and it seems natural to conclude that these isolated ova are the direct descendants of the primitive ova of the earlier stage. It seems a fair deduction that in some cases primitive ova undergo a direct metamorphosis into permanent ova by a modification of their nucleus, and the assumption of a granular character in their protoplasm, without ever forming the constituent part of a nest.

It is not quite clear to me that in all nests the coalescence of the protoplasm of the ova necessarily takes place, since some nests are to be found at all stages in which the ova are distinct. Nevertheless, I am inclined to believe that the fusion of the ova is the normal occurrence.

The mode of formation of the permanent ova may then, according to my observations, take place in two ways:—1. By the formation of granular protoplasm round the nucleus in a nest, and the

separation of the nucleus with its protoplasm as a distinct ovum. 2. By the direct metamorphosis of an isolated primitive ovum into a permanent ovum. The difference between these two modes of formation does not, from a morphological point of view, appear to be of great importance.

The above results appear clearly to show that *the primitive ova in the female are not to be regarded as true ova, but as the parent sexual cells which give rise to the ova :* a conclusion which completely fits in with the fact that cells exactly similar to the primitive ova in the female give rise to the spermatic cells in the male.

Slightly after the period of their first formation the permanent ova become invested by a very distinct and well-marked, somewhat flattened, follicular epithelium (Pl. XVIII, fig. 3). Where the ova lie in the deeper layers of the germinal epithelium, the follicular epithelium soon becomes far more columnar on the side turned inwards, than on that towards the surface, especially when the inner side is in contact with the stroma (Pl. XVII, fig. 7, and Pl. XVIII, figs. 24 and 26). This is probably a special provision for the growth and nutrition of the ovum.

There cannot be the smallest doubt that the follicular epithelium is derived from the general cells of the germinal epithelium—a point on which my results fully bear out the conclusions of Ludwig and Semper.

The larger ova themselves have a diameter of about 0·06 mm., and their nucleus of about 0·04 mm. The vitellus is granular, and provided with a distinct, though delicate membrane, which has every appearance of being a product of the ovum itself rather than of the follicular epithelium. The membrane would seem indeed to be formed in some instances even before the ovum has a definite investment of follicle cells. The vitellus is frequently vacuolated, but occasionally the vacuoles appear to be caused by a shrinking due to the hardening reagent. The nucleus has the same peculiar reticulate character as at first. Its large size, as compared with the ovum, is very noticeable.

With this stage the embryonic development of the ova comes to a close, though the formation of fresh ova continues till comparatively late in life. I have, however, two series of sections of ovaries preserved in osmic acid, from slightly larger embryos than the one last described, about which it may be well to say a few words before proceeding to the further development of the permanent ova.

The younger of these ovaries was from a Scyllium embryo 10 centimètres long, preserved in osmic acid.

A considerable number of nests were present (Pl. XVIII,

fig. 13), exhibiting, on the whole, similar characters to those just described.

A series of measurement of the nuclei in them were made, leading to the following results:—

0·014 mm.
0·014 mm.
0·016 mm.
0·016 mm.
0·018 mm.
0·018 mm.

Thus, if anything, the nuclei were slightly smaller than in the younger embryo. It is very difficult in the osmic specimens to make out clearly the exact outlines of the various structures, the nuclei in many instances being hardly more deeply stained than in the protoplasm around them. The network in the nuclei is also far less obvious than after treatment with picric acid. The permanent ova were hardly so numerous as in the younger ovary before described. A number of these were measured with the following results:—

Ovum.	Nucleus.
0·03 mm.	0 014 mm.
0·034 mm.	0·018 mm.
0·028 mm.	0·016 mm.
0·03 mm.	0·02 mm.
0 04 mm.	0·02 mm.
0·04 mm.	0·02 mm.
0·048 mm.	0·02 mm.

These figures show that the nuclei of the permanent ova are smaller than in the younger embryo, and it may therefore be safely concluded that, in spite of the greater size of the embryo from which it is taken, the ovary now being described is in a more embryonic condition than the one last dealt with.

Though the permanent ova appeared to be formed from the nests in the manner already described, it was fairly clear from the sections of this ovary that many of the original primitive ova, after a metamorphosis of the nucleus and without coalescing with other primitive ova to form nests, become converted directly into the permanent ova. Many large masses of primative ova, or at least of ova with the individual outlines of each ovum distinct, were present. The average size of ova composing these was however small, the body measuring about 0·016 mm., and the nucleus 0·012 mm. Isolated ova with metamorphosed nuclei could also be found measuring 0·022, and their nuclei about 0·014 mm.

The second of the two ovaries, hardened in osmic acid, was somewhat more advanced than the ovary in which the formation of permanent ova was at its height. Fewer permanent ova were in

the act of being formed, and many of these present had reached a considerable size, measuring as much as 0·07 mm. Nests of the typical forms were present as before, but the neuclei in them were more granular than at the earlier period, and on the average slightly smaller. A series measured had the following diameters:—

0·01 mm.
0.012 mm.
0·014 mm.
0·016 mm.

One of these nests is represented on Pl. XVIII, fig 20. Many nests with the outlines of the individual ova distinct were also present.

On the whole it appeared to me, that the second mode of formation of permanent ova, viz. that in which the nest does not come into the cycle of development, preponderated to a greater extent than in the earlier embryonic period.

Post-embryonic development of the ova.—My investigations upon the post embryonic growth and development of the ova, have for the most part been conducted upon preserved ova, and it has been impossible for me, on this account, to work out, as completely as I should have wished, certain points, more especially those connected with the development of the yolk.

Although my ovaries have been carefully preserved in a large number of reagents, including osmic acid, picric acid, chromic acid, spirit, bichromate of potash, and Müller's fluid, none of these have proved universally successful, and bichromate of potash and Müller's fluid are useless. Great difficulties have been experienced in distinguishing the artificial products of these reagents. My investigations have led me to the result, that in the gradual growth of the ova with the age of the individual the changes are not quite identical with those during the rapid growth which takes place at periods of sexual activity, after the adult condition has been reached—a result to which His has also arrived, with reference to the ova of Osseous Fish. I propose dealing separately with the several constituents of the egg-follicle.

Egg membranes.—A vitelline membrane has been described by Leydig[1] in Raja, and an albuminous layer of the nature of a chorion[2] by Gegenbaur[3] in Acanthias—the membranes described in these two ways being no doubt equivalent.

[1] 'Rochen u. Haie.'

[2] By *chorion* I mean following E. van Beneden's nomenclature, a membrane formed by the follicular epithelium, and, by *vitelline membrane*, one formed by the vitellus or body of the ovum.

[3] "Bau und Entwicklung d. Wirbelthiereier," &c., 'Mull. Archiv.,' 1861.

Dr. Alex. Shultz[1] has more recently investigated a considerable variety of genera and finds three conditions of the egg membranes. (1.) In Torpedo, a homogeneous membrane, which is of the nature of a chorion. (2.) In Raja, a homogeneous membrane which is, however, perforated. (3). In Squalidæ, a thick homogeneous membrane, internal to which is a thinner perforated membrane. He apparently regards the perforated inner membrane as a specialised part of the simple membrane found in Torpedo, and states that this membrane is of the nature of a chorion.

My own investigations have led me to the conclusion that though the egg-membranes can probably be reduced to single type for Elasmobranchs, yet that they vary with the stage of development of the ovum. Scyllium (stellare and canicula) and Raja have formed the objects of my investigation. I commence with the two former.

It has already been stated that in Scyllium, even before the follicular epithelium becomes formed, a delicate membrane round the ovum can be demonstrated, which appears to me to be derived from the vitellus or body of the ovum, and is therefore of the nature of a vitelline membrane. It becomes the vitelline membrane of Leydig, the albuminous membrane of Gegenbaur, and homogeneous membrane of Schultz.

In a young fish (not long hatched) with ova of not more than 0·12 mm., this membrane, though considerably thicker than in the embryo, is not thick enough to be accurately measured. In ova of 0·5 mm. from a young female (Pl. XVIII, fig. 21) the vitelline membrane has a thickness of 0·002 mm. and is quite homogeneous.[2] Internally to it may be observed very faint indications of the differentiation of the outermost layer of the vitellus into the perforated or radially striated membrane of Schultz, which will be spoken of as *zona radiata*.

In an ovum of 1 mm. from the nearly full grown though not sexually mature female, the zona radiata has increased in thickness and definiteness, and may measure as much as 0·004 mm. It is always very sharply separated from the vitelline membrane, but appears to be more or less continuous on its inner border with the body of the ovum, at the expense of which it no doubt grows in thickness.

In ova above 1 mm. in diameter, both vitelline membrane and zona radiata, but especially the latter, increase in thickness.

[1] "Zur Entwicklungsgeschichte d. Selachier," 'Arch. f. mikr. Anat.,' vol. xi.

[2] The apparent structure in the vitelline membrane in my figure is merely intended to represent the dark colour assumed by it on being stained. The Zona radiata has been made rather too thick by the artist.

The zona becomes marked off from the yolk, and its radial striæ become easy to see even with comparatively low powers. In many specimens it appears to be formed of a number of small columns, as described by Gegenbaur and others. The stage of about the greatest development of both the vitelline membrane and zona radiata is represented on Pl. XVIII, Fig. 22.

At this time the vitelline membrane appears frequently to exhibit a distinct stratification dividing it into two or more successive layers. It is not, however, acted on in the same manner by all reagents, and with absolute alcohol appears at times longitudinally striated.

From this stage onwards, both vitelline membrane and zona gradually atrophy, simultaneously with a series of remarkable changes which take place in the follicular epithelium. The zona is the first to disappear, and the vitelline membrane next becomes gradually thinner. Finally, when the egg is nearly ripe, the follicular epithelium is separated from the yolk by an immeasurably thin membrane—the remnant of the vitelline membrane—only visible in the most favorable sections (Pl. XVIII, fig. 23 v t.). When the egg becomes detached from the ovary even this membrane is no longer to be seen.

Both the vitelline membrane and the zona radiata are found in Raja, but in a much less developed condition than in Scyllium. The vitelline membrane is for a long time the only membrane present, but is never very thick (Pl. XIX, fig. 31). The zona is not formed till a relatively much later period than in Scyllium, and is always delicate and difficult to see (Pl. XIX, fig. 32). Both membranes atrophy before the egg is quite ripe; and an apparently fluid layer between the follicular epithelium and the vitellus, which coagulates in hardened specimens, is probably the last remnant of the vitelline membrane. It is, however, much thicker than the corresponding remnant in Scyllium.

Though I find the same membranes in Scyllium as Alexander Schultz did in other Squalidæ, my results do not agree with his as to Raja. Torpedo I have not investigated.

It appears to me probable that the ova in all Elasmobranch Fishes have at some period of their development the two membranes described at length for Scyllium. Of these the inner one, or zona radiata, will probably be admitted on all hands to be a product of the peripheral protoplasm of the egg.

The outer one corresponds with the membrane usually regarded in other Vertebrates as a chorion or product of the follicular epithelium, but, by tracing it back to its first origin, I have been led to reject this view of its nature.

The follicular epithelium.—The follicular epithelium in the

eggs of Raja and Acanthias has been described by Gegenbaur.[1] He finds it flat in young eggs, but in the larger eggs of Acanthias more columnar, and with the cells wedged in so as to form a double layer. These observations are confirmed by Ludwig.[2]

Alexander Schultz[3] states that in Torpedo, the eggs are at first enclosed in a simple epithelium, but that in follicles of ·008 mm. there appear between the original large cells of the follicle (which he describes as granulosa cells and derives from the germinal epithelium) a number of peculiar small cells. He states that these are of the same nature as the general stroma cells of the ovary, and believes that they originate in the stroma. When the eggs have reached 0·1—0·15 mm., he finds that the small and large cells have a very regular alternating arrangement.

Semper records but few observations on the follicular epithelium, but describes in Raja the presence of a certain number of large cells amongst smaller cells. He believes that they may develope into ova, and considers them identical with the larger cells described by Schultz, whose interpretations he does not, however, accept.

My own results accord to a great extent with those of Dr. Schultz, as far as the structure of the follicular epithelium is concerned, but I am at one with Semper in rejecting Schultz's interpretations.

In Scyllium, as has already been mentioned, the follicular epithelium is at first flat and formed of a single layer of uniform cells, each with a considerable amount of clear protoplasm and a granular nucleus. It is bounded externally by a delicate membrane—the membrana propria folliculi of Waldeyer—and internally by the vitelline membrane. In the ovaries of very young animals the cells of the follicular epithelium are more columnar on the side towards the stroma than on the opposite side, but this irregularity soon ceases to exist.

In many cases the nuclei of the cells of the follicular epithelium exhibit a spindle modification, which shows that the growth of the follicular epithelium takes place by the division of its cells. No changes of importance are observable in the follicular epithelium till the egg has reached a diameter of more than 1 mm.

It should here be stated that I have some doubts respecting the completeness of the history of the epithelium recorded in the sequel. Difficulties have been met with in completely elucidating the chronological order of the occurrences, and it is possible that some points have escaped my observation.

[1] Loc. cit. [2] Loc. cit. [3] Loc. cit.

The first important change is the assumption of a palisade-like character by the follicle cells, each cell becoming very narrow and columnar and the nucleus oval (Pl. XIX, fig. 28). In this condition the thickness of the epithelium is about 0·025 mm. The epithelium does not, however, become uniformly thick over the whole ovum, but in the neighbourhood of the germinal vesicle it is very flat and formed of granular cells with indistinct outlines, rather like the hypodermis cells of many Annelida. Coincidently with this change in the follicular epithelium the commencement of the atrophy of the membranes of the ovum, described in the last section, becomes apparent.

The original membrana propria folliculi is still present round the follicular epithelium, but is closely associated with a fibrous layer with elongated nuclei. Outside this there is now a layer of cells, very much like an ordinary epithelial layer, which may possibly be formed of cells of the true germinal epithelium (fig. 28, *fé*). This layer, which will be spoken of as the secondary follicle layer, might easily be mistaken for the follicular epithelium, and it is possible that it has actually been so mistaken by Eimer, Clark, and Klebs, in Reptilia, and that the true follicular epithelium (in a flattened condition) has been then spoken of as the *Binnenepithel.*

In slightly older eggs the epithelial cells are no longer uniform or arranged as a single layer. The general arrangement of these cells is shown in (Pl. XIX, fig. 29.) A considerable number of them are more or less flask-shaped, with bulky protoplasm prolonged into a thin stem directed towards the vitelline membrane, with which, in many instances if not all, it comes in contact. These larger cells are arranged in several tiers. Intercalated between them are a number of elongated small cells with scanty protoplasm and a deeply staining nucleus, not very dissimilar to, though somewhat smaller than, the columnar cells of the previous stage. There is present a complete series of cells intermediate between the larger cells and those with a deeply stained nucleus, and were it not for the condition of the epithelium in Raja, to be spoken of directly, I should not sharply divide the cells into two categories. In surface views of the epithelium the division into two kinds of cells would not be suspected. There can, it appears to me, be no question that both varieties of cell are derived from the primitive uniform follicle cells.

The fibrous layer bounding the membrana propria folliculi is thicker than in the last stage, and the epithelial-like layer (*fé*), which bounds it externally is more conspicuous than before. Immediately adjoining it are vascular and lymph sinuses. The thickness of the follicular epithelium at this stage may reach as

much as 0·04 mm., though I have found it sometimes considerably flatter. The cells composing it are, however, so delicate that it is not easy to feel certain that the peculiarities of any individual ovum are not due to handling. The absence of the peculiar columnar epithelium on the part of the surface adjoining the germinal vesicle is as marked a feature as in the earlier stage. When the egg is nearly ripe, and the vitelline membrane has been reduced to a mere remnant, the follicular epithelium is still very columnar (Pl. XVIII, fig. 23). The thickness is greater than in the last stage, being now about 0·045 mm., but the cells appear only to form a single definite layer. From the character of their nuclei, I feel inclined to regard them as belonging to the category of the smaller cells of the previous stage, and feel confirmed in this view by finding certain bodies in the epithelium, which have the appearance of degenerating cells with granular nuclei, which I take to be the flask-shaped cells which were present in the earlier stage.

I have not investigated the character of the follicular epithelium in the perfectly ripe ovum ready to become detached from the ovary. Nor can I state for the last-described stage anything about the character of the follicular epithelium in the neighbourhood of the germinal vesicle.

As to the relation of the follicular epithelium to the vitelline membrane, and the possible processes of its cells continued into the yolk, I can say very little. I find in specimens teased out after treatment with osmic acid, that the cells of the follicular epithelium are occasionally provided with short processes, which might possibly have perforated the vitelline membrane, but have met with nothing so clear as the teased out specimens figured by Eimer. Nothing resembling the cells within the vitelline membrane, as described by His,[1] in Osseous Fish, and Lindgren in Mammalia, has been met with.[2]

My observations in Raja are not so full as those upon Scyllium, but they serve to complete and reconcile the observations of Semper and Schultz, and also to show that the general mode of growth of the follicular epithelium is fundamentally the same in my representatives of the two divisions of the Elasmobranchii. In very young eggs, in conformity with the results of all previous observers, I find the follicular epithelium approximately uniform. The cells are flat, but extended so as to appear of an unexpected size in views of the surface of the follicle. This condition does not, however, last very long. A certain number of the cells enlarge considerably, others remaining smaller and flat. The differences between the larger and the smaller cells are more con-

[1] 'Das Ei bei Knochenfischen.'
[2] 'Arch. f. Anat. Phys.,' 1877.

spicuous in sections than in surface views, and though the distribution of the cells is somewhat irregular, it may still be predicted as an almost invariable rule that the smaller cells of the follicle will line that part of the surface of the ovum, near to which the germinal vesicle is situated. On Pl. XIX, fig. 30, is shown in section a fairly average arrangement of the follicle cells. Semper considers the larger cells of such a follicle to be probably primitive ova destined to become permanent ova. This view I cannot accept: firstly, because these cells only agree with primitive ova in being exceptionally large—the character of their nucleus, with its large nucleolus, being not very like that of a primitive ovum. Secondly, because they shade into ordinary cells of the follicle; and thirdly, because no evidence of their becoming ova has come before me, but rather the reverse, in that it seems probable that they have a definite function connected with the nutrition of the egg. To this point I shall return.

In the next stage the small cells have become still smaller. They are columnar, and are wedged in between the larger ones. No great regularity in distribution is as yet attained (Pl. XIX, fig. 31). Such a regularity appears in a later stage (Pl. XIX, fig. 32), which clearly corresponds with fig. 8 on Pl. XXXIV of Schultz's paper, and also with the stage of Scyllium in Pl. XIX, fig. 29, though the distinction between the two kinds of cells is here far better marked than in Scyllium. The big cells have now become flask-shaped like those in Scyllium, and send a process down to the vitelline membrane. The smaller cells are arranged in two or three tiers, but the larger cells in a single layer. The distribution of the larger and smaller cells is in some instances very regular, as shown in the surface view on Pl. XIX, fig. 33. There can, it appears to me, be no doubt that Schultz's view of the smaller cells being lymph-cells which have migrated into the follicle cannot be maintained.

The thickness of the epithelium at this stage is about 0·04 mm. In the succeeding stages, during which the egg is rapidly growing to the colossal size which it eventually attains, the follicular epithelium does not to any great extent alter in constitution. It grows thicker on the whole, and as the vitelline membrane gradually atrophies, its lower surface becomes irregular, exhibiting somewhat flattened prominences, which project into the yolk. At the greatest height of the prominences the epithelium may reach a thickness of 0·06 mm., or even more. The arrangement of the tissues external to the follicular epithelium is the same in Raja as in Scyllium.

The most interesting point connected with the follicle, both in Scyllium and Raja and presumably in other Elasmobranchs is that its epithelium at the time when the egg is rapidly approaching

maturity is composed with more or less of distinctness of two forms of cells. One of these is large flask-shaped and rich in protoplasm, the other is small, consisting of a mere film of protoplasm round a nucleus. Considering that the larger cells appear at the time of rapid growth, it is natural to interpret their presence as connected with the nutrition of the ovum. This view is supported by the observations of Eimer and Braun, on the development of Reptilian ova. In many Reptilian ova it appears from Eimer's[1] observations, that the follicular epithelium becomes several layers thick, and that a differentiation of the cells, similar to that in Elasmobranchs, takes place. The flask-shaped cells eventually undergo peculiar changes, becoming converted into a kind of beaker-cell, with prolongations through the egg membranes, which take the place of canals leading to the interior of the egg. Braun also expresses himself strongly in favour of the flask-shaped cells functioning in the nutrition of the egg.[2] That these cells in the Reptilian ova really correspond with those in Elasmobranch's appears to me clear from Eimer's figures, but I have not myself studied any Reptilian ovum. My reasons for dissenting from both Semper's and Schultz's views on the nature of the two forms of follicular cells have already been stated.

The vitellus and the development of the yolk-spherules.—Leydig, Gegenbaur, and Schultz, have recorded important observations on this head. Leydig[3] chiefly describes the peculiar characters of the yolk-spherules.

Gegenbaur[4] finds in the youngest eggs fine granules, which subsequently develop into vesicles, in the interior of which the solid oval spheres, so characteristic of Elasmobranchs, are developed.

Schultz describes in the youngest ova of Torpedo the minute yolk-sperules arranged in a semi-lunar form around the excentric germinal vesicle. In older ova they spread through the whole. He also gives a description of their arrangement in the ripe ovum. Dr. Schultz further finds in the body of the ovum peculiar protoplastic striæ, arranged as a series of pyramids, with the bases directed outwards. In the periphery of the ovum a protoplastic network is also present, which is continuous with the above-mentioned pyramidal structures.

My observations do not very greatly extend those of Gegenbaur and Schultz with reference to the development of the

[1] 'Archiv. fur mikr. Anat.,' vol. viii.

[2] "Braun Urogenital system d. Amphibien, Arbeiten a. d.," 'Zool.-zoot. Institut Wurzburg,' Bd. iv. He says, in reference to the flask-shaped cell, p. 166, "Höchstens würde ich die Funktion der grossen Follikelzellen als *einzellige Drüsen* mehr betonen."

[3] Loc. cit.

[4] Loc. cit.

yolk, and closely agree with what Gegenbaur has given in the paper above quoted more fully for Aves and Reptilia than for Elasmobranchii.

In very young ova the body of ovum is simply granular, but when it has reached about 0·5 mm. the granules are seen to be arranged in a kind of network, or spongework (Pl. XVIII, fig 21), already spoken of in my monograph on Elasmobranch Fishes.

This network becomes more distinct in succeeding stages, especially in chromic acid specimens (Pl. XVIII, fig. 22), probably in part owing to a granular precipitation of the protoplasm. In the late stages, when the yolk spherules are fully developed, it is difficult to observe this network, but, as has been shown in my monograph above quoted, it is still present after the commencement of embryonic development. An arrangement of the protoplasmic striæ like that described by Schultz has not come under my notice.

The development of the yolk appears to me to present special difficulties, owing to the fact pointed out by His[1] that the conditions of development vary greatly according to whether the ovary is in a state of repose or of active development. I do not feel satisfied with my results on this subject, but believe there is still much to be made out. Observations on the yolk spherules may be made either in living ova, in ova hardened in osmic acid, or in ova hardened in picric or chromic acids. The two latter reagents, as well as alcohol, are however unfavorable for the purpose of this study, since by their action the yolk spherules appear frequently to be broken up and otherwise altered. This has to some extent occurred in Pl. XVIII, fig. 21, and the peculiar appearance of the yolk of this ovum is in part due to the action of the reagent. On the whole I have found osmic acid the most suitable reagent for the study of the yolk, since without breaking up the developing spherules, it stains them of a deep black colour. The yolk spherules commence to be formed in ova, of not more than 0·06 mm. in the ovaries of moderately old females. In young females they are apparently not formed in such small ova. They arise as extremely minute, highly refracting particles, in a stratum of protoplasm *some little way below the surface, and are always most numerous at the pole opposite the germinal vesicle.* Their general arrangement is very much that figured and described by Allen Thomson in Gasterosteus,[2] and by Gegenbaur and Eimer in young Reptilian ova. In section they naturally appear as a ring, their general mode of distribution being fairly typically represented on Pl. XIX, fig. 27. The ovum represented

[1] 'Das Eie bei Knochenfischen.'

[2] "Ovum," in 'Todd's Cyclopædia,' fig. 69.

in fig. 27, was 0·5 mm. in diameter, and the yolk spherules were already largely developed; in smaller ova they are far less numerous, though arranged in a similar fashion. The developing yolk spherules are not uniformly distributed but are collected in peculiar little masses or aggregations (Pl. XVIII, fig. 21). These resemble the granular masses, figured by His (loc. cit. Pl. IV, fig. 33) in the Salmon, and may be compared with the aggregations figured by Gotte in his monograph on *Bombinator igneus* (Plate I, fig. 9). It deserves to be especially noted, that when the yolk spherules are first formed, the *peripheral layer of the ovum* is entirely free from them, a feature which is however apt to be lost in ova hardened in picric acid (Pl. XVIII, fig. 21). Two points about the spherules appear clearly to point to their being developed in the protoplasm of the ovum, and not in the follicular epithelium. (1) That they do not make their appearance in the superficial stratum of the ovum. (2) That no yolk spherules are present in the cells of the follicular epithelium, in which they could not fail to be detected, owing to the deep colour they assume on being treated with osmic acid.

It need scarcely be said that the yolk spherules at this stage are not cells, and have indeed no resemblance to cells. They would probably be regarded by His as spherules of fatty material, unrelated to the true food yolk.

As the ova become larger the granules of the peripheral layer before mentioned gradually assume the character of the yolk spheres of the adult, and at the same time spread towards the centre of the egg. Not having worked at fresh specimens, I cannot give a full account of the growth of the spherules; but am of opinion that Gegenbaur's account is probably correct, according to which the spheres at first present gradually grow and develop into vesicles, in the interior of which solid bodies (nuclei of His?) appear and form the permanent yolk spheres. When the yolk spheres are still very small they have the typical oblong form[1] of the ripe ovum, and this form is acquired while the centre of the ovum is still free from them.

The growth of the yolk appears mainly due to the increase in size and number of the individual yolk spheres. Even when the ovum is quite filled with large yolk spheres, the granular protoplastic network of the earlier stages is still present, and serves to hold together the constituents of the yolk. In the cortical layer of nearly ripe ova, the yolk has a somewhat different character to that which it exhibits in the deeper layers, chiefly

[1] The peculiar oval, or at times slightly rectangular and striated yolk spherules of Elasmobranchs are mentioned by Leydig and Gegenbaur (pl. xi, fig. 20), and myself, 'Preliminary Account of Development of Elasmobranch Fishes,' and by Filippi and His in 'Osseous Fishes.'

owing to the presence of certain delicate granular (in hardened specimens)bodies, whose nature I do not understand, and to special yoke spheres rather larger than the ordinary, provided with numerous smaller spherules in their interior, which are probably destined in the course of time to become free and to form ordinary yolk spheres.

