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THE EARLY EMBRYOLOGY OF CIONA INTESTINALIS,
FLEMMING (L.).

By W. E. CASTLE.

WITH THIRTEEN PLATES.

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No. 7. — *The Early Embryology of Ciona intestinalis, Flemming (L.).*¹ By W. E. CASTLE.

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I. INTRODUCTION.

So long ago as 1866, Kowalevsky wrote, "Die Entwicklungsgeschichte der Ascidien wurde schon vielfach studirt." If this statement was true

¹ Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, under the direction of E. L. Mark, No. LII.

then, it is doubly so now, for the literature of the subject has since that time multiplied many fold. Nevertheless there still remain many unsettled questions regarding the embryology of the Tunicates. Concerning so fundamental a point as the derivation of the primary germ layers in the embryo, quite contradictory opinions have been expressed within the last ten years by observers of world-wide reputation.

I undertook the inquiry, the results of which are recorded in the following pages, in the hope of being able to throw light on this disputed question by the study of other forms than those which had been most carefully examined, and by the application of new methods to the problem. A short experience convinced me that the only method which could yield positive conclusions was that of cell lineage, a method which has been applied so successfully to the study of annelid and molluscan embryology by a number of observers, and had already been employed to a limited extent in the study of ascidian embryology by Van Beneden et Julin ('84), Seeliger ('85), and Chabry ('87).

It soon became clear to me that some of the conflicting statements made by my predecessors arose from errors on their part due to incorrect orientation of certain stages. The nature of these errors I have fully explained in a preliminary communication (Castle, '94). A further study of the embryonic history, cell by cell, through the periods of cleavage and gastrulation, and even down to the differentiation of the several larval organs, has led me to conclusions somewhat at variance with those of earlier investigators regarding the origin of the primary germ layers and the organs derived from them. One of the most important of these conclusions is that the mesoderm of Ascidians — and probably also that of Amphioxus and the Vertebrates — is derived in part from the primary entoderm and in part from the primary ectoderm. The grounds on which this conclusion rests are set forth in the later portions of this paper; in the earlier part of the paper I have recorded some observations on the maturation and fertilization of the ascidian egg.

It gives me pleasure to acknowledge in this place my very great obligations to Professor E. L. Mark for direction and kindly criticism of my entire work. My best thanks are also due to Dr. Alexander Agassiz, in whose laboratory at Newport the material for my studies was chiefly collected, and to Colonel Marshall McDonald for numerous courtesies extended to me at the United States Fish Commission Station at Wood's Holl.

II. MATERIAL, LIFE HISTORY.

The material for this study was collected in the months of August and September of two successive seasons, 1893 and 1894. The species employed seems to be, beyond question, the *Ciona intestinalis* of Flemming, a classical object of study on the other side of the Atlantic. It was made the subject of an extensive monograph by Roule ('84); its larval history has been studied by Kowalevsky ('66 and '71) and by Willey ('93); its cleavage stages by Samassa ('94); its fertilization stages by Boveri ('90); and the formation of its egg envelopes by Fol ('84). Loeb ('91) also has employed it in certain physiological investigations. The specimens which I collected at Newport answer fully to Roule's detailed descriptions of the species. The large size (8-10 cm. long) attained by individuals at Newport under favorable conditions confirms Roule's conjecture that the forms described from the United States as *Ascidia ocellata* by Louis Agassiz, as *A. tenella* by Stimpson ('52), and as *Ciona tenella* by Verrill ('71) were only small-sized individuals of *Ciona intestinalis*.

Specimens were obtained by me from two different localities just within the entrance of Narragansett Bay. The animals were usually found adhering to the under side of stones at a depth of from a few inches to a few feet below low-water mark. Upon removal to the laboratory they were carefully washed and placed in aquaria whose water was kept fresh by a jet of air. Once a day the water was changed, and the aquaria thoroughly cleaned, to prevent the accumulation of bacteria or other possibly injurious organisms. This painstaking treatment was probably unnecessary, for the animals are very hardy and bear ill-treatment well. For example, I have kept specimens for weeks at a time in small glass aquaria without change of water, and the only signs of misuse which they exhibited were a slight shrinkage in size and a greatly diminished production of eggs, — both symptoms referable to an insufficient food supply.

Ciona, like all other Tunicates, is hermaphroditic, and the number of eggs produced by a single adult individual in the course of a season must be enormous. Often hundreds are deposited in a single night. Under normal conditions each adult individual, during the summer months, lays eggs once in every twenty-four hours, with the regularity of the sunrise.

Korschelt u. Heider ('93, p. 1267) state that in most cases among the *Ascidians* self-fertilization appears to be prevented by the ripening of the

male and female sexual elements at different periods; although in some cases, where the sexual products mature simultaneously, self-fertilization is not excluded. Neither of these statements holds good for *Ciona*. Although in the adult period it produces both sexual elements throughout the spawning season, and discharges them simultaneously, self-fertilization rarely occurs, — a conclusion to which I have been led by repeated experiments. The most complete series of these experiments will be briefly described.

The observation had been made that an individual accidentally left overnight in an aquarium by itself laid eggs which failed to develop; whereas, when two or more individuals were placed together in an aquarium, all other conditions being the same, the eggs laid developed almost without exception.

First Experiment.

Acting on the suggestion thus offered, I placed together in an aquarium two or three small, clear individuals (evidently young); in another aquarium was placed an equal number of very large-sized (old) individuals. A greater number of eggs was laid by the large individuals, as one would naturally expect, but the eggs in both aquaria were perfectly fertile. This experiment showed that ripe eggs and sperm are produced both by young and by old individuals.

Second Experiment.

Twenty rather large-sized individuals were selected for experimentation and divided into two lots, A and B, of ten individuals each. The animals of each lot were carefully washed and placed in clean glass dishes filled with fresh sea-water. The individuals of lot A (Table I.) were placed each in a separate dish, those of lot B (Table II.) were placed two in a dish. The next morning a careful examination of each aquarium was made to determine what proportion of the eggs laid had been fertilized. The experiment was repeated on five successive days; on the sixth day, as a control experiment, the lots were interchanged, the animals of lot A (Table II.) being paired, and those of lot B (Table I.) isolated. The results for the six days are embodied in Tables I. and II.

Taking an average of the fifty-eight cases in which eggs were laid by isolated individuals (Table I.), we find that 4.8% of the eggs were fertilized. The occurrence on a single day in two cases of fertilization of 90% of the eggs laid makes me suspect that the dishes were not properly cleaned on that day, and that live spermatozoa may have remained clinging to the sides of the dish after the previous day's experiment. If so, and if

TABLE I.—CLOSE FERTILIZATION.

Day.	Lot A.					Lot B.	
	1st.	2d.	3d.	4th.	5th.	6th.	Total of Cases.
90% fertilized			2				2
25% fertilized	1	1					2
10% fertilized		1	1		1		3
5% fertilized	1	1		1			3
4% fertilized				1			1
None fertilized	8	7	7	7	8	10	47
No eggs laid				1	1		[2]
Total							58 [60]

Average fertility = 4.8%.

TABLE II.—CROSS FERTILIZATION.

Day.	Lot B.					Lot A.	
	1st.	2d.	3d.	4th.	5th.	6th.	Total of Cases.
100% fertilized	5	4	4	5	5	5	28
20% fertilized			1				1
None fertilized		1					1
Total							30

Average fertility = 94%.

the dishes were by chance interchanged, a certain amount of cross fertilization may of course have been possible.

On the three succeeding days (fourth, fifth, and sixth) greater precautions were taken, and the jars were dried as well as washed before the experiment was repeated. It will be observed that the proportion of eggs fertilized on those days was distinctly less than on the first three days.

Taking an average of the thirty cases in which eggs were laid in aquaria containing each *two* individuals (Table II.), we find that 94% of the eggs laid were fertilized.

The single instance in which none of the eggs laid in one aquarium were fertilized may be explained by a failure on the part of one of the two animals confined together to emit the sexual products on that particular occasion. Table I. indicates that such cases sometimes occur; for in two instances out of sixty no eggs at all were laid.

Comparing the results of the two tables, we see that under conditions allowing of only close (self-) fertilization (Table I.), less than 5% of the eggs developed; whereas under conditions permitting of cross fertilization (Table II.) at least 90% of the eggs developed.

The question now arose, Do eggs laid by isolated individuals fail to develop because the parent does not discharge sperm at the proper time (perhaps for want of stimulation by another individual), or do the eggs fail to develop because they are *incapable* of fertilization by sperm from the same parent? To settle this point if possible, resort was had to artificial fertilization.

Third Experiment.

The same animals employed in the second experiment were also used in this one. Half of the individuals of each lot were taken for an attempt at close fertilization, the other half being reserved for an attempt at cross fertilization. Each animal was dipped in 90% alcohol to kill any spermatozoa which might be adhering to it; the fingers and instruments used were treated in the same way. Eggs and sperm were removed from the sexual ducts of the animal, and thoroughly mixed in a dish of clean fresh sea-water, the dish having been previously carefully washed and then dried.

The second ten were treated in exactly the same way, except that the sexual products — both male and female — of *two* individuals were mixed together in a single dish.

The proportion of fertilized eggs in each dish was subsequently carefully observed. The results are given in Tables III. and IV.

TABLE III.—ARTIFICIAL CLOSE FERTILIZATION.

	Cases.
50 % fertilized	1
4 % "	2
1 % "	1
$\frac{1}{2}$ % "	2
None "	4
Total	10

Average for ten cases, 6% = proportion of eggs fertilized.

TABLE IV.—ARTIFICIAL CROSS FERTILIZATION.

	Cases.
100 % fertilized	4
50 % "	1
Total	5

Average for five cases, 90% = proportion of eggs fertilized.

As the animals employed in the above experiment had been confined in the laboratory for some days, and the production of the sexual elements had in consequence considerably diminished, it was thought desirable to repeat the experiment on animals freshly collected. This accordingly was done with the following equally conclusive results.

TABLE III. a.—ARTIFICIAL CLOSE FERTILIZATION.

	Cases.
50% fertilized	1
12½% "	2
10% "	1
5% "	1
2% "	2
None "	3
Total	10

Average for ten cases, 9.4% = proportion of eggs fertilized.

TABLE IV. a.—ARTIFICIAL CROSS FERTILIZATION.

100% fertilized in every case!

The proportion of close fertilized eggs was greater in this experiment than in the preceding. Many of the eggs so fertilized, however, never developed beyond the 2- or 4-cell stage. The cross fertilized eggs all developed normally and at the same rate.

Combining the results of Tables III. and III. *a*, and those of IV. and IV. *a*, we get an average of 7.7% of the eggs developing after close fertilization, and 95% developing after cross fertilization. These averages agree fairly well with those obtained from Experiment 2, which were 4.8% and 94% respectively.

Experiment 3 shows conclusively that, in the case of *Ciona*, eggs are to a large extent incapable of fertilization by sperm from the same individual as the eggs. Cross fertilization must, therefore, be the rule, and close fertilization the exception under natural conditions. The rare occurrence of close fertilization is probably due to a lack of mutual attraction between eggs and sperm produced by the same individual, an attraction invariably existing between the eggs of one individual and the sperm of another, and probably chemical in its nature. This case is paralleled in certain flowering plants, whose pollen will not germinate when placed on the stigma of the flower from which it was taken, though on the stigma of other flowers of the same species of plant it germinates readily.

