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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 Filippi (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 ($\frac{1}{1\,0}$ 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 ($\frac{1}{1\,8}\frac{0\,9}{7\,8}$), 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 eingechlossenen Kern eine länglich-kugelige Form, es tritt eine membran auf."

Ludwig ($\frac{\text{L}\,\text{L}\,\text{L}}{0\,8}$) accepts this view and extends the same to other Hirudinea. Leuckart ($\frac{1\,0\,6}{6\,7\,8}$), on the other hand, speaks of the egg as arising from a ready formed cell. Robin ($\frac{1\,4\,2}{5}$) 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 ($\frac{1\,0\,6}{0\,8\,0}$) 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 ($^{1}\frac{4}{1}^{3}$) 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 ($\frac{1}{1}\frac{0}{2}\frac{9}{8}$), 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 (355). 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 (36) and some other naturalists mentioned by Moquin Tandon (112), 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 (106) thinks it probable that copulation and reciprocal fecundation take place. According to Robin (143) 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 ($\frac{59}{3}$), for those species which lay their eggs in sacs. Leuckart ($\frac{106}{683}$) 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 ($\frac{1}{7}\frac{3}{0}\frac{6}{0}$).

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 ($\frac{2}{6}\frac{7}{}$) Nephelis,) Frommann (49, blood-cells), Schwalbe (149, ganglionic cells), Flemming (38, Unio and Anodonta; 39, connective tissue, endothelium, muscle, nerve, cartilage, and epithelium), Flemming ($\frac{3}{7}\frac{0}{0}\frac{}{9}$) and Giard ($\frac{5}{4}\frac{2}{3}\frac{}{4}$, Echinus miliaris), van Beneden ($\frac{1}{1}\frac{6}{7}\frac{}{0}$), Asteracanthion and rabbit), O. Hertwig ($\frac{7}{3}\frac{0}{5}\frac{}{1}\frac{}{8}$) Toxo-

[1] This reticulum was, so far as I am aware, first described by Kleinenberg in his well-known work on 'Hydra' ($\frac{5}{4}\frac{2}{1}$).

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 ($\frac{1}{3}\frac{2}{7}$) is inclined to believe, is evident from the fact that it is to be seen in living nuclei, according to the testimony of Hertwig ($\frac{7}{3}\frac{0}{5}$), 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 ($\frac{40}{476}$), lays particular stress on the star-shaped poles. He maintains ($\frac{42}{667-8}$) 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 ($\frac{21}{98}$), 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 ($\frac{40}{476}$) and Strasburger ($\frac{154}{206}$) 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 ($\frac{3}{292-292}$), they are produced by innumerable fine streams of nuclear fluid from the ends of the spindle. Butschli ($\frac{27}{192202}$), 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 ($\frac{58}{85-95}$) 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 ($\frac{38}{195-207}$) 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 ($\frac{42}{667}$), 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 ($\frac{154}{25}$) 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 ($\frac{32}{308}\frac{23}{592}$) maintains that these appearances are called forth by amœba-like pseudopodia of the nucleus itself.

Villot ($\frac{156}{10}$) 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 ($\frac{145}{466}$) 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 ($\frac{34}{90}$) in 1847. He described each nucleus as a *centre d'une radiation une peu confuse.* Krohn ($\frac{91}{314}$, Ascidia, 1852) described *Irradiations centren* around which the yolk was arranged in *radienförmigen Streifen;* Remak ($\frac{143}{132}$) (Rana esculenta 1855) found *radiale Striefen* in one of the upper cleavage-spheres; Gegenbaur (54 Sagitta, 1857); Leuckart ($\frac{107}{90}$, Nematoidea, 1867—1876); Kowalevsky ($\frac{83}{4}$, 85, $\frac{86}{609}$, Ascidia, 1866; Euaxes and Lumbricus, 1871; Pyrosoma, 1875); Kupffer ($\frac{62}{123}$, Ascidia, 1870); Bütschli (24, Nematoidea, 1873); Fol ($\frac{40}{476}$, Geryonia, 1873; Pteropod, 1875; Echinoderms, 1877); Metschnikoff ($\frac{147}{15}$, Geryonia, Polyxenia, 1874); Auerbach ($\frac{13}{8}\frac{8}{9}\frac{1}{1}$ Nematoidea, 1874); van Beneden ($\frac{15}{48}$, 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 ($\frac{3}{2}\frac{7}{7}\frac{}{6}$) and other Mollusca, and on the egg of Euaxes ($\frac{8}{1}\frac{5}{4}$). 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 ($\frac{1}{4}\frac{4}{9}^{3}$).