The mode of formation of the yolk spheres above described, appears to me to be the normal, and possibly the only one. Certain peculiar structures have, however, come under my notice, which may perhaps be connected with the formation of the yolk. One of these resembles the bodies described by Eimer[1] as "Dotterschorfe." I have only met these bodies in a single instance in ova of 0·6 mm., from the ovary (in active growth) of a specimen of *Scy. canicula* 23 inches in length. In this instance they consisted of homogeneous clear bodies (not bounded by any membrane) of somewhat irregular shape, though usually more or less oval, and rarely more than 0·02 mm. in their longest diameter. They were very numerous in the peripheral layer of the ovum, but quite absent in the centre, and also not found outside the ovum (as they appear to be in Reptilia). Yolk granules formed in the normal way, and staining deeply by osmic acid, were present, but the "Dotterschorfe" presented a marked contrast to the remainder of the ovum, in being absolutely unstained by osmic acid, and indeed they appeared more like a modified form of vacuole than any definite body. Their general appearance in Scyllium may be gathered from Eimer's figure 8, Pl. XI, though they were much more numerous than represented in that figure, and confined to the periphery of the ovum.

Dr. Eimer describes a much earlier condition of these structures, in which they form a clear shell enclosing a central dark nucleus. This stage I have not met with, nor can I see any grounds for connecting these bodies with the formation of the yolk, and the fact of their not staining with osmic acid is strongly opposed to this view of their function. Dr. Eimer does not appear to me to bring forward any satisfactory proof that they are in any way related to the formation of the yolk, but wishes to connect them with the peculiar body, well known as the yolk nucleus, which is found in the amphibian ovum.[2]

Another peculiar body found in the ova may be mentioned here, though it more probably belongs to the germinal vesicle than to the yolk. It has only been met with in the vitellus of some of the medium sized ova of a young female. Examples of this body are represented on Pl. XVIII, fig. 25. A *x*). As a rule there

[1] "Untersuching über die Eier d. Reptilian," 'Archiv. f. mikros. Anat.,' vol. viii.

[2] Vide Allen Thomson, article "Ovum," Todd's 'Encyclopædia,' p. 95.

is only one in each of the ova in which they are present, but there may be as many as four. They consist of small vesicles with a very thick doubly contoured membrane, which are filled with numerous deeply staining spherical granules. At times they contain a vacuole. Some of the larger of them are not very much smaller than the germinal vesicle of their ovum, while the smallest of them present a striking resemblance to the nucleoli (fig. 25 B), which makes me think that they may possibly be nucleoli which have made their way out of the germinal vesicle. I have not found them in the late stages or large ova.

The following measurements show the size of some of these bodies in relation to the germinal vesicle and ovum:—

Diameter of Ovum.	Diameter of Germinal Vesicle.	Diameter of Body in Vitellus.
0·096 mm.	0·03 mm.	0·009 mm.
0·064 mm.	0·025 mm.	0·012 mm.
0·096 mm.	0·03 mm.	0·019 mm. 0·003 mm.

Germinal vesicle.—Gegenbaur[1] finds the germinal vesicle completely homogeneous and without the trace of a germinal spot. In Raja granules or vesicles may appear as artificial products, and in Acanthias even in the fresh condition isolated vesicles or masses of such may be present. To these structures he attributes no importance.

Alexander Schultz[2] states that there is nothing remarkable in the germinal vesicle of the Torpedo egg, but that till the egg reaches 0·5 mm., a single germinal spot is always present (measuring about 0·01 mm.), which is absent in larger ova.

The bodies described by Gegenbaur are now generally recognised as germinal spots and will be described as such in the sequel. I have very rarely met with the condition with the single nucleolus described by Schultz in Torpedo.

My own observations are confined to Scyllium. In very young females, with ova not larger than 0·09 mm., the germinal vesicle has the same characters as during the embryonic periods. The contents are clear but traversed by a very distinct and deeply staining reticulum of fibres connected with the several nucleoli which are usually present and situated close to the membrane.

In a somewhat older female in the largest ova of about 0·12 mm., the germinal vesicle measures about 0·06 mm., and usually occupies an excentric position. It is provided with a distinct though delicate membrane. The network, so conspicuous during the embryonic period, is not so clear as it was, and has the appearance of being formed of lines of granules rather

[1] Loc. cit.
[2] Loc. cit.

than of fibres. The fluid contents of the nucleus remain as a rule, even in the hardened specimens, perfectly clear, though they become in some instances slightly granular. There are usually two, three, or more nucleoli generally situated, as described by Eimer, close to the membrane of the vesicle, the largest of which may measure as much as 0·006 mm. They are highly refracting bodies, containing in most instances a vacuole, and very frequently a smaller spherical body of a similar nature to themselves.[1] Granules are sometimes also present in the germinal vesicle, but are probably only extremely minute nucleoli.

In ova of 0·5 mm. the germinal vesicle has a diameter of 0·12 mm. (Pl. XVIII, fig. 21). It is usually shrunk in hardened specimens though nearly spherical in the living ovum. Its contents are rendered granular by reagents though quite clear when fresh, and the reticulum of the earlier stages is sometimes with difficulty to be made out, though in other instances fairly clear. In all cases the fibres composing it are very granular. The membrane is thick. Peculiar highly refracting nucleoli, usually enclosing a large vacuole, are present in considerable numbers, and are either arranged in a circle round the periphery, or sometimes aggregated towards one side of the vesicle; and in addition, numerous deeply staining smaller granular aggregations, probably belonging to the same category as the nucleoli (from which in the living ovum they can only be distinguished by their size), are scattered close to the inner side of the membrane over the whole or only a part of the surface of the germinal vesicle. In a fair number of instances bodies like that figured on Pl. XIX, fig. 27, are to be found in the germinal vesicle. They appear to be nucleoli in which a number of smaller nucleoli are originating by a process of endogenous growth, analogous perhaps to endogenous cell-formation. The nucleoli thus formed are, no doubt, destined to become free. The above mode of increase for the nucleoli appears to be exceptional. The ordinary mode is, no doubt, that by simple division into two, as was long ago shewn by Auerbach.

Of the later stages of the germinal vesicle and its final fate, I can give no account beyond the very fragmentary statements which have already appeared in my monograph on Elasmobranch Fishes.

Formation of fresh ova and ovarian nests in the post embryonic stages.—Ludwig,[2] was the first to describe the formation of

[1] Compare, with references to several points, the germinal vesicle at this stage with germinal vesicle of the frog's ovum figured by O. Hertwig, 'Morphologische Jahrbuch,' vol. iii, pl. iv., fig. 1.

[2] Loc. cit.

ova in the post-embryonic periods. His views will be best explained by quoting the following passage :—

"The follicle of Skates and Dog-fish, with the ovum it contains, is to be considered as an aggregation of the cells of the single-layered ovarian epithelium which have grown into the stroma, and of which one cell has become the ovum and the others the follicular epithelium. The follicle, however, draws in with it into the stroma a number of additional epithelial cells in the form of a stalk connecting the follicle with the superficial epithelium. At a later period the lower part of the stalk at its junction with the follicle becomes continuously narrowed, and at the same time a rupture takes place in the cells which form it. In this manner the follicle becomes at last constricted off from the stalk, and so from its place of origin in the superficial epithelium, and subsequently lies freely in the stroma of the ovary."

He further explains that the separation of the follicles from the epithelium takes place much earlier in Acanthias than in Raja, and that the sinkings of the epithelium into the stroma may have two or three branches each with a follicle.

Semper gives very little information with reference to the post-embryonic formation of ova. He expresses his agreement on the whole with Ludwig, but, amongst points not mentioned by Ludwig, calls attention to peculiar aggregations of primitive ova in the superficial epithelium, which he regards as either rudimentary testicular follicles or as nests similar to those in the embryo.

My observations on this subject do not agree very closely with those either of Ludwig or Semper. The differences between us partly, though not entirely, depend upon the fundamentally different view we hold about the constitution of the ovary and the nature of the epithelium covering it (vide p. 389 and 390).

In very young ovaries (Pl. XVII, fig. 8) nests of ova (in my sense of the term) are very numerous, but though usually superficial in position are also found in the deeper layers of the ovary. They are especially concentrated in their old position, close to the dorsal edge of the organ. In some instances they do not present quite the same appearance as in the embryo, owing to the outlines of the ova composing them being distinct, and to the presence between the ova of numerous interstitial cells derived from the germinal epithelium, and destined to become follicular epithelium. These latter cells at first form a much flatter follicular epithelium than in the embryonic periods, so that the smaller adult ova have a much less columnar investment than ova of the same size in the embryo. A few primitive ova may still be found in a very superficial position, but occasionally

also in the deeper layers. I am inclined to agree with Semper that some of these are freshly formed from the cells of the germinal epithelium.

In the young female with ova of about 0·5 mm. nests of ova are still fairly numerous. The nests are characteristic, and present the various remarkable peculiarities already described in the embryo. In many instances they form polynuclear masses, not divided into separate cells, generally, however, the individual ova are distinct. The ova in these nests are on the average rather smaller than during the embryonic periods. The nests are frequently quite superficial and at times continuous with the pseudo-epithelium, and individual ova also occasionally occupy a position in the superficial epithelium. Some of the appearances presented by separate ova are not unlike the figures of Ludwig, but a growth such as he describes has, according to my observations, no existence. The columns which he believes to have grown into the stroma are merely trabeculæ connecting the deeper and more superficial parts of the germinal epithelium; and his whole view about the formation of the follicular epithelium round separate ova certainly does not apply, except in rare cases, to Scyllium. It is, indeed, very easy to see that most freshly formed ova are derived from nests, as in the embryo; and the formation of a follicular epithelium round these ova takes place as they become separated from the nests. A few solitary ova, which have never formed part of a nest, seem to be formed in this stage as in the embryo; but they do not grow into the stroma surrounded by the cells of the pseudo-epithelium, and only as they reach a not inconsiderable size is a definite follicular epithelium formed around them. The follicular epithelium, though not always formed from the pseudo-epithelium, is of course always composed of cells derived from the germinal epithelium.

In all the ova formed at this stage the nucleus would seem to pass through the same metamorphosis as in the embryo.

In the later stages, and even in the full-grown female of Scyllium, fresh ova seem to be formed and nests also to be present. In Raja I have not found freshly formed ova or nests in the adult, and have had no opportunity of studying the young forms.

Summary of observations on the development of the ovary in Scyllium and Raja.

(1.) The ovary in the embryo is a ridge, triangular in section, attached along the base. It is formed of a core of stroma and a covering of epithelium. A special thickening of the epithelium on the outer side forms the true germinal epithelium, to which the ova are confined (Pl. XVII, fig. 1).

In the development of the ovary the stroma becomes differentiated into an external vascular layer, especially developed in the neighbourhood of the germinal epithelium, and an internal lymphatic portion, which forms the main mass of the ovarian ridge (Pl. XVII, figs. 2, 3, and 6).

(2.) At first the thickened germinal epithelium is sharply separated by a membrane from the subjacent stroma (Pl. XVII, figs. 1, 2, and 3), but at about the time when the follicular epithelium commences to be formed round the ova, numerous strands of stroma grow into the epithelium, and form a regular network of vascular channels throughout it, and partially isolate individual ova (Pl. XVII, figs. 7 and 8). At the same time the surface of the epithelium turned towards the stroma becomes irregular (Pl. XVII, fig. 9), owing to the development of individual ova. In still later stages the stroma ingrowths form a more or less definite tunic close to the surface of the ovary. External to this tunic is the superficial layer of the germinal epithelium, which forms what has been spoken of as the pseudo-epithelium. In many instances the protoplasm of its cells is produced into peculiar fibrous tails which pass into the tunic below.

(3.) *Primitive ova.*—Certain cells in the epithelium lining the dorsal angle of the body cavity become distinguished as primitive ova by their abundant protoplasm and granular nuclei, at a very early period in development, even before the formation of the genital ridges. Subsequently on the formation of the genital ridges these ova become confined to the thickened germinal epithelium on the outer aspect of the ridges (Pl. XVII, fig. 1).

(4.) *Conversion of primitive ova into permanent ova.*—Primitive ova may in Scyllium become transformed into permanent ova in two ways—the difference between the two ways being, however, of secondary importance.

(*a.*) A nest of primitive ova makes its appearance, either by continued division of a single primitive ovum or otherwise. The bodies of all the ova of the nest fuse together, and a polynuclear mass is formed, which increases in size concomitantly with the division of its nuclei. The nuclei, moreover, pass through a series of transformations. They increase in size and form delicate vesicles filled with a clear fluid, but contain close to one side a granular mass which stains very deeply with colouring reagents. The granular mass becomes somewhat stellate, and finally assumes a reticulate form with one more highly refracting nucleoli at the nodal points of the reticulum. When a nucleus has reached this condition the protoplasm around it has become slightly granular, and with the enclosed nucleus is seg-

mented off from the nest as a special cell—a permanent ovum (figs. 13, 14, 15, 16). Not all the nuclei in a nest undergo the whole of the above changes; certain of them, on the contrary, stop short in their development, atrophy, and become employed as a kind of pabulum for the remainder. Thus it happens that out of a large nest perhaps only two or three permanent ova become developed.

(*b.*) In the second mode of development of ova the nuclei and protoplasm undergo the same changes as in the first mode; but the ova either remain isolated and never form part of a nest, or form part of a nest in which no fusion of the protoplasm takes place, and all the primitive ova develop into permanent ova. Both the above modes of the formation continue through a great part of life.

(5.) *The follicle.*—The cells of the germinal epithelium arrange themselves as a layer around each ovum, almost immediately after its separation from a nest, and so constitute a follicle. They are at first flat, but soon become more columnar. In Scyllium they remain for a long time uniform, but in large eggs they become arranged in two or three layers, while at the same time some of them become large and flask-shaped, and others small and oval (fig. 29). The flask-shaped cells have probably an important function in the nutrition of the egg, and are arranged in a fairly regular order amongst the smaller cells. Before the egg is quite ripe both kinds of follicle cells undergo retrogressive changes (Pl. XVIII, fig. 23).

In Raja a great irregularity in the follicle cells is obsrveable at an early stage, but as the ovum grows larger the cells gradually assume a regular arrangement more or less similar to that in Scyllium (Pl. XIX, fig. 30—33).

(6.) *The egg membranes.*—Two membranes are probably always present in Elasmobranchs during some period of their growth. The first formed and outer of these arises in some instances before the formation of the follicular epithelium, and would seem to be of the nature of a vitelline membrane. The inner one is the zona radiata with a typical radiately striated structure. It is formed from the vitellus at a much later period than the proper vitelline membrane. It is more developed in Scyllium than in Raja, but atrophies early in both genera. By the time the ovum is nearly ripe both membranes are very much reduced, and when the egg (in Scyllium and Pristiurus) is laid, no trace of any membrane is visible.

(7.) *The vitellus.*—The vitellus is at first faintly granular, but at a later period exhibits a very distinct (protoplasmic) network of fibres, which is still present after the ovum has been laid.

The yolk arises, in the manner described by Gegenbaur, in ova

of about 0·06 mm. as a layer of fine granules, which stain deeply with osmic acid. They are at first confined to a stratum of protoplasm slightly below the surface of the ovum, and are most numerous at the pole furthest removed from the germinal vesicle. They are not regularly distributed, but are aggregated in small masses. They gradually grow into vesicles, in the interior of which oval solid bodies are developed, which form the permanent yolk-spheres. These oval bodies in the later stages exhibit a remarkable segmentation into plates, which gives them a peculiar appearance of transverse striation.

Certain bodies of unknown function are occasionally met with in the vitellus, of which the most remarkable are those figured at *x* on Pl. XVIII, fig. 25, A.

(8). *The germinal vesicle.*—A reticulum is very conspicuous in the germinal vesicle in the freshly formed ova, but becomes much less so in older ova, and assumes, moreover, a granular appearance. At first one to three nucleoli are present, but they gradually increase in number as the germinal vesicle grows older, and are frequently situated in close proximity to the membrane.

The Mammalian Ovary (Pl. XIX, figs. 35—41).

The literature of the mammalian ovary has been so often dealt with that it may be passed over with only a few words. The papers which especially call for notice are those of Pflüger,[1] Ed. van Beneden,[2] and especially Waldeyer,[3] as inaugurating the newer view on the nature of the ovary, and development of the ova; and of Foulis[4] and Kölliker[5], as representing the most recent utterances on the subject. There are, of course, many points in these papers which are touched on in the sequel, but I may more especially here call attention to the fact that I have been able to confirm van Beneden's statement as to the existence of polynuclear protoplasmic masses. I have found them, however, by no means universal or primitive; and I cannot agree in a general way with van Beneden's account of their occurrence. I have found no trace of a germogene (Keimfache) in the sense of Pflüger and Ed. van Beneden. My own results are most in accordance with those of Waldeyer, with whom I agree in the fundamental propositions that both ovum and follicular epithelium are derived from the germinal epithelium, but I cannot

[1] 'Die Eierstöcke d. Säugethiere u. d. Menschen,' Leipzig, 1863.
[2] 'Composition et Signification de l'œuf Acad. v. de Belgique,' 1868.
[3] 'Eierstock u. Ei.' Leipzig, 1870.
[4] 'Trans. of Roy. Society, Edinburgh,' vol, xxvii, 1875, and this Journal, vol. xvi.
[5] 'Verhandlung d. Phys. Med. Gesellschaft,' Würzburg, 1875, N. F. Bd. viii.

accept his views of the relation of the stroma to the germinal epithelium.

In the very interesting paper of Foulis, the conclusion is arrived at, that while the ova are derived from the germinal epithelium, the cells of the follicle originate from the ordinary connective tissue cells of the stroma. Foulis regards the zona pellucida as a product of the ovum and not of the follicle. To both of these views I shall return, and hope to be able to show that Foulis has not traced back the formation of the follicle through a sufficient number of the earlier stages. It thus comes about that though I fully recognise the accuracy of his figures, I am unable to admit his conclusions. Kölliker's statements are again very different from those of Foulis. He finds certain cords of cells in the hilus of the ovary, which he believes to be derived from the Wolffian body, and has satisfied himself that they are continuous with Pflüger's egg-tubes, and that they supply the follicular epithelium. To the general accuracy of Kölliker's statements with reference to the relations of these cords in the hilus of the ovary I can fully testify, but am of opinion that he is entirely mistaken as to their giving rise to the follicular epithelium, or having anything to do with the ova. I hope to be able to give a fuller account of their origin than he or other observers have done.

My investigations on the mammalian ovary have been made almost entirely on the rabbit—the type of which it is most easy to procure a continuous series of successive stages; but in a general way my conclusions have been controlled and confirmed by observations on the cat, the dog, and the sheep. My observations commence with an embryo of eighteen days. A transverse section, slightly magnified, through the ovary at this stage, is represented on Pl. XIX, fig. 35, and a more highly magnified portion of the same in fig. 35A. The ovary is a cylindrical ridge on the inner side of the Wolffian body, composed of a superficial epithelium, the germinal epithelium (*g. e.*), and of a tissue internal to this, which forms the main mass of it. In the latter two constituents have to be distinguished—(1) an epithelial-like tissue (*t*), coloured brown, which forms the most important element, and (2) vascular and stroma elements in this.

The germinal epithelium is a layer about 0·03—0·04 mm. in thickness. It is (*vide* fig. 35A, *g. e.*) composed of two or three layers of cells, with granular nuclei, of which the outermost layer is more columnar than the remainder, and has elongated rather than rounded nuclei. Its cells, though they vary slightly in size, are all provided with a fair amount of protoplasm, and cannot be divided (as in the case of the germinal epithelium of

Birds, Elasmobranchii, &c.), into primitive ova, and normal epithelial cells. Very occasionally, however, a specially large cell, which, perhaps, deserves the appellation primitive ovum, may be seen. From the subjacent tissue the germinal epithelium is in most parts separated by a membrane-like structure (fluid coagulum); but this is sometimes absent, and it is then very difficult to determine with exactness the inner border of the epithelium. The tissue (*t*), which forms the greater mass of the ovary at this stage, is formed of solid columns or trabeculæ of epithelial-like cells, which present a very striking resemblance in size and character to the cells of the germinal epithelium. The protoplasm of these cells stains slightly more deeply with osmic acid than does that of the cells of the germinal epithelium, so that it is rather easier to note a difference between the two tissues in osmic acid than in picric acid specimens. This tissue approaches very closely, and is in many parts in actual contact with the germinal epithelium. Between the columns of it are numerous vascular channels (shown diagrammatically in my figures) and a few normal stroma cells. This remarkable tissue continues visible through the whole course of the development of the ovary, till comparatively late in life, and during all the earlier stages might easily be supposed to be about to play some part in the development of the ova, or even to be part of the germinal epithelium. It really, however, has nothing to do with the development of the ova, as is easily demonstrated when the true ova begin to be formed. In the later stages, as will be mentioned in the description of those stages, it is separated from the germinal epithelium by a layer of stroma; though at the two sides of the ovary it is, even in later stages, sometimes in contact with the germinal epithelium.

In most parts this tissue is definitely confined within the limits of the ovary, and does not extend into the mesentery by which the ovary is attached. It may, however, be traced *at the anterior end* of the ovary into connection with the walls of the Malpighian bodies, which lie on the inner side of the Wolffian body (*vide* fig. 35B), and I have no doubt that it grows out from the walls of these bodies into the ovary. In the male it appears to me to assist in forming, together with cells derived from the germinal epithelium, the seminiferous tubules, the development of which is already fairly advanced by this stage. I shall speak of it in the sequel as tubuliferous tissue. The points of interest in connection with it concern the male sex, which I hope to deal with in a future paper, but I have no hesitation in identifying it with the segmental cords (*segmentalstränge*) discovered by Braun in Reptilia, and described at

length in his valuable memoir on their urogenital system.[1] According to Braun the segmental cords in Reptilia are buds from the outer walls of the Malpighian bodies. The bud from each Malpighian body grows into the genital ridge before the period of sexual differentiation, and sends out processes backwards and forwards, which unite with the buds from the other Malpighian bodies. There is thus formed a kind of trabecular work of tissue in the stroma of the ovary, which in the Lacertilia comes into connection with the germinal epithelium in both sexes, but in Ophidia in the male only. In the female, in all cases, it gradually atrophies and finally vanishes, but in the male there pass into it the primitive ova, and it eventually forms, with the enclosed primitive ova, the tubuli seminiferi. From my own observations in Reptilia I can fully confirm Braun's statements as to the entrance of the primitive ova into this tissue in the male, and the conversion of it into the tubuli seminiferi. The chief difference between Reptilia and Mammalia, in reference to this tissue, appears to be that in Mammalia it arises only from a few of the Malpighian bodies at the anterior extremity of the ovary, but in Reptilia from all the Malpighian bodies adjoining the genital ridge. More extended observations on Mammalia will perhaps show that even this difference does not hold good.

It is hardly to be supposed that this tissue, which is so conspicuous in all young ovaries, has not been noticed before; but the notices of it are not so numerous as I should have anticipated. His[2] states that the parenchyna of the sexual glands undoubtedly arises from the Wolffian canals, and adds that while the cortical layer (Hulle) represents the earlier covering of a part of the Wolffian body, the stroma of the hilus, with its vessels, arises from a Malpighian body. In spite of these statements of His, I still doubt very much whether he has really observed either the tissue I allude to or its mode of development. In any case he gives no recognisable description or figure of it.

Waldeyer[3] notices this tissue in the dog, cat, and calf. The following is a free translation of what he says, (p. 141): —"In a full grown but young dog, with numerous ripe follicles, there were present in the vascular zone of the ovary numerous branched elongated small columns (Schläuche) of epithelial cells, between which ran blood-vessels. They were only separated from the egg columns of the cortical layer by a row of large follicles. There can be no doubt that we have here remains of the sexual part of the Wolffian body—the canals

[1] 'Arbeiten a. d. Zool. Zoot.,' Institut. Wurzburg, Bd. iv.
[2] 'Archiv f. mikros. Anat.,' vol. i, p. 160.
[3] Loc. cit.

of the parovarium—which in the female sex have developed themselves to an extraordinary extent into the stroma of the sexual gland, and perhaps are even to be regarded as *homologues of the seminiferous tubules* (the italics are my own). I have almost always found the above condition in the dog, only in old animals these seminiferous canals seem gradually to atrophy. Similar columns are present in the cat, only they do not appear to grow so far into the stroma." Identical structures are also described in the calf.

Romiti gives a very similar description to Waldeyer of these bodies in the dog.[1] Born also describes this tissue in young and embryonic ovaries of the horse as the *Keimlager*.[2] The columns described by Kölliker,[3] and believed by him to furnish the follicluar epithelium, are undoubtedly my tubuliferous tissue, and, as Kölliker himself points out, are formed of the same tissue as that described by Waldeyer.

Egli gives a very clear and accurate description of this tissue, though he apparently denies its relation with the Wolffian body.

My own interpretation of the tissue accords with that of Waldeyer. In addition to the rabbit, I have observed it in the dog, cat, and sheep. In all these forms I find that close to the attachment of the ovary, and sometimes well within it, a fair number of distinct canals with a large lumen are present, which are probably to be distinguished from the solid epithelial columns. Such large canals are not as a rule present in the rabbit. In the dog solid columns are present in the embryo, but later they appear frequently to acquire a tubular form, and a lumen. Probably there are great variations in the development of the tissue, since in the cat (not as Waldeyer did in the dog) I have found it most developed.

In the very young embryonic ovary of the cat the columns are very small and much branched. In later embryonic stages they are frequently elongated, sometimes convoluted, and are very similar to the embryonic tubuli seminiferi. In the young stages these columns are so similar to the egg tubes (which agree more closely with Pflüger's type in the cat than in other forms I have worked at) that to any one who had not studied the development of the tissue an embryo cat's ovary at certain stages would be a very puzzling object. I have, however, met with nothing in the cat or any other form which supports Kölliker's views.

My next stage is that of a twenty-two days' embryo. Of this

[1] 'Archiv f. mikr. Anat.,' vol. x.
[2] 'Archiv f. Anatomie. Physiologie, u. wiss. Medicine.' 1874.
[3] Loc. cit.

stage I have given two figures corresponding to those of the earlier stage (fig. 36 and 36A).

From these figures it is at once obvious that the germinal epithelium has very much increased in bulk. It has a thickness 0·1—0·09 mm. as compared to 0·03 mm. in the earlier stage. Its inner outline is somewhat irregular, and it is imperfectly divided into lobes, which form the commencement of structures nearly equivalent to the nests of the Elasmobranch ovary. The lobes *are not* separated from each other by connective tissue prolongations; the epithelium being at this stage perfectly free from any ingrowths of stroma. The cells constituting the germinal epithelium have much the same character as in the previous stage. They form an outer row of columnar cells internal to which the cells are more rounded. Amongst them a few large cells with granular nuclei, which are clearly primitive ova, may now be seen, but by far the majority of the cells are fairly uniform in size, and measure from 0·01—0·02 mm. in diameter, and their nuclei from 0·004—0·006 mm. The nuclei of the columnar outer cells measure about 0·008 mm. They are what would ordinarily be called granular, though high powers show that they have the usual nuclear network. There is no special nucleolus. The rapid growth of the germinal epithelium is due to the division of its cells, and great masses of these may frequently be seen to be undergoing division at the same time. Of the tissue of the ovary internal to the germinal epithelium, it may be noticed that the tubuliferous tissue derived from the Malpighian bodies is no longer in contact with the germinal epithelium, but that a layer of vascular stroma is to a great extent interposed between the two. The vascular stroma of the hilus has, moreover, greatly increased in quantity.