There seems to be a particular time of day in the case of each species of simple Ascidian for the discharge of the sexual products. Different aquaria, in which are placed individuals of the same species, if they are subjected to the same conditions of temperature, etc., invariably contain eggs in exactly the same stage of development. This shows conclusively that the time of egg-laying has been the same in the case of each aquarium. For, on account of the rapidity of development, a slight difference in the time of egg-laying would be readily detected by a difference in the stage of development exhibited by the eggs in different aquaria. In the case of *Ciona* the sexual products are discharged about an hour or an hour and a half before sunrise. The stimulus to their discharge is probably the increasing light of daybreak.

If at about the time mentioned one approaches the aquarium with a lighted lamp, he will see the animals suddenly contract violently two or three times in succession, then resume their accustomed tranquillity. A careful examination will then reveal the eggs floating as little golden specks in the thoroughly agitated water. Soon they begin to settle to the bottom of the aquarium and can then be collected in convenient quantities by means of a pipette. The violent expulsion of the contents

of the atrium simultaneously with the release of the sexual products from their respective ducts, must secure under natural conditions a wider distribution and more thorough mixing of the eggs and spermatozoa than would otherwise occur.¹

The season of spawning of *Ciona* probably extends in this country, as in Europe, from spring to autumn. I have never collected adult specimens which did not contain mature eggs and spermatozoa, though I have taken them as early in the season as the 10th of June and as late as the 22d of September.

The development of the ovum is very rapid, as I shall show further on, and the larval period brief. The growth of the metamorphosed individual must also be very rapid, as the following facts indicate. In the summer of 1892 specimens of *Ciona* were abundant in a certain locality at Newport. But the succeeding winter was a cold one, and seems to have killed off those individuals which were situated in very shallow water. In the summer of 1893 specimens were to be found only at a depth of over two feet below low-water mark. In August and September of the next year, however, they occurred in abundance just below low-water mark. But those so situated were rather small, not exceeding 7 cm. in length, very clear, and free from dirt or parasitic growths, thus giving evident signs of youthfulness. They cannot have been over fifteen months old, and may have been much younger. Yet they were sexually mature, and produced eggs in abundance.

¹ The time of egg-laying is about the same — viz. just before daybreak — in the case of *Molgula Manhattensis*, on which I made some observations in the United States Fish Commission Laboratory at Wood's Holl, Mass., in June and July, 1894. *Cynthia*, whose habits I studied at the same place, lays its eggs with equal clock-like regularity, but toward nightfall instead of at daybreak. The late afternoon is also the time of spawning for *Amphioxus* (Wilson '93, Willey '94). The manner of egg-laying is the same in *Molgula* as in *Ciona*. Herein my observations differ from those of Kingsley ('83), who states that in *Molgula* fertilization occurs within the atrium, and that the eggs are for some time afterward retained there. I have never found embryos within the atrial chamber, though I have often seen them adhering to the bodies of the parent individuals, where some eggs had probably settled at the time of spawning. My observations regarding the manner of cleavage in *M. Manhattensis* also differ from those of Professor Kingsley. He states that the cleavage is unequal, much as in certain Mollusks, and results in the formation of a cap of very small micromeres resting on a few very large macromeres. According to repeated observations of my own, made both on naturally and on artificially fertilized eggs, the cleavage progresses very much as in other Ascidians, the first two cleavages being equal. I think Professor Kingsley must have been misled by appearances in immature eggs obtained by dissecting out the ovaries for artificial fertilization.

Allusion has been made to the rapidity of development of the egg. Within twelve hours after fertilization the larval form is attained, the tail being coiled round the trunk within the egg membranes. Hatching usually occurs within the next twelve hours, i. e. in the first night after the laying of the eggs. It is brought about by twitchings of the larval tail, which finally rupture the egg membranes. Under certain conditions the larva does not succeed in breaking through the egg membranes. Metamorphosis then sets in almost immediately, and is completed within the egg membranes, a functionally free-swimming stage being wholly suppressed. This is regularly the case in *Molgula Manhattensis*, where hatching of the larva is exceptional, the new, metamorphosed individual arising just where the egg settled after it was thrown out into the water and fertilized. However, in *Ciona* the more primitive course of events is usually pursued. The larva then escapes from the egg membranes as a miniature tadpole, the "test cells" clinging to its thin and adherent covering of homogeneous, non-cellular mantle substance secreted by the ectoderm. These test cells are soon brushed off as the tadpole swims about; they have no connection, as is now well known, with the cells to be found later in the mantle of the adult.

The larvæ avoid the daylight and swim toward the least brightly illuminated side of the aquarium.¹ Here they attach themselves, usually near the surface of the water, to the side of the aquarium. Sometimes the attachment is by the head end, as it is commonly said to be, but I have more often observed the larvæ attached by the sticky mantle substance at the tip of the tail, the body then hanging head downward against the side of the aquarium.

The larval stage varies in duration from twenty-four hours to several days. It is terminated by the beginning of metamorphosis, whose successive steps are well known through the description of Kowalevsky ('66 and '92), Willey ('93), and others.

¹ I have observed that the larvæ of *Amarœcium* also avoid the daylight, i. e. are negatively phototactic; but the larvæ of *Botryllus* are strongly *positively* phototactic, swarming *toward* ordinary daylight. This difference may perhaps be explained by the difference in habitat of the parent organisms. *Botryllus*, whose larvæ seek the light, is commonly found in well illuminated places, e. g. adhering to floating eel-grass. On the other hand, *Ciona* and *Amarœcium*, whose larvæ avoid the light, more often occur in darkened places, the former on the under side of stones, the latter adhering to piles underneath wharves, or on the sea bottom in sheltered spots near shore.

III. METHODS.

1. Killing, Preservation.

Whenever it was desired to kill a lot of eggs, a sufficient quantity of them was collected in a pipette from the bottom of an aquarium and transferred to a watch-glass, or directly to a small vial of two drams' capacity, in which the eggs were ultimately stored. After the eggs had settled to the bottom of the dish, the water was carefully removed and the killing reagent applied.

The eggs were ultimately preserved in 90% alcohol, and the vials tightly corked, or preferably stoppered with cotton plugs and stored in tightly sealing glass jars. When the latter method is employed, the jars must be kept right side up in transportation, otherwise the small eggs will settle into the cotton plugs and be lost. However, the extra trouble which this method necessitates is well worth taking, for it entirely avoids the injurious effects on preserved material sometimes caused by the tannin which alcohol will extract from corks, if they are used.

Several killing reagents were employed, viz. Flemming's fluid, Hermann's fluid, picro-nitric, corrosive-acetic, and Perenyi's fluid.¹ The blackening effects of the first two reagents made material killed in them unfit for use in the study of eggs as whole objects. Likewise in the case of sections the results from them were disappointing. The only real service rendered by either of these two reagents was in demonstrating in the egg by their blackening effects the character and distribution of the fatty yolk granules. Most serviceable of all the reagents employed on the eggs and embryos up to the period of hatching was Perenyi's fluid. It renders the abundant yolk clear and transparent, and preserves all structures perfectly, without distortion by either swelling or shrinking. Its use does not in my experience interfere in the least with sharp differential staining. The fluid was allowed to act for about twenty minutes, then followed by 70% alcohol, which, to insure removal of every trace of the killing reagent, was changed once or twice in the course of the next twenty-four hours, and replaced at the end of that time with 90% alcohol. A longer treatment with the killing reagent, extending to three or four hours, seemed to give no added advantage, but to interfere slightly with subsequent staining.

Picro-nitric also gave good results, but for the pre-larval stages not so good as Perenyi's fluid, its clearing effects being less. It seems, however,

¹ For the composition of the killing reagents and stains mentioned in this paper, see Lee's "The Microtome's Vade Mecum," 3d edition, London, 1893.

to have been for the larval stages the best reagent which I employed. Davidoff's corrosive-acetic mixture, which has been much used of late by workers on ascidian embryology, is in my experience less faithful in its preservation than Perenyi's fluid, for it shows a tendency to swell certain structures, and lacks the instantaneous hardening effects of that reagent.

2. Decortication, Staining, Mounting.

The egg of *Ciona* is surrounded by a series of egg membranes, a correct idea of which is given by the figure of the mature egg of *Ascidia canina*, reproduced after Kupffer ('72) in Korschelt u. Heider's "Lehrbuch d. vergl. Entwicklungsgeschichte," Figure 736. The egg cell is seen to be surrounded by a clear space — probably occupied by jelly — bounded by the test cells, which are arranged in a rather compact layer one cell deep, so that they seem almost to form an epithelium underneath the chorion. The chorion is a structureless transparent membrane, upon which, as on a basement membrane, the follicle cells ("Schaumzellen") rest. In the egg of *Ciona*, after it is thrown out into the water, these highly vacuolated cells are even more conspicuous than in the egg of *Ascidia* as figured by Kupffer. They extend out radially about twice as far as indicated by Kupffer's figure, forming a sort of halo round the egg. The highly refractive nuclei are carried out to the pointed outer ends of the tapering follicle cells.

The presence of the follicle cells and test cells did not interfere seriously with the study of the early stages of cleavage in the living egg, since the clear space between the egg cell and the layer of test cells allows one, with a sufficiently strong illumination, to make out perfectly the outline of the blastomeres and sometimes even nuclear figures in them. But upon preservation in alcohol the envelope formed by the test cells, chorion, and follicle cells collapses, obliterating the clear space and becoming closely applied against the egg cell, thus forming a very serious obstacle to the study of the egg as a whole object. This obstacle I was able to remove by following in a modified form a very ingenious method devised by Chabry ('87, p. 169) for the removal of the follicle cells from the living egg of *Ascidella*, a process which he called "decor-tication." It consisted in simply sucking the eggs into a fine capillary glass tube too small to admit the eggs without the removal of their follicle cells, yet large enough to allow the passage uninjured of the egg itself.

In applying this method to preserved material, I first stained the eggs, as a rule, so that they might be more easily seen. Upon transferring

them to alcohol of a low grade, or to water, the egg envelopes would again stand out clear of the ovum, as in the living egg. By then sucking the eggs one at a time into a glass tube of the proper calibre, the entire envelope, consisting of follicle cells, test cells, and chorion, could be removed with considerable facility, and in the majority of cases without injury to the egg itself. Eggs thus decorticated and then mounted afforded excellent surface views.

The eggs are rather opaque, on account of the large amount of yolk which they contain, so that any stain except a very faint one is an obstacle in the study of whole preparations. Excellent results were obtained by mounting in balsam, without any staining whatever, eggs which had been killed in Perenyi's fluid and decorticated.¹ But for the 64-cell and later stages staining was found desirable. Many carmine and hæmatoxylin stains were tried; the one which gave by far the best results being Orth's picro-carminate of lithium. The eggs were treated with a small amount of this stain in a watch-glass for from six to twenty-four hours, then washed thoroughly in water. By this method resting nuclei are stained bright rose-color, while all other structures take only a faint yellow color from the picric acid, and give up even this if the washing is sufficiently prolonged. But the carmine stain in the nuclei is extremely tenacious, and does not fade in the least upon prolonged washing in water or preservation for months in strong alcohol. After the eggs had been stained and decorticated, they were dehydrated, cleared in xylol or cedar oil, then mounted in balsam, the cover glass being supported with small glass rollers made from fine capillary tubes. These served the double purpose of preventing the crushing of the egg and allowing it to be rolled into any desired position by movement of the cover glass. Changing the position of the egg, however, is not often necessary, for at an early stage it takes on a flattened form, which causes it to come to rest with the dorsal or the ventral surface uppermost. This is the case at all periods between the 24-cell stage and that at which the neural tube begins to close, except for a brief period, when the embryo consists of from forty-six to sixty-four cells, and the vertical axis becomes equal to or even greater than the longitudinal axis. Then there is no single position of stable repose for the embryo, and rolling is often necessary to bring it into the positions desired.