(*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 ($\frac{38}{109}$, Anodonta), "the attempts to divide" reported by Brandt ($\frac{22}{37}$, Ascaris), the "form-changes" noted by Schultz ($\frac{145}{66}$, 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 ($\frac{27}{9}$), 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 ($\frac{149}{88}$), is proved by such sections as that given in fig. 63, Plate XIII. This section shows the second polar globule in

[1] Fol ($\frac{45}{2}$, $\frac{46}{11}$), 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 aftrr 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 ($\frac{3}{2\,0\,2}$).

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, ($\frac{2}{3}\frac{7}{4}\frac{}{7}$, $\frac{7}{2}\frac{1}{2}$). Hertwig has studied the same phenomena in the egg of Toxopneustes, Asteracanthion and Sphaerechinus, and has here described as "Spermakern" ($\frac{7}{3}\frac{0}{8}\frac{}{4}$, $\frac{7}{2}\frac{2}{7}\frac{}{4}$) and "Eikern" ($\frac{7}{3}\frac{0}{5}\frac{}{7}$, $\frac{7}{2}\frac{2}{7}\frac{}{4}$) 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 ($\frac{1}{4}\frac{5}{8}\frac{3}{3}\frac{3}{0}\frac{0}{9}$).

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 ($\frac{9}{2}\frac{5}{1}\frac{}{3}$), 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 ($\frac{3}{5}\frac{5}{7}$) and Zeller ($\frac{1}{3}\frac{5}{6}\frac{8}{0}$, 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 ($\frac{5}{2}\frac{8}{5}\frac{}{6}\frac{}{1}$), are opposed to the view that they are nuclei. Oellacher ($\frac{1}{4}\frac{2}{1}\frac{4}{6}$) 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 ($\frac{3\,7}{1\,9\,8}$). The peculiar clusters of nuclei found by Balfour ($\frac{1\,1}{3\,4\,6}$) 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 ($\frac{3\,7}{1\,9\,8}$) that these clusters are nuclei in process of coalescing, has not the slightest shade of probability in its favour.

Van Beneden ($\frac{1\,6}{1\,5\,6}$) 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 ($\frac{3}{2\,0\,3}$), 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 ($\frac{3}{2\,0\,4}$) 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" ($\frac{5\,6}{6\,1}$).

The clear central spaces (ca) that arise in the poles of the amphiaster (fig. 73) probably correspond to Götte's "Lebenskeim" ($\frac{3\,8}{3\,1}$).