My next stage is that of a twenty six days embryo, but the characters of the ovary at this stage so closely correspond with those of the succeeding one at twenty eight days that, for the sake of brevity, I pass over this stage in silence.

Figs. 37 and 37A are representative sections of the ovary of the twenty-eighth day corresponding with those of the earlier stages.

Great changes have become apparent in the constitution of the germinal epithelium. The vascular stroma of the ovary has grown into the germinal epithelium precisely as in Elasmobranchs. It appears to me clear that the change in the relations between the stroma and epithelium is not due to a mutual growth, but entirely to the stroma, so that, as in the case of Elasmobranchs, the result of the ingrowth is that the germinal epithelium is honeycombed by vascular stroma. The vascular growths generally take the paths of the lines which separated the nests in an earlier condition, and cause these nests to become the egg

tubes of Pflüger. It is obvious in figure 37 that the vascular ingrowths are so arranged as imperfectly to divide the germinal epithelium into two layers separated by a space with connective tissue and blood-vessels. The outer part is relatively thin, and formed of a superficial row of columnar cells, and one or two rows of more rounded cells; the inner layer is much thicker, and formed of large masses of rounded cells. The two layers are connected together by numerous trabeculæ, the stroma between which eventually gives rise to the connective tissue capsule, or tunica albuginea, of the adult ovary.

The germinal epithelium is now about 0·19—0·22 mm. in thickness. Its cells have undergone considerable changes. A fair number of them (fig. 37 *a*, *p.o.*), especially in the outer layer of the epithelium, have become larger than the cells around them, from which they are distinguished, not only by their size, but by their granular nucleus and abundant protoplasm. They are in fact undoubted primitive ova with all the characters which primitive ova present in Elasmobranchs, Aves, &c. In a fairly typical primitive ovum of this stage the body measures 0·02 mm. and the nucleus 0·014 mm. In the inner part of the germinal epithelium there are very few or no cells which can be distinguished by their size as primitive ova, and the cells themselves are of a fairly uniform size, though in this respect there is perhaps a greater variation than might be gathered from fig. 37A. The cells are on the average about 0·016 mm. in diameter, and their nuclei about 0·008—0·001mm., considerably larger, in fact, than in the earlier stage. The nuclei are moreover more granular, and make in this respect an approach to the character of the nuclei of primitive ova.

The germinal epithelium is still rapidly increasing by the division of its cells, and in fig. 37A there are shown two or three nuclei in the act of dividing. I have represented fairly accurately the appearance they present when examined with a moderately high magnifying power. With reference to the stroma of the ovary, internal to the germinal epithelium, it is only necessary to refer to fig. 37 to observe that the tubuliferous tissue (*t*) forms a relatively smaller part of the stroma than in the previous stage, and is also further removed from the germinal epithelium.

My next stage is that of a young rabbit two days after birth, but to economise space I pass on at once to the following stage five days after birth. This stage is in many respects a critical one for the ovary, and therefore of great interest. Figure 38 represents a transverse section through the ovary (on rather a smaller scale than the previous figures) and shows the general relations of the tissues.

The germinal epithelium is very much thicker than before

—about 0·38 mm. as compared with 0·22 mm. It is divided into three obvious layers: (1) an outer epithelial layer which corresponds with the pseudo-epithelial layer of the Elasmobranch ovary, average thickness 0·03 mm. (2) A middle layer of small nests, which corresponds with the middle vascular layer of the previous stage; average thickness 0·1 mm. (3) An inner layer of larger nests; average thickness 0·23 mm.

The general appearance of the germinal epithelium at this stage certainly appears to me to lend support to my view that the whole of it simply constitutes a thickened epithelium interpenetrated with ingrowths of stroma.

The cells of the germinal epithelium, which form the various layers, have undergone important modifications. In the first place a large number of the nuclei—at any rate of those cells which are about to become ova—have undergone a change identical with that which takes place in the conversion of the primitive into the permanent ova in Elasmobranchs. The greater part of the contents of the nucleus becomes clear. The remaining contents arrange themselves as a deeply staining granular mass on one side of the membrane, and later on as a somewhat stellate figure: the two stages forming what were spoken of as the granular and stellate varieties of nucleus. To avoid further circumlocution I shall speak of the nucleus undergoing the granular and the stellate modifications. At a still later period the granular contents form a beautiful network in the nucleus.

The pseudo-epithelium (fig. 38A) is formed of several tiers of cells, the outermost of which are very columnar and have less protoplasm than in an earlier stage. In the lower tiers of cells there are many primitive ova with granular nuclei, and others in which the nuclei have undergone the granular modification. The primitive ova are almost all of the same size as in the earlier stage. The pseud-epithelium is separated from the middle layer by a more or less complete stratum of connective tissue, which, however, is traversed by trabeculæ connecting the two layers of the epithelium. In the middle layer there are comparatively few modified nuclei, and the cells still retain for the most part their earlier characters. The diameter of the cells is about 0·012 mm., and that of the nucleus about 0·008 mm. In the innermost layer (fig. 38B), which is not sharply separated from the middle layer, the majority of the cells, which in the previous stage were ordinary cells of the epithelium, have commenced to acquire modified nuclei. This change, which first became apparent to a small extent in the young two days after birth, is very conspicuous at this stage. In some of the cells the nucleus is modified in the granular manner, in others in the stellate, and in a certain

number the nucleus has assumed a reticular structure characteristic of the young permanent ovum.

In addition, however, to the cells which are becoming converted into ova, a not inconsiderable number may be observed, if carefully looked for, which are for the most part smaller than the others, generally somewhat oval, and in which the nucleus retains its primitive characters. A fair number of such cells are represented in fig. 38B. In the larger ones the nucleus will perhaps eventually become modified; but the smaller cells clearly correspond with the interstitial cells of the Elasmobranch germinal epithelium, and are destined to become converted into the epithelium of the Graafian follicle. In some few instances indeed (at this stage very few), in the deeper part of the germinal epithelium, these cells commence to arrange themselves round the just formed permanent ova as a follicular epithelium. An instance of this kind is shown in fig. 38B, *o*. The cells with modified nuclei, which are becoming permanent ova, usually present one point of contrast to the homologous cells in Elasmobranchs, in that they are quite distinct from each other, and not fused into a polynuclear mass. They have around them a dark contour line, which I can only interpret as the commencement of the membrane (zona radiata?), which afterwards becomes distinct, and which would thus seem, as Foulis has already insisted, to be of the nature of a vitelline membrane.

In a certain number of instances the protoplasm of the cells which are becoming permanent ova appears, however, actually to fuse, and polynuclear masses identical with those in Elasmobranchs are thus formed (cf. E. van Beneden).[1] These masses become slightly more numerous in the succeeding stages. Indications of a fusion of this kind are shown in fig. 38B. That the polynuclear masses really arise from a fusion of primitively distinct cells is clear from the description of the previous stages. The ova in the deeper layers, with modified granular nuclei, measure about 0·016—0·02 mm., and their nuclei from 0·01—0·012 mm.

With reference to the tissue of the hilus of the ovary, it may be noticed that the tubuliferous tissue (*t*) is relatively reduced in quantity. Its cells retain precisely their previous characters.

The chief difference between the stage of five days and that of two days after birth consists in the fact that during the earlier stage comparatively few modified nuclei were present, but the nuclei then presented the character of the nuclei of primitive ova.

I have ovaries both of the dog and cat of an equivalent stage, and in both of these the cells of the nests or egg tubes may be divided into two categories, destined respectively to become ova and follicle cells. Nothing which has come under my notice tends

[1] Loc. cit.

to show that the tubuliferous tissue is in any way concerned in supplying the latter form of cell.

In a stage, seven days after birth, the same layers in the germinal epithelium may be noticed as in the last described stage. The outermost layer or pseudo-epithelium contains numerous developing ova, for the most part with modified nuclei. It is separated by a well marked layer of connective tissue from the middle layer of the germinal epithelium. The outer part of the middle layer contains more connective tissue and smaller nests than in the earlier stage, and most of the cells of this layer contain modified nuclei. In a few nests the protoplasm of the developing ova forms a continuous mass, not divided into distinct cells, but in the majority of instances the outline of each ovum can be distinctly traced. In addition to the cells destined to become ova, there are present in these nests other cells, which will clearly form the follicular epithelium. A typical nest from the middle layer is represented on Plate XIX, fig. 39 A.

The nests or masses of ova in the innermost layer are for the most part still very large, but, in addition to the nests, a few isolated ova, enclosed in follicles, are to be seen.

A fairly typical nest, selected to show the formation of the follicle, is represented on Plate XIX, fig. 39 B.

The nest contains (1) fully formed permanent ova, completely or wholly enclosed in a follicle. (2) Smaller ova, not enclosed in a follicle. (3) Smallish cells with modified nuclei of doubtful destination. (4) Small cells obviously about to form follicular epithelium.

The inspection of a single such nest is to my mind a satisfactory proof that the follicular epithelium takes its origin from the germinal epithelium and not from the stroma or tubuliferous tissue. The several categories of elements observable in such a nest deserve a careful description.

(1) *The large ova in their follicles.*—These ova have precisely the character of the young ova in Elasmobranchs. They are provided with a granular body invested by a delicate, though distinct membrane. Their nucleus is large and clear, but traversed by the network so fully described for Elasmobranchs. The cells of their follicular epithelium have obviously the same character as many other small cells of the nest. Two points about them deserve notice—(*a*) that many of them are fairly columnar. This is characteristic only of the first formed follicles. In the later formed follicles the cells are always flat and spindle-shaped in section. In this difference between the early and late formed follicles Mammals agree with Elasmobranchs. (*b*) The cells of the follicle are much more columnar towards the inner side than towards the outer. This point also is common to Mammals and Elasmobranchs.

Round the completed follicle a very delicate membrana propria folliculi appears to be present.[1]

The larger ova, with follicular epithelium, measure about 0·04 mm., and their nucleus about 0·02 mm., the smaller ones about 0·022 mm., and their nucleus about 0·014 mm.

(2) *Medium sized ova.*—They are still without a trace of a follicular epithelium, and present no special peculiarities.

(3) *The smaller cells with modified nuclei.*—I have great doubt as to what is the eventual fate of these cells. There appear to be three possibilities.

(*a*) That they become cells of the follicular epithelium; (*b*) that they develop in ova; (*c*) that they are absorbed as a kind of food by the developing ova. I am inclined to think that some of these cells may have each of the above-mentioned destinations.

(4) *The cells which form the follicle.*—The only point to be noticed about these is that they are smaller than the indifferent cells of the germinal epithelium, from which they no doubt originate by division. This fact has already been noticed by Waldeyer.

The isolated follicles at this stage are formed by ingrowths of connective tissue cutting off fully formed follicles from a nest. They only occur at the very innermost border of the germinal epithelium. This is in accordance with what has so often been noticed about the mammalian ovary, viz. that the more advanced ova are to be met with in passing from without inwards.

By the stage seven days after birth the ovary has reached a sufficiently advanced stage to answer the more important question I set myself to solve, nevertheless, partly to reconcile the apparent discrepancy between my account and that of Dr. Foulis, and partly to bring my description up to a better known condition of the ovary, I shall make a few remarks about some of the succeeding stages.

In a young rabbit about four weeks old the ovary is a very beautiful object for the study of the nuclei, &c.

The pseudo-epithelium is now formed of a single layer of columnar cells, with comparatively scanty protoplasm. In it there are present a not inconsiderable number of developing ova.

A layer of connective tissue—the albuginea—is now present below the pseudo-epithelium, which contains a few small nests with very young permanent ova. The layer of medium sized nests internal to the albuginea forms a very pretty object in well stained sections, hardened in Kleinenberg's picric acid. The ova in it have all assumed the permanent form, and are provided with

[1] Loc. cit., Waldeyer, p. 23, denies the existence of this membrane for Mammalia. It certainly is not so conspicuous as in some other types, but appears to me nevertheless to be always present.

beautiful reticulate nuclei, with, as a rule, one more especially developed nucleolus, and smaller granular bodies. Their diameter varies from about 0·028 to 0·04 mm. and that of their nucleus from 0·016 to 0·02 mm. The majority of these ova are not provided with a follicular investment, but amongst them are numerous small cells, clearly derived from the germinal epithelium, which are destined to form the follicle (*vide* fig. 40 A and B). In a few cases the follicles are completed, and are then formed of very flattened spindle-shaped (in section) cells. In the majority of cases all the ova of each nest are quite distinct, and each provided with a delicate vitelline membrane (fig. 40 A). In other instances, which, so far as I can judge, more common than in the previous stages, the protoplasm of two or more ova is fused together.

Examples of this are represented in Pl. XIX, fig. 40 A. In some of these the nuclei in the undivided protoplasm are all of about the same size and distinctness, and probably the protoplasm eventually becomes divided up into as many ova as nuclei; in other cases, however, one or two nuclei clearly preponderate over the others, and the smaller nuclei are indistinct and hazy in outline. In these latter cases I have satisfied myself as completely as in the case of Elasmobranchs, that only one or two ova (according to the number of distinct nuclei) will develop out of the polynuclear mass, and that the other nuclei atrophy, and the material of which they were composed, serves as the nutriment for the ova which complete their development. This does not, of course, imply that the ova so formed have a value other than that of a single cell, any more than the development of a single embryo out of the many in one egg capsule implies that the embryo so developing is a compound organism.

In the innermost layer of the germinal epithelium the outlines of the original large nests are still visible, but many of the follicles have been cut off by ingrowths of stroma. In the still intact nests the formation of the follicles out of the cells of the germinal epithelium may be followed with great advantage. The cells of the follicle, though less columnar than was the case at an earlier period, are more so than in the case of follicles formed in the succeeding stages. The previous inequality in the cells of the follicles is no longer present.

The tubuliferous tissue in the zona vasculosa appears to me to have rather increased in quantity than the reverse; and is formed of numerous solid columns or oval masses of cells, separated by strands of connective tissue, with typical spindle nuclei.

It is partially intelligible to me how Dr. Foulis might from an examination of the stages similar to this, conclude that the

follicle cells were derived from the stroma; but even at this stage the position of the cells which will form the follicular epithelium, their passage by a series of gradations into obvious cells of the germinal epithelium and the peculiarities of their nuclei, so different from those of the stroma cells, supply a sufficient series of characters to remove all doubt as to the derivation of the follicle cells. Apart from these more obvious points, an examination of the follicle cells from the surface, and not in section, demonstrates that general resemblance in shape of follicle cells to the stroma cells is quite delusory. They are in fact flat, circular, or oval, plates not really spindle-shaped, but only apparently so in section. While I thus fundamentally differ from Foulis as to the nature of the follicle cells, I am on this point in complete accordance with Waldeyer, and my own results with reference to the follicle cannot be better stated than in his own words (pp. 43, 44).

At six weeks after birth the ovary of the rabbit corresponds very much more with the stages in the development of the ovary, which Foulis has more especially studied, for the formation of the follicular epithelium, than during the earlier stages. His figure ('Quart. Journ. Mic. Sci.,' Pl. XVII, fig. 6) of the ovary of a seven and a half months' human fœtus is about the corresponding age. Different animals vary greatly in respect to the relative development of the ovary. For example, the ovary of a lamb at birth about corresponds with that of a rabbit six weeks after birth. The points which may be noticed about the ovary at this age are first that the surface of the ovary begins to be somewhat folded. The appearances of these folds in section have given rise, as has already been pointed out by Foulis, to the erroneous view that the germinal epithelium (pseudo-epithelium) became involuted in the form of tubular open pits. The folds appear to me to have no connection with the formation of ova, but to be of the same nature as the somewhat similar folds in Elasmobranchs. A follicular epithelium is present around the majority of the ova of the middle layer, and around all those of the inner layer of the germinal epithelium. The nests are, morever, much more cut up by connective tissue ingrowths than in the previous stages.

The follicle cells of the middle layers are very flat, and spindle-shaped in section, and though they stain more deeply than the stroma cells, and have other not easily characterised pecularities, they nevertheless do undoubtedly closely resemble the stroma cells when viewed (as is ordinarily the case) in optical section.

In the innermost layer many of the follicles with the enclosed ova have advanced considerably in development and are formed of columnar cells. The somewhat heterodox view of these cells propounded by Foulis I cannot quite agree to. He says ('Quart.

Mic., Sci.,' p. 210): "The protoplasm which surrounds the vesicular nuclei acts as a sort of cement substance, holding them together in the form of a capsular membrane round the young ovum. This capsular membrane is the first appearance of the membrana granulosa." I must admit that I find nothing similar to this, nor have I met with any special peculiarities (as Foulis would seem to indicate) in the cells of the germinal epithelium or other cells of the ovary.

Figure 41 is a representation of an advanced follicle of a six weeks rabbit, containing two ova, which is obviously in the act of dividing into two. Follicles of this kind with more than one ovum are not very uncommon. It appears to me probable that follicles, such as that I have figured, were originally formed of a single mass of protoplasm with two nuclei; but that instead of one of the nuclei atrophying, both of them eventually developed and the protoplasm subsequently divided into two masses. In other cases it is quite possible that follicles with two ova should should rather be regarded as two follicles not separated by a septum of stroma.

On the later stages of development of the ovary I have no complete series of observations. The yolk spherules I find to be first developed in a peripheral layer of the vitellus. I have not been able definitely to decide the relation of the zona radiata to the first formed vitelline membrane. Externally to the zona radiata there may generally be observed a somewhat granular structure, against which the follicle cells abut, and I cannot agree with Waldeyer (loc. sit., p. 40) that this structure is continuous with the cells of the discus, or with the zona radiata. Is it the remains of the first formed vitelline membrane? I have obtained some evidence in favour of this view, but have not been successful in making observations to satisfy me on the point, and must leave open the question whether my vitelline membrane becomes the zona radiata or whether the zona is not a later and independent formation, but am inclined myself to adopt the latter view. The first formed membrane, whether or no it becomes the zona radiata, is very similar to the vitelline membrane or Elasmobranchs and arises at a corresponding stage.

Summary of observations on the mammalian ovary.—The general results of my observations on the mammalian ovary are the following:—

(1) The ovary in an eighteen days' embryo consists of a cylindrical ridge attached along the inner side of the Wolffian body, which is formed of two parts; (*a*) an external epithelium —two or three cells deep (the germinal epithelium); (*b*) a hilus or part forming in the adult the vascular zone, at this stage

composed of branched masses of epithelial tissue (tubuliferous tissue) derived from the walls of the anterior Malpighian bodies, and numerous blood-vessels, and some stroma cells.

(2) The germinal epithelium gradually becomes thicker, and after a certain stage (twenty-three days) there grow into it numerous stroma ingrowths, accompanied by blood-vessels. The germinal epithelium thus becomes honeycombed by strands of stroma. Part of the stroma eventually forms a layer close below the surface, which becomes in the adult the tunica albuginea. The part of the germinal epithelium external to this layer becomes reduced to a single row of cells, and forms what has been spoken of in this paper as the pseudo-epithelium of the ovary. The greater part of the germinal epithelium is situated internal to the tunica albuginea, and this part is at first divided up by strands of stroma into smaller divisions externally, and larger ones internally. These masses of germinal epithelium (probably sections of branched trabeculæ) may be spoken of as nests. In the course of the development of the ova they are broken up by stroma ingrowths, and each follicle with its enclosed ovum is eventually isolated by a layer of stroma.

(3) The cells of the germinal epithelium give rise both to the permanent ova and to the cells of the follicular epithelium. For a long time, however, the cells remain indifferent, so that the stages, like those in Elasmobranchs, Osseous Fish, Birds, Reptiles, &c., with numerous primitive ova embedded amongst the small cells of the germinal epithelium, are not found.

(4) The conversion of the cells of the germinal epithelium into permanent ova commences in an embryo of about twenty-two days. All the cells of the germinal epithelium appear to be capable of becoming ova: the following are the stages in the process, which are almost identical with those in Elasmobranchs:—

(*a*) The nucleus of the cells loses its more or less distinct network, and becomes very granular, with a few specially large granules (nucleoli). The protoplasm around it becomes clear and abundant—primitive ovum stage. It may be noted that the largest primitive ova are very often situated in the pseudo-epithelium. (*b*) A segregation takes place in the contents of the nucleus within the membrane, and the granular contents pass to one side, where they form an irregular mass, while the remaining space within the membrane is perfectly clear. The granular mass gradually develops itself into a beautiful reticulum, with two or three highly refracting nucleoli, one of which eventually becomes the largest and forms the germinal spot *par excellence*. At the same time the body of the ovum becomes slightly granular. While the above changes, more especially

those in the nucleus, have been taking place, the protoplasm of two or more ova may fuse together, and polynuclear masses be so formed. In some cases the whole of such a polynuclear mass gives rise to only a single ovum, owing to the atrophy of all the nuclei but one, in others it gives rise by subsequent division to two or more ova, each with a single germinal vesicle.

(5) All the cells of a nest do not undergo the above changes, but some of them become smaller (by division) than the indifferent cells of the germinal epithelium, arrange themselves round the ova, and form the follicular epithelium.

(6) The first membrane formed round the ovum arises in some cases even before the appearance of the follicular epithelium, and is of the nature of a vitelline membrane. It seems probable, although not definitely established by observation, that the zona radiata is formed internally to the vitelline membrane, and that the latter remains as a membrane, somewhat irregular on its outer border, against which the ends of the follicle cells abut.

General Observations on the Structure and Development of the Ovary.

In selecting Mammalia and Elasmobranchii as my two types for investigation, I had in view the consideration that what held good for such dissimilar forms might probably be accepted as true for all Vertebrata with the exception of Amphioxus.

The structure of the ovary.—From my study of these two types, I have been led to a view of the structure of the ovary, which differs to a not inconsiderable extent from that usually entertained. For both types the conclusion has been arrived at that the whole egg-containing part of the ovary is really *the thickened germinal epithelium*, and that it differs from the original thickened patch or layer of germinal epithelium, mainly in the fact that it is broken up into a kind of meshwork by growths of vascular stroma. If the above view be accepted for Elasmobranchii and Mammalia, it will hardly be disputed for the ovaries of Reptilia and Aves. In the case also of Osseous Fish and Amphibia, this view of the ovary appears to be very tenable, but the central core of stroma present in the other types is nearly or quite absent, and the ovary is entirely formed of the germinal epithelium with the usual strands of vascular stroma.[1] It is obvious that according to the above view Pflüger's egg-tubes are merely trabeculæ of germinal epithelium, and have no such importance as has been attributed to them. They are present in a more or less modified form in all types of

[1] My view of the structure of the ovary would seem to be that held by Götte, 'Entwicklungsgeschichte d. Unke,' p. 14 and 15.

ovaries. Even in the adult Amphibian ovary, columns of cells of the germinal epithelium, some indifferent, others already converted into ova, are present, and, as has been pointed out by Hertwig,[1] represent Pflüger's egg-tubes.

The formation of the permanent ova.—The passage of primitive ova into permanent ova is the part of my investigation to which the greatest attention was paid, and the results arrived at for Mammalia and Elasmobranchii are almost identical. Although there are no investigations as to the changes undergone by the nucleus in other types, still it appears to me safe to conclude that the results arrived at hold good for Vertebrates generally.[2] As has already been pointed out the transformation which the so-called primitive ova undergo is sufficient to shew that *they are not to be regarded as ova but merely as embryonic sexual cells.* A feature in the transformation, which appears to be fairly constant in Scyllium, and not uncommon in the rabbit, is the fusion of the protoplasm of several ova into a syncytium, the subsequent increase in the number of nuclei in the syncytium, the atrophy and absorption of a portion of the nuclei, and the development of the remainder into the germinal vesicles of ova; the vitellus of each ovum being formed by a portion of the protoplasm of the syncytium.

As to the occurrence of similar phenomena in the Vertebrata generally, it has already been pointed out that Ed. van Beneden has described the polynuclear masses in Mammalia, though he does not appear to me to have given a complete account of their history. Götte[3] describes a fusion of primitive ova in Amphibia, but he believes that the nuclei fuse as well as the bodies of the ova, so that one ovum (according to his view no longer a cell) is formed by the fusion of several primitive ova with their nuclei. I have observed nothing which tends to support Götte's view about the fusion of the nuclei, and regard it as very improbable. As regards the interpretation to be placed upon the nests formed of fused primitive ova, Ed. van Beneden maintains that they are to be compared with the upper ends of the egg tubes of Insects, Nematodes, Trematodes, &c. There is no doubt a certain analogy between the two, in that in both cases certain nuclei of a polynuclear mass increase in size, and with the protoplasm around them become segmented off from the remainder of the mass as ova, but the analogy cannot be pressed. The primitive ova, or even the general germinal epithelium, rather

[1] Loc. cit., 36.

[2] Since writing the above I have made out that in the Reptilia the formation of the permanent ova takes place in the same fashion as in Elasmobranchii and Mammalia.

[3] 'Entwicklungsgeschichte, d Unke.'

than these nests, must be regarded as giving origin to the ova, and the nests should be looked on, in my opinion, as connected more with the nutrition than with the origin of the ova. In favour of this view is the fact that as a rule comparatively few ova are developed from the many nuclei of a nest; while against the comparison with the egg tubes of the Invertebrata it is to be borne in mind that many ova appear to develop independently of the nests.

In support of my view about the nests there may be cited many analogous instances from the Invertebrata. In none of them, however, are the phenomena exactly identical with those in Vertebrata. In the ovary of many Hydrozoa (*e.g. Tubularia mesembryanthemum*,) out of a large number of ova which develop up to a certain point, a comparatively very small number survive, and these regularly feed upon the other ova. During this process the boundary between a large ovum and the smaller ova is indistinct: in the outermost layer of a large ovum a number of small ova are embedded, the outlines of the majority of which have become obscure, although they can still be distinguished. Just beyond the edge of a large ovum the small ova have begun to undergo retrogressive changes; while at a little distance from the ovum they are quite normal. An analogous phenomenon has been very fully described by Weismann[1] in the case of Leptodera, the ovary of which consists of a germogene, in which the ova develop in groups of four. Each group of four occupies a separate chamber of the ovary, but in summer only one of the four eggs (the third from the germogene) developes into an ovum, the other three are used as pabulum. In the case of the winter eggs the process is carried still further, in that the contents of the alternate chambers, instead of developing into ova, are entirely converted, by a series of remarkable changes, into nutritive reservoirs. Fundamentally similar occurrences to the above are also well known in Insects. Phenomena of this nature are obviously in no way opposed to the view of the ovum being a single cell.

With reference to the origin of the primitive ova, it appears to me that their mode of development in Mammals proves beyond a doubt that they are modified cells of the germinal epithelium. In Elasmobranchü their very early appearance, and the difficulty of finding transitional forms between them and ordinary cells of the germinal epithelium, caused me at one time to seek (unsuccessfully) for a different origin for them. Any such attempts appear to me, however, out of the question in the case of Mammals.