The self-orientation of the egg during most of the early stages was of

¹ I find that Lillie ('95) has obtained good results in the case of the eggs of the mollusk *Unio* by mounting, without staining, material killed in Perenyi's fluid. He, however, used glycerine instead of balsam as a mounting medium.

great service in sectioning. When this was desired, the egg, previously studied as a whole object, was returned to xylol. The transfer was accomplished by placing the slide on which it was mounted in a shallow porcelain dish containing a little xylol. This soon dissolved away the balsam, and left the egg free and clearly visible against the white background. The egg was next removed to a shallow watch-glass with a perfectly flat bottom, which was previously smeared with a thin layer of glycerine. Any superfluous xylol was removed from about the egg with filter paper, and a small amount of melted paraffine poured over it, enough to fill the watch-glass to a depth of 3 to 5 mm. The whole was then set over the paraffine bath for fifteen or twenty minutes, when it was placed floating on a dish of water to cool. This being accomplished, the paraffine block was removed from the watch-glass, and the egg, which of course had settled to the bottom and lay with its long axis parallel to the surface of the block, was oriented under the compound microscope in any manner desired. The thinness of the block generally allowed plenty of light to pass through it for this purpose, and it was usually not difficult, owing to the shape of the embryo, to determine its axes. Sections were usually cut $6\frac{2}{3} \mu$ in thickness.

The staining which was found most advantageous for the study of the egg as a whole object was altogether too faint for sections. These were accordingly given a further staining after fixation to the slide. Ehrlich's hæmatoxylin was employed, diluted one half with water. After immersion in the stain for from twenty minutes to an hour, the sections were washed in water to remove the superfluous stain, then to decolorize were placed in 35% alcohol containing 0.1% hydrochloric acid. Here they were allowed to remain until quite pale in color, usually for about five minutes. They were then rinsed in 35% alcohol and held for an instant over the unstoppered mouth of an ammonia bottle, a treatment which gave the hæmatoxylin remaining in the sections a deep blue color, and insured the permanency of the stain. The sections were then passed through the grades of alcohol, cleared in xylol, and mounted in balsam. This process, when properly conducted, resulted in a beautiful and sharply differential double stain. The nuclei retained the light rose tint given them by the carminate of lithium, for the superadded hæmatoxylin stain had been entirely removed from them, except in the chromatic elements, which possessed a deep black color. Cell boundaries, attraction spheres, and other cytoplasmic structures, were clearly brought out, and the fundaments of various organs, as, for example, chorda, mesoderm, and definitive endoderm, were distinguished one from another with great sharpness

by the different tints of blue which they exhibited. Iron hæmatoxylin was sometimes employed instead of Ehrlich's, but the results were no better — indeed not so good — for the differentiation of organs or their fundaments.

For studying the processes of maturation and fertilization sections alone could be employed on account of the opacity of the eggs. In making sections of these stages orientation was of course impossible, so that a large number of the eggs was embedded together, without previous decortication, and cut at random. The egg membranes, so far from being an obstacle, were at these stages a positive advantage, since they served to protect and hold the polar globules in place. The material employed in the study of maturation and fertilization stages was killed either in Perenyi's or in Hermann's fluid, the best results being obtained from the former. For convenience the killing of each day will be referred to as a series (A, B, or C), made up of lots (1, 2, 3, etc.) which were killed at intervals of about ten minutes, the first lot being killed as soon after the laying as a sufficient number of eggs could be collected, usually about five or ten minutes.

IV. MATURATION AND FERTILIZATION.

The eggs of series A, lot 1, show an early stage in the process of maturation, namely, the formation of the first polar globule. Figure 1 represents a section through one of the eggs of this lot most advanced in development. The egg envelopes, which rest close down upon the egg, are left out in this and all the other figures. Already at this stage we recognize that the egg is made up of two unlike hemispheres, one richer in yolk, the other richer in protoplasm. The former occupies the future *dorsal* or *endodermal* side of the egg, and at the centre of its surface, as stated in my preliminary communication ('94), the polar globules form. The cell division which will give rise to the first polar globule is seen in this figure to be already well advanced, the chromatin being accumulated at the two ends of the spindle. About the deeper end of the spindle there is a small space free from yolk granules and occupied by a finely granular deeply staining mass of protoplasm, of which we shall have more to say. The entire remainder of the dorsal hemisphere, except that small portion of it occupied by the spindle itself, is filled with rounded yolk granules (cf. Fig. 2) of a rather uniform size, closely packed together, but with slender films of staining protoplasm passing between and around them. Davidoff's ('89) beautiful figures, particularly his *Tafel VI. Fig. 33*,

give a correct idea of this "Schaumwerk" structure, if one imagines the yolk granules many times smaller and the protoplasmic films much more slender than in the egg of *Distaplia* as represented by Davidoff.

The ventral hemisphere also is filled with yolk granules, but here the protoplasmic packing between them is more abundant and less uniformly distributed. It is most conspicuous at the surface, where it forms a thin layer nearly free from yolk granules spreading over almost the whole hemisphere. Within this layer it fades away gradually, but often, as in the case figured (Fig. 1), again becomes prominent at a little deeper level as a series of irregular blotches among the yolk granules; then it once more grows fainter toward the centre of the egg, attaining the condition described for the dorsal hemisphere.

The presence of a spermatozoön cannot be detected in the eggs of this lot. In those of Series B, lot 3, however, its influence is clearly visible. (See Figs. 2 and 4.) About fifteen minutes is estimated to have elapsed between the stage just discussed and the one here presented. At this stage we see in the ventral hemisphere, at some point just beneath the surface, a spherical region entirely free from yolk granules. (See Fig. 2.) Its central portion is occupied by a finely granular substance, which stains in hæmatoxylin an intense blue, shading off somewhat gradually into the more faintly and lightly colored protoplasm occupying the outer portion of the area and continuous with the similarly stained films of the Schaumwerk. At one point the yolk-free region extends out to the surface of the egg. This probably represents the place of entrance of the spermatozoön, which we have reason to believe produces the clear area. The deeply staining substance at the centre of this area is the *male archoplasm* or attraction sphere. It is undoubtedly similar in nature, as it is in optical appearance, to the darkly stained substance seen at the deep end of the maturation spindle in Figure 1, and which may therefore be called the *female archoplasm*. The male pronucleus cannot be made out in the egg a portion of which is shown in Figure 2. In other eggs of the same lot, however, it can be clearly seen; for example, in Figure 4, which represents a stage a little more advanced than the one seen in Figure 2. The area free from yolk is seen in Figure 4 to have enlarged somewhat; the attractive influence of the archoplasm at its centre has manifestly been extended over the greater portion of the hemisphere in which it lies. This fact is indicated *diagrammatically* by the dotted lines in the figure. They are meant simply to indicate that those films of the protoplasmic Schaumwerk which run radially with reference to the attraction sphere have become thicker and more promi-

nent than those running in other directions. Along them as radii doubtless protoplasm is passing to augment the yolk-free area. Nothing in the nature of "fibres" has been observed in them. Excentrically situated in the yolk-free area (Fig. 4) is seen the male pronucleus, a perfectly clear oval body, with a delicate but sharp boundary. Its long axis lies radially with reference to the attraction sphere, which manifestly exerts on it a directive influence. Figure 4 represents the eleventh of a series of eighteen sections. The sixteenth section of the series is shown in Figure 3. It contains the second maturation spindle, at either end of which is an attraction sphere in the centre of a slight accumulation of protoplasm. The chromosomes cannot be clearly made out, but perhaps lie aggregated in a small dark mass close down against the attraction spheres. It is evident that the amount of chromatin involved in this division is less than in the case of the first maturation division (cf. Fig. 1). The obliquity of the plane of sectioning to the dorso-ventral axis of the egg makes this spindle appear to lie quite a little below the surface of the egg. Such, however, is not the case; it comes close up to the surface, but obliquely, not vertically, as did the first maturation spindle. Indeed, an examination of other specimens, less advanced, shows that it first appears in a horizontal position, i. e. at right angles to the direction of the first maturation spindle as seen in Figure 1, but later rotates so that one end of the spindle lies deeper in the egg than the other.¹ The first polar globule does not really lie in this section, but has been projected there from its real position on the margin of the next section, the seventeenth of the series.

In Figure 5 is represented a section, the fifth of a series of sixteen, through an egg of Series A, lot 3, killed twenty minutes later than lot 1 of the same series (cf. Fig. 1). The section passes obliquely in a dorso-ventral direction, unlike those shown in Figures 2-4, which were more nearly horizontal. On the ventral margin of the section is seen the cap of protoplasm which as early at least as the beginning of maturation covered that side of the egg. The male archoplasm has moved deeper into the egg, and its attractive influence has been extended so that it is now manifested over the greater portion of the section. In consequence of this attraction on the protoplasm the area free from yolk has con-

¹ A rotation of the maturation spindles from an original tangential to a radial position has been observed repeatedly in other animals; in the case of the second spindle, the tangential position is doubtless correlated with the derivation of its two archoplasmic masses from the single archoplasmic mass left in the egg after the completion of the first maturation division.

siderably enlarged. The male pronucleus has also increased in size and followed the lead of its attraction sphere toward the centre of the egg. In the dorsal half of the section is seen the female pronucleus, already grown to considerable size. In it can be discerned small chromatic granules, and behind it and deeper in the section the female archoplasm. This archoplasm seems to be much less energetic than that of the male element, for its influence is scarcely perceptible, even on the portion of the egg in which it lies, and it does not appear to modify either the shape or course of the female pronucleus, which, as we shall see, moves toward the male archoplasm leaving its own behind. The polar globules represented at the margin of this section do not as a matter of fact occur in that position, but at the margin of the preceding section. If that section were properly superposed on this, the polar globules would lie over, but a little to the left of the female pronucleus.

A stage somewhat later than the one just described, though found in the same lot of eggs, is shown in Plate II. Figures 7-10, which represent the fourth, seventh, tenth, and twelfth sections respectively of a series of sixteen. In Figure 7 is seen the male pronucleus with its archoplasm now divided; in Figure 8, the female pronucleus; in Figure 9, the female archoplasm; and in Figure 10, the polar globules marking both the centre of the future dorsal surface of the embryo, and the point from which the female pronucleus starts in its journey through the egg toward the male pronucleus. The position of these various bodies with relation to one another can be most clearly illustrated by two reconstructions (Figs. 11 and 12) upon planes perpendicular to the plane of sectioning and at right angles to each other. Suppose the sections piled one above another in their original order and position, the first section of the series being uppermost and the egg thus reconstructed to be viewed as a transparent object in the direction of the arrow at the left of Figure 7. One would then see the appearance shown in Figure 11, which is a projection of the egg and the most important bodies in it upon a plane parallel to the line *ab* (Fig. 7), and perpendicular to the plane of Figure 7.

If the egg be viewed in the direction of the arrow at the top of Figure 7, one gets the appearance shown in Figure 12, which is a projection upon a plane parallel with the line *a'b'* in Figure 7, and perpendicular to the plane of that figure.

A comparison of the stage under discussion with that represented in Figure 5 shows that considerable changes have occurred in the interval between them. The male pronucleus (Fig. 7) has grown to much greater size and contains several conspicuous chromatic granules. In-

stead of a single attraction sphere, there are two, both well defined and at a considerable distance apart. An examination of other eggs of the same lot shows how the condition here existing has come about. The male archoplasm moving in advance of its pronucleus (cf. Fig. 5) has gradually elongated transversely to its line of progress, arranged itself about two centres instead of one, and finally constricted itself into two distinct spherical masses, which move apart, and by their combined action on the male pronucleus draw it forward to a position midway between them, so that its long axis lies in the line joining their centres. The female pronucleus (Fig. 8) has approached to within a short distance (about one fifth the diameter of the egg) of the male pronucleus.