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 quiescent 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 ($\frac{1 2 3}{1 7}$, 1852), Leydig ($\frac{1 1 0}{2 8}$, $\frac{1 1 0}{1 0 3}$, 1854), Gegenbaur ($\frac{5 3}{6 9}$, 1854), Leuckart ($\frac{1 0 5}{6 7}$, 1858, $\frac{1 0 7}{3 4 6}$), Keferstein ($\frac{8 0}{1}$, 1868), Häckel ($\frac{8 5}{2 1}$, 1869), van Beneden ($\frac{1 3}{3 6}$, 1870), Kowalevsky ($\frac{8 5}{4 1}$, 1871), Frey ($\frac{4 5}{6 3}$, 1873), and v. Baer ($\frac{7}{2 0 3}$, 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 ($\frac{6}{3 8}$, 1846), Reichert ($\frac{1 3 8}{2 0 1}$, 1846), Leuckart ($\frac{1 0 4}{9 2 7}$, 1853), Weismann ($\frac{1 5 7}{1}$, 1863), Kupffer ($\frac{0 3}{1 6 9}$, 1870), Kleinenberg ($\frac{5 1}{4 2}$, 1872), Oellacher ($\frac{1 2 4}{1 2 1 3}$, 1872), Auerbach ($\frac{3}{7 9 9}$, 1874), Robin ($\frac{1 4 3}{8 7}$, 1875), Flemming ($\frac{3 8}{1 8 2}$, 1875), Götte ($\frac{3 8}{1 8}$, 1875), Balfour (Monogr. Develop. Elasmobranch Fishes, 1876), Kölliker ($\frac{0 6}{5 3}$, 1876), and Bischoff ($\frac{1 2}{3 4}$, 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 ($\frac{19}{218}$) 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 ($\frac{16}{153}$). "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 ($\frac{16}{175}$), 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 ($\frac{30}{234}$, Neritina fluviatilis) corroborate all this, and are all the more convincing as they compel Bütschli to abandon the opinion previously expressed ($\frac{19}{234}$), 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 ($\frac{154}{302}$). 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 ($\frac{3}{2\,0\,2-2\,1\,4}$). 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 ($\frac{2\,4}{1\,0\,1}\ \frac{}{4}$) 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 ($\frac{2\,3}{2\,0\,1}$), containing results obtained in the study of several genera of nematoids (Tylenchus, Cepholobus, 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 ($\frac{1\,5\,4}{2\,1\,2}$, Phallusia mamillata). Shortly after (1875) came the very important investigations of Oscar Hertwig on *Toxopneustes lividus*. Auerbach ($\frac{3}{2\,4\,8}$) had already compared the union of the pronuclei with the "*Copulation zweier Individiduen, oder wenigstens 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 ($\frac{7\,0}{3\,8\,3}$): "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 ($\frac{7}{2}\frac{9}{7}\frac{4}{8}$-$\frac{9}{2}\frac{7}{7}\frac{8}{8}$) 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 ($\frac{1}{1}\frac{6}{5}\frac{}{4}$) 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 ($\frac{1}{1}\frac{6}{5}\frac{}{4}$) 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 ($\frac{1}{9}\frac{5}{}$). 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 ($\frac{1}{1}\frac{6}{7}\frac{}{8}$) 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 $\frac{77}{84}$ 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 ($\frac{68}{5684}$, Pedicellina) has called attention to the universality and early appearance of polar differentiation in the egg; and Ray Lankester ($\frac{10}{38}$) 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 ($\frac{8.5}{1\,2-1\,3}$) 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 ($\frac{2\,3}{3\,9\,6}$, 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 ($\frac{1\,1\,8}{4\,7\,4}$).

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 ($\frac{8\,1}{4\,9}$) 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 ($\frac{1\,1\,7}{4\,6}$) 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 ($\frac{1\,3\,7}{3\,4\,5}$) 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 ($\frac{1\,3\,2}{1\,3\,4}$).

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 ($\frac{4\,1}{3\,8}$) 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 ($\frac{7}{8}\frac{1}{4}\frac{1}{5}$) 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 ($\frac{71}{59}$, $\frac{72}{276}$) 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 ($\frac{1}{4}\frac{1}{1}\frac{4}{1}$), 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 ($\frac{1}{1}\frac{5}{0}\frac{8}{11}$) has done, with the polar globules, since they arise not only before fecundation ($\frac{9}{1}\frac{2}{2}\frac{4}{4}$), but also before the transformation of the germinal vesicle ($\frac{9}{3}\frac{3}{6}\frac{6}{6}$, $\frac{8}{6}\frac{6}{0}\frac{7}{7}$). 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 ($\frac{1}{1}\frac{3}{5}\frac{5}{8-9}$, 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 ($\frac{1}{2}\frac{4}{6}\frac{3}{-5}\frac{3}{4}$) 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 ($\frac{1}{3}\frac{2}{3}\frac{1}{1}$) 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 $\frac{1}{3}\frac{5}{2}\frac{9}{2}$). 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 ($\frac{1}{1}\frac{2}{2}$) and Villot ($\frac{1}{3}\frac{5}{4}\frac{6}{4}$).