The egg membranes.—The homologies of the egg membranes in the Vertebrata are still involved in some obscurity. In Elasmobranchii there are undoubtedly two membranes present. (1) An outer and first formed membrane—the albu-

[1] 'Zeit. für wiss., Zool,' Bd., xxvii.

minous membrane of Gegenbaur—which, in opposition to previous observers, I have been led to regard as a vitelline membrane. (2) An inner radiately striated membrane, formed as a differentiation of the surface of the yolk at a later period. Both these membranes usually atrophy before the ovum leaves the follicle. In Reptilia[1] precisely the same arrangement is found as in Elasmobranchii, except that as a rule the zona radiata is relatively more important. The vitelline membrane external to this (or as it is usually named the chorion) is, as a rule, thin in Reptilia; but in Crocodilia is thick (Gegenbaur), and approaches the condition found in Scyllium and other Squalidæ. It appears, as in Elasmobranchs, to be formed before the zona radiata. A special internal differentiation of the zona radiata is apparently found (Eimer) in many Reptilia. No satisfactory observations appear to be recorded with reference to the behaviour of the two reptilian membranes as the egg approaches maturity. In Birds[2] the same two membranes are again found. The first formed and outer one is, according to Gegenbaur and E. van Beneden, a vitelline membrane; and from the analogy of Elasmobranchii I feel inclined to accept their view. The inner one is the zona radiata, which disappears comparatively early, leaving the ovum enclosed only by the vitelline membrane, when it leaves the follicle. All the large-yolked vertebrate ova appear then to agree very well with Elasmobranchs in presenting during some period of their development the two membranes above mentioned.

Osseous fish have almost always a zona radiata, which it seems best to assume to be equivalent to that in Elasmobranchs. Internal to this is a thin membrane, the equivalent, according to Eimer, of the membrane found by the same author within the zona in Reptilia. A membrane equivalent to the thick vitelline membrane of Elasmobranchii would seem to be absent in most instances, though a delicate membrane, external to the zona, has not infrequently been described; Eimer more especially asserts that such a membrane exists in the perch within the peculiar mucous covering of the egg of that fish.

In Petromyzon, a zona radiata appears to be present,[3] which is divided in the adult into two layers, both of them perforated. The inner of the two perhaps corresponds with the membrane internal to the zona radiata in other types. In Amphibia the single late formed and radiately striated (Waldeyer) membrane would appear to be a zona radiata. If the suggestion on page 431 turns out to be correct the ova of Mammalia possess

[1] Gegenbaur, loc. cit.; Waldeyer, loc. cit.; Eimer, loc. cit.; and Ludwig, loc. cit.

[2] Gegenbaur, Waldeyer, E. van Beneden, Eimer.

[3] 'Carlherla, 'Zeit. f. wiss. Zool.,' Bd. xxx.

both a vitelline membrane and zona radiata. E. Van Beneden[1] has, moreover, shown that they are also provided at a certain period with a delicate membrane within the zona.

The reticulum of the germinal vesicle.—In the course of description of the ovary it has been necessary for me to enter with some detail into the structure of the nucleus, and I have had occasion to figure and describe a reticulum identical with that recently described by so many observers. The very interesting observations of Dr. Klein in the last number of this Journal have induced me to say one or two words in defence of some points in my description of the reticulum. Dr. Klein says, on page 323, "I have distinctly seen that when nucleoli are present—the instances are fewer than is generally supposed; they are accumulations of the fibrils of the network." I have no doubt that Klein is correct in asserting that nucleoli are fewer than is generally supposed; and that in many of these instances what are called nucleoli are accumulations, "natural or artificial," of the fibrils of the network; but I cannot accept the universality of the latter statement, which appears to me most certainly not to hold good in the case of ova, in which nucleoli frequently exist in the absence of the network.

Again, I find that at the point of intersection of two or more fibrils there is, as a rule, a distinct thickening of the matter of the fibrils, and that many of the dots seen are not merely, as Dr. Klein would maintain, optical sections of fibrils.

It appears to me probable that both the network and the nucleoli are composed of the same material—what Hertwig calls nuclear substance—and if Dr. Klein merely wishes to assert this identity in the passage above quoted, I am at one with him.

Although a more or less distinct network is present in most nuclei (I have found it in almost all embryonic nuclei) it is not universally so. In the nuclei of primitive ova I have no doubt that it is absent, though present in the unmodified nuclei of the germinal epithelium; and it is present only in a very modified form in the nuclei of primitive ova undergoing a transformation into permanent ova. The absence of the reticulum does not, of course, mean that the substance capable of forming a reticulum is absent, but merely that it does not assume a particular arrangement.

One of the most interesting points in Klein's paper, as well as in those of Heitzmann and Eimer, is the demonstration of a connection between the reticulum of the nucleus and fibres in the body of the cell. Such a connection I have not found in ova, but may point out that it appears to exist between the subgerminal nuclei in Elasmobranchs and the protoplasmic network in the yolk

[1] Loc. cit.

in which they lie. This point is called attention to in my 'Monograph on Elasmobranch Fishes,' page 39, where it is stated that "the network in favorable cases may be observed to be in connection with the nuclei just described. Its meshes are finer in the vicinity of the nuclei, and the fibres in some cases appear almost to start from them." The nuclei in the yolk are knobbed bodies divided by a sponge work of septa into a number of areas each with a nucleolar body.

The Reproduction *of* Lichens *and the* Sexuality *of the* Ascomycetes.

By Sydney H. Vines. M.A., Christ's College, Cambridge. With Plate XX.

In a previous number of this Journal[1] an account was given of the present state of the "lichen-gonidia question," in which especial reference was made to the researches of Stahl into the significance of the gonidia occurring in the hymenium of various lichens. Those researches, it was pointed out, afforded strong evidence in favour of Schwendener's theory of the nature of lichens. Stahl's further researches,[2] of which a brief *résumé* will here be given, into the processes of their reproduction, seem to place the soundness of this theory beyond reasonable doubt.

No less than four distinct organs have been discovered at various times in the thallus of lichens, all of which have been regarded as being connected with their reproduction, viz. (1) Soredia, (2) Spermogonia, bearing Spermatia, (3) Apothecia, containing asci in which spores are formed, (4) Pycnidia, giving rise to Stylospores.

The Soredia were first mentioned by Acharius,[3] their structure was accurately described by Tulasne,[4] and their development was fully made out by Schwendener.[5] Their significance appears to have been also completely understood by Wallroth and Meyer. A soredium consists of one or more gonidia surrounded by a mass of hyphæ, and when it is

[1] April, 1878.

[2] 'Ueb. die Geschlechtliche Fortpflanzung der Collemaceen,' 1877.

[3] 'Lichenographia Universalis,' 1810.

[4] L. R. Tulasne, 'Mém. sur les Lichens,' 1852, p. 24.

[5] In Nägeli's 'Beitr. zur Wiss. Bot,' Heft. ii, 1860; also, 'Flora,' 1863.

thrown off from the thallus, it directly grows into a new individual.

The other organs are of more complicated structure, and until recently, their significance was by no means fully understood. The Spermogonia were first recognised as being distinct organs by Itzigsohn,[1] who believed them to be antheridia containing motile antherozoids. Tulasne, however, pointed out that the spermatia with which they are filled are not motile, and that the movement observed by Itzigsohn was merely molecular; he considered that they were comparable to the non-motile antherozoids of the Florideæ. More recently Cornu[2] has shown that spermatia may be induced to germinate when they are placed under favorable conditions, and he is therefore inclined to regard them as conidia developed in special receptacles, which differ from the ordinary conidia in size only. In the introductory paragraphs of his paper Stahl argues in opposition to the views of Cornu that the fact of their having germinated is no absolute proof of the non-sexual nature of the spermatia, and he appeals to the growth of pollen-tubes in solutions of sugar in support of his argument.[3] He also points out that Cornu was unsuccessful in inducing the germination of spermatia derived from lichens. The facts upon which Stahl bases his argument in favour of the sexual nature of the spermatia of lichens will be stated in considering the development of the apothecia.

The structure of the Apothecia had been carefully investigated and described by Tulasne in his above-mentioned work. Schwendener[4] had shown that the hyphæ from which the asci were developed were independent of those which bore the paraphyses, and Fuisting[5] had distinguished the former group of hyphæ as "ascogenous hyphæ," and had shown that the excipulum and the paraphyses were developed from the same kind of hyphal filaments, but no accurate knowledge as to the very earliest stages of their development had been obtained until the publication of Stahl's researches. Possibly, as he himself points out, the early stages of development

[1] 'Bot. Zeit.,' 1850. Up to this time the Spermogonia had been regarded as distinct genera of Lichens, Pyrenotheca (Fries), Thrombium (Wallroth), just as the Soredia had been regarded by Acharius as forming the genus Variolaria.

[2] 'Ann. d. Sci. Nat.,' sér. vi, t. iii, 1876.

[3] See the recent observations of Tomaschek, "Ueb. die Entwick. der Pollenpflänzchen des Colchicum Autumnale." 'Sitzber. d. k. Akad. d. Wiss.,' lxxvi, 1877, Wien.

[4] 'Flora,' 1864.

[5] 'Bot. Zeit.,' 1868.

which he describes, had been seen, but had been misinterpreted by Gibelli.[1]

In the thallus of *Collema microphyllum*, Stahl detected certain organs to which he gives the name of Carpogonia. Each carpogonium consists of a hyphal filament deeply placed in the thallus, which is coiled on itself (generally two or three times), and is then continued straight to the free surface of the thallus, above which its terminal cell projects (Fig. 1). To the spirally wound portion he gives the name of Ascogonium, to the straight portion that of Trichogyne.

Not unfrequently spermatia could be seen adhering in considerable number to the projecting (Fig. 2, *a*) cell of the trichogyne. It will be readily understood that they must have been conveyed there by means of water. In some cases Stahl was able to detect (Fig. 2, *b*) a canal which placed the contents of the spermatium in connection with those of the terminal cell of the trichogyne. The result of this is that the cells of the trichogyne wither and disappear, and that certain processes of growth of the cells of the ascogonium are initiated, which may be briefly described as follows:

The cells first of all increase in size, and then they undergo division. As a result of this the spiral arrangement of the cells becomes less and less conspicuous, for the cells gradually separate from one another. Whilst these changes have been taking place in the ascogonium, it has become invested by a dense felt-work of hyphæ formed by the active growth of the hyphæ of the thallus. From this investing layer hyphæ grow inwards between the separating coils of the ascogonium and bear paraphyses, which form the rudimentary hymenium. At the same time outgrowths have been formed from the cells of the ascogonium, which either are asci or grow into hyphal filaments, which bear asci as lateral branches (Fig. 3). The asci, whether derived directly or indirectly from the cells of the ascogonium, come to lie in the hymenium among the paraphyses. At this period of its development the apothecium possesses all the elements which usually enter into its composition. Most externally is a dense layer of interlaced hyphæ forming the hypothecium, the inner portion of which consists of a layer of pseudo-parenchymatous tissue, the excipulum proprium, within this is the layer of ascogenous filaments, and most internally lies the hymenium consisting of paraphyses derived more or less directly from the hypothecium and of asci derived more or less directly from the ascogonium.

In various species of Physma, Mass. (Lempholemma,

[1] 'Nuov-giorn. Bot. Ital.,' 1870.

Körber), the ascogonia are formed in immediate proximity to the spermogonia, but in other respects they resemble those of Collema, and their subsequent history is the same.

It appears from these observations that the development of the apothecia of lichens is the result of the fertilisation of carpogonia by means of spermatia. *Collema pulposum* affords negative evidence in support of this statement, for in large thalli of this Lichen Stahl was unable to find any spermogonia, and at the same time he failed to detect any indications of developing apothecia. His investigation of *Synechoblastus conglomeratus*, of *Leptogium Hildenbrandii* and *microscopicum*, and of such heteromerous lichens as *Parmelia stellaris* and *pulverulenta* completely confirmed the above conclusions.

The resemblance existing between the apothecia of lichens and the fructifications of ascomycetous fungi had long been remarked. Now that the development of the apothecia has been completely traced, and their origin from the colourless filaments only of the thallus (except in so far as the gonidia of the thallus give rise to hymenial gonidia) has been placed beyond doubt, there is sufficient evidence to justify a classification which places lichens among the Ascomycetes in the class Carposporeæ. Collateral evidence is obtained by a comparison of the mode of fertilisation of the carpogonia of lichens with that of the Floridea. In both groups the sexual cells are completely differentiated, and in both the male cells are not motile. Stahl very naturally suggests the propriety of calling the male cells of the Florideæ not antherozoids, but spermatia.

The evident sexuality of the lichens has an important bearing upon the vexed question of the sexuality of the whole group of the Ascomycetes, to which they are now attached. The carpogonium of *Ascobolus furfuraceus*[1], one of the Discomycetes, resembles in some respects that of Collema, and the resemblance of the ascogonium of Eurotium[2] and of Sordaria[3] to that of Collema is most striking, but the carpogonium of the lichen is peculiar in that it possesses a trichogyne. In the above-mentioned Ascomycetes fertilisation is effected not by spermatia, but by a kind of conjugation of the ascogonium with another filament, the

[1] Janczewski, "Morph. Unters. üb. Ascobolus furfuraceus." 'Bot. Zeitg.,' 1871.

[2] De Bary, 'Eurotium, etc., nebst Bemerkungen über die Geschlechtsorgane der Ascomyceten,' 1870.

[3] Gilkinet, 'Rech. Morphol. sur les Pyrénomycétes,' J. Sordariées, 1874.

pollinodium; consequently the formation of a trichogyne is unnecessary.

Doubt has, however, been cast upon this interpretation of the coalescence of these two filaments. Van Tieghem[1] considers that, in Eurotium at any rate, this apparently sexual act is merely an instance of a coalescence of hyphal filaments which is by no means uncommon, and his observations upon Chætomium lead him to conclude that not only is such a coalescence unnecessary for the formation of a fructification, but that it tends rather to prevent it. Brefeld[2] also pronounces against the sexuality of the Ascomycetes. He found that ascogenous hyphæ removed from the developing fructification and cultivated in solutions of salts did not form asci, but simply grew out into ordinary hyphal branches. Stahl, in reply to Brefeld's arguments, points out that the development of the one generation from the other is not necessarily connected with the formation of sexual cells or of spores. In mosses, for instance, Stahl[3] and Pringsheim[4] have shown that the cells of the sporogoniam will, under certain circumstances, grow out into protonemal filaments upon which new moss-plants are developed. Here the transition from the one generation (sporophore) to the other (oophore) is effected independently of the spores. In the ferns it appears, from the researches of Farlow,[5] that the prothallus (oophore) may produce a young fern (sporophore) by simple budding without any formation of archegonia. These facts suffice to overthrow the argument founded by Brefeld upon his observations.

The forms of ascomycetous fungi, studied by Van Tieghem and by Brefeld (Peziza, Morchella), apparently do not present that distinct origin from separate groups of hyphæ of the paraphyses and of the asci, which is so prominent a feature in the development of the apothecium of a lichen, and their sexual organs are undeveloped. In the lichens, and some other Ascomycetes, which may be regarded as being the most perfectly developed forms of this group, the evident differentiation accompanying the first formation of the apothecium is accompanied by a well-marked sexuality.

[1] 'Sur le dév. du fuit des Chætomeum et la préteudue sexualité des Ascomycetes,' 1876.

[2] "Die Entwickelungsgeschichte der Basidiomyceten." 'Bot. Zeit.,' 1876.

[3] "Ueb. Protonemabildung an dem Sporogonium der Laubmoose." 'Bot. Zeitg.,' 1876.

[4] "Ueb. Sprossung der Moosfrüchte, &c." 'Jahrb. f. Wiss. Bot.,' 1877.

[5] See this Journal, vol. xiv, 1874.

In a second group, including the Erysipheæ, Eurotium, and others, the differentiation of paraphyses and asci is not so evident, and the sexuality is less marked. In a third group, including Morchella, Peziza, and others, this differentiation does not present itself, and the sexuality cannot be detected.

A very striking instance of this has been described by Bauke[1] in *Pleospora herbarum*, one of the commonest of the Sphæriaceæ (Pyrenomycetes). The formation of the perithecium commences with the enlargement and subsequent division of usually several adjacent cells of a hypha, which may be regarded as a female organ, a carpogonium, but not an ascogonium. In this way a rounded mass of cells is produced, which becomes of a deep brown colour on the exterior. This stage is usually reached in four or five days from the commencement of development, but it is three or four weeks before the formation of the "nucleus," more accurately, of the paraphyses, begins. A number of thin, closely-packed hyphæ, grow out from certain of the parenchymatous cells which lie at about the same level near the base of the young perithecium. These hyphæ absorb not only the contents of the cells forming its central portion, but their thickened cell-walls as well; they seem to perform the same function as the delicate hyphæ, which grow out from the ascogonium of Penicillium. The perithecium may undergo a period of inactivity, but this is by no means necessary, before the asci are formed. They arise as outgrowths from the basal cells of the paraphyses. Preparations which show the development of the young asci very clearly present no appearance which could possibly indicate anything like a sexual process.

It appears, therefore, that certain Ascomycetes are distinctly sexual, whereas others are distinctly asexual. The absence of distinct sexual organs may be accounted for either by considering the members of the group in which they do not occur as primitive forms which have not reached a sufficiently high stage of development for such a differentiation, or (and this is, perhaps, the more probable assumption) by regarding them as degraded forms which have lost the sexuality which more primitive forms still retain.

It remains to consider briefly the significance of the pycnidia. Their existence in lichens was first discovered by Tulasne[2], and he points out that the presence of them

[1] "Zur Entwickelungsgeschichte der Ascomyceten." 'Bot. Zeit.,' 1877

[2] Loc. cit., p. 107.

affords fresh proof of the close affinity which exists between these plants and the ascomycetous fungi. He had already shown that his predecessors were mistaken in regarding the pycnidia of these fungi as distinct individuals, forming the genera Phoma, Diplodia, Sphæropsis, &c., and had suggested that, since they occur upon the mycelium with the perithecia, they are probably organs of some kind belonging to the fungus. De Bary's researches upon Cicinnobolus[1] threw doubt upon the correctness of Tulasne's suggestion. It seemed to be absolutely certain that Erysiphe and Cicinnobolus belonged to the same mycelium, that they were in fact, different organs of the same plant; but De Bary proved that this was really a case of parasitism, that the mycelium of Cicinnobolus was parasitic upon that of Erysiphe. Under these circumstances it was still possible to argue that the pycnidia might after all be distinct fungi parasitic upon the mycelium bearing them.

An elaborate investigation into the development of the pycnidia has recently been made by Bauke[2] with the most conclusive results. Mycelia formed by the germination of the ascospores of *Pleospora polytricha*, *Cucurbitaria elongata*, and *Leptosphæria* (*Pleospora*) *Doliolum*, regularly produced pycnidia, and the connection of the pycnidium with the ascospore was in each case satisfactorily ascertained. It must, therefore, be concluded that the pycnidia do not form a distinct group of fungi, but that they belong to the Ascomycetes.

These pycnidia, in the course of their development, presented appearances which recalled the formation of the perithecium of Ascobolus and of other ascomycetous fungi. In *Pleospora herbarum* the mode of development of the pycnidium, so far as the formation of the stylospeors, is essentially the same as that of the perithecium. These facts suggest, as Bauke[3] points out, that a genetic relatio nship exists between these two forms of fructification, and he goes on to inquire if the pycnidia and the perithecia may not be regarded as alternate generations. According to the views recently propounded by Pringsheim,[4] as to the alternation of generations in Thallophytes, the morphological analogues of the two alternate generations of Cormophytes, are to be found in the independent neutral and sexual indi-

[1] De Bary and Woronin, 'Beit. z. Morphol. und Physiol. d. Pilze,' iii, Heft, 1870.

[2] 'Beitr. z. Kenntniss der Pycniden, Nova Acta. d. k. Leop.-Carol Akad.,' 1876.

[3] 'Bot. Zeitung,' 1877.

[4] 'Jahrb. f. Wiss. Bot.,' d. xi, Heft 1, 1877

viduals of Thallophytes. If this be so, the mycelium and the "fruit" of the Ascomycetes cannot be regarded as the two distinct generations, but the fruit which has been produced sexually belongs to the same generation as the mycelium bearing it, and the second neutral generation begins, instead of ending, with the spore. Pringsheim suggests that the pycnidia represent the neutral generation of the Ascomycetes, and that probably the irregular succession of these two forms indicates an incomplete, as it were, preparatory form of the alternation of generations. These suggestions seem to be supported by Bauke's observations upon *Pleospora herbarum*, for here perithecia and pycnidia appear to be distinct generations, and their succession is extremely irregular. Bauke explained the similarity in the development of pycnidium and perithecium on the hypothesis that the pycnidium was derived by adaptation from the perithecium at a time when the latter had already attained its present development, and that an alternation of generations became apparent at a later period. Pringsheim prefers to consider that both pycnidium and perithecium—in fact all the sexual and neutral fructifications of Thallophytes, are simply the result of modifications taking place in particular directions of a single primary fructification, the neutral sporangium.

EXPLANATION OF PLATE XX.

FIG. 1.—Section of thallus of *Collema Microphyllum*.

a. The projecting cell of the trichogyne.

FIG. 2.—*a*. Spermatia surrounding the terminal cell of the trichogyne.

b. Coalescence of a spermatium with the terminal cell of the trichogyne.

FIG. 3.—Young apothecium of *Collema Microphyllum*.

a. Excipulum thallodes.

b. Excipulum proprium.

c. Hypothecium.

The hymenium consists principally of paraphyses, between which young asci are being formed from ascogenous hyphæ.

NOTES AND MEMORANDA.

Recent Observations on Botrydium granulatum.—In an interesting memoir lately published in the 'Botanische Zeitung' (Nos. 41 and 42, 1877) by Profs. J. Rostafinski and M. Woronin, the history of a geographically widely diffused, but hitherto not at all understood, little Chlorophyllaceous alga—*Botrydium granulatum*—has just been brought to light. The investigations had first been begun by Professor Rostafinski and Count H. zu Solms Laubach in Strassburg. They found, however, that Professor Woronin in Finland was occupied in the same research, and that he had already published some of his results in the 'Naturforschende Gesellschaft' in St. Petersburg; subsequently Count Solms withdrew, and the two other observers completed their investigations in conjunction, and have just published their results.

After giving an historical *résumé* of the literature on Botrydium—from Ray, Dillen, Linnæus, onwards—the authors proceed to describe their observations on its development-history.

Having alluded to its well-known mode of occurrence and the form of this little plant, they take up the account of its history by describing the zoospores. If a plant be placed in water, its contents become modified at the later part of the day or at night into these. The first indication is the formation of numerous vacuoles in the chlorophyll-containing parietal stratum of contents, these vacuoles gradually increasing in quantity until the latter acquires a reticulate aspect. The wall swells up, which exerts a pressure on the fluid contents, whereby the wall bursts somewhere at the top, and the zoospores, meantime resulting from the division of the parietal stratum, issue forth. If the plant be only moistened —which by no means seldom happens in nature—the zoospores do not swarm out, but come to rest within the col-

lapsed wall of the Botrydium. Such were known to previous observers as "germ-cells" or "gonidia."

The zoospores are elongato-oviform, 5—8 mm. in breadth, and about 20 mm. long. They are furnished with two to four chlorophyll-granules, and bear, at the colourless, scarcely pointed apex, a single long flagellum.

Having once swarmed out they soon come to rest, lose the flagellum, become surrounded by a membrane, increase in size, and on damp earth soon germinate.

This they do by giving off from the downward side a short hyaline process, which penetrates the soil, the opposite end becoming elevated upwards, and remaining the only bearer of the chlorophyll. In this stage they represent the so-called *Protococcus botryoides*.

Reverting to the large zoosporanges, which the authors call *ordinary*, they found them capable of yet another modification. If one be allowed to dry, its membrane begins to collapse, loses its colour in time, and becomes soon empty. The membrane is covered by fine calcareous granules, is dry, brittle, hyaline; the whole protoplasmic contents have now passed down into the ramifications of the root. Here they break up into a number of cells, all nearly alike, only in the thicker neck-part they lie two or three side by side, whilst elsewhere they present a continuous simple chain. Each of these root-cells is surrounded by a special membrane.

These are capable of a threefold mode of development.

If removed from the soil and brought into a drop of water the membrane swells, bursts the wall of the root, and becomes a subterranean zoosporange; the formation of the zoospores is independent of the light at any hour of the day or night. The zoospores are quite similar to those above described, and in germinating behave in the same way.

If a chain of these root-cells, still inclosed in a ramification of the root itself, be laid on moist earth, they each put forth a hyaline process, which presses into the soil, the opposite end arising aloft, thus each root-cell becoming a vegetative plant.

But if the root-cells are not removed and the culture be kept equably moist, in time another modification ensues; they now begin to germinate in the earth. They become inflated, putting forth a hyaline root-process, the wall of which becomes very much thickened on the inner side below the inflated upper portion. This thickening advances almost to the obliteration of the cell-cavity. By intercalary growth of the root-portion the upper part becomes raised aloft, so that

the apex is carried up above the surface of the soil. These products of the modification of the root-cells the authors name *hypnosporanges*. They represent the so-called *Botrydium Wallrothii*. The portion above ground is exactly globular, scarcely 0·5 mm. in diameter, dark- almost black-olive-green, and not tapering downwards. For a considerable distance downwards the root is constantly unbranched, and, as said, its wall much thickened. The secondary ramifications are thin-walled. Dried, the hypnosporanges maintain their power of germination during the whole year in which they originate, and, when brought into water, form zoospores independently of the hour of day or might. The zoospores, as in the former instances, are uniflagellate, germinate, and form young plants in the same manner.

Young plants formed therefrom can increase by cell division. At any point of the aerial part of the plant a protuberance is formed, into which a portion of the plasma and of the chlorophyll collects. When the protuberance has acquired the size of the mother-plant, it puts forth a colourless hyaline process, which penetrates the earth as a root. The connection with the mother-plant is shut off by a septum, and finally the two cells separate, in order to lead an independent existence. Several such projections may be simultaneously given off, giving origin to so many daughter-individuals. But this process cannot be followed out under the microscope; if a young plant be placed in a drop of water it becomes in the evening or at night modified into a *vegetative zoosporange*.

These uniflagellate zoospores germinate in the manner described when brought upon a moist substratum. On ordinary garden earth or on sand they thrive but badly, and form no ordinary zoosporanges. They succeed better on muddy or clayey soil. In water they never germinate. In this medium the zoospores, when they come to rest, become surrounded by a double membrane and lie dormant for months. If such be brought upon a clayey soil the contents increase, burst the wall, and begin to form a vegetative plant.

If the zoospores be sparingly distributed upon the soil, and the whole kept under an equable degree of moisture, the vegetative plants in time become ordinary zoosporanges. The little plants may sometimes become directly modified into hypnosporanges.