It has grown to an equal size with the male pronucleus, and, like it, contains large chromatic granules. No trace of an archoplasmic body can be seen in connection with it, nor in either of the adjacent sections. However, what are unmistakably the remains of one are visible three sections behind the female pronucleus. (See Fig. 9; compare also Figs. 11 and 12.) This archoplasmic body shows signs of disintegration, being rather diffuse and exerting apparently no attractive influence on the egg protoplasm. The female pronucleus has clearly passed beyond its control, and is now advancing rapidly to unite with the male pronucleus. One might doubt that the body described is identical with a female archoplasm, were it not perfectly constant in its appearance at this stage *behind the female pronucleus in the path of the latter from the point where the polar globules were formed toward the male pronucleus*. Moreover, though diligent search has been made, a similar body has never been found at this stage in any other portion of the ovum, except in connection with the male pronucleus.

In from five to ten minutes after the stage just described the two pronuclei are seen to have come together (Plate III. Fig. 13, and Plate I. Fig. 6). They are indistinguishable from each other so far as size and optical appearance are concerned, and are flattened against each other, but their nuclear membranes remain intact, and there is no mingling of their substance until the first cleavage is about to take place. (See Plate III. Fig. 14.) At the stage shown in Plate II. Fig. 7, we saw that the male pronucleus was already elongated between its two attraction spheres. The female pronucleus is seen in Figure 13 (Plate III.) to have joined it while it is still in that condition. Both have further increased in size. Very soon the nuclear membranes disappear, the attraction spheres move farther apart (cf. Figs. 13 and 14, Plate III.), and a spindle forms between them, on whose equator are seen the chromosomes.

To recapitulate. *In the impregnated egg of Ciona two archoplasmic masses can be recognized, one in connection with each of the pronuclei. That derived from the spermatazoön is much the more energetic of the two, and is alone concerned in bringing the pronuclei together. While the pronuclei are still a considerable distance apart, the male archoplasm divides into two distinct attraction spheres, between which the first cleavage spindle later forms. The female archoplasm degenerates, taking no part whatever in the formation of the first cleavage spindle. There is accordingly in the fertilization of Ciona no union of male and female archoplasms.*

Let us compare briefly these conclusions with those of other recent observers on the subject of the attraction sphere in fertilization.

The fertilization of the Tunicate egg has been studied hitherto by Boveri ('90) and Julin ('93). Boveri's observations, made on *Ciona intestinalis* and *Ascidia mentula*, were, as he states, incomplete on account of an accident to his preserved material. It was his opinion that no astral radiations ("Polstrahlungen") are present in the maturation of the egg, and that the two asters of the first cleavage spindle are derived by division from a single one arising in connection with the spermatazoön soon after its entrance into the egg. Julin was able to confirm on *Styelopsis grossularia* the observations of Boveri, and to supplement them, as he says, by demonstrating at the centre of each aster of the first cleavage spindle a centrosome. No figures, however, accompany Julin's paper; moreover, he states that his observations were restricted to two stages, corresponding to those shown in Boveri's Tafel XII. Figs. 27 and 29.

Though my own conclusions are in entire agreement with those of Boveri and Julin as to the derivation of the attraction spheres of the first cleavage spindle exclusively from the spermatazoön, my observations differ from theirs regarding certain minor points, as the reader may learn by consulting the papers cited.

On the subject of fertilization in groups of animals other than the Tunicata there is an enormous literature. I shall refer to only a few of the most recent papers.

In 1891 Fol described the famous "quadrille of the centres" as occurring in the fertilization of the sea-urchin egg. According to his account, there arises in the egg from the tip of the spermatazoön, a centre of attraction ("spermocentre"), which later divides. In connection with the egg nucleus appears another centre of attraction ("ovocentre"), which likewise divides. Upon the meeting of the pronuclei, each half-spermocentre unites with a half-ovocentre to form an astrocentre. The

two astrocentres arise on opposite sides of the cleavage nucleus, and between them the first cleavage spindle forms.

A short time after the publication of Fol's paper, Guignard ('91) described as occurring in the fertilization of a flowering plant a similar union of male and female centres of attraction ("sphères directrices"). More recently Conklin ('94) has observed its occurrence in the case of a mollusk, *Crepidula*.

Fol's observations, however, are flatly contradicted by the careful studies of Wilson and Mathews ('95) on three different genera of Echinoderms. They find that "the central archoplasm sphere ('attraction sphere') of the cleavage amphiaster is derived by direct and unbroken descent from the central mass of the sperm-aster without visible participation of an egg-aster."

Fick ('93) also observed that in the fertilization of a Vertebrate, *Axolotyl*, the centrosomes of the first cleavage spindle are derived exclusively from the spermatozoön. Brauer ('92) arrived at a similar conclusion regarding the fertilization of a crustacean, *Branchipus*, and Mead ('95) regarding a worm, *Chætopteris*. These observations are in entire agreement with those made prior to Fol's announcement of the "Quadrille" by Boveri ('88) on *Ascaris* and *Sagitta*, and by Vejdovsky ('88) on *Rhynchelmis*. Boehm ('88) had also expressed with some caution a similar view regarding *Petromyzon*.

On the other hand, Wheeler ('95), in a paper published simultaneously both with that of Wilson and Mathews and with that of Mead, states that in *Myzostoma* both centres of attraction arise in connection with the *egg nucleus*, none whatever being produced by the spermatozoön.

Summary on Maturation and Fertilization.

(1) *In a majority of the animals in which fertilization has been most recently studied the attraction centres of the first cleavage spindle are derived from the spermatozoön and from the spermatozoön only.*

(2) *But in the fertilization of at least one animal, and undoubtedly in all cases of parthenogenetic development, the attraction centres arise solely in connection with the egg nucleus.*

(3) *Both these facts prove conclusively that the archoplasm, or "organ of division," is not a bearer of heredity, since in fertilization it may be derived from the sexual product of one parent only, whereas it is a well recognized law that heritable substance is contributed to the offspring by both parents equally.*

(4) *If the archoplasm is furnished in some cases by the sperm only*

and in others by the ovum only, it is not inconceivable that in yet other cases *both* may contribute to its formation. Therefore the observations of Guignard and Conklin are not necessarily irreconcilable with those more recently made by Wilson and Mathews, Mead, Wheeler, and myself, as well as the earlier observations of others. In any case, however, the theoretical conclusions based on Fol's "quadrille," as to the share which the attraction centres enjoy in the phenomena of heredity, may now be definitely set aside.¹

V. POLARITY OF THE EGG.

Attention has already been called to the fact that even before fertilization one axis of the egg, the vertical, has been determined. The point where the polar globules form is its dorsal pole, which lies at the centre of the surface of the *less richly protoplasmic* hemisphere. At some point on the surface of the opposite hemisphere, the spermatozoön usually enters the egg, and there is reason to believe that its point of entrance determines the median plane of the embryo, and so its antero-posterior axis.

After the two pronuclei have met, they move toward the centre of the egg, and in that region the first cleavage spindle arises (Plate III. Fig. 14). It invariably lies parallel to a tangent at the point of formation of the polar globules. The first cleavage plane, which in accordance with a general law is perpendicular to the spindle at its equator, passes through the point where the polar globules arose and divides the egg into two equal blastomeres (Plate III. Fig. 15; cf. Plate V. Fig. 27).

¹ Boveri ('95), in a paper recently received, completely confirms the observations of Wilson and Mathews regarding the source of the attractive bodies of the first cleavage spindle of the sea-urchin egg. He for the first time in his published writings, so far as I know, gives a formal definition of the centrosome, applying the term to what Wilson and Mathews call the "archoplasm." Boveri, if I rightly understand him, recognizes an archoplasm surrounding the centrosome, at least at certain stages, and specifically different both from the centrosome and from the general cytoplasm.

What in the foregoing pages I have called indifferently *archoplasm* and *attraction sphere* undoubtedly corresponds with what Boveri in his latest paper ('95) defines as the centrosome. A centrosome in the sense of Heidenhain, that is, a simple, distinct granule staining black in iron-hæmatoxylin, I have not been able to detect in the egg of *Ciona*; nor have I observed a substance (Boveri's archoplasm) specifically distinct from the egg cytoplasm, enveloping the attractive body (Boveri's centrosome). As the reader will glean from the earlier pages of this chapter, I regard the substance forming the radiations about the attractive body as identical with the egg cytoplasm. — June, 1895.

The section seen in Figure 15 shows that cleavage has progressed more rapidly from the ventral than from the dorsal surface. This is to be explained by the richer supply of protoplasm on the ventral surface.

A study by reconstruction or otherwise of a series of sections through an egg in this stage *invariably* shows that cleavage has also progressed with unequal rapidity from the two *ends* of the embryo.

That end at which cleavage is more advanced is destined to become the posterior end. In this case also the inequality in rate of cleavage is attended (probably caused) by an inequality in the distribution of protoplasm. The protoplasmic cap of the ventral hemisphere is always thicker at the future posterior end of the embryo than at the anterior end, and as the first cleavage plane cuts the egg, this accumulation of protoplasm migrates in between the two blastomeres, its presence probably being the accelerating force in the separation of the blastomeres.

After the first cleavage is completed, the protoplasm, which had migrated in between the blastomeres, again returns to the surface and takes up a very definite position on the adjacent faces of the blastomeres just below the equator of the egg. (See Plate III. Fig. 17, x.)

This region appears in the living egg as a clear area, and marks the spot where arise later the small flattened posterior cells found so useful in orientation by Van Beneden et Julin and others. That this clear area is the region of their formation I have been able to establish by continuous observations of the living egg, controlled and completely supported by the study of preparations. *The thickened spot in the protoplasmic cap of the ventral hemisphere at the beginning of cleavage*, which seems to determine the posterior end of the embryo, *I believe to be caused by the entrance of the spermatozoön*. It is evident that the spermatozoön, unless it enters exactly at the ventral pole of the vertical axis, must lie upon entrance nearer to one end of the egg than to the other, supposing that it is in the median plane and ventral hemisphere of the embryo.

The *nearer* end, I believe, becomes the posterior end of the embryo, and is determined for that fate by the accumulation of protoplasm in the region of entrance of the spermatozoön. It is impossible to say in any particular case exactly where the spermatozoön has entered the egg, for its presence there cannot be detected until it has begun to form a yolk-free area in the egg. However, I have never observed a case in which the spermatozoön did not give evidence from its position of having entered the egg excentrically with reference to the lower pole of the vertical axis. Hence I conclude that cases of entrance at that pole, if they occur, are extremely rare.

Summary on Polarity of the Egg.

(1) *The dorso-ventral axis of the embryo is predetermined in the egg before fertilization; the polar globules invariably form at its dorsal pole.*

(2) *The spermatozoön may enter the egg at any point on its ventral hemisphere, that point probably determining, however, the median plane and posterior end of the embryo.*

(3) If we adopt the commonly employed terms *animal* and *vegetative* for the two poles of the unfertilized egg, we must call the ventral the animal pole, and the dorsal the vegetative pole. For it is the *ventral* half of the egg which contains a richer supply of protoplasm, and which consequently cleaves more rapidly and becomes the ectodermal side of the embryo; whereas the dorsal half of the egg contains less protoplasm, cleaves less rapidly, and forms the endodermal portion of the embryo.