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 ($\frac{30}{237}$) 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 ($\frac{2}{2}\frac{8}{16}\frac{-7}{19}$, $\frac{30}{237}$) 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 $\frac{70}{386}$, Engelmann $\frac{35}{630}$, Bütschli $\frac{27}{216}$), and during their temporary union a reciprocal fecundation takes place, the "nucleolus-segments" with a little protoplasm being interchanged (according to Engelmann). Bütschli ($\frac{27}{83}$) 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 ($\frac{26}{431}$, $\frac{27}{71}$), who first advanced the idea of the nuclear character of the "nucleolus," admits ($\frac{37}{3\,3\,1}$) 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 ($\frac{3 \cdot 5}{6\,1\,3}$), and in Vorticella nebulifera ($\frac{2\,7}{3\,3\,9}$), 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

($\frac{2}{2}\frac{7}{3}\frac{}{0}$), 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* ($\frac{3}{2}\frac{0}{3}\frac{}{7}$).

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 ($\frac{2}{3}\frac{7}{3}\frac{}{1}$), 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 (154 155) 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 30) 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 (87), 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" (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 *e* 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 ($\frac{59}{1821}$) 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 ($\frac{143}{138-140}$) 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 ($\frac{143}{129}\frac{143}{132}$) from three of the primary blastomeres, without the participation of the fourth.

Bütschli ($\frac{27}{9\ 10}$), 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 ($\frac{30}{240}$) 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.

(e) 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 ($\frac{1\,4\,3}{1\,6\,3}$) 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 ($\frac{1\,4\,3}{1\,6\,6}$) 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 ($\frac{5}{34}$–$\frac{9}{51}$) 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 ($\frac{1}{93},\frac{3}{98},\frac{6}{99}$) 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 ($^{1}\frac{4}{9}^{3}$) 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 ($\frac{1}{5}\frac{6}{5}\frac{6}{6}-\frac{6}{5}\frac{6}{6}\frac{0}{0}$) 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 ($\frac{1\,1}{3-9}$). "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 ($\frac{77}{45}$) 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 ($\frac{8}{17}$) 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 margiu 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 around 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 ensheathed 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 ($\frac{17}{45}$) 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 ($\frac{3\,0}{4\,5}$) 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 ($\frac{8 5}{1 8 - 1 9}$) 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* ($\frac{1 4 0}{1 5 3 - 1 7 7}$) 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 ($_{a}\frac{1 2 8}{0 3 - 4}$) 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 ($\frac{2 1}{1 4 0, 1 5 9}$), 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 ($\frac{8}{1}\frac{1}{3}\frac{1}{1}\frac{1}{0}\frac{1}{0}$), 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 ($\frac{1}{6}\frac{0}{7}\frac{5}{}$) 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*" ($\frac{9}{3}\frac{8}{9}\frac{}{4}\frac{}{1}$). "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 ($\frac{866}{009}$) 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 ($\frac{855}{456}$) 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 ($\frac{855}{354}$) 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 ($\frac{8\,3\,3^1}{1\,1\,8}$) 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 (94, 17, Gasterosteus, Spinachia), Bambecke (8, Cyprinoids), Götte (56, 74), Oellacher (125, 12), His (74, 38), and Klein (82, Salmo; Balfour (326–329, Mustelus) and Schultz (145–471, 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 (311, 27): "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 (57–8), Rauber (131, 134, 134–576), and Balfour (19, 8). Götte saw what he interpreted as a process of segmentation about these nuclei. His (67, 71) and Disse (160, 78) 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 (50, 46), Rathke (136, 67), and Hoffman (77, 45) all derive the entoderm by delamination from the blastoderm. According to Robin (143–495) 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 ($\frac{18}{39}$) 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 ($\frac{18}{42}$) 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 ($\frac{106}{67}$), 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 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.

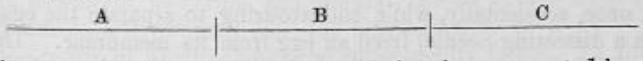
General Considerations.

a. Axial Differentation.