Thus, the vegetative plants of Botrydium can be increased by cell-division, directly form zoospores, become ordinary

zoosporanges, with all their consequences, such as root-cells, &c., or they may be directly modified into hypnosporanges. But they can also carry on existence in yet another way.

If exposed to drought the following series of phenomena may be observed:—The wall collapses more or less, and the protoplasmic chlorophyll-bearing contents break up into a number of cells, their number depending on the size of the mother-plant. Each is surrounded by a delicate membrane, its contents homogeneous, at first green, with time and continued dryness or sunshine passing into red. These are the *spores* of Botrydium, and have been known as *Protococcus coccoma, palustris* and *botryoides*.

These spores, be they green or red, become changed in water to zoosporangia, their protoplasmic contents giving rise to zoospores in the way already described. If the spores be still green, their zoospores have a distinct fusiform figure. At the apex of the shorter end they possess two cilia. They consist of protoplasm which is slightly coloured, with exception of a lenticular-shaped region, stretching at one side a certain distance backwards, which remains uncoloured. These zoospores conjugate in twos, sometimes several together. They come in contact by their ciliated ends, then come to touch laterally by the uncoloured portions, when the fusion of the conjugating zoospores takes place, immediately after which they present a cordate figure, and in the middle is noticeable a colourless vacuole. Finally the *isospore* thus originating becomes globular, the vacuole occupying the centre.

If the zoospores be isolated before conjugation, they finally break up, without presenting any products capable of germination.

The zoospores, likewise sexual, originating from the red spores, have a different figure, their posterior end being rounded off; otherwise they have the same structure, and behave, as regards conjugation, in the same way as the green ones.

The red spores maintain their germinative powers for years, but after two years their zoospores are languid and, what is more important, they offer a parthenogenesis of a peculiar kind. The red spores, if they be kept moist only, become no more altered even after weeks, whilst the green, under these circumstances (as already pointed out by Cienkowski), may directly germinate into vegetative plants. Whether these present ordinary or sexual zoospores the authors did not follow out.

To return to the isospore. It is at first globular and

capable of immediate germination, during which the central vacuole becomes removed to the end towards the substratum, whilst the green-coloured protoplasm becomes mainly collected in the upper part, the colourless end tapering off in order to penetrate the soil. A couple of weeks' continued culture may again give vegetative plants capable of division and of zoospore-formation.

The isospores also present resting-stages, the form of the originally globular cell becoming modified. Soon after conjugation these are flattened, with irregular lateral boundaries; these become on the following day exactly hexagonal. The membrane of the isospore becomes thickened, and, on the lateral margins, present some tuberculations, but there is no secondary membrane formed. Brought upon damp earth they soon become globular and otherwise behave as normal isospores. In very young plantlets traces of the tuberculations can be seen, but these soon disappear. The further growth leads to the same result as that of the globular isospores, that is, the formation of a vegetative plant.

In order to distinguish that which appertains to the cycle of alternation of generation from the remainder, the simple way is to start from the fertilized germ (Ei) and see what are the modifications of the plant, thence originating, *actually essential*, in order to arrive again at the same reproductive process.

We have the isospore—it germinates—produces the vegetative plant, which needs neither to divide nor to produce a sexual zoospore, nor to become an ordinary sporange—it can directly produce spores. These close the first sporophore generation. The second oophore generation becomes presented in the germination of these spores in the form of sexual zoospores, which directly lead to the formation of the isospore—the limits of two generations. All the rest are but phenomena of adaptation.

Thus, in nature, the vegetative plants in spring almost all become zoosporangia and spread the growth over considerable areas. Zoospores which fall into water are not lost; they acquire a double membrane, and lie dormant until they chance mechanically to arrive on moist soil. If drought sets in, the plasma retreats to the roots. If the earth be some time a little moist, the root-cells become hypnospores, awaiting the rain in order to develope multitudes of zoospores. But if the earth become rapidly dried the root-cells remain unaltered until a moistening excites the formation of zoospores. A great many of the root-cells can manifestly accidentally reach the surface of the soil, and thus, according

to the state of the moisture of the earth or of the air, sometimes germinate directly, sometimes become zoospores.

All this occurs, as said, mostly in the spring; the hotter months favour the formation of spores, but at that period only the vegetative plants are mostly to be found, either undergoing cell-division or spore-formation. They can, as said, also furnish uniciliate zoospores without becoming modified into the ordinary zoosporanges.

The formation of the ordinary zoospores can thus come to pass in a fourfold way—(1) from the vegetative plant, (2) from the ordinary zoosporange, (3) from the root-cell, (4) from the hyposporange. As further modes of increase there are likewise to be noted—(5) cell-division, (6) formation of spores, (7) formation of zoospores. Botrydium possesses also fivefold resting stages—(1) of the asexual zoospores laid in water—for months; (2) of the root-cells—the year through, in which they have originated; (3) of the hypnosporanges—the year through, in which they have originated; (4) of the spores—for years; (5) of the isospores—at least over the year, in which they have originated.

On comparing the development of Botrydium with that of other Chlorosporeæ—as touching the alternation of generation—the fact at once strikes us that Botrydium presents that kind of alternation in which the existence of the vegetative plant occurs in the post-embryonal period of life, as in the ferns. All other Chlorosporeæ behave otherwise, that is, like a moss; the vegetative plant originates from the spore, and not from the fertilised germ (Ei).

The sexual cells show no sexual differences. Their mother-cells can in their youth directly germinate; they form at once a vegetative plant, and so disturb the cycle of alternation. In later age they are incapable of this modification. The products of their division behave in a different manner. If they originate from young mother-cells they conjugate promptly, without it they perish; with increasing age of the spore they become more languid in these functions, the conjugation lasts longer, and finally the spore reaches an age in which the sexual cells, without conjugating, directly germinate. In this case the cycle of the alternation becomes again broken, but this time at the other end.

Referring to the author's previous mention that the formation of zoospores takes place sometimes favoured by darkness (vegetative plants, ordinary zoosporangia), and sometimes quite independently of the light, as well in the night as in the day hours. In explanation of this phenomenon, notice-

able in many instances in the algal world, the authors adduce the following considerations.

In order to the formation of zoospores all the products of assimilation collected in the chlorophyll-granules are in solution, and equably distributed in the protoplasm of the cell. But in assimilation, which is in the most intimate dependence with the light, an opposite process is set up. If, thus, a cell be still capable of assimilation—if it be, so to say, still in a vegetative state—it can arrive at zoospore formation only in the night hours. But if, on the other hand, an organ be in a resting state—if all the cell-substances be equably distributed in the plasma—it then forms zoospores, after being moistened with water, quite independently of the light at any hour of the day or night.

Touching the affinities of Botrydium, the authors regard it as the type of a family (Botrydiaceæ) of Isosporeæ, equivalent to Panderineæ and Hydrodictyeæ, with the following as its characters:

Isosporeæ presenting in germination a vegetative plant, the contents of this becoming modified into an indefinite number of resting-spores; spore-contents, in germination, becoming modified into a number of sexual zoospores, conjugating and forming isospores.

Botrydium (Wallroth).

Vegetative plants unicellular, increasing by cell-division and zoospore-formation; asexual zoospores uniciliate, sexual biciliate; isospores sometimes globular, and alike capable of germination, sometimes compressed and hexagonal, furnished with a few tuberculate thickenings.

B. granulatum (L.), Grev.

Vegetative plants elongated, with a hyaline end penetrating the soil, the opposite chlorophyll-containing end projecting into the air, inflated or sometimes subdividing, the contents becoming modified during dryness into a number of spores, becoming red; these, on their part, giving rise to the sexual zoospores, conjugating and furnished with two cilia. Vegetative plants increasing also by cell-division and formation of asexual uniciliate zoospores, these germinating only on moist earth, in water becoming surrounded by a double membrane and passing into rest. Vegetative plants becoming modified by increase of volume of their exposedinflated upper portion, and simultaneous copious ramification of the subterranean root into an almost globular, light green, ordinary zoosporange, tapering off downwards, its contents under

water becoming rejuvenised into an immense number of uniciliated zoospores; during prolonged drought, on the other hand, passing down into the subterranean root-ramifications, and there breaking up into a number of root-cells, surrounded by a special membrane, these becoming modified either into subterranean zoosporanges, or directly into vegetative plants, or into rooted hypnosporanges. Hypnosporanges black-olive-green, globular, the neck portion of the root having the wall thickened almost to the closing up of its cavity, for a long distance simple, secondary ramifications sparing, thin-walled

W. Archer.

Motility of the Spermatozoids of Limulus. — Through the kindness of Mr. Carrington F.L.S. of the Westminster Aquarium, I received some months since a specimen of the American King crab in the living condition. My principal object was to study the colouring matter of the blood. Concerning it I will here only remark, that the blood when first shed is almost colourless having but a faint opalescence of a blueish tint. This however, rapidly gives way to a blue colour which increases in intensity for three hours after the blood has been shed, and finally attains a deep indigo tint. The colour is due either to the chemical change, or to the solution of, a substance contained in the corpuscles. The specimen from which I collected the blood was fortunately a male, and was in the full vigour of the breeding season. A quantity of white cream-like sperm was discharged by the animal from the orifices of the seminal ducts, placed on the protected surface of the opercular plate. I was thus able to examine some of the spermatozoa in the mature and living condition. The simple fact which I wish now to record and which I believe has not been previously recorded is, that the spermatozoa of Limulus are actively motile. They are, like the other histological elements of that animal, of very large size. Each presents a lemon-shaped head provided with a delicate tail of great length. At the moment of writing I am unable to lay my hand on the measurements. The vibratile character of the tail is, however, the important matter. Thousands of the spermatozoids were seen swarming together on the field of the microscope, whilst the travelling of isolated individuals over considerable distances was watched.

Any fact, however small, which bears upon the question of the affinities of Limulus is important. Usually Limulus is considered as an Entomostracous Crustacean; by some naturalists it is placed in a class alone, in close relationship with the other branchiferous Arthropoda, others again would associate it most intimately with the Arachnida and in fact with the Scorpions. The spermatozoids of the various classes of Arthropoda have, so far as they have been

studied, given various results as to the possession of motility. In the Crustacea, excepting the Cirrhipedia, it appears to be well established that the spermatozoids are incapable of automatic movements. In the Arachnida on the other hand this is not so well established, whilst in the Insecta alone does it appear that movements of the spermatozoids have been habitually observed. So far then the motile character of the spermatozoids of Limulus tends to separate it from the Crutascea, though our knowledge of the spermatozoa of the Arachnida does not enable me to add that there is any special approximation in this matter between Limulus and the members of that group. A comparison of the morphology and development of Limulus and of Scorpio renders it absolutely necessary in my opinion to class Limulus and its fossil allies under the Arachnida as a sub-class, "Branchiopulmonata."

E. Ray Lankester.

The Early Developmental Changes in the Reptilian Ovum.—Professors Kuppfer and Benecke have recently published a very interesting note on this subject. Their observations were made on *Lacerta agilis* and on *Emys Europea*, in both of which types they found the embryonic changes to be closely alike. Segmentation takes place exactly as in birds, and the resulting blastoderm, which is thickened at its edge, spreads rapidly over the yolk. A small embryonic shield makes its appearance in the centre of the blastoderm shortly before the yolk is half enclosed. It is somewhat pyriform, and mainly distinguished from the remainder of the blastoderm by the more columnar character of its constituent epiblast cells. At the narrower end of the shield, which may be spoken of as posterior in relation to the future embryo, an invagination takes place, and gives rise to a sack, the blind end of which is directed forwards. The opening of the sack may be spoken of as the blastopore. A linear thickening of the epiblast arises in front of the blastopore, along which the medullary groove soon appears. In the cephalic region the medullary groove flattens out, and posteriorly the two medullary folds diverge, and enclose between them the blastopore, behind which they again meet. On the completion of the medullary canal the blastopore becomes obliterated; but there can be but little doubt, although the authors of the paper do not appear to touch on this point, that on the closure of the medullary folds the medullary canal remains in direct communicatian with the cavity of the invaginated sack, so that the latter has exactly the same relation to the medullary canal as the embryonic structure, which I have called "the postanal section of the alimentary canal" in Elasmobranchii.

At the time when the above-described invagination appears,

the blastoderm is already constituted of two layers, the lower of which is the true hypoblast, and gives rise to the epithelium of the alimentary tract, in the formation of which the invaginated sack has no share. According to the authors, the mesoblast grows out from the lips of the blastopore as four masses. Two of these are lateral; a third is anterior and median, and although at first independent of the epiblast, soon attaches itself to it, and forms with it a kind of axis cord; a fourth mass applies itself to the invaginated sack. The true alimentary canal is formed by a series of folds similar to those in Aves; but the tail fold is so situated that the invaginated sack lies on the ventral aspect of the hind end of the alimentary tract. The authors suggest that the invaginated sack gives rise to the Allantois, a view which fits in very well with Gasser's recent observations on the primitive streak of Aves, as well as with the presence of a vesicle at the end of the postanal section of the alimentary tract in Elasmobranchs, and with Kuppfer's own suggestion about the similar vesicle at the end of the alimentary tract in Osseous Fish.

The head fold of the Amnion is formed very early, and grows over the head before the closure of the cephalic medullary folds.

F. M. Balfour.

Recent Researches on Bacteria.—The progress of knowledge with regard to the life-history of Bacteria has been carefully recorded in the pages of this Journal during the past ten years, sometimes by original memoirs, sometimes by more or less extended notices of foreign publications:—'The Origin and Development of Bacterium Termo,' by Frau Lüders, vol. viii, p. 32. 'Spontaneous Generation,' by Professor Dyer, vol. x, p. 333. 'Relations of Torula, Penicillium, and Bacterium,' by Prof. Huxley, vol. x, p. 355. 'Bacteria and their Relations to Putrefaction and Contagion,' by Prof. Cohn, vol. xii, p. 207. 'Natural History of the Vibriones,' by Oscar Grimm, vol. xii, p. 411. 'The Origin of Bacteria,' by Prof. Sanderson, vol xi, p. 323. 'Bacteria and the Germ Theory,' by Prof. Lister, vol. xiii, p. 380. 'Researches on Bacteria' (first series), by Prof. Cohn, vol. xiii, p. 156. 'A Peach-Coloured Bacterium,' by E. Ray Lankester,' vol. xiii, p. 408. 'The Theory of Fermentations,' by Louis Pasteur, vol. xiii, p. 351. 'Bacteria in Malignant Pustule,' by Drs. Fränkel and Orth, vol. xiv, p. 288. 'Bacterium Rubescens,' by Wm. Archer, vol. xv, p. 206. 'Bacteria in Disease,' by Dr. Payne, vol. xv, p. 327. 'A Pink-Coloured Spirillum,' by Dr. Klein, vol. xv, p. 381. 'Account of Cohn's Researches into the History of Bacteria' (second series), vol. xvi, p. 259. 'Further Observations on Bacterium Rubescens,' by E. Ray Lankester, vol. xvi, p. 27. 'Account of Cohn's Researches on Bacteria

(third series), vol. xvii, p. 81. 'Atmospheric Bacteria,' by G. F. Dowdeswell, vol. xviii, p. 83. 'Life-History of Bacillus Anthracis, by Dr. Cossar Ewart, vol. xviii, p. 161. 'The Nature of Fermentation,' by Prof. Lister, vol. xviii, p. 177.

During the present year an important memoir by the eminent botanist, Cienkowski, has been published in vol. xxv. of the 'Memoirs of the Imp. Acad. of Sciences of St. Petersburg,' entitled 'Zur Morphologie der Bacterien.' The Memoir is illustrated by two plates. Cienkowski holds—(1) that just as a Palmella-condition develops from diverse chlorophyll-green Algæ so do Zoogloea-forms arise from several colourless filamentous Algæ. (2) Among such generators of Zoogloea are *Crenothrix*, *Leptothrix*, and *Cladothrix dichotoma*. From the last-named Alga, in all probability, arise the commonest Zoogloea formations; those, namely, of *Bacterium termo* and *lineola*. (3) The Bacteria are transformed by repeated sub-division into *Micrococcus*; the latter also arises directly from Leptothrix-like filaments. (4) *Micrococcus*, *Bacterium*, *Torula*-forms, *Bacteria*-chains, are not generically different, since they often occur together in the same mass of Zoogloea, which can be proved to have been developed from a colourless Alga, and from which they can be freed and brought into the motile phase.

Cienkowski's views agree, therefore, to some extent, with those of Hofmann, Lüders, and Billroth, and are diametrically opposed to those of Cohn, whose services in this field of research he nevertheless admits are of the highest value. Hofmann, in 'Bot. Zeitüng,' 1869, p. 253, had, it appears, observed the development of *Micrococcus* from filamentous forms. Cienkowski is apparently unacquainted with my observations on *Bacterium rubescens*, by which the connection of filamentous forms with a variety of other forms (biscuit-shaped, spherical, rod-like, aggregated as Zoogloea, or in the form of networks or of pavements) is demonstrated.

In the 'Proceedings of the Royal Society,' No. 188, 1878, Ewart and Geddes describe and figure the various forms present in a growth of a brownish tint which they observed in a tank at University College, London.

I have no doubt from their description and from observation of the same growth that the organism present was identical with my *Bacterium rubescens*. During the phase in which they observed it the production of *Spirillum*-forms was exceedingly active. The *Spirillum*-form observed by Ewart and Geddes and the filaments related to it appear to be identical with those described and figured by Warming, 'Observations sur quelques Bacteries qui se rencontrent sur les côtes du Danemark.' Société d'Hist. Nat. de Copenhague, 1875. Professor Giard, of Lille, has

also figured the same *Spirillum*-form of *Bacterium rubescens* in the 'Revue des Sciences Naturelles, tom v, 1877. I do not feel satisfied from the account given by Ewart and Geddes that the bodies which I have called "loculi" and which they term "spores" have any characters which justify the use of the latter term in regard to them. They do *not* appear to be the same kind of bodies in origin as the so-called "spores" discovered by Cohn in Bacillus, and it is not at all certain that they germinate, as Ewart and Geddes have inferred, though their observations lend a certain amount of probability to that suggestion.

In a second paper in the same journal Dr. Ewart discusses the 'Life-history of Bacterium termo and Micrococcus.' According to his observations *Bacterium termo* elongates under certain conditions into small filaments in which micrococcus-like bodies ("spores") are formed as in *Bacillus anthracis*. At the same time Ewart is inclined from certain experiments to hold that besides the micrococcus-like spores of Bacteria there are independent *micrococci* which do not give rise to Bacteria. Interesting notes on conditions affecting the life of Bacteria are also recorded.

Mr. Dallinger, who with Dr. Drysdale was the first to discover (Sept. 1875) the flagella (one at each end) of *Bacterium termo*, has recently measured the thickness of these filaments. He finds it to be a little less than the $\frac{1}{200},\frac{1}{000}$th of an inch.

Messrs Dallinger and Drysdale are, I believe, the only observers who have succeeded in seeing the flagella of *Bacterium termo*. They have done so by the use of the best objectives and the most carefully contrived methods of illumination which the microscopists of this country have devised. Other naturalists had inferred the presence of such flagella, and Cohn had seen and drawn them in the case of the large *Spirillum volutans*. The larger Bacteria (such as the large forms of *B. rubescens* erroneously assigned to the genus Monas with which they have no relation, under the names *Monas Okeni, Monas vinosa, Rhabdomonas rosea*), possessing thicker flagella, were studied, and their flagella recognised after the English observers had made their discovery on the much more difficult object, *B. termo*. In the journal of the Royal Microscopical Society for September, 1878 (vol. i, No. 4), Mr. Dallinger gives large figures drawn to scale, and showing the flagella of *Bacterium termo, B. lineola, Bacillus subtilis, B. ulna, Vibrio rugala, Spirillum undula,* and *Spirillum volutans*.

E. Ray Lankester.

The Development of Calcareous Sponges.—Professor Franz Eilhard Schulze returns to this subject in the 'Zeitsch. wiss. Zool.,

2nd part, 1878. It will be remembered that since the appearance of Haeckel's monograph on the group, there has been a continuous series of papers by Metschnikoff, Oscar Schmidt, F. E. Schulze, Barrois, and Keller, giving the most contradictory accounts of the formation of the diblastula. No one had succeeded since Metschnikoff in observing the transition from the free-swimming to the fixed condition. According to Metschnikoff, the blastula of *Sycon ciliatum* consists of a hemisphere of long ciliate cells, and a hemisphere of large, rounded, granular cells ; the ciliate cells become invaginated to form the endoderm—whilst the large granular cells form the ectoderm and skeleton. The blastopore closes, and a new osculum forms by rupture. (See this Journal, vol. xv., p. 78.) Metschnikoff's account seemed very improbable and for a time embryologists were completely led astray by the "pseudo-gastrula," described by F. E. Schulze, as the true gastrula or diblastula. (See this Journal, vol. xvi, p. 65.) Curiously enough, the embryo-sponge, whilst still in the brood-cavities of the parent, exhibits a temporary concavo-convex form, in which it is *the large round cells which are invaginated*, and *not* the ciliate cells. This invagination was very naturally mistaken by Schulze for the endodermal invagination. The definite result of Schulze's last paper is, however, fully to confirm Metschnikoff's account as to the permanent invagination. Schulze has succeeded in keeping the embryos under observation whilst they fix themselves. His observations show that the blastopore becomes the pole of attachment.

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Fig. 2.

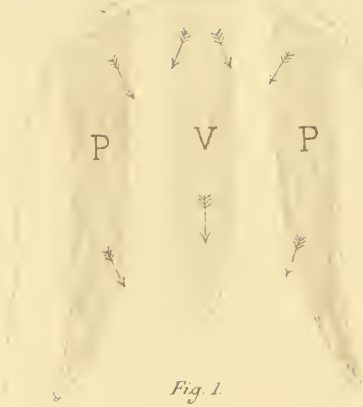


Fig. 1.

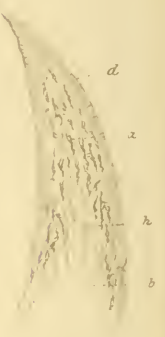


Fig. 3.

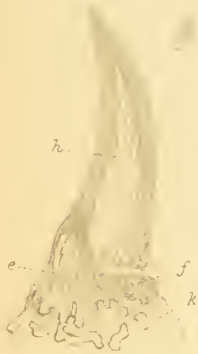


Fig. 4

Fig. 5.

Fig. 6.

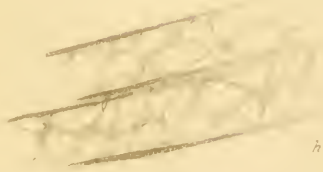


Fig. 7

Fig. 8

DESCRIPTION OF PLATE I,

Illustrating Mr. C. S. Tomes' Paper on the Teeth of Pike.

Fig. 1.—Diagram of roof of pike's mouth, showing the directions in which alone the several teeth forming the vomerine and palatine bands are capable of being bent.

Fig. 2.—Anchylosed tooth of pike, somewhat diagrammatic.
a, central core of osteo-dentine; *d*, layer of fine-tubed dentine.

Fig. 3.—Hinged tooth of pike, somewhat diagrammatic.
b, empty pulp cavity; *h*, fine rods of calcified tissue.

Fig. 4.—Section of hinged tooth *in situ*.
e, fibrous hinge; *f*, buttress of bone on which the tooth is received; *k*, bone of jaw.

Fig. 5.—Section of hinged tooth *in situ*, more highly magnified. The fine bands (*h*) which tie the tooth down to the bone are seen to be continuous both with the dentine and the bone.

Fig. 6.—Point of attachment of one of the bands (*h*) with the bone (*k*), the surface of which is clothed with osteoblasts (*o*). × 300.

Fig. 7.—Calcified portion of the axial bands, from high up in the tooth; between the rigid rods (*h*) is a fine network of cobweb-like tenuity (*l*). × 400.

Fig. 8.—Point of attachment of one of the rods to the subjacent bone, showing that it is made up of many fibres (*i*). × 500.

DESCRIPTION OF PLATES II & III,

Illustrating Dr. A. Milnes Marshall's Paper on the Development of the Cranial Nerves in the Chick.

All the figures were drawn with a Hartnack camera. The numbers attached indicate the magnifying power, in diameters, employed in drawing each figure. All the drawings are from single sections, except fig. 21, in which two consecutive sections were employed. A large number of the figures are semi diagrammatical, there being no object to be gained by representing the mesoblast or the histological details; the outlines are, however, strictly accurate in all cases.

ALPHABETICAL LIST OF REFERENCES.

a, Point at which the secondary attachment of the nerve to the neural canal occurs; *al.*, alimentary canal; *aor.*, dorsal aorta; *aud.*, auditory epithelium; *b*, primary attachment of nerve to summit of neural canal; *br.* 1, first branchial arch; *br.* 2, second branchial arch; *c. f.*, choroidal fissure; *c. h.*, cerebral hemispheres; *com.*, longitudinal nerve commissure; *ep.*, external epiblast; *f. b.*, fore-brain; *f. g.*, fore-gut; *f. m. b.*, constriction between fore- and mid-brain; *h. b.*, hind-brain; *Hy.*, hyoid arch; *hy.*, hypoblast; *inf.*, infundibulum; *i. v.*, investing mass; *l.*, lens; *n.*, neural ridge; *m. b.*, mid-brain; *m. g.*, mid-gut; *m. h. b.*, constriction between mid- and hind-brain; *mn.*, mandibular arch; *m. p.*, muscle-plate; *mx.*, maxillary process; *n.*, notochord; *o. c.*, optic cup; *olf.*, olfactory pit; *o. v.*, optic vesicle; *pit.*, pituitary diverticulum; *p.v.*, protovertebræ; *r. e.*, rectus externus; *r. i.*, rectus internus; *r. s.*, rectus superior; *sp.*, spinal ganglion; *v. c.*, visceral cleft; I—X, cranial nerves (Sömmering); V 1, ophthalmic nerve; V 2, superior maxillary nerve; V 3, inferior maxillary nerve.

PLATE II.

Fig. 1.—Transverse section through the mid-brain of a twenty-seven hours' chick, × 90. Specimen hardened in ¼ p. c. chromic acid, to which a few drops of a 1 p. c. solution of osmic acid was added; allowed to remain twenty-four hours.

Figs. 2-5.—Transverse sections through the brain of a twenty-four hours' chick. Hardened in same manner as fig. 1. × 90.

Fig. 2.—Through anterior part of fore-brain; two halves quite separate; no mesoblast.

Fig. 3.—Through posterior part of optic vesicles. The section is imperfect, especially at its lower part, where the roof only of the fore-gut is present.

Fig. 4.—Through widest part of mid-brain; also imperfect.

Fig. 5.—Through hind-brain.

Figs. 6-10.—Transverse sections through brain of a twenty-nine hours chick. Specimen hardened in picric acid for three hours. × 90.

Fig. 6.—Through anterior part of optic vesicles.

Fig. 7.—Through constriction separating fore- and mid-brains.

Fig. 8.—Through middle of mid-brain.

FIG. 9.—Anterior part of hind-brain.

FIG. 10.—Hind-brain and thickening auditory epithelium.

FIG. 11.—Transverse section through hind-brain of a forty-seven hours' chick, passing through seventh nerve. Picric acid three and a half hours. × 90.