(4) We may say, accordingly, that *the form changes accompanying maturation occur, in Ciona at least, and presumably in Ascidians in general, at the pole of the egg opposite to that at which they occur in Amphioxus*, and, so far as known, in all other animals producing eggs with polar differentiation; for the changes connected with maturation are uniformly reported to take place at the *animal*, i. e. at the more richly protoplasmic pole, whereas in *Ciona* they take place at the vegetative pole.

VI. CELL LINEAGE OF THE EMBRYO.

The statement made in the preceding paragraph presents a condition of affairs so directly contrary to that found in other groups of animals, as well as to what has been assumed by all previous writers to be the case in Ascidians, that it requires the presentation of unmistakable evidence in its support. Such evidence I have to offer, both from the study of the living egg and from that of preparations. Before passing, however, to the consideration of this evidence, a word of explanation is necessary concerning the system of nomenclature to be employed.

1. Nomenclature.

In any extended work on cell lineage it is desirable to have some system of naming the individual cells which will indicate readily the exact history of each, — from what part of the matured ovum it has been derived, by how many divisions it is removed from the ovum, and from what other cells these divisions have separated it. In this paper I shall

follow with some modifications the system introduced by Kofoid ('94) in his work on *Limax*.

1. Each cell will be designated by a letter with two exponents.

2. The letter indicates the quadrant of the egg from which the cell in question has been derived, or in other words that cell of the 4-cell stage from which it is descended. Viewing the egg from the ventral or animal pole (the one *opposite* that at which the polar cells are formed), the left anterior quadrant is *A*, the right anterior *B*, the right posterior *C*, and the left posterior *D*. In dorsal views, *A* and *D* are of course the right quadrants, and *B* and *C* the left.

3. The first exponent indicates the *generation* to which a cell belongs; that is, the number of cell divisions by which it is removed from the ovum. The ovum is generation one, the 2-cell stage two, the 4-cell stage three, etc. (See the Table of Cell Lineage on page 275.)

4. The second exponent indicates the *number* of a cell in a generation, the cells of each quadrant being numbered independently from the animal toward the vegetative pole.¹ If in any case two cells of common descent lie in an equatorial position, that one which is nearer the sagittal plane is given the lower numeral.

To ascertain the designation of the mother cell of any particular cell, its *first* exponent must be diminished by one; and its *second* exponent, if an even number, must be divided by two, but if an odd number it must first be increased by one and then divided by two.

In order to determine the daughter cell of a particular cell, simply reverse this process; that is, increase the first exponent by one, and double the second exponent. To determine the other daughter cell, diminish this second exponent by one. For example, the daughter cells of $a^{5.4}$ are $a^{6.8}$ and $a^{6.7}$.

2. Cleavage.

A. EARLY STAGES OF CLEAVAGE.

(a) To 24-cell Stage.

Figures 19–26 (Plate IV.) show eight views of a living egg, drawn by means of an Abbé camera lucida at successive stages, the egg remaining undisturbed in position under the microscope throughout the period of observation. The left side of the egg is, as I shall show, towards the

¹ In gastrulation, the cells about the vegetative pole are depressed to a lower level than the margin of the blastopore. In naming cells it is considered that the vegetative pole is also depressed at that period, and lies constantly on the dorsal surface at the common point of meeting of the cells derived from the four quadrants.

observer. In Figure 19 the process of maturation is seen to be completed, the polar globules lying in a slight depression on the dorsal surface of the egg. The 2-cell stage is shown in Figure 20. The 4-cell stage is seen in Figure 21 to be approaching, and has been reached at the stage shown in Figure 22. The two blastomeres on the side toward the observer appear to be of equal size, the other two are hid from sight. A view of the egg immediately after the next division is shown in Figure 23; the appearance nine minutes later is shown in Figure 24. These both represent the 8-cell stage, and show that *the four cells which lie nearest the polar globules are smaller than those more remote*. They also show that division has occurred in such a manner that the pair of cells occupying the upper right-hand corner of the figure is in contact with the diagonally opposite pair of cells in the lower left-hand corner of the figure, whereas the pair of cells in the upper left-hand corner is entirely separated from that diagonally opposite it. This arrangement is due to no accidental shoving of cells one over another, but is found *invariably* occurring at the 8-cell stage. The diagonally opposite cells which are in contact form respectively the posterior dorsal and anterior ventral portions of the embryo. This arrangement of the cells of the 8-cell stage has up to the present time been overlooked by all writers on tunicate embryology except Chabry ('87). He both distinctly recognized and clearly figured it. (See his Planche XVIII. Fig. 9.) But, as I pointed out in a previous paper ('94), that hemisphere of the egg which he, following Van Beneden et Julin, called *dorsal*, was really the *ventral* hemisphere, so that he wrongly calls the cells in contact the *anterior* dorsal and *posterior* ventral. If we correct his naming of the hemispheres, his observations on *Ascidiella* are brought into complete agreement with mine on *Ciona* regarding this point. In both cases the posterior dorsal and anterior ventral cells of the 8-cell stage are in contact. Though Seeliger ('85) apparently overlooked the fact, his figures (Taf. I. Figs. 7, 8, and 10), when their orientation is corrected as I ('94) have shown to be necessary for other reasons, present precisely the same arrangement of cells in the 8-cell stage of *Clavelina*. This condition is therefore probably of general occurrence among the simple and social *Ascidians*.

The 16-cell stage immediately after its formation is shown in Plate IV. Fig. 25, and half an hour later in Figure 26. In the stage represented by Figure 26, spindles, directed as indicated by the arrows, were already visible in the large cells, occupying the lower half of the figure, though none had yet appeared in the smaller cells composing the upper half of the figure. This fact foreshadows an earlier division on the part of the

cells of the lower hemisphere, which would lead to a stage of twenty-four cells. Such a stage was figured in my preliminary paper ('94, Plate I. Figs. 1 and 2; here reproduced in Plate IX. Figs. 51 and 52), and it was there demonstrated that the hemisphere in which division is earliest, as the egg passes from the 16-cell stage, becomes later the ventral or ectodermal hemisphere of the embryo.

Accordingly the series of observations illustrated by Figures 19-26 goes to prove that the four larger cells of the 8-cell stage, which are more remote from the polar globules, form the ventral or ectodermal half of the embryo, whereas the four smaller cells, on which the polar globules rest, become the dorsal or endodermal half of the embryo.

The same thing is shown by Figures 27-34 (Plates V. and VI.), a series of drawings of an egg viewed from its anterior end. In Figures 27-29 are seen successive phases of the 2-cell stage. Figure 30 shows the 4-cell stage, and Figures 31 and 32 two phases of the 8-cell stage. At the 8-cell stage in this series, as well as in the series previously examined, the four cells nearest the polar globules are smaller than the other four; they will form, as we shall see, the dorsal hemisphere. There has been no shoving of cells across the median plane, but shoving has occurred among the cells of the right and left halves of the embryo separately, as was seen also at this stage in the series previously examined. (See Plate IV. Fig. 23.) According to the rule already stated, we should find in contact with each other the diagonally opposite pairs of cells which are to form respectively the posterior dorsal and anterior ventral portions of the embryo; while the other two pairs of cells should be completely separated. If this is true in the case before us (Figs. 31 and 32), we are looking at the anterior end of the embryo, for the pair of *ventral* cells *nearest* the observer is seen to be in contact with the most *remote* pair of *dorsal* cells.

Figure 33 (Plate VI.) shows the 16-cell stage, and Figure 34 the 24-cell stage in process of formation. In this egg also the cells of the hemisphere most remote from the polar globules were first to divide in passing from the 16-cell stage. Those of the other hemisphere divided in this case about twelve minutes later. Therefore *by this series also the hemisphere more remote from the polar globules is shown to be the ventral or ectodermal*. That one is looking in this series at the anterior end of the embryo, as already suggested, and not at the posterior end, is shown by a comparison of Figure 34 (Plate VI.) with Figure 51 (Plate IX.), both of which represent the 24-cell stage. The posterior end of the embryo is seen in Figure 51 to be marked by a noticeably small pair of

cells, the like of which does not appear in Figure 34, but may be supposed to lie hidden from view at the more remote end of the embryo. Moreover, the cells $A^{6.1}$, $A^{6.2}$, $B^{6.1}$, $B^{6.2}$ of Figure 51, which are situated at the anterior end of the embryo, correspond well in size with the four cells nearest the observer in Figure 34. Therefore the rule previously stated for the orientation of the 8-cell stage is exemplified in this series also.

In Figures 45-50 (Plate VIII.) is shown another series of drawings illustrating what has been said regarding the clear protoplasmic region (x) which throughout cleavage marks the posterior end of the embryo. In this series one looks down obliquely on the dorsal surface of the embryo from its posterior end. The polar globules are not visible, for the reason that they do not come into profile at the margin of the egg, a circumstance which is necessary for an exact determination of their position.

In Figure 45, a 2-cell stage, the clear region appears in each blastomere at x . During each successive cell division it bulges out as represented in Figure 46, and again in Figure 47, just as if it were the most plastic portion of the egg and responded most readily to the internal tension which accompanies cell division. Such indeed is probably the case, for this region is free from yolk granules, consisting of protoplasm only, as has been already pointed out.

In Figure 48, the 8-cell stage is seen to be completely formed. Applying our rule for the orientation of the egg at this stage, we decide that the pair of cells occupying the centre of the figure and nearest to the observer is to form the posterior dorsal portion of the embryo; for (1) it belongs to the set of four smaller cells formed by the first equatorial plane of cleavage, and (2) it is in contact with the diagonally opposite pair of cells of the other hemisphere. The sequel justifies our conclusion. Figure 49 represents the 16-cell stage, and Figure 50 the 24-cell stage.¹ In Figure 50 it is seen that the small posterior cells of the ectodermal hemisphere, unmistakably identical with $C^{6.3}$ and $D^{6.3}$ of Figure 51 (Plate IX.), have appeared just where the clear portions forming prominences at the time of cell division have all the time been. These portions have become a part of the small cells in question, which contain less yolk than any other cells of the egg at this stage, and subsequently cleave less rapidly than any other cells of the ventral

¹ It will be observed that between the stages represented in Figures 49 and 50 there has been a slight rotation of the egg, so that the latter figure exhibits an exactly dorsal view instead of an obliquely dorsal one.

hemisphere. The persistence of this clear polar region in stages later than that of 24 cells was shown in certain figures of my preliminary paper (reproduced in Plate IX. Figs. 54 and 55). It finally passes into the small flattened cells $C^{7.5}$, $D^{7.5}$ (Plate XI. Fig. 71), of whose later history we shall have more to say.

Chabry ('87) observed in *Ascidella* at the beginning of the 8-cell stage the formation of polar prominences such as I have described, and spoke of them as a sure means of orienting the egg at this stage. On page 203 he says: "Il est encore une marque propre aux cellules P et $P[D^{4.1}, C^{4.1}]$ que permet de les distinguer de toutes les autres, elle consiste en une petite saillie en forme de mamelon, saillie qui est dirigée horizontalement en arrière et que montrent les figures 2 et 23 de la planche XVIII. Cette saillie n'est visible qu'au début du stade VIII. [8-cell] et surtout durant la segmentation qui produit P et $P[D^{4.1}, C^{4.1}]$." Apparently Chabry overlooked the formation of the prominences at other than the 4- and 8-cell stages, and failed to recognize their true significance. For he explains them as merely foreshadowing the form and direction of the next cell division, and as referable to a supposed general phenomenon, which, stated in his own words, is as follows: "Que les blastomères ont à l'instant où ils viennent de se produire et mieux encore *durant leur individualisation* des formes spécifiques qu'ils perdent peu d'instant après. Ces formes spécifiques paraissent être en rapport avec les segmentations dont ces blastomères seront plus tard le siège . . . la segmentation a donc lieu dans tous les cas, perpendiculairement au plus grand axe que possédait le blastomère durant son individualisation."