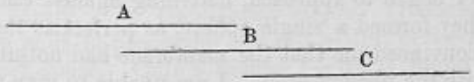
The early appearance of structural axes in the developing egg is a very significant fact, and deserves special mention. Among those authors who have called attention to this point may be mentioned Auerbach ($\frac{3}{1\,9\,6}$, Ascaris and Strongylus), Hatschek ($\frac{1\,6\,8}{5\,0\,8}$, Pedicellina), Selenka ($\frac{1\,5\,1}{1\,7\,4}$, Holothuria), A. Agassiz ($\frac{3}{3\,6\,4}$, Ctenophora), His ($\frac{7\,4}{6}$, Salmo), and Rauber ($\frac{1\,3\,3}{1\,8\,9}$, 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 ($\frac{6\,5}{4\,1\,2}$) has called *ontogenetic acceleration* (heterochrony), and it finds an explanation in the principle of *precocious segregation* (Lankester ($^{1}\frac{0\,1}{1\,5}$)). 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 (Blastocœl, Huxley.)

A morula, as defined by Häckel ($\frac{6\,5}{4\,2\,1}$), 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 ($\frac{5}{4}\frac{5}{8}\frac{5}{5}$) supposed this cavity to be the place originally filled by the germinal vesicle. According to Häckel ($\frac{6}{3}\frac{2}{7}\frac{2}{7}\frac{2}{8}$, $\frac{6}{4}\frac{5}{2}\frac{5}{2}$), 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 ($\frac{5\,8}{1\,2\,2}$), 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 ($\frac{1\,3\,1}{6}$) 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, $\frac{6\,8}{5\,0\,5}$), and at about the same time in Holothuria (Selenka, $\frac{1\,5\,1}{1\,5\,9}$).

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 ($\frac{1\,4\,6}{2\,0\,9}$) 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 ($\frac{8}{4}\frac{5}{2}$) 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 ($\frac{9}{3}\frac{7}{4}$) 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, $\frac{6}{5}\frac{8}{0}\frac{}{7}$) 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 ($\frac{30}{243-4}$) 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 ($\frac{85}{4}$) did not succeed in tracing the mesoderm in the Hirudinea. In Euaxes ($\frac{85}{15}$) 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, $\frac{21}{119}$). Bobretzky derived the mesoderm from the ectoderm. Rabl ($\frac{128}{2023-4}$) found a double symmetrical arrangement of the mesoderm in Lymnæus, and derived it from entoderm and ectoderm. According to Bütschli ($\frac{29}{340}$, $\frac{306}{220}$) 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 ($\frac{99}{1600}$) refers the origin to both ectoderm and entoderm. Langerhans ($\frac{95}{172,1725-8}$) 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 ($\frac{30}{2386}$, 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, $\frac{85}{847}$) the mesoderm arises as lateral diverticula of the entoderm.[1] Kowalevsky, in a very valuable paper ($\frac{87}{1806-7}$), has demonstrated the same thing for Amphioxus, and at the same time proved the correctness of Balfour's explanation ($\frac{129}{4294}$) of a fact noted in the development of the Selachians ($\frac{111}{3447}$)—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 ($\frac{1\,2}{2\,2\,3}$), 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, $\frac{1\,1}{3\,3\,5\,6}$) has been confirmed by His ($\frac{7\,5}{1\,1\,8}$). 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 ($\frac{8\,7}{1\,9\,0}$) furthermore states that the origin of mesoderm in the Brachiopods is similar to that of Sagitta and Amphioxus. Metschnikoff ($\frac{1\,2\,3}{1\,4\,7}$) 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 ($\frac{1\,5}{2\,3,4\,1}$) has found also that the mesoderm goes with the entodermic segment in the rabbit. Selenka has observed the same (Holothuria, $\frac{1\,5\,1}{1\,5\,9}$). 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, $\frac{21}{109}$).

(*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 ($\frac{65}{54-5}$). 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, $\frac{17}{191}$; Gastrophysema, $\frac{165}{420}$; Sycandra raphanus, $\frac{146}{271}$).