FIGS. 12, 13.—Transverse sections through hind-brain of a fifty hours' chick. Picric acid. × 90.

FIG. 12.—Through auditory pit on left side; seventh nerve on right.

FIG. 13.—Through glosso-pharyngeal nerve on left side; auditory pit on right.

FIG. 14.—Transverse section through hind-brain and roots of seventh nerve of a sixty-seven hours' chick. Picric acid. × 90.

FIGS. 15, 16.—Transverse sections through hind-brain of a ninety-three hours' chick. Picric acid. × 27.

FIG. 15.—Through fifth nerve.

FIG. 16.—Through seventh nerve.

FIGS. 17-20.—Horizontal sections (*vide* text for exact plane) through head of a ninety-three hours' chick. Picric acid. × 17.

FIG. 17.—Through olfactory pit, eye, third and ophthalmic nerves; fore- and hind-brains.

FIG. 18.—Through olfactory and third nerves, and through constriction between mid- and hind-brain.

FIG. 19.—Through point of origin of olfactory nerves from fore-brain.

PLATE III.

FIG. 20.—Through point of origin of third nerves from mid-brain.

FIGS. 21, 22.—Longitudinal sections through head of a ninety-six hours' chick. Picric acid. × 15.

FIG. 21.—Through fifth, seventh, and auditory nerves. In the section from which the drawing was made the auditory nerve and vesicle were not so well shown; they were accordingly filled in from the next section.

FIG. 22.—Through olfactory pit; third, ninth, and tenth nerves.

FIG. 23.—Longitudinal section through hind-brain and visceral arches of a 100 hours' chick. Picric acid. × 17. Showing fifth, seventh, eighth, ninth, and tenth nerves, and five visceral arches.

FIG. 24.—Longitudinal section through the posterior part of the head and the neck of a ninety-six hours' chick. Picric acid. × 15. Showing ninth and tenth nerves, with commissure and spinal ganglia and nerves.

FIG. 25.—Longitudinal section through posterior part of head and anterior part of neck of a 122 hours' chick. Picric acid. × 19. Showing investing mass, infundibulum, mouth with pituitary diverticulum; sixth nerve, commissure, and spinal nerves and ganglia.

FIG. 26.—Longitudinal section through head of a 122 hours' chick. Picric acid. × 17. Showing second, third, fifth, eighth, ninth, and tenth nerves; with some of the eye muscles, the mouth, and pituitary diverticulum.

FIG. 27.—Horizontal section through the first five protovertebræ of a ninety-three hours' chick. Picric acid. × 40. Showing spinal ganglia and connecting commissure.

FIG. 28.—Horizontal section through fifth, sixth, and seventh protovertebræ of a ninety-three hours' chick. Picric acid. × 60. Shows muscle-plates, spinal ganglia, and commissure.

EXPLANATION OF PLATE IV,

Illustrating Prof. Ed. Van Beneden's Memoir on the Development of Osseous Fish.

Fig. 1.—Egg of Gadoid fish, observed at Villafranca at seven a.m., showing the blastodisc divided into two enucleate spheres—the granular "intermediate layer" and the homogeneous "deutoplasmic globe" with eccentric oil-drop.

Fig. 2.—A similar egg later in the day (eleven o'clock). The blastodisc consists of many large nucleated cells; the intermediate layer is still granular.

Fig. 3.—Optical section of a similar egg at a still later hour of the same day (five o'clock). The superficial cells of the blastodisc are hexagonal. In the intermediate layer are seen numerous "free-formed" oval nuclei.

Fig. 4.—Egg from the same mass on the following day, in optical section.

Fig. 5.—The same egg focussed, so as to give a surface view of the intermediate layer with its free-formed nuclei and the radiating granular striæ around them.

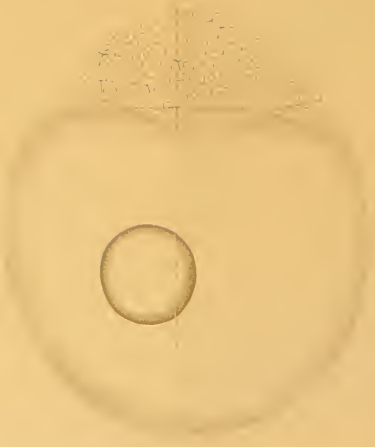
Fig. 6.—Optical section of a considerably later stage, showing the blastodisc, subjacent layer of large cells, peripheral welt of the intermediate layer and cells on the floor and on the roof of the segmentation cavity.

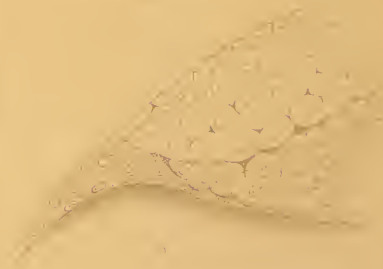
Fig. 7.—A portion of the egg drawn in fig. 4, showing the flattening form of the enveloping cells of the blastodisc, and the complete delimitation of cells in the intermediate layer together with the presence of free oval nuclei.

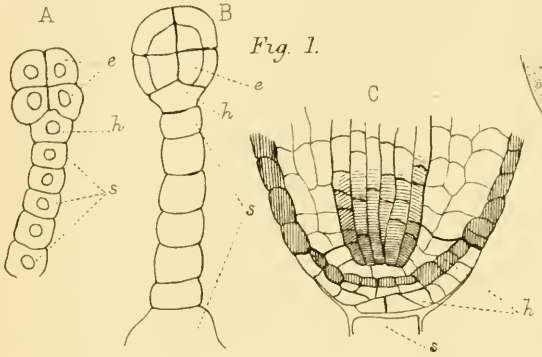
Fig. 8.—A portion of the intermediate layer as seen in fig. 5, in order to show the radiating striæ around the nuclei and the presence of nucleoli.

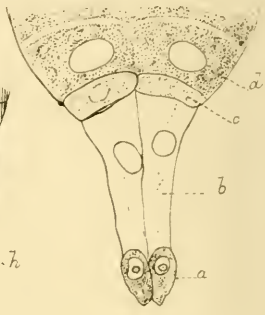
Fig. 9.—A portion of the view given in fig. 6 more highly magnified, showing on the one hand the similarity of the cells of the layer subjacent to the blastodisc to those of the intermediate layer, from which they are probably derived; and on the other hand the very different character of the cells of the blastodisc.

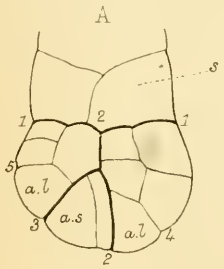
Fig. 10.—A portion of the floor of the segmentation cavity of fig. 6, showing the pinching off of cells.

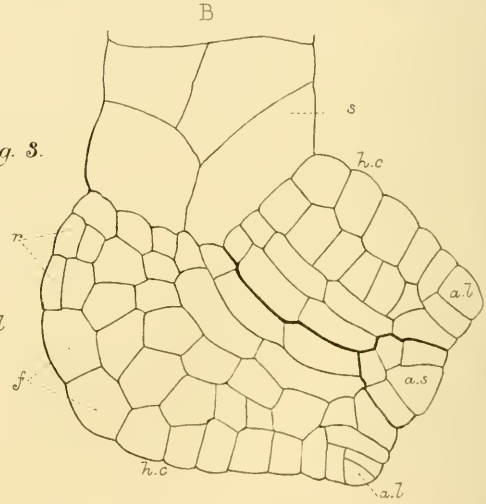


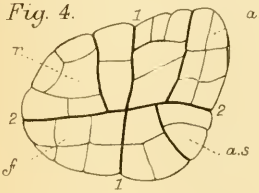


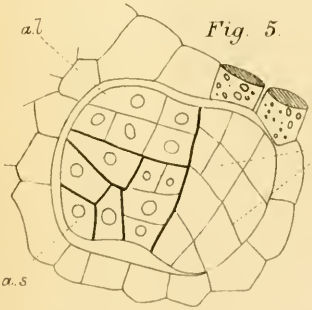


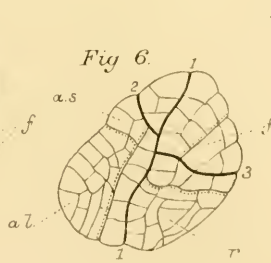












McFarlane & Erskine, Lith^rs Edin^r

EXPLANATION OF PLATE V,

Illustrating Mr. Sydney H. Vines' Paper on the Homologies of the Suspensor.

FIG. 1.—A, B, C. Stages in the development of *Capsella bursa-pastoris* (after Hanstein).
e. Embryo. *s.* Suspensor. *h.* Hypophysis, and tissues derived from it.

FIG. 2.—Development of embryo of *Pinus sylvestris* (after Strasburger).
a. Embryonic cell. *b.* Elongated cell of suspensor. *c.* "Rosette cell." *d.* Cell subsequently formed, taking no part in development of embryo, lying in lower portion of germinal vesicle.

FIG. 3.—A. B. Stages in the development of Selaginella (after Pfeffer).
1, 3, 3, 4, 5. The walls formed in the embryo.
s. Suspensor. *a. s.* Apical cell of stem. *a. l.* Apical cell of leaf.
h. c. Hypocotyledonary portion of stem.
f. Cells giving rise to so-called foot.
r. Cells giving rise to root.

FIG. 4.—Embryo of Marsilia (after Hanstein).
1, 2. Primary septa.
a. l. Apical cell of leaf. *a. s.* Apical leaf of stem. *r*, Root.
f. Fost.

FIG. 5.—Embryo of Salvinia (after Pringsheim).
a. s. Apical cell of stem. *a. l.* Segment giving rise to scutiform leaf. *f.* Segment giving rise to foot (caulicle).

FIG. 6.—Embryo of *Pteris aquilina* (after Hofmeister).
1, 2, 3. Primary septa.
a. l. Apical cell of leaf.
a. s. Apical stem of stem (both in anterior inferior segment of the embryo).
f. posterior superior segment, forming foot, together with anterior superior segment, according to Hofmeister.
r. Posterior inferior segment, giving rise to root.

FIG. 7.—Embryo of a Liverwort, *Grimaldia barbifrons* (after Kienitz-Gerloff).
s. Upper half of embryo forming sporogonium.
f. Lower half forming seta and foot.
1. Primary septum.

EXPLANATION OF PLATE VI,

Illustrating the Abstract of Reichenbach's Paper on the Development of the Crayfish.

References.

A. Thoracico-abdominal process.
At. I. Antennules.
At. II. Antennæ.
an. Anus.
B. Rudiment of carapace.
G. Blastopore.
H. Endodermal plug which partly fills up the blastopore in stages II and III.
N. Position of heart.
K. Procephalic lobes.
KS. Head-discs.
Ml. Mandible.
Oe. Mouth.
R. Medullary groove.
V. Optic fossæ or depressions in the head-discs which form the optic ganglia.

Fig. 1.—Embryo in the first stage, with horse-shoe shaped blastopore.

Fig. 2.—Embryo in the second stage, with annular blastopore.

Fig. 3.—Embryo in the third stage, with procephalic lobe, optic fossæ, medullary groove, and decussing blastopore.

Fig. 4.—Embryo in the fourth stage, with increased thoracico-abdominal process and greatly reduced blastopore.

Fig. 5.—Embryo in the fifth stage, with commencement of fore and hind-gut, rudiments of mandibles and of carapace, and closed blastopore.

Fig. 6.—Embryo in the sixth or Nauplius stage, with antennules, antennæ, and mandibles, well-formed mouth and anus, and no trace of blastopore.

Fig. 1

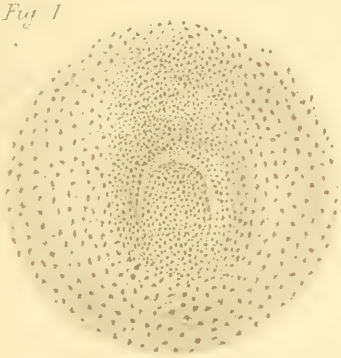


Fig. 2

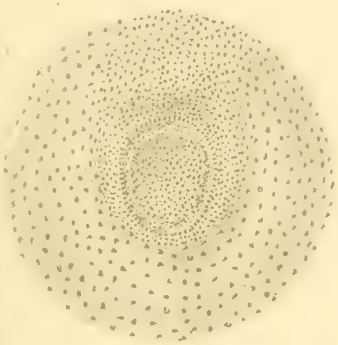


Fig. 3.

Fig. 4

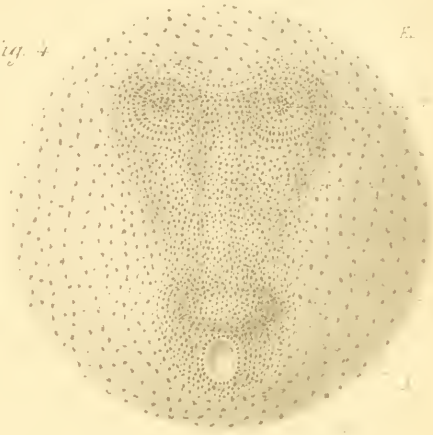
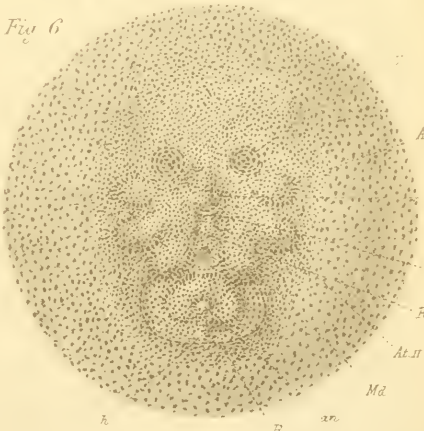
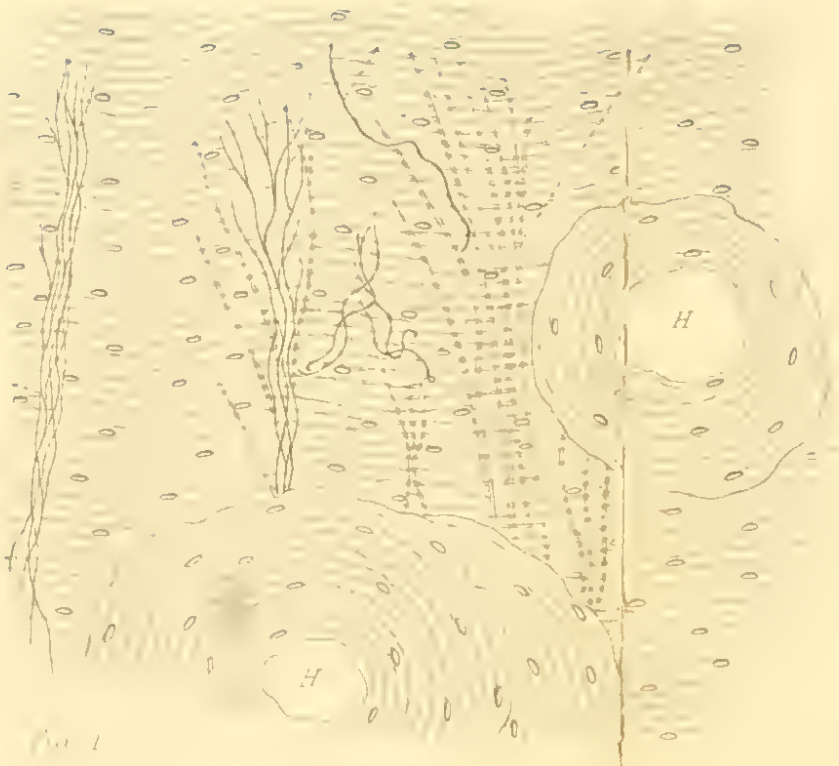


Fig. 5

Fig. 6

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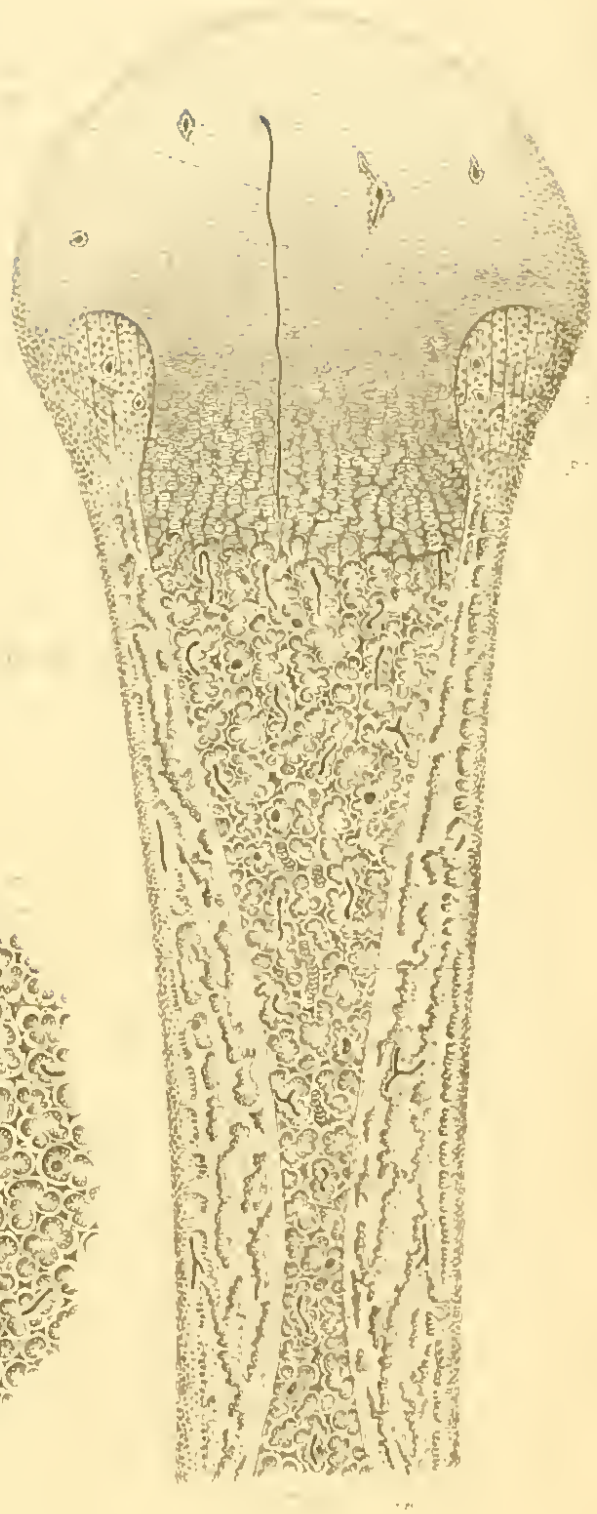


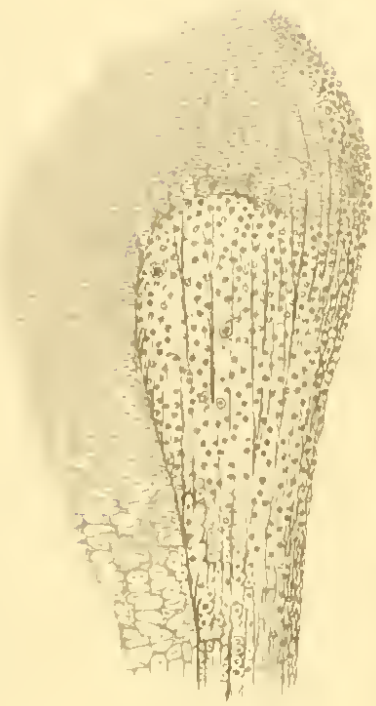


Fig. 2.

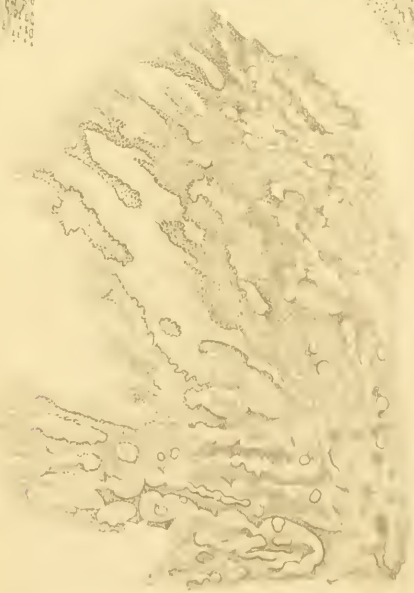


Fig. 1.

DESCRIPTION OF PLATES VII & VIII,

Illustrating Mr. E. A. Schäfer's observations on the Structure and Development of Osseous Tissue.

PLATE VII.

Fig. 1.—Transverse section of human tibia, softened with picric acid and stained with magenta glycerine. From near the surface of the bone. H H, Haversian canals, with their systems of concentric lamellæ; in all the rest of the figure the lamellæ are circumferential. *p f*, ordinary perforating fibres; *e e*, elastic perforating fibres. Drawn under a power of about 150 diameters.

Fig. 2.—Section through the middle of the humerus of a fœtal sheep. The entire bone was about 1 inch long. *i c*, The part of the shaft which was primarily ossified in cartilage; what remains of the primary bone is represented dark, and enveloped by the clear secondary deposit. The areolæ of the bone are occupied by osteoblasts, with blood-vessels variously cut, and represented as dark lines. One long straight vessel (*br*) passes in advance of the line of ossification far into the cartilaginous head; most of the others loop round close to the cartilage. At one or two places in the older parts of the bone (*c*) elongated groups of cartilage cells may still be seen, which have hitherto escaped absorption. *i m*, The part of the bone that has been ossified in membrane, *i. e.* in the osteoblastic tissue under the periosteum. It is well marked off from the central portion, and is bounded, peripherally, by a jagged edge, the projections of which are indistinctly seen to be prolonged by bunches of osteogenic fibres. A row of osteoblasts covers the superficial layer of the bone. The subperiosteal layer is prolonged above into the thickening (*p*), which encroaches upon the cartilage of the head of the bone, and in which are seen, amongst numerous osteoblasts and a few blood-vessels, the straight, longitudinal osteogenic fibres (*o f*) and some other fibres (*p f*) crossing their direction and probably representing the perforating fibres of Sharpey. The main outlines of the figure were taken from a specimen that had been stained lightly with carmine, but the details, which have been represented with as near an approach to accuracy as possible, were chiefly filled in from sections which had been stained with logwood,

hence the dark appearance of the cartilage, especially near the advancing ossification. The secondary osseous deposit near the centre of the bone has not been represented of sufficient relative thickness. Drawn under a magnifying power of about 30 diameters.

Fig. 3.—Longitudinal section through the subperiosteal thickening (encoche d'ossification). Drawn under a power of about 200 diameters. The preparation was stained with logwood and eosin. The osteoblastic tissue and the osteogenic fibres are shown. One or two blood-vessels are seen cut across. On the left is the cartilage, which is quite sharply marked off from the tissue of the encoche. Only the more superficial fibres are traceable into the superficial part of the cartilaginous head; the rest terminate for the most part before reaching the cartilage.

Fig. 4.—Represents a very small piece of the foregoing preparation under a higher magnifying power (400 diameters). The mode in which the angular osteoblasts fit in between and are applied to the osteogenic fibres is shown. The adjacent cartilage is on the left hand of the figure. *b*, bone; *c*, cartilage; *o*, *f*, osteogenic fibres.

Fig. 5.—Transverse section of a bone similar to Fig. 2, and drawn to the same scale; (*i c*), part ossified in cartilage; (*i m*), part ossified in membrane; (*p*), sub-periosteal layer; (*o*), osteogenetic fibres.

PLATE VIII.

Fig. 1.—A piece of the growing parietal of a fœtal cat, 1½ inch long. The blood-vessels and investing membranes were removed, but the osteogenic fibres and osteoblasts were left. The osteoblasts were not apparent, on account of the lowness of the magnifying power employed. The growing edge is seen to be fringed with osteogenic fibres; the intercrossing of these can be seen. One or two islands of ossified tissue are seen united to the main bony mass by bridges of osteogenic fibres. Slightly magnified.

Fig. 2.—The extremities of two osseous spicules, terminating in bunches of nearly straight, stiff-looking, osteogenic fibres. Some of the fibres of the two spicules are continuous into one another; others take directions oblique to one another, and tending in the progress of growth to intercross. The osteoblasts appear as if entangled amongst the fibres; many of them are seen to be applied to the fibres along a part of their course. The newly-deposited earthy matter is in the form of minute globules, giving a granular appearance to the new bone; as the intermediate substance between the globules becomes filled up by calcific deposit, the bone acquires a clearer aspect (as in the lower part of the figure. From a part of the growing margin of the bone represented in Fig. 1. Drawn under a magnifying power of 400 diameters.

Fig. 1.

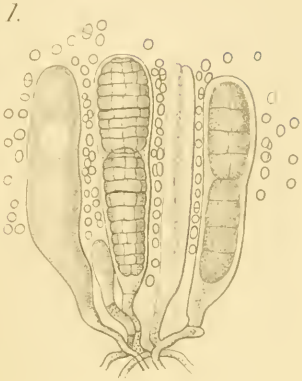


Fig. 2.

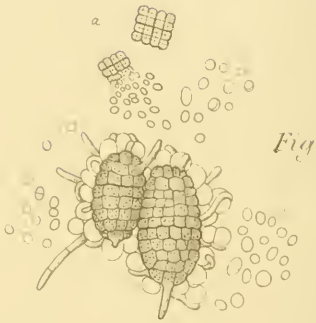


Fig. 3

Fig. 4.

Fig. 5.

EXPLANATION OF PLATE IX,

Illustrating Mr. Vines' Paper on Recent Researches into the Nature of Lichens.

Fig. 1.—A portion of the hymenium of Endocarpon pusillum. To the left, an ascus with as yet undivided contents; next to it, one with two ripe spores; still more to the right, an empty ascus. The interspaces are filled with spherical hymenial gonidia. $\frac{320}{1}$.

Fig. 2.—Two recently extruded spores with attached hymenial gonidia. $\frac{320}{1}$.

Fig. 3.—The hymenial gonidia have, in consequence of their investment by the hyphæ, increased in size, as a comparison of them with the free gonidia at once shows. At *a*, two Pleurococcus colonies derived each from a single hymenia gonidium. $\frac{320}{1}$.

Fig. 4.—A young thallus from a culture on porous earthenware, with two spores still attached. The mass of gonidia is invested by a layer of cells. $\frac{320}{1}$.

Fig. 5.—Two young thalli of Endocarpon five months old, cultivated on earthenware. $\frac{5}{1}$.

DESCRIPTION OF PLATE X,

Illustrating Prof. Lankester's observations on the Blood-corpuscles (see p. 68), and Mr. D'Arcy Power's on the Endothelia of the Common Earthworm (*Lumbricus terrestris*).

Figs. 1 to 6 are from drawings by Mr. Lankester.

Figs. 7, 8, and 9 are from drawings by Mr. D'Arcy Power.

Fig. 1.—Small blood-vessel from testis of earthworm, treated with osmic acid and subsequently stained with picrocarmine. *x*, The structureless blood-clot, coloured naturally by hæmoglobin; *n*, nuclei of the wall; *c*, corpuscles (free nuclei).