It is hardly necessary, I suppose, to say anything at this late day in refutation of Chabry's generalization. My own observations indicate that cells tend to assume at the time of their formation ("individualisation") a *spherical* form,¹ if they are homogeneous in structure, and that the departure from an evenly rounded contour at the posterior end of the ventral hemisphere is explicable by the presence there of a region peculiar in its constitution, containing as it does less yolk than the other superficial portions of the egg.²

¹ Mutual pressure of cells may modify this form, in which case the direction of the next division may perhaps be predicted, as Chabry states, at the time of the "individualisation" of cells. For, *other things being equal*, it is true that the spindle arises in the longest axis of the cell.

² I am aware that Van Beneden et Julin ('84) have offered an entirely different explanation for certain phenomena probably related to those under discussion, which they observed in the cleaving egg of *Clavelina*. Their explanation implies

Let us examine still another series of drawings (Figs. 35-42, Plates VI. and VII.) made from the living egg, which in this case is viewed from the ventral side and a little obliquely. The polar globules of course are not seen, since they lie on the opposite side of the egg. Neither is the point of view a favorable one to bring the posterior polar regions clearly into profile as in the series last examined.

Figure 35 (Plate VI.) shows the 4-cell stage; Figures 36 and 37, successive views of the 8-cell stage; and Figure 38, a 12-cell stage, the four cells of the ventral hemisphere having divided in this case a little earlier than those of the dorsal hemisphere. This is unusual, for the difference in rate of cleavage of the cells of the two hemispheres commonly first appears, as we have seen in the three series previously examined, in passing from the 16-cell stage to one of 24 cells.

Figure 39 (Plate VII.) gives a view of the egg five minutes after the stage shown in Figure 38 had been reached. It represents the 16-cell stage. A drawing made five minutes later still is shown in Figure 40, and one made ten minutes after that is shown in Figure 41.

In the last mentioned figure, the cells of this uppermost hemisphere are seen to have again become rounded in outline preparatory to the next cell division. Spindles are already visible in them, as indicated by the arrows, those last to appear being the ones in the small cells ($C^{5.2}$, $D^{5.2}$) at the lower margin of the figure. The subsequent division was about a minute later in these two cells than in the others of the same hemisphere; this is regularly the case in the cell division which leads to the 24-cell stage.

Figure 42, the last of the series, will be at once recognized, by one who has read my preliminary paper, as a ventral view of the 24-cell stage. (Cf. Plate IX. Fig. 51.) The posterior end is clearly marked by the small cells $C^{6.3}$, $D^{6.3}$. A re-examination of Figures 36 and 37 (Plate VI.) shows that the rule previously stated for orienting the egg at the 8-cell stage is again exemplified in the case of this series, for in

the existence during karyokinesis of *astral fibres* which attach to the cell wall at particular points and by their contraction depress its surface.

Such an explanation seems to me inadequate, at least for this case; first, because I have seen no evidence of the existence of *astral fibres* in karyokinesis; secondly, because at successive cleavages the prominences appear in the same structurally peculiar region, whether the karyokinetic spindle is directed *toward* that region — as the explanation of Van Beneden et Julin would imply — or not (see Plate VIII. Fig. 47); thirdly, because *astral fibres*, if present, should appear in every blastomere at karyokinesis, but I have been able to discover these peculiar prominences only in the particular regions already described.

Figures 36 and 37 we see in contact cells which we know, from an examination of Figure 42, eventually become the anterior ventral and posterior dorsal portions of the embryo.

We have now followed the cleavage cell by cell to the 24-cell stage. We have seen that cleavage is from the very beginning bilateral, and progresses in a very definite manner and at a very definite rate. This we shall find is true in the further development of the egg, even until the complete closure of the blastopore. Wilson ('94) observed that the cleavage of *Amphioxus* showed all gradations between a perfectly radial, a bilateral, and even a spiral form; and he raised a query whether the same might not be found to be true for *Ascidians*. In *Ciona* at least this does not seem to be the case. I have never observed an instance of deviation from the regular mode of cleavage described in the foregoing paper, unless one so construes the occasional very slight difference in the time of cleavage of the cells of the two hemispheres in passing from the 8-cell stage, a matter to which allusion was made on page 232. No rotation of the cells of one hemisphere over those of the other even in the slightest degree has ever been observed. In having a perfectly definite and stereotyped manner of cleavage, the ascidian egg resembles more closely the egg of Annelids, Mollusks, and the great majority of Invertebrates, than it does that of *Amphioxus* and the Vertebrates, notwithstanding that the *end product* of cleavage shows unmistakably the now generally admitted closer affinity of Tunicates with the latter group of animals.

It remains to call attention to some of the internal phenomena accompanying the early cleavage stages. The first cleavage spindle arises, as has been stated, not far from the centre of the egg. (See Plate III. Fig. 14.) As its first cleavage is nearing completion, however, the attraction spheres and nuclei begin to move toward the dorsal surface of the egg, *away from* its more richly protoplasmic (animal) pole, from which the plane of separation cuts in more rapidly. (See Plate III. Fig. 15.) The attraction sphere of each blastomere grows more diffuse as the nuclei pass into a resting condition; it then elongates in a horizontal direction and parallel to the first plane of cleavage, and finally divides. The parts separate and the nucleus moves out to a position between them. (See Plate III. Fig. 16.) By this time the attraction spheres and nuclei unmistakably lie closer to the dorsal (maturation) surface of the egg. (Plate III. Fig. 16; cf. Plate IV. Figs. 20, 21, and Plate V. Figs. 27-29; also Van Beneden et Julin's ['84] Figs. 2 and 4*b*,

Planche VII., remembering that dorsal and ventral are reversed in Van Beneden et Julin's figures.) The yolk-free protoplasm trails downward from the attraction spheres forming a sort of crescent in each blastomere. (See Plate III. Fig. 16.)

During the second and third cleavages the nuclei remain somewhat nearer the dorsal (vegetative) pole. (Plate IV. Figs. 20-22, and Plate V. Fig. 30; cf. Van Beneden et Julin's ['84] Figs. 4 *b* and 5, Planche VII.) It follows naturally that when the 8-cell stage is formed by the first equatorial plane of cleavage (third cleavage), an inequality is observed in the size of the newly formed blastomeres, the four nearer to the *dorsal* pole being *smaller* than their sister cells, though the latter are richer in protoplasm. (See Plate IV. Fig. 23, and Plate III. Fig. 18.)

(*b*) *Summary on Early Cleavage Stages.*

1. The future posterior end of the embryo is marked at the 2-cell stage by an accumulation of protoplasm free from yolk in each blastomere at contiguous regions. This accumulation persists throughout cleavage, and forms at each cell division a pair of protuberances beyond the general contour of the blastomeres.

Subsequently to the 8-cell stage, in each of the two blastomeres in which these accumulations lie, the spindle at three successive cell divisions is directed *toward* the protoplasmic accumulation of that cell and lies *nearer* to it than to the opposite side of the cell. In consequence the newly formed cell, which contains the region in question, is in each case *smaller* than its sister cell. (Cf. $D^{5.1}$ and $D^{5.2}$, Fig. 38, Plate VI.; $D^{6.2}$ and $D^{6.3}$, Fig. 51, Plate IX.; and $D^{7.5}$ and $D^{7.6}$, Fig. 62, Plate X.)

At each of these divisions also cleavage occurs *later* in the cells containing the protoplasmic accumulations than in their sister cells.

2. The first cleavage plane is vertical, and passes through the point of formation of the polar globules. It coincides with the future median plane of the embryo, and divides the egg into two blastomeres equal in size and similar in every particular. They form respectively the right and left halves of the embryo. The fate, as just stated, of the first two blastomeres of the ascidian egg was first pointed out in the case of *Clavelina* by Van Beneden et Julin ('84).

3. The second cleavage plane is also vertical, and at right angles to the first. Like the first, it passes through the point of formation of the polar globules. It divides the egg into four blastomeres, among which no difference of size can be recognized.

4. The third cleavage plane is at right angles to both the preceding, i. e. equatorial in position. It separates four smaller cells lying nearer to the polar globules and more abundantly supplied with yolk, from four larger ones more remote from the polar globules and richer in protoplasm.

The former are destined to give rise to the dorsal or endodermal hemisphere of the embryo; the latter, to the ventral or ectodermal hemisphere.

The protoplasmic accumulations mentioned under paragraph 1 always fall in the posterior pair of cells of the ventral hemisphere (viz. $D^{4.1}$, $C^{4.1}$) close to its line of contact with the dorsal hemisphere. This pair of cells is never in contact with the anterior pair of cells of the dorsal hemisphere, but the *anterior* pair of cells of the ventral hemisphere (viz. $A^{4.1}$, $B^{4.1}$) is *invariably* in contact with the posterior pair of cells of the dorsal hemisphere (viz. $c^{4.2}$, $d^{4.2}$).

The fact just stated affords a ready and unfailing means of orienting the 8-cell stage. This was recognized by Chabry in the case of *Asci-diella*, and is shown by an examination of Seeliger's figures to be equally true for *Clavelina*. It probably holds good among all the simple and social Ascidians.

5. The 16-cell stage is usually reached by simultaneous divisions in both hemispheres. Sometimes, however, the cells of the ventral hemisphere at this cleavage divide sooner than those of the dorsal hemisphere, thus giving rise to a 12-cell stage, but this very soon changes to a 16-cell stage by the cleavage of the cells of the dorsal hemisphere.

6. As the egg passes from the 16-cell stage, cleavage *invariably occurs earlier* in the cells of the ventral hemisphere, i. e. the descendants of the four larger cells of the 8-cell stage, than it does in the cells of the dorsal hemisphere. A 24-cell stage results, in which the cells of the ventral hemisphere, being twice as numerous as those of the dorsal hemisphere, cover more surface and begin the process of overgrowth (epiboly), forcing the cells of the dorsal hemisphere into a somewhat columnar form. (See Plate VII. Fig. 44.)

B. LATER STAGES OF CLEAVAGE.

(a) *From 24-cell to 46-cell Stage.*

The 24-cell stage was taken as the point of departure in my preliminary paper ('94), and the cell lineage was traced in detail through a stage of 46 cells. I shall not repeat except in the form of a brief *résumé* what was there said regarding those stages, but shall content myself

with reproducing (in Plate IX.) the figures of Plate I. accompanying that paper, which were executed to illustrate this period of the developmental history. These figures present dorsal and ventral views of the 24-cell stage (Plate IX. Figs. 51 and 52), the 32-cell stage (Figs. 53 and 54), and the 46-cell stage (Figs. 55 and 56).

The interpretation, as given in my preliminary paper, of the lineage through the 46-cell stage rested upon the strongest possible evidence, viz. the observation of karyokinetic figures for every cell division which was represented as having occurred. Moreover, it was shown that these observations made it possible to reconcile the conflicting statements of others who had studied the cleavage of the ascidian egg. Such excellent observers as Van Beneden et Julin, on the one hand, and Seeliger, on the other, held contrary opinions as to which was the dorsal side and which the anterior end of the embryo in its early stages in one and the same genus, *Clavelina*.

It was shown in my paper, both from an examination of the authors' own figures and from a comparison with the lineage of *Ciona*, that their conflicting statements arose from a fundamental error on the part of each, Van Beneden et Julin being correct in their determination of the *ends* of the embryo, and Seeliger in his determination of the dorsal and ventral *surfaces* of the early stages. Upon correcting these mistakes, it was found that the observations of the writers mentioned were brought into harmony, and were then in agreement with my own observations on *Ciona*.