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 ($\frac{98}{104-108}$, 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."

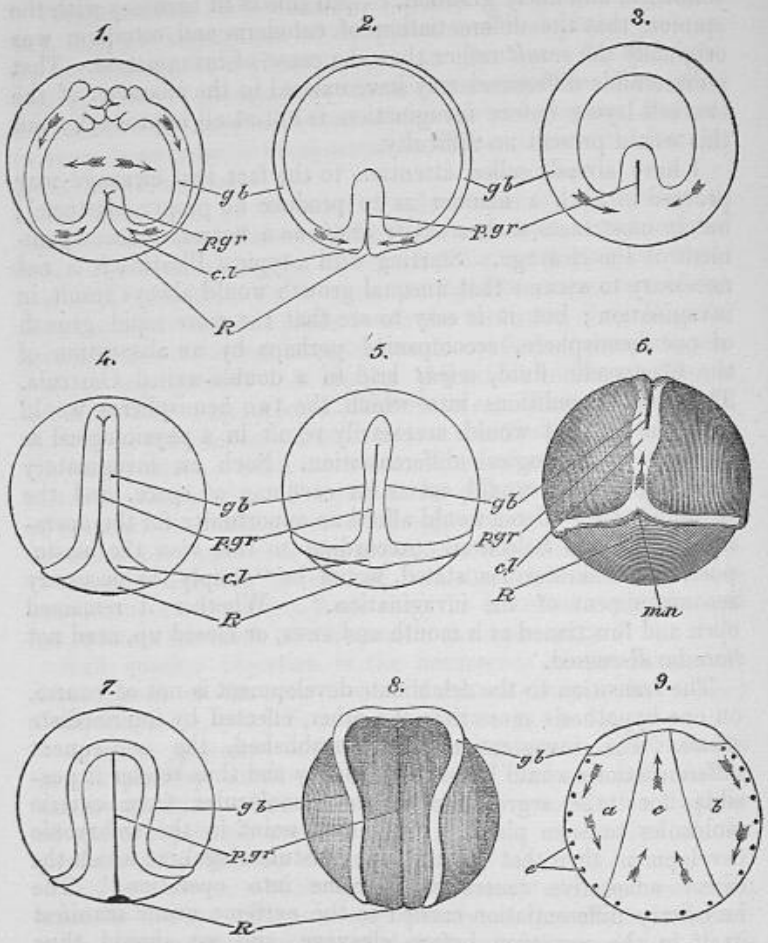
With this process "a change in the character of the two cell-layers goes hand in hand. The cells of the outer layer become larger . . . quite clear and transparent. Those of the inner layer, on the other hand, do not grow, but become darker, yellowish, and finely granular." All this is in harmony with 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.
FIG. 2 = Pristiurus (after His).
FIGS. 3 and 6 = Chick (after Rauber).
FIG. 5 = Salmo (after His).
FIG. 7 = Accipenser (after Kowalevsky).
FIG. 8 = Frog (after Rusconi).

g. b. = Germ-band.
p. gr. = Primitive groove.
c. l. = Caudal lobes.
R. = Anus of Rusconi.
m. n. = Marginal notch.
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($\frac{67}{7}$) 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 ($\frac{89}{176}$). 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" (75 109). Compare also 74 91). 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 (75 94, 76 118) 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" (133 64). 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 (83 173) by Kowalevsky, according to a citation by Rauber (134 15), has been confirmed by Balfour

($\frac{1\,1}{3\,3\,8}$) and by His ($\frac{7\,5}{1\,1\,3}$). Kowalevsky ($\frac{3\,9}{1\,7\,5}$) 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 ("retro-central") 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 ($\frac{1\,6\,0}{8\,6}$) 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 ($\frac{76}{153}$).

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 ($\frac{1}{5}\frac{3}{7}\frac{2}{0}$). 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 " ($\frac{1}{1}\frac{7}{2}\frac{}{0}\frac{}{2}$).

The same view is more fully given in an article published in this Journal January, 1878. (161).

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[1] My opinion was formed entirely independently of these remarks.

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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 nuelei, 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.