Fig. 2.—A similar blood-vessel of smaller calibre.

Fig. 3.—Some of the free nuclei or blood-corpuscles drawn to a larger scale. The actual short diameter of these corpuscles is $\frac{1}{3000}$th of an inch.

Fig. 4.—An Amœboid corpuscle from the perivisceral fluid of the earthworm, drawn from a specimen preserved by treatment with osmic acid and picrocarmine. This corpuscle is represented on the same scale of amplification as those given in fig. 3.

Fig. 5.—Two vascular swellings or globules, from a capillary of a segmental organ or "nephridium." *A* is represented as seen after silver-treatment, showing the cell-outlines of the capillary and globule; *B* is from a spirit-specimen stained with Kleinenberg's hæmatoxylin. The centre of *B* is occupied by the corpuscles first noticed by Gegenbaur. They appear to be somewhat smaller than those found in the vessels elsewhere. *n*, Nuclei of the wall-cells forming the wall.

Fig. 6.—Portion of a larger vessel from a muscular septum (osmic acid and picrocarmine). *n*, Nuclei; *c*, corpuscles (free nuclei); *in*, inner coat of the vessel; *ad*, outer coat.

Fig. 7.—Two blood-vessels from the side of a nephridium or segmental tube: probably an arteriole and a venule. The outlines of the cells constituting the vascular wall are rendered evident by silver-staining, and are seen to differ markedly in the two vessels. The outlines are accurately copied from the preparation, and consequently *some* of the divisions between adjacent cells are not given, not having taken up the staining.

Fig. 8.—Cell-outlines from silver-staining of a larger blood-vessel.

Fig. 9.—Retiform tissue from the earthworm, closely similar to that of the mammalian omentum. This tissue is found supporting the coils of the successive nephridia or renal tubes. The cell outlines are brought into view by silver-staining.

Fig. 10.—Endothelial cell-outlines from the surface of retractor muscle of Sipunculus nudus, stained with silver. From a drawing made by Mr. Lankester in 1872.

Fig 1

Fig 4

Fig 3

Fig 2

Fig 5

Fig 6

Fig 9

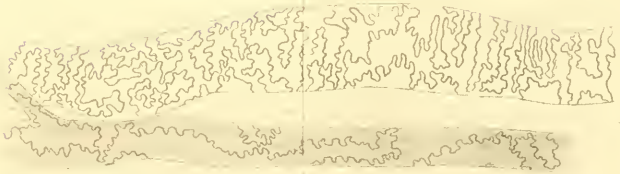


Fig 7

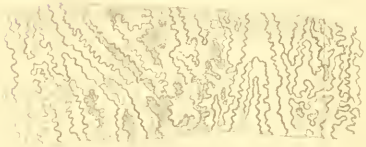


Fig 8

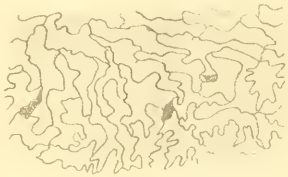
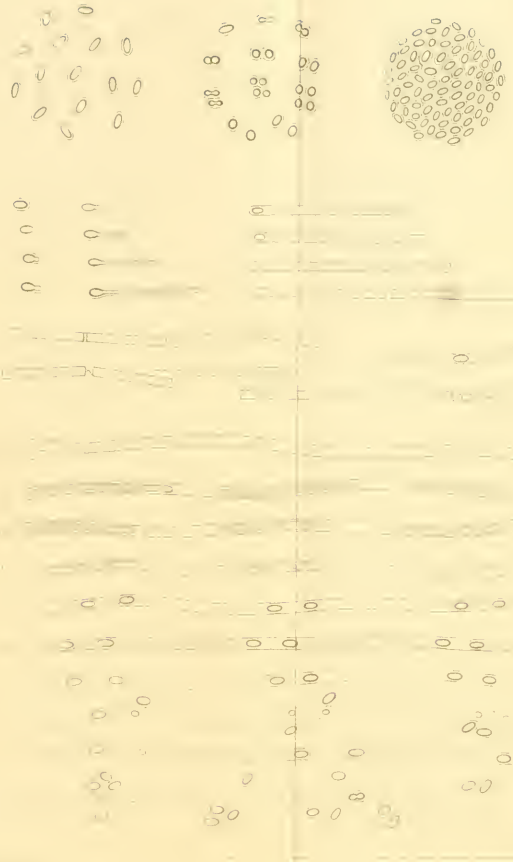


Fig 10



EXPLANATION OF PLATE XI,

Illustrating Mr. J. Cossar Ewart's observations on the Life-History of *Bacillus anthracis*.

Fig. 1.—Spores which have escaped from the filaments.

Fig. 2.—One spore dividing into four sporules.

Fig. 3.—Sporules forming a zoogloea.

Figs. 4 and 5.—A sporule developing into a rod, which in Fig. 5 is beiginning to divide into two segments.

Fig. 6.—A rod undergoing segmentation.

Fig. 7.—Rods with bodies in them, which may be looked upon as vacuoles or nuclei.

Fig. 8.—A newly developed filament.

Fig. 9.—Filament in which the protoplasm has divided into somewhat long segments.

Fig. 10.—A filament in which the protoplasm has undergone further segmentation.

Fig. 11.—The first appearance of the spores in the form of minute specks at the adjacent ends of the segments.

Fig. 12.—Shows the fully developed spores which have been formed by the contraction of the protoplasm.

Fig. 13.—The spaces between the spores have become granular, an indication that the filament is disintegrating.

Fig. 14.—The granular appearance has gone, and only the faintest possible indication of the filament is visible.

Fig. 15.—A filament from which nearly all the spores have escaped. Minute clear spots are seen at the points formerly occupied by the spores.

Fig. 16.—A filament which has broken into short segments, in some of which spores still persist.

Fig. 17.—A filament still more disintegrated; in one of the segments a dumb-bell-shaped spore is visible.

Fig. 18.—Matted rods in the subcutaneous tissue, from which rods extend an rows between the connective-tissue fibres.

Fig. 19.—Rods forming a zoogloea.

Fig. 20.—A rod undergoing segmentation.

Fig. 21.—A rod lengthening into a filament.

Fig. 22.—A filament containing spores, becoming granular at one end dshowing transverse lines between the spores.

Fig. 23.—Spore-bearing filaments forming a rope work.

Fig. 24.—Part of a filament containing a spore in process of division.

Fig. 25.—Shows the different stages through which a spore passes in its development into a rod.

Fig. 26.—Short filaments containing spores.

Figures A to E,

Illustrating Dr. Klein's Paper, "Experimental Contribution to the Etiology of Infectious Diseases with special reference to the Doctrine of Contagium vivum."

Fig. A.—Bacillus of infectious *Pneumo-enteritis* of the pig, cultivated

in humor aqueous of rabbit, showing spores germinating into rods, isolated rods, and series of rods.

Fig. R.—From a similar specimen as in fig. 1, at a later stage; most of the rods have grown into long filaments.

Fig. C.—Showing the formation of bright cylindrical spores in the filaments at a later stage.

Fig. D.—The filaments in which the spores are formed become very indistinct.

Fig. E.—Zoogloea of spores; its segments a hyaline gelatinous matrix, in which the spores are embedded; the spores are seen to possess a linear arrangement.

The drawings are represented as the objects appear when seen under a Zeiss's F objective, and Hartnack's III eye-piece, fitted to a Hartnack's small stand.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EXPLANATIONS OF PLATE XII—XV.

Illustrating C. O. Whitman's paper on "The Embryology of Clepsine."

(All the figures are outlined with the camera lucida.)

a, *b*, *c*. Permanent blastomeres. *ar*. Anal ring of dorsal blood-vessel. *b.cal*. Base of calotte. *cp*. Central part of polar area (amphiaster). *cg*. Cerebral ganglia. *c*. Cæca of stomach. *cal*. Calotte. *Cb*. Cephalic branch. *d*. Terminal disc. *d.t*. Dorsal trunk. *ea*. External orifice of segmental organ. *Ent*. Entoderm. *Ep*. Epidermis. *f.pn*. Female pronucleus. *f.pnl*. Female pronucleolus. *gb*. Germ-bands. *ia*. Internal orifice of segmental organ. *id*. Diverticula of intestine. *i*. Intestine. *m*. Mesoderm. *m.Pn*. Male pronucleus. *m.Pnl*. Male pronucleolus. *Med.S* Median sinus. *ms*. Marginal sinus. *n*. Nerve-cells. *o*. Œsophagus. *oa*. Pharyngeal atrium. *o.c*. Pharyngeal clefts. *P*. Pharynx. *Pa*. Polar area of amphiaster. *Pa*. Pharyngeal aperture (mouth, figs. 37—49). *Pb*. Pharyngeal branch. *Pf*. Polar figure. *Pg*. Polar globules. *Pgr*. Primitive groove. *Pr*. Polar ring (upper). *Pr'*. Polar ring (lower). *Pz*. Protoplasm-zones. *s*. Segment-cells. *Seg.c*. Segmentation cavity. *Seg.O*. Segmental organ. *Sep*. Septa. *S O.G*. Subœsophageal ganglia. *St*. Stomach. *t.b g*. Terminal body-ganglia. *vt*. Ventral trunk. *x & xy*. Mesoblasts. x^1—x^6. Neuroblasts. *z*. Problematic cells.

PLATE XII.

(Figs. 1—55 magnified about 25 diameters.)

Figs. 1—7.—A peristaltic constriction, resembling the first cleavage constriction, passes from the middle of the egg (fig. 2) towards the polar figure (*Pf*), and is followed by the extrusion of the first polar globule (fig. 7, *Pg*). Time from fig. 1 to fig. 7, 10—15 min.

Fig. 8.—Form of egg after appearance of the second pol. globule. (1 h. 25 min. after deposit.)

Fig. 9.—First ring appears around the shaded area of fig. 8. (1 h. 30 min.)

Fig. 10.—Second ring (*Pr'*) appears, and at about the same time ring-rays from the upper ring. (1 h. 35 min.)

Fig. 11.—The calotte much reduced (*cal*). The lower ring-substance covers the aboral pole in the form of a disc. Rays of the ring-substance seen at both poles. (

Fig. 12.—Just before the appearance of the cleavage furrow. Calotte has approached one side of the ring, and the rays have nearly disappeared. (2 h. 45 min.)

Figs. 13 and 14.—Two views of the same egg as the cleavage begins. *Pr'* is reduced to a mere point. *Pr* has assumed a crescentic shape. (3 h.)

Fig. 15.—The first meridional cleavage completed. The cleavage walls close up sooner on the upper than on the lower pole. (3 h. 30 min.)

Fig. 16.—Thirty minutes later. Seen from above. *Pr* begins to disappear.

FIG. 17.—The second meridional division begins to cut the larger of the two spheres, passing from the centre outwards. (5 h.)

FIG. 18.—Ten minutes later the cleavage begins on the smaller sphere, advancing in the opposite direction.

FIG. 19.—First two meridional divisions completed. (5 h. 30 min.)

FIG. 20.—The first ectoblast is in process of formation from the upper pole of the largest blastomere (*x*). (6 h. 30 min.)

FIG. 21.—The second ectoblast in process of formation from the blastomere (*b*). The first ectoblast has been pushed to the left into the blastomere (*a*). (7 h.)

FIG. 22.—The third and fourth ectoblasts are forming simultaneously from *a* and *c*. (7 h. 30 min.)

FIG. 23.—The formation of the four primary ectoblasts is completed, and *x* begins to divide. (8 h. 30 min.)

FIG. 24.—Thirty minutes later, from below. The division of *x* is well advanced. (9 h.)

FIG. 25.—The egg is seen from below. The division of *x* into *x'* and *x* has been completed. (12 h.)

FIG. 26.—The same from above. Three or four cells have been added to the primary ectoblasts from *a* and *b*.

FIG. 27.—Two hours later, from below. *x* is in process of division. (14 h.)

FIG. 28.—Thirty minutes later. (14 h. 30 min.)

FIG. 29.—Seen from below. The division of the original blastomere (*x*) (fig. 22) into the two mesoblasts (*x* and *xy*), and the primary neuroblast (*x'*) is accomplished. (14 h. 45 min.)

FIG. 30.—Seen from above. Ten to twelve cells have been added to the four ectoblasts. *x'* begins to divide, the plane of cleavage passing from above downward. (21 h.)

FIG. 31.—The four primary ectoblasts are completely encircled with cells derived from *a*, *b*, and *c*. The division of x^1 into x^2 and x^2 is completed. (24 h.)

FIG. 32.—The same from below.

FIG. 33.—The two cells (x^2) are in process of cleavage. (24 h. 15 min.)

FIG. 34.—The division of the two cells (x^2) into the four cells (x^3) is complete. The four primary ectoblasts have divided each into four parts. (24 h. 30 min.)

FIG. 35.—The two median cells (x^3) have pushed each other apart by nipple-like protrusions of their contiguous faces. On the left, just above (*xy*), are seen four cells in a row. They have been produced from the upper pole of the mesoblast (*xy*). (26 h. 30 min.)

FIG. 36.—The nipple-like protrusions have been constricted off as two small median cells (x^4). The mesoblast (*x*), which occupies the lower pole in fig. 29, has changed its position, passing backward and upward, followed by the ventral blastomere (*c*). It now lies just under the two cells (x^4). This egg is seen from the left side. (26 h. 40 min.)

FIG. 37.—The two cells x^4 have broken up into smaller cells. The two median cells (x^3) have produced the two cells (x^5), and the two outer cells (x^3) have produced x^6. This completes two symmetrically placed groups of neuroblasts, each group consisting of four cells (x^3, x^5, x^6, x^3). The line of mesoblastic cells produced by *xy* is still to be seen. The nuclei of *a*, *b*, and *c* have each divided into three or four parts, which are now seen in the surface of the blastomeres. These free nuclei are spoken of in the text as "entoplasts." The mesoblast (*x*) has completely disappeared, being covered by the posterior ends of *a*, *b*, and *c*. *xy* has also sunk deeply into *a*, but is still plain to be seen. (28 h. 30 min.)

FIG. 38.—The two neuroblasts x^5 are covered with ectodermic cells. The

germ-bands appear as two thickened lateral margins of the germinal disc. The place of the future mouth (*pa*) is seen in the centre of the cephalic mass; this point marks nearly the centre of the four primary ectoblasts. The pharyngeal clefts (*oc*) are marked by slight depressions at the line of junction of the germ-bands with the cephalic portion. (36 h.)

Fig. 39.—The same from below. The entoplasts have increased in number.

Fig. 40.—Only the thickened margins of the germinal disc are represented. The four superficial lines of cells produced by the neuroblasts give the bands a striated appearance. The fore ends of the bands rise above the level of the cephalic portion, as is plainly seen when the egg is tilted. The pharyngeal clefts (*oc*) are now nearly parallel with the linear depression leading backward from the mouth (*Pa*). (42 h.)

Fig. 41.—The anterior ends of the germ-bands are already in contact, and the pharyngeal clefts are nearly at right angles to the postoral depression, which is seen to be continuous with the primitive groove (*p.gr*). (48 h.)

Fig. 42.—The same, tilted on the hind end, to show that the ventral blastomere (*c*) is being driven up between the lateral blastomeres (*a*, *b*).

Fig. 43.—The same seen from behind. The entoplasts, which are not represented in the following figures, are quite numerous, and scattered over the entire surface of *a*, *b*, and *c*.

Fig. 44.—Seen from behind. The germ-bands are about two thirds closed. The ventral blastomere (*c*) is now wider on the dorsal than on the ventral side. (60 h.)

Fig. 45.—The same, seen from before. The metameric formation already begun.

Fig. 46.—The germ-bands most closed. The pharyngeal clefts have encircled the pharyngeal portion. The ganglionic formation is quite distinct. A few hours before hatching. (72 h.)

Fig. 47.—The same. *xy* is seen as a dark area in the right side.

Fig. 48.—The neuroblasts, which are much reduced in size, form a semicircle around the posterior ends of the fully closed bands. The two mesoblasts appear as ill-defined dark spots, lying near the surface of *a* and *c*. (One day old.)

Fig. 49.—The same, seen in profile. The segment-cells are seen between each metamere.

Fig. 50.—The pharynx protrudes a little. *xy* is less distinct. The septa are lengthening towards the dorsal side. The boundary lines of *a*, *b*, and *c* have disappeared. (3 days old.)

Fig. 51.—The same, from the ventral side.

Fig. 52.—The dorsal side is still shorter than the ventral. The three main divisions of the digestive tract with their cæca are now recognisable. The septa have not yet reached the dorsal side. (6 days old.)

Fig. 53.—Of the same age, but farther advanced.

Fig. 54.—Also of the same age, but still farther advanced. The dorsal and ventral sides are of about equal length.

Fig. 55.—The worm has nearly attained its definitive form. The eyes appear about this time as orange-coloured spots. (7 days old.)

PLATE XIII.

FIG. 56. —Circulatory apparatus, digestive tract, and one of the segmental organs. Magnified circa 50 diam. (14 days old.)

FIG. 57.—A transverse section of the egg-string, showing the central nucleated protoplasm (rhachis) and the peripheral layer of primitive egg-cells. Magnified 440 diam.

FIG. 58.—A longitudinal, optical section of the egg-string. *a*, *b*, *c*, and *d* = successive stages in the growth of the egg-cell. The rhachis is here much larger comparatively than in fig. 57. Magnified 160 diam.

FIG 59.—A portion of the egg-string at a time when the eggs have come to lie externally to it. Magnified 50 diam.

FIG. 60.—Three vesiculæ germinativæ. *a* represents the vesicle at the time the egg is liberated from the string, *b* is the vesicle of the full-grown egg, and *c* is probably an incipient reticular stage. Magnified 160 diam.

FIG. 61.—Nucleus corresponding nearly with the stage represented in fig. 58. The homogeneous nucleolus lies in the centre of the reticulum. Magnified 440 diam.

FIG. 62.—Section of a fresh-laid egg, showing the archiamphiaster. Only a part of the section is represented. Magnified 160 diam.

FIG. 63.—The second polar globule is in process of elimination. Magnified 160 diam.

FIG. 64.—One pole of the amphiaster in fig. 62 more highly magnified. (440 diam.).

FIG. 65.—A. Section of the egg just after the formation of the polar globules. The male (*m.Pn*) and female (*f.Pn*) pronuclei are present. Magnified 50 diam.

B. The polar globules highly magnified. (Zeiss J. and the camera nach Oberhäuser.)

FIG. 66.—C is a section of the egg at the time the first polar ring begins to form. The pronuclei are approaching each other. In the female pronucleus two pronucleoli are seen in close contact. The same are more highly magnified in D. The male pronucleus (magnified 160 diam. in E) contains only one nucleolus. F represents the united pronuclei.

FIG. 67.—G is a section of the egg just after the union of the pronuclei. The path of the female pronucleus is indicated by the shaded line. Magnified 50 diam. H and I are two primary cleavage-nuclei. Magnified 160 diam.

PLATE XIV.

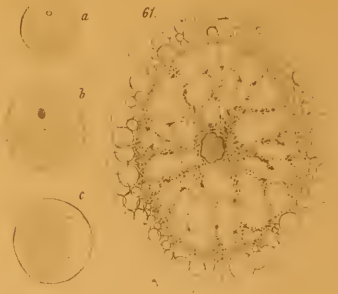
FIG. 68.—K is a section of the egg thirty minutes after the appearance of the first polar ring. The second ring already covers the lower pole. L represents the cleavage-nucleus of another section. Magnified 160 diam. N = the three pronucleoli.

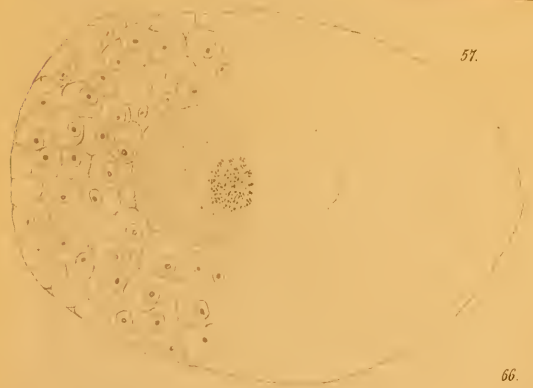
FIGS. 69, 70, and 71 (O) represent sections of the egg forty-five minutes, sixty minutes, and sixty minutes respectively after the appearance of the first polar ring, showing the concentration of the rings. In fig. 71 (O) the nucleus is spindle-shaped, but the nucleoli are still unchanged. P is another nucleus of the same date, magnified 160 diam.

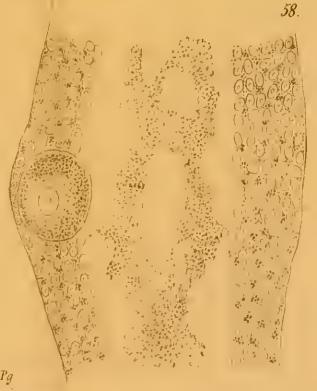
FIG. 72 —The nucleus has assumed the amphiastral form, and the ring-substance has plunged deeper into the egg. This section bears the same date as figs. 70 and 71, but is evidently farther advanced. Magnified 50 diam.

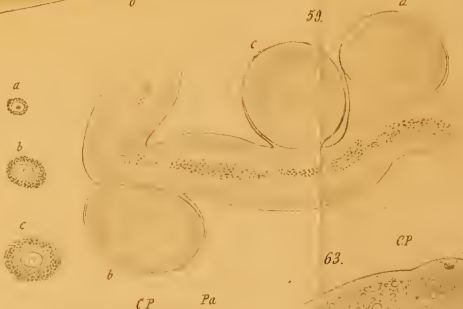
FIG. 73.—A little farther advanced than fig. 72, although of the same date. Magnified 50 diam.

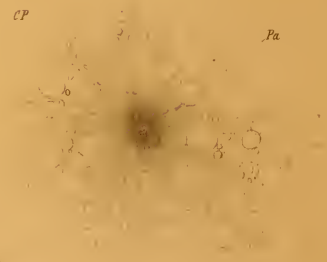
FIG. 74.—A moment before the first cleavage begins.

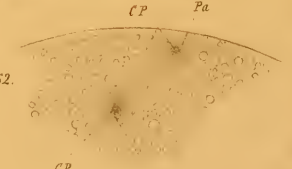


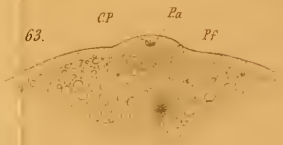


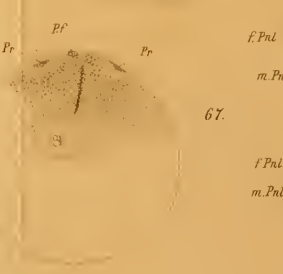


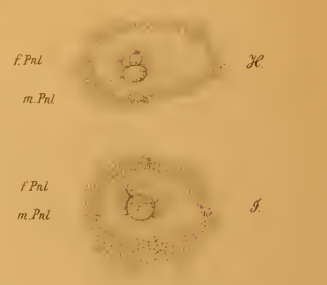


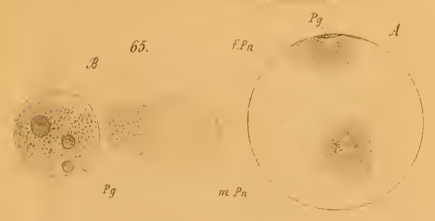


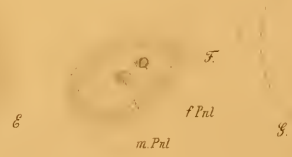










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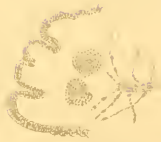


FIG. 75.—The cleavage has begun. The length of the amphiaster has reached its maximum.

FIG. 76.—First cleavage completed, but the two nuclei are still connected by a slender band of nucleoplasm.

FIG. 76 R.—One of the two nuclei in fig. 62, magnified 160 diam.

FIG. 77.—A section of the upper polar ring, taken about 1 mm. under the surface. The more darkly shaded ring around the base of the calotte is seldom seen. Magnified 50 diam.

FIG. 78.—One pole of the amphiaster (fig. 73), magnified 440 diam.

FIG. 79.—A transverse section through the middle of an egg arrived at stage 26, Pl. XII. T represents the nucleus of one of the ectoblasts at about the same date, magnified 440 diam.

FIG. 80.—A horizontal section of stage 36, Pl. XII, taken just below the germinal disc.

FIG. 81.—A transverse section of stage 37, Pl. XII, taken just behind the germinal disc, to show the positions of the mesoblasts (*x* and *xy*). Cells are seen leading from the nuclear poles of the mesoblasts up to the germinal disc.

FIG. 82.—A longitudinal section of the same date, which passes through the middle of the left mesoblast (*x*).

FIG. 83.—A horizontal section of the same date, below the germinal disc. The nuclei lie near the surface of the blastomeres.

FIG. 84.—A transverse section just in front of the neuroblasts (stage 41, Pl. XII). A line of cells leads from each mesoblast to the germ-band of the corresponding side.

FIG. 85.—A transverse section at a little later date.

FIG. 86.—A median transverse section (stage 44, Pl. XII). *x* and x_1 have changed their positions with reference to each other in consequence of the invagination. Compare with fig. 84.

FIG. 87.—A horizontal section (stage 43, Pl. XII). In the anterior part (lower part of the figure) the germ-bands have united, but in the posterior part they are still unclosed.

FIG. 88.—A median transverse section at the time of hatching, showing the nerve-cells (*n*) and a pair of segment-cells (*s*). Magnified 160 diam.

FIG. 89.—A similar section two days later.

PLATE XV.

FIG. 90.—A sagittal section, showing the origin of the nerve-cells and the segment-cells. Just hatched. Zeiss. A, A. and camera.

FIG. 91.—Embryo freed from the yolk, showing the number of body-segments and the position of the segment-cells (*s*). (2½ days old.) Magnified 50 diam.