In order to demonstrate that I had correctly determined the dorsal and ventral faces of the egg for the 46-cell and earlier stages, in contradiction to the interpretation of Van Beneden et Julin, I figured a single older stage described as one of 66 cells (Castle '94, Plate II. Figs. 11 and 12). Its presentation was intended to bridge the gap between the 46-cell stage and gastrulation. This purpose it fulfilled, for it showed gastrulation already commenced, and so proved beyond question which was to be the oral (dorsal) and which the aboral (ventral) surface.

A desire to give completeness to my figures led me to state the *lineage* of this stage as I then understood it. I have since found, from the study of more complete series of embryos than I had at that time secured, that I was mistaken as to the time of cell division in one pair of cells (*C^{7.s}*, *D^{7.s}*, Fig. 56, Plate IX.). I supposed it had already occurred at the stage represented in Figures 11 and 12 (Plate II.) of my former paper. Consequently the lineage there given for this stage is incorrect. Though this fact does not affect the main conclusions of my preliminary

paper, it necessitates modification of several minor statements, as will be indicated in detail later.

The 24-cell stage, it has been seen, arises from the 16-cell stage by an earlier division on the part of the cells of the ventral hemisphere than occurs in those of the dorsal hemisphere. Accordingly, we find that at the 24-cell stage the ventral hemisphere consists of sixteen cells, whereas the dorsal hemisphere is made up of only eight. These eight are compressed into a columnar form by the overgrowth of the cells of the ventral hemisphere already begun. (See Plate VII. Fig. 44.) Their nuclei lie in a superficial position, while their deep ends are heavily laden with unassimilated yolk. They retain this columnar form up to and throughout gastrulation. In number, they are soon brought up to an equality with the cells of the ventral hemisphere by division, which leads to the 32-cell stage (Plate IX. Figs. 53 and 54) and places all the cells of the egg in the sixth generation.

Presently the cells of the ventral hemisphere again anticipate in division those of the dorsal hemisphere, this time by a still longer interval. Among the cells of the ventral hemisphere differences in the time of division could, as we have seen, be detected at the preceding cleavage. At the present cleavage the differences become more pronounced. In particular, the small posterior cells, $C^{6.3}$, $D^{6.3}$ (Plate IX. Figs. 53 and 54), divide enough later than their fellows to allow us to recognize a 46-cell stage (Plate IX. Figs. 55 and 56), made up as follows:—

Ventral hemisphere,	28 cells in the seventh generation,
	2 cells ($C^{6.3}$, $D^{6.3}$) in the sixth generation.
Dorsal hemisphere,	16 cells in the sixth generation.

46

When the two small cells $C^{6.3}$, $D^{6.3}$, divide, which they do earlier than the cells of the dorsal hemisphere, a stage of forty-eight cells is reached, all the cells of the ventral hemisphere (thirty-two in number) being in the seventh generation, and those of the dorsal hemisphere (sixteen in number) being in the sixth generation. Such a stage is shown in Plate X. Figs. 57 and 58.

(b) 48-cell Stage.

The embryo shown in Figures 57 and 58 has a vertical axis the length of which is equal to that of its antero-posterior axis, if not greater. Accordingly it has been found easier to maintain this axis in a horizontal position, and hence more convenient to represent the egg as viewed

from the anterior and posterior ends respectively, rather than from the dorsal and ventral surfaces, as in most of the other stages figured. This stage (48-cell) is made up as follows:—

Ventral hemisphere, 32 cells in the seventh generation.

Dorsal hemisphere, 16 cells in the sixth generation.

48

It will be observed that the cells of the ventral hemisphere, though all in the seventh generation, are not all equally advanced in their preparations for division, which evidently is again about to set in. For while the cells occupying the centre of the ventral hemisphere, or, in other words, lying nearest to the animal pole of the egg, are about to pass into the next generation, the cells occupying the margin of the ventral hemisphere, and more remote from the animal pole, contain nuclei entirely quiescent, like those seen in the cells of the dorsal hemisphere. This is contrary to the statement made in my preliminary notice ('94), in which I said that at this division those cells of the ectodermal hemisphere which were marginal and in contact with cells of the endodermal hemisphere were *first* to divide. This erroneous statement arose from the wrong interpretation given to Figures 11 and 12 ('94 Plate II.) in describing the cell lineage of that stage, a matter to which attention has already been directed.

In the embryo shown in Plate X. Figs. 57 and 58 (48-cell stage) one may readily distinguish three regions, each composed of sixteen cells. The first region is the dorsal hemisphere, with its sixteen cells all in the sixth generation ($a^{6.5}$ – $a^{6.8}$, $d^{6.5}$ – $d^{6.8}$, and the corresponding cells in quadrants *B* and *C*). These cells are destined to form the endoderm of the larva, the chorda, and a portion of the mesoderm. The second group of sixteen cells occupies the centre of the ventral hemisphere ($A^{7.1}$, $A^{7.2}$, $A^{7.3}$, $A^{7.5}$, $A^{7.7}$, $D^{7.1}$, $D^{7.2}$, and $D^{7.3}$, with the corresponding cells in quadrants *B* and *C*). They are in the seventh generation, but already contain spindles, showing that they are soon to pass into the eighth generation. This group of cells will form the ectoderm of the larva. The remaining sixteen cells of this embryo, also belonging to the ventral hemisphere, form the third group ($A^{7.4}$, $A^{7.6}$, $A^{7.8}$, and $D^{7.4}$ – $D^{7.8}$, with the corresponding cells in quadrants *B* and *C*). They too are in the seventh generation, but their nuclei are quiescent, showing that these cells will be later in dividing than the other cells of the ventral hemisphere. They are arranged in an equatorial band between the other two groups

of cells. This band is interrupted at only one point on each side of the embryo, where a single cell ($A^{7.5}$, $B^{7.5}$) of the ectodermal group reaches up into contact with the cells of the dorsal hemisphere. From the equatorial band just described are derived chiefly nerve cells and mesoderm cells.

(c) *64-cell Stage.*

The completion of the divisions foreshadowed by spindles in the ectodermal group of cells of the stage last discussed (Plate X. Figs. 57 and 58) doubles the number of cells in that group, and brings the number in the entire embryo up to sixty-four, distributed as follows.

Ventral hemisphere (designated by the letters *A, B, C, D*):—

32 cells in the 8th generation = the ectodermal group.

16 " 7th " = the equatorial band.

48

Dorsal hemisphere (designated by *a, b, c, d*):—

16 cells in the 6th generation.

64

Such a stage is shown in Plate X. Figs. 59 and 60, the former representing a ventral and the latter a dorsal view. The egg has again assumed the flattened form which it had at the 32-cell stage.

Examining first the ventral surface (Fig. 59), we see that the divisions foreshadowed in the 48-cell stage (Figs. 57 and 58) have in every instance occurred in a direction perpendicular to that of the spindle in the mother cell, though a slight displacement is in some cases appearing among the daughter cells, on account of the mitoses arising in the equatorial band. The cells of the ectodermal group, on account of their recent division, now number thirty-two, as many as are found in both the other groups put together. They are in the eighth generation, one generation in advance of the cells of the equatorial band, and two generations in advance of the cells of the dorsal hemisphere. They are $A^{8.1}$ – $A^{8.6}$, $A^{8.9}$, $A^{8.10}$, $A^{8.13}$, $A^{8.14}$, and $D^{8.1}$ – $D^{8.6}$, together with the corresponding cells in quadrants *B* and *C*.

The equatorial band is, as at the last stage, composed of sixteen cells all in the seventh generation, but six of them (three on each side of the median plane, Fig. 60, $A^{7.4}$, $A^{7.8}$, and $D^{7.4}$) now show signs of approaching division. Four of these mitotic cells form the anterior segment of the equatorial band, and are destined to produce a considerable portion of

the nervous system of the larva. (See Fig. 60, $A^{7.4}$, $A^{7.8}$, $B^{7.4}$, and $B^{7.8}$.) The two remaining mitotic cells of the equatorial band are situated laterally one in each of the posterior quadrants (Figs. 59 and 60, $D^{7.4}$, $C^{7.4}$). The ten remaining cells of the equatorial band all contain resting nuclei. Eight of these cells are grouped at the extreme posterior end of the equatorial band in a region where, from the 16-cell stage on, we have found cleavage to be more tardy than in any other part of the ventral hemisphere. These eight cells are $D^{7.5}$, $D^{7.6}$, $D^{7.7}$, $D^{7.8}$, and the corresponding cells in quadrant C . (Figs. 59 and 60. Compare Fig. 57.) The two remaining cells of the equatorial band which still show no signs of division are $A^{7.6}$ and $B^{7.6}$ (Fig. 60), situated about midway between the anterior and posterior ends of the embryo.

Of the sixteen cells comprising the dorsal hemisphere (Fig. 60), six, which lie in contact with the equatorial band ($a^{6.5}$, $a^{6.7}$, $a^{6.8}$, with their mates in quadrants B and C), are in mitosis. Four of them, the most anterior of the cells of the dorsal hemisphere, lie in a transverse row across the dorsal surface of the embryo (Fig. 60, $a^{6.7}$, $a^{6.5}$, $b^{6.5}$, and $b^{6.7}$). They will ultimately form the greater portion of the chorda. We will call them the *anterior chorda fundament*. The two other mitotic cells of the dorsal hemisphere are $d^{6.6}$ and $c^{6.6}$, in the posterior half of the embryo (Fig. 60). The spindles in these cells are directed obliquely forward, upward, and outward, so that, taking into consideration the superficial position of the nuclei of the dorsal hemisphere, we may predict that the coming division will result in cutting off in each case a smaller more superficial cell from a larger cell extending deeper; the small cell will also lie anterior and lateral to its sister cell. The unequal divisions in these two cases will separate cells of unlike fate; the two smaller cells will constitute the *posterior chorda fundament*, the two larger ones will form mesoderm.

The ten remaining cells of the dorsal hemisphere (Fig. 60, $a^{6.6}$, $a^{6.8}$, $d^{6.5}$, $d^{6.7}$, $d^{6.8}$, and the corresponding cells in quadrants B and C) show as yet no signs of division. They are grouped about the vegetative pole of the egg, the point of origin of the polar cells, and *will form the whole of the definitive endoderm of the larva, and nothing else.*

(d) 76-cell Stage.

Upon the completion of division in the twelve mitotic cells of the embryo represented in Plate X. Figs. 59 and 60, a stage of seventy-six cells would be reached. An embryo in this stage is shown in Plate X. Figs. 61 and 62. It contains in the

Ventral hemisphere (designated by the letters *A, B, C, D*):

32 cells in the eighth generation = the ectodermal group.

12 cells in the " " } = the equatorial band.

10 cells in the seventh " }

54 —

Dorsal hemisphere (designated by *a, b, c, d*): —

2 mesoderm cells in the seventh generation.

10 chorda cells in the " "

10 endoderm cells in the sixth "

22 —

76

The ectodermal group of the ventral hemisphere contains the same number of cells as at the 64-cell stage, viz. thirty-two, — sixteen on each side of the median plane, ten of them being derived from an anterior quadrant (*A*), six from a posterior quadrant (*D*). They cover nearly the entire ventral surface of the egg. (See Plate X. Fig. 61, $A^{8.1}-A^{8.6}$, $A^{8.9}$, $A^{8.10}$, $A^{8.13}$, $A^{8.14}$, and $D^{8.1}-D^{8.6}$, as well as the corresponding cells in the right half of the figure.) All the cells of this group are in the eighth generation.