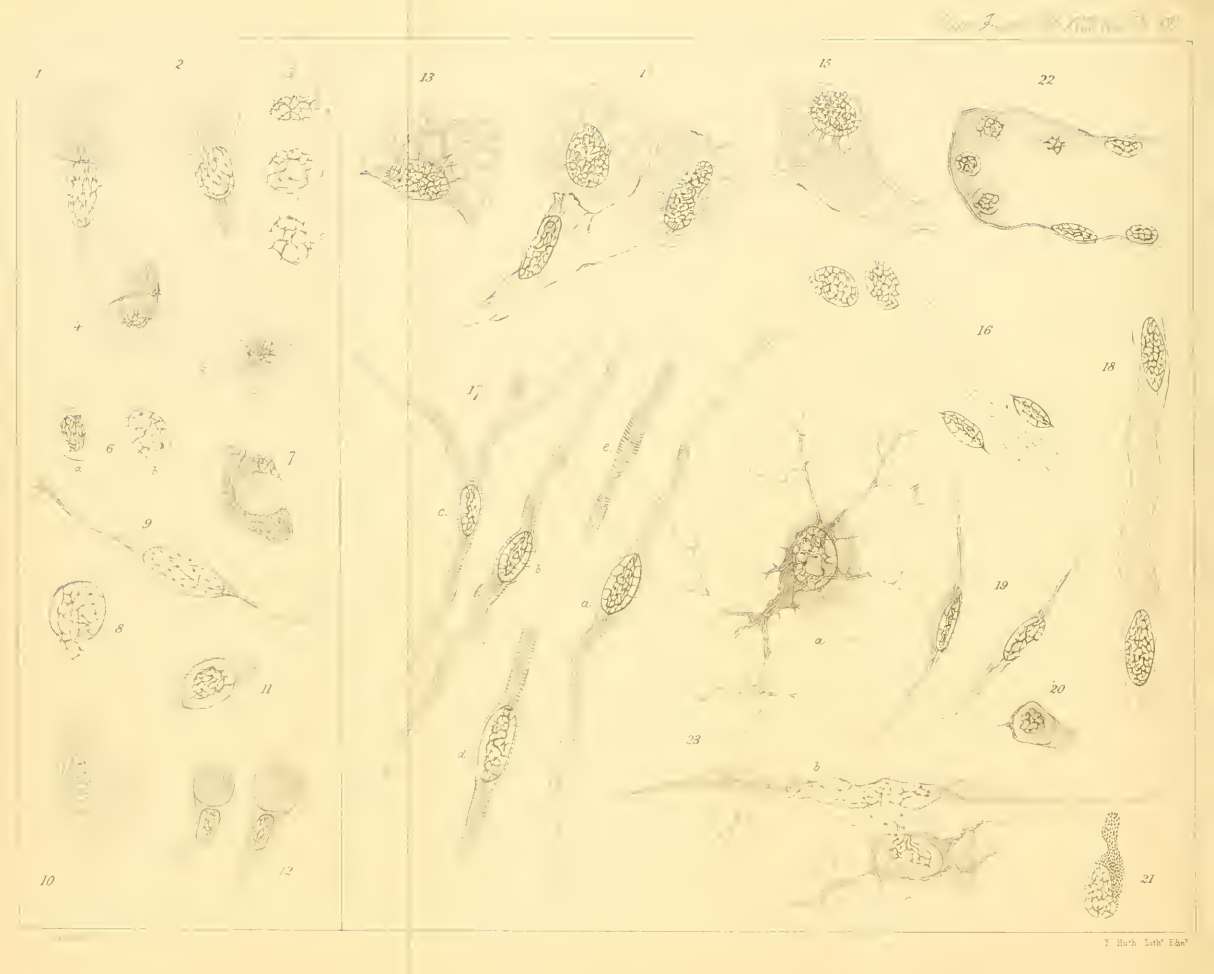
FIG. 92.—A segmental organ and one of the segment-cells 3½ days after exclusion. Magnified 440 diam.

FIG. 93.—A sagittal section (constructed from two successive sections) showing the condition of the entoderm eight days after exclusion. Magnified about 25 diam.

FIG. 94.—A portion of a horizontal section, one day later. The entodermic cells are arranged in a single compact layer. Magnified 160 diam.

FIG. 95.—A portion of fig. 93 magnified 160 diam. The entodermic cells are loosely arranged in the periphery of the yolk.

FIG. 96.—A horizontal section, passing near the middle of the pharynx. About three days old. Magnified 50 diam.



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EXPLANATION OF PLATE XVI,

Illustrating Dr. Klein's paper, "Observations on the Structure of Cells and Nuclei."

Figures 1—12 (incl.) refer to preparations of stomach, 13—23 to those of mesentery of newt. For method of preparing see text. All figures are represented as seen on Hartnack's small stand with eye-piece III, and Zeiss's objective F, except 17 *e*, which is drawn with Hartnack's No. 10 Immersion.

Figs. 1 and 2 represent goblet-cells; the intranuclear network is well shown, and also the fibrils passing from this into the upper and lower part of the cell.

Fig. 3, *a*, *b*, and *c*.—The intranuclear network separated from the membrane of the nucleus.

Fig. 4.—A goblet-cell (like that of 1 and 2) as seen obliquely from above, showing the intracellular network.

Fig. 5.—The intracellular network looked at vertically from above.

Fig. 6.—Two isolated nuclei showing the intranuclear network; this has shrunk to a considerable extent.

Fig. 7.—A gland-cell, showing the dense network of fine fibrils of the cell-substance; the nucleus has escaped, but there are still left a few fibrils, probably connecting the two networks, viz. the intranuclear and the intracellular.

Fig. 8.—An isolated nucleus of a gland-cell; the wall of the nucleus is broken at one place and the intranuclear fibrils are seen passing outwards.

Fig. 9.—A connective-tissue corpuscle—endothelial plate—seen in profile; both the intracellular fibrils and those of the intranuclear network are well shown.

Fig. 10.—A similar cell seen from its broad surface.

Fig. 11.—Portion of a cell; the intranuclear network shrunk.

Fig. 12.—Two epithelial cells; the intracellular fibrils are well shown. The top of both cells is seen in an oblique direction, and the network of fibrils is therefore brought into view. Preparation treated with Müller's fluid and then with mixture of chromic acid and spirit; see text.

Figs. 13, 14, 15.—Isolated endothelial plates of surface of mesentery. The intracellular and intranuclear networks of fibrils are well shown.

Fig. 16.—A capillary blood-vessel; the two upper nuclei are seen from their broad side, the two lower in profile. They all show the network of fibrils. In the lower portion of the wall of the vessel an imperfect network of fibrils may be perceived.

Fig. 17, *a* and *b*.—Two unstriped muscle-fibres. The intranuclear network of fibrils is well seen; these are in connection with fibrils of the substance of the muscle-fibre; there are seen numerous transverse markings along almost the whole length of the muscle-fibre. That these transverse markings, corresponding to rings which constitute the cortical part, *i. e.* the sheath, is well shown in *c*, *d*, and *e*.

Fig. 18.—A non-medullated nerve-fibre of mesentery of newt; the nerve-fibre has a delicate sheath, the nuclei of which contain a distinct network of fibrils.

Fig. 19.—Two connective-tissue corpuscles seen side-ways. The nucleus contains a network of fibrils, in connection with that of the cell-substance.

Fig. 20 and 21.—Two migratory cells; 20, a common pale one, 21, a coarsely granular one. Their nucleus shows the network of fibrils very well.

Fig. 22.—A cæcal dilatation of a lymphatic vessel of the same membrane, showing the nuclei of the endothelial cells forming the wall of the lymphatic, and also nuclei of lymph-corpuscles.

Fig. 23. *a* and *c*.—Two connective-tissue corpuscles; the distinction between 'ground-plate' and 'fibrillar substance' is well shown; the 'fibrillar substance' is in connection with the intranuclear fibrils.

b, a nucleated plate ensheathing a minute non-medullated nerve-fibre. The intranuclear network is seen in connection with the processes and fibrils of the connective-tissue corpuscle *c*.

EXPLANATION OF PLATES XVII, XVIII & XIX.

Illustrating Mr. Balfour's memoir on "Structure and Development of the Vertebrate Ovary."

EXPLANATION OF PLATE XVII

List of Reference Letters.

p o. Primitive ovum. *d o.* Permanent ovum in the act of being formed. *o.* Permanent ovum. *f e.* Follicular epithelium. *ov r.* Ovarian portion of ovarian ridge. *ps e.* Pseudo-epithelium of ovarian ridge. *e p.* Non-ovarian epithelium of ovarian ridge. *n n.* Nests of nuclei of ovarian region. *str.* Stroma of ovarian ridge. *v. str.* Vascular region of stroma adjoining ovarian ridge. *l. str.* Lymphatic region of stroma. *v.* Blood-vessel. *d v.* Developing blood-vessels. *x.* Modified nucleus.

Fig. 1.—Transverse section of the ovarian ridge of an embryo of *Scy. canicula*, belonging to stage P, showing the ovarian region with thickened epithelium and numerous primitive ova. Zeiss, C, ocul. 2. *Picric acid.*

Fig. 2.—Transverse section of the ovarian ridge of an embryo of *Scyllium canicula*, considerably older than stage Q. Zeiss, C, ocul. 2. *Picric acid.* Several nests, some with distinct ova, and others with the ova fused together, are present in the section (*n. n.*), and several examples of modified nuclei in still distinct ova are also represented. One of these is marked *x.* The stroma of the ovarian ridge is exceptionally scanty.

Fig. 3.—Transverse section through part of the ovarian ridge, including the ovarian region of an almost ripe embryo of *Scyllium canicula.* Zeiss, C, ocul. 2. *Picric acid.* Nuclear nests (*n. n.*), developing ova (*d. o.*), and ova (*o.*), with completely formed follicular epithelium, are now present. The ovarian region is still well separated from the subjacent stroma, and does not appear to contain any cells except those of the original germinal epithelium.

Fig. 4.—Section through ovarian ridge of the same embryo as fig. 3, to illustrate the relation of the stroma (*str.*) and ovarian region. Zeiss, *a a*, ocul. 2. *Picric acid.*

Fig. 5.—Section through the ovarian ridge of an embryo of *Scyllium canicula*, 10 cm. long, in which the ovary was slightly less advanced than in fig. 3. To illustrate the relation of the ovarian epithelium to the subjacent vascula stroma. Zeiss, A, ocul. 2. *Osmic acid. y.* points to a small separated portion of the germinal epithelium.

Fig. 6.—Section through the ovarian ridge of an embryo of *Scyllium canicula*, slightly older than fig. 5. To illustrate the relation of the ovarian epithelium to the subjacent vascular stroma. Zeiss. A, ocul. 2. *Osmic acid.*

Fig. 7.—More highly magnified portion of the same ovary as fig. 6. To illustrate the same points. Zeiss, C, ocul. 2. *Osmic acid.*

Fig. 8.—Section through the ovarian region (close to one extremity, where it is very small) from a young female of *Scy. canicula.* Zeiss, C, ocul. 2. *Picric acid.* It shows the vascular ingrowths amongst the original epithelial cells of the ovarian region.

Fig. 9.—Section through the ovarian region of the same embryo as fig. 8, at its point of maximum development. Zeiss, A, ocul. 2. *Picric acid.*

Fig. 10.—Section through superficial part of the ovary of an embryo, showing the pseudo-epithelium; the cells of which are provided with tails prolonged into the general tissue of the ovary. At *f e.* is seen a surface view of the follicular epithelium of an ovum. Zeiss, C, ocul. 2. *Picric acid.*

EXPLANATION OF PLATE XVIII.

List of Reference Letters.

o. Permanent ovum. *d o.* Developing ovum. *g v.* Germinal vesicle. *f e.* Follicular epithelium. *yk.* Yolk. *d yk.* Developing yolk. *vt.* Vitelline membrane. *z n.* Zona radiata. *d n.* Modified nucleus of primitive ovum. *ps e.* Pseud-epithelium of ovarian region *str.* Stroma ingrowths into ovarian epithelium.

Fig. 11.—Section through part of an ovary of *Scyllium canicula* of stage Q, with three primitive ova, the most superficial one containing a modified nucleus.

Fig. 12.—Section through part of an ovary of an example of *Scyllium canicula*, 8 c. m. long. The section passes through a nest of ova with modified nuclei, in which the outlines of the individual ova are quite distinct. Zeiss, E, ocul. 2. *Picric acid.*

Fig. 13.—Section through part of ovary of the same embryo as in fig. 5. The section passes through a nest of nuclei, with at the least two developing ova, and also through one already formed permanent ovum. Zeiss, E, ocul. 2. *Osmic acid.*

Figs. 14, 15, 16, 17, 18.—Sections through parts of the ovary of the same embryo as fig. 3, with nests of nuclei and a permanent ova in the act of formation. Fig 14 is drawn with Zeiss, D D, ocul. 2. Figs. 15, 16, 17, with Zeiss, E, ocul. 2. *Picric Acid.*

Fig. 19.—Two nuclei from a nest which appear to be in the act of division. From ovary of the same embryo as fig. 3.

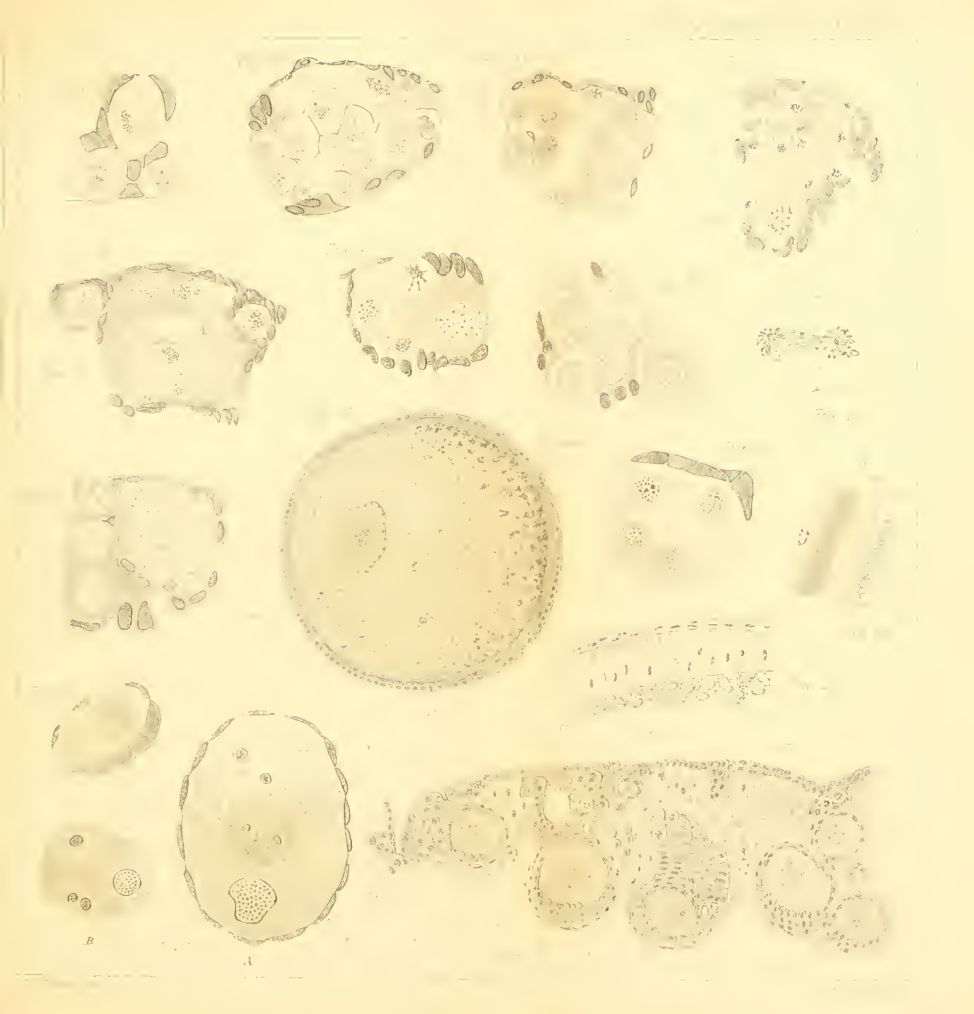
Fig. 20.—Section through part of an ovary of the same embryo as fig. 6, containing a nest of nuclei. Zeiss F, ocul. 2. *Osmic acid.*

Fig. 21.—Ovum from the ovary of a half-grown female, containing isolated deeply stained patches of developing yolk granules. Zeiss, B. ocul. 2. *Picric acid.*

Fig. 22.—Section through a small part of the ovum of an immature female of *Scyllium canicula*, to show the constitution of the yolk, the follicular epithelium, and the egg membranes. Zeiss, E, ocul. 2. *Chromic acid.*

Fig. 23.—Section through part of the periphery of a nearly ripe ovum of *Scy. canicula.* Zeiss, C, ocul. 2. It shows the remnant of the vitelline membrane (*v. t.*) separating the columnar but delicate cells of the follicular epithelium (*f. e.*) from the yolk (*yk.*). In the yolk are seen yolk-spherules in a protoplasmic network. The transverse markings in the yolk spherules have been made oblique by the artist.

Fig. 24.—Fully formed ovum containing a second nucleus (*x*), probably about to be employed as pabulum; from the same ovary as fig. 5. The follicular epithelium is much thicker on the side adjoining the stroma than on the upper side of the ovum. Zeiss, F, ocul. 2. *Osmic acid.*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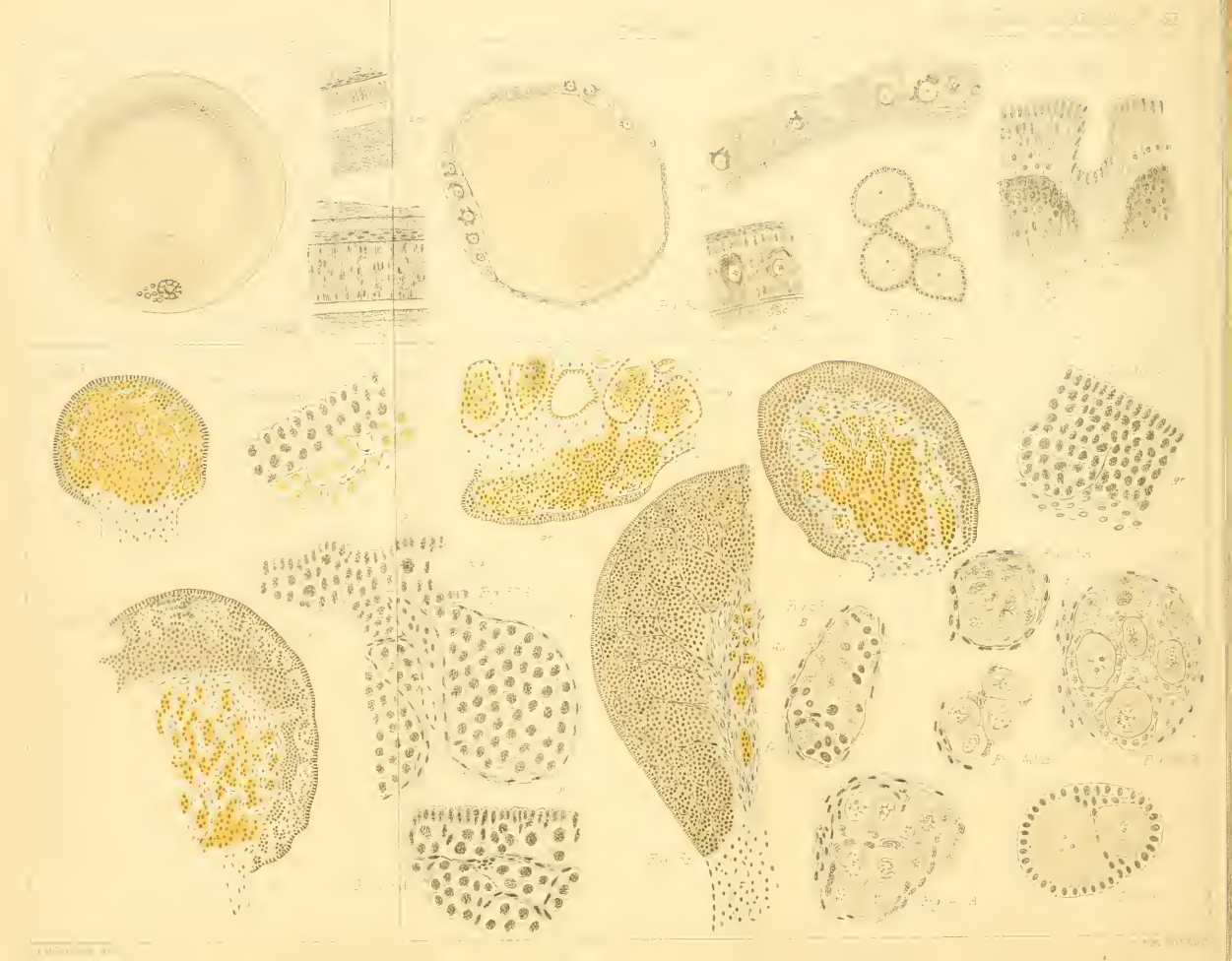


FIG. 25.—A. Ovum from the same ovary as fig. 21, containing in the yolk three peculiar bodies, similar in appearance to the two small bodies in the germinal vesicle. B. Germinal vesicle of a large ovum from the same ovary, containing a body of a strikingly similar appearance to those in the body of the ovum in A. Zeiss, E, ocul. 2. *Picric acid.*

FIG. 26.—Section of the ovary of a young female of *Scyllium stellare* 16½ centimetres in length. The ovary is exceptional, on account of the large size of the stroma ingrowths into the epithelium. Zeiss, C, ocul. 2. *Osmic acid.*

EXPLANATION OF PLATE XIX.

LIST OF REFERENCE LETTERS FOR FIGS. 27—34.

fe. Follicular epithelium. *fe'.* Secondary follicular epithelium. *vt.* Vitelline membrane. *z.n.* Zona radiata. *yk.* Yolk (vitellus). *g.v.* Germinal vesicle.

FIG. 27.—Ovum of *Scyllium canicula*, 0.5 mm. in diameter, treated with osmic acid. The figure illustrates the development of the yolk and a peculiar mode of proliferation of the germinal spots. Zeiss, A, ocul. 2.

FIG. 28.—Small part of the follicular epithelium and egg membranes of a somewhat larger ovum of *Scyllium canicula* than fig. 22. Zeiss. D D, ocul. 2.

FIG. 29.—The same parts as in fig. 28, from a still larger ovum. Zeiss, D D, ocul. 2.

FIG. 30.—Ovum of Raja with follicular epithelium. Zeiss, C, ocul. 2.

FIG. 31.—Small portion of a larger ovum of Raja than fig. 30. Zeiss, D D, ocul. 2.

FIG. 32.—Follicular epithelium, &c., from an ovum of Raja still larger than fig. 31. Zeiss, D D, ocul. 2.

FIG. 33.—Surface view of follicular epithelium from an ovum of Raja of about the same age as fig. 33.

FIG. 34.—Vertical section through the superficial part of an ovary of an adult Raja to show the relation of the pseud-epithelium to the subjacent stroma. Zeiss, D D, ocul. 2.

COMPLETE LIST OF REFERENCE LETTERS FOR MAMMALIAN OVARY.

ov. Ovary. *ge.* Germinal epithelium. *t.* Tubuliferous tissue, derived from Malpighian bodies. *po.* Primitive ovum. *n.* Nest of cells of the germinal epithelium. *nd.* Nuclei in the act of dividing. *do.* Developing ovum. *o.* Permanent ovum. *fe.* Follicular epithelium. *fc.* Cells which will form the follicular epithelium. *mg.* Malpighian body.

FIG. 35.—Transverse section through the ovary of an embryo rabbit of eighteen days, hardened in osmic acid. The colours employed are intended to render clear the distinction between the germinal epithelium (*g e.*) and the tubuliferous tissue (*t.*), which has grown in from the Wolffian body, and which gives rise in the male to parts of the tubuli seminiferi. Zeiss, A, ocul. 2.

FIG. 35 A.—Transverse section through a small part of the ovary of an embryo from the same female at fig. 35, hardened in picric acid, showing the relation of the germinal epithelium to the subjacent tissue. Zeiss, D D, ocul. 2.

Fig. 35 B.—Longitudinal section through part of the Wolffian body and the anterior end of the ovary of an eighteen days' embryo, to show the derivation of tubuliferous tissue (*t.*) from the Malpighian bodies, close to the anterior extremity of the ovary. Zeiss, A, ocul. 1.

Fig. 36.—Transverse section through the ovary of an embryo rabbit of twenty-two days, hardened in osmic acid. It is coloured in the same manner as fig. 35. Zeiss, A, ocul. 2.

Fig. 36 A.—Transverse section through a small part of the ovary of an embryo, from the same female as fig. 36, hardened in picric acid, showing the relation of the germinal epithelium to the stroma of the ovary. Zeiss, D D, ocul, 2.

Figs. 37 and 37 A.—The same parts of an ovary of a twenty-eight days' embryo as figs. 36 and 36 A of a twenty-two days' embryo.

Fig. 38.—Ovary of a rabbit five days after birth, coloured in the same manner as figs. 35, 36, and 37, but represented on a somewhat smaller scale. *Picric acid.*

Fig. 38 A.—Vertical section through a small part of the surface of the same ovary as fig. 38. Zeiss, D D, ocul. 2.

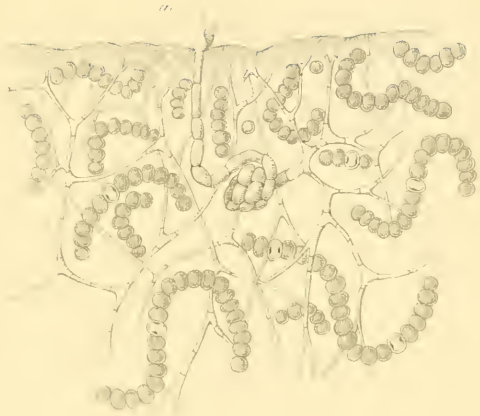
Fig. 38 B.—Small portion of the deeper layer of the germinal epithelium of the same ovary as fig. 38. The figure shows the commencing differentiation of the cells of the germinal epithelium into true ova and follicle cells. Zeiss, D D, ocul. 2.

Fig. 39 A.—Section through a small part of the middle region of the germinal epithelium of a rabbit seven days after birth. Zeiss, D D, ocul. 2.

Fig. 39 B.—Section through a small part of the innermost layer of the germinal epithelium of a rabbit seven days after birth, showing the formation of Graafian follicles. Zeiss, D D, ocul. 2.

Figs. 40 A and 40 B.—Small portions of the middle region of the germinal epithelium of a rabbit four weeks after birth. Zeiss, D D, ocul. 2.

Fig. 41.—Graafian follicle with two ova, about to divide into two follicles, from a rabbit six weeks after birth. Zeiss, D D, ocul. 2.



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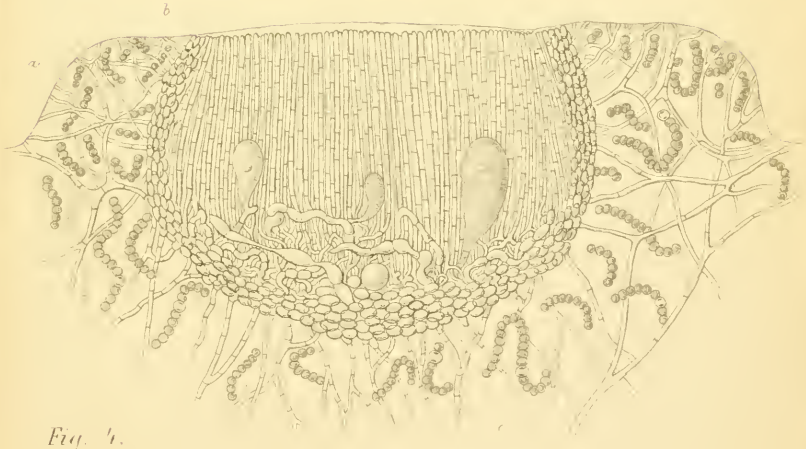

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