The equatorial band now contains six more cells than at the 64-cell stage, in consequence of the completion of divisions foreshadowed at that stage in the cells $A^{7.4}$, $A^{7.8}$, $B^{7.4}$, $B^{7.8}$, $C^{7.4}$, and $D^{7.4}$ (Fig. 60). It now consists of twenty-two cells, which, in passing from the posterior end forward, are $D^{7.5}$, $D^{7.6}$, $D^{7.7}$, $D^{7.8}$, $D^{8.7}$, $D^{8.8}$, $A^{7.6}$, $A^{8.16}$, $A^{8.15}$, $A^{8.8}$, and $A^{8.7}$, with the corresponding cells in quadrants *B* and *C* (Fig. 62). Six of the cells on each side of the median plane are derived from a posterior and five from an anterior quadrant. Signs of approaching division have at this stage become visible in four of the cells of this equatorial band, viz. $A^{7.6}$, $B^{7.6}$, $D^{7.7}$, and $C^{7.7}$. In the case of the first two cells mentioned the spindles stand vertically (cf. Plate X. Fig. 67, $A^{7.6}$); in the other two cells ($C^{7.7}$, $D^{7.7}$) the spindles are nearly horizontal in position, though their antero-lateral ends lie at a slightly higher level than the opposite ends.

There are only six cells remaining in the equatorial band which neither have passed into the eighth generation nor show any signs of immediately doing so. They are grouped at the posterior end of the embryo, which has been repeatedly pointed out as the region of slowest cleavage among the cells of the ventral hemisphere. The six cells in question are $D^{7.5}$, $D^{7.6}$, $D^{7.8}$, and the corresponding cells in quadrant *C* (Fig. 62).

Although for convenience I shall continue to use the term equatorial band, it is clear that the cells composing it are no longer strictly equatorial in position, but now lie on the flattened dorsal surface (Fig. 62). This change of position has come about in consequence of the more rapid cell division in the ventral hemisphere. How considerable the difference in rate of division has been between the cells of the two hemispheres, one readily appreciates if he stops to consider that the cells of the ventral hemisphere now number fifty-four, whereas those of the dorsal hemisphere number only twenty-two.

In the dorsal hemisphere (Fig. 62) the divisions foreshadowed by spindles at the 64-cell stage (Fig. 60) have taken place, but no new ones are approaching. The number of cells in this hemisphere is now twenty-two; twelve of them (the chorda and mesoderm cells, cf. description of Fig. 62 in the explanation of Plate X.) are in the seventh generation, and ten (the endoderm fundament), in the sixth generation, no divisions having occurred in the last named group of cells since the 32-cell stage. Of the ten chorda cells, eight derived from the anterior quadrants are arranged in a crescent-shaped band capping the anterior end of the dorsal hemisphere; they are $a^{7.9}$, $a^{7.10}$, $a^{7.13}$, $a^{7.14}$, and the corresponding cells in quadrant *B*. They form the anterior chorda fundament. The other two chorda cells, which are derived from the posterior quadrants, are $d^{7.11}$ and $c^{7.11}$. They form the posterior chorda fundament, and are at present separated from the anterior chorda cells by two cells of the equatorial band, viz. $A^{7.6}$ and $B^{7.6}$.

The sister cells of $d^{7.11}$ and $c^{7.11}$, viz. $d^{7.12}$ and $c^{7.12}$, are the sole contribution of the dorsal hemisphere to the mesoderm of the larva, for the greater part of the mesoderm is, as we shall see, derived from the equatorial band.

Among the endoderm cells it is noticeable that $d^{6.5}$ and its mate $c^{6.5}$ have been shoved forward out of their own quadrants to a position beside the endoderm cells derived from the anterior quadrants.

(e) *Summary on Later Cleavage Stages.*

1. In the cleaving ovum one can recognize, in passing from the animal to the vegetative pole successive zones, in each of which cleavage takes place less rapidly than in the preceding. At the 64-cell stage (Plate X. Figs. 59, 60) there are three such zones: first, a group of thirty-two cells encircling the animal pole, all of them in the *eighth* generation; second, an equatorial zone of sixteen cells, all in the *seventh* generation; third, a group of sixteen cells encircling the vegetative pole,

all in the *sixth* generation. The first two zones are descended from the four ventral cells of the 8-cell stage, i. e. from the four cells most remote from the point of formation of the polar globules. The third zone is descended from the four dorsal cells of the 8-cell stage. The ectoderm is derived chiefly from the first zone, — that is, the zone encircling the animal pole; — the mesoderm is derived chiefly from the second zone, and the endoderm exclusively from the third zone.

This zonal arrangement persists throughout cleavage and the early stages of gastrulation, but its symmetry is at each succeeding stage disturbed to an increasing extent by the fact that cell division is less rapid at the posterior than at the anterior end of the embryo.

2. Although, as just stated, cleavage progresses with unequal rapidity at the two poles of the antero-posterior axis, as well as at those of the dorso-ventral axis, it is *equal* in rate at the two poles of the third axis of the egg, viz. the transverse. The last mentioned fact serves to maintain the perfectly bilateral form of the embryo.

The differentiation of the poles of the dorso-ventral and antero-posterior axes, the reader will recall, was already recognizable by structural cytoplasmic differences in the unsegmented ovum. *The form and rate of cleavage are therefore manifestly predetermined by the internal constitution of the ovum.*

3. Gastrulation.

A. EARLY STAGES OF GASTRULATION.

(a) 112-cell Stage.

An embryo a little more advanced in development than the one represented in Figures 61 and 62 (Plate X.) is shown in dorsal view in Figure 71 (Plate XI.). No new divisions have occurred in the dorsal hemisphere, which accordingly consists, as at the last stage, of twenty-two cells. In the equatorial band, the four cells which were preparing for division at the 76-cell stage (Plate X. Fig. 62, $D^{7.7}$, $C^{7.7}$, $A^{7.6}$, and $B^{7.6}$) are seen in Figure 71 to have divided, though in the case of $A^{7.6}$ and $B^{7.6}$, on account of the vertical position of the spindles (cf. Fig. 67), only the more superficial daughter cell is in each instance visible ($A^{8.12}$, $B^{8.12}$, Fig. 71). No further divisions have occurred in the equatorial band, which therefore consists at this stage of twenty-six cells, all in the eighth generation except the group of six cells arranged in crescent form at the posterior end of the embryo, viz. $D^{7.5}$, $D^{7.6}$, $D^{7.8}$, and the corresponding cells in quadrant C. These cells have lingered in the seventh generation later than all other cells of the ventral hemisphere.

The equatorial band as a whole has now moved to a position distinctly within the margin of the dorsal surface (Fig. 71), so that a row of cells from the ectodermal group of the ventral hemisphere has come into view outside it round almost the entire periphery of the embryo (*cf.* Fig. 62). This change has come about in consequence of additional divisions in the ectodermal group of cells, which now not only has spread over the entire ventral surface of the embryo, but is encroaching upon its dorsal surface.

Division has occurred nearly synchronously in all the cells of the ectodermal group, though somewhat sooner in those nearest the animal pole. (See Plate X. Figs. 63-70.) The strongest possible confirmation of my own observation regarding the simultaneousness of division in the cells of the ectodermal group in this period of development is afforded by Samassa's ('94) Figures 10 and 11, Taf. II. These represent respectively a dorsal and a lateral view of a stage intermediate between those shown in my Figures 62 and 71. In Samassa's Figures 10 and 11, all the cells of what I have called the equatorial band are figured as containing quiescent nuclei, except the four seen to be mitotic in my Figure 62 (Samassa's cells 3 and 6, Fig. 10). The other cells of the ventral hemisphere visible in Samassa's figures are without exception in process of division. It may accordingly be confidently assumed that at the stage shown in Figure 71 the cells of the ectodermal group of the ventral hemisphere have all passed into the ninth generation. If so, they number sixty-four; this agrees well with the approximate count which one can make from dorsal and ventral views, though it is impossible to be sure about the exact number of ectoderm cells lying at this stage in an equatorial position between the dorsal and ventral surfaces. Accordingly I shall not attempt to give for this and subsequent stages the lineage of the individual cells of the ectodermal group. This would be a work of great difficulty and of some uncertainty, for in this case the cells entirely lack those marked differences of size, stainability, and arrangement which make the lineage for the cells of the equatorial band and dorsal hemisphere a matter of perfect definiteness.

If the estimate given of the number of cells in the ectodermal group is correct, the embryo shown in Plate XI. Fig. 71 represents a stage of one hundred and twelve cells distributed as follows.

Ventral hemisphere : —

64 cells in the 9th generation = the ectodermal group,
20 " 8th " }
6 " 7th " } = the equatorial band.
90 —

Dorsal hemisphere : —

2 mesoderm cells in the 7th generation.			
10 chorda	"	7th	"
10 endoderm	"	6th	"
22 —			
90 (in ventral hemisphere).			
<u>112</u>			

The process of gastrulation has at this stage already set in. Not only is the ectoderm growing over so as to envelop the dorsal hemisphere, but the latter is at the same time sinking down and becoming saucer-shaped. (Cf. Figs. 66 and 77.) Accordingly, gastrulation may be said to take place by a combination of the two processes of epiboly and invagination.

(b) *Differentiation of the Principal Organs as seen at the 112-cell Stage.*

a. TOPOGRAPHICAL.

We will now consider this same embryo (Plate XI. Fig. 71) with reference to the ultimate fate of its cells. At the depressed centre of its dorsal surface, surrounding the point of formation of the polar globules, we find the ten cells of the definitive endoderm, all in the sixth generation and containing each a very large nucleus. They are $a^{6.6}$, $a^{6.8}$, $d^{6.5}$, $d^{6.8}$, $d^{6.7}$, and the corresponding cells in quadrants *B* and *C*. Two of them are derived from each of the anterior quadrants (*A* and *B*), and three from each of the posterior quadrants (*C* and *D*). Together they constitute the entire fundament of the definitive larval endoderm.

The endoderm fundament is surrounded by two concentric rows of cells from which are derived some of the most important organs of the larva. The inner row or ring of cells we will call the *chorda-mesenchyme ring*, because it is destined to produce the chorda and mesenchyme. In it we must include the small flattened cells, $C^{7.5}$, $D^{7.5}$, but not their sister cells, $C^{7.6}$, $D^{7.6}$, which, though in contact superficially with endoderm cells, really belong, as their fate shows, in the second or outer ring.

The chorda mother cells, all of which are included in the chorda-mesenchyme ring, are derived, as has been already stated, in part from the anterior and in part from the posterior quadrants. Those derived from the anterior quadrants are at this stage eight in number. They form the anterior segment of the chorda-mesenchyme ring (Fig. 71, $a^{7.9}$, $a^{7.10}$, $a^{7.12}$, $a^{7.14}$, and the corresponding cells on the left of the median plane). The posterior chorda cells are only two in number

($c^{7.11}$, $d^{7.11}$, Fig. 71), one in the right and one in the left half of the embryo. They are now separated both from each other and from the anterior chorda cells. We shall see later how they are brought into contact with each other, in the median plane, and with the anterior chorda cells.

The mesenchyme mother cells are also ten in number, but, unlike the chorda cells, they are derived chiefly from the *posterior* quadrants. They are $A^{8.12}$, its deep-lying sister cell, $A^{8.11}$, $d^{7.12}$, $D^{7.8}$, and $D^{7.5}$, with the corresponding cells in the left half of the embryo, all indicated by a flat tint in the Figures.¹ It will be observed that *the mesenchyme fundament is made up of cells derived from both hemispheres and all four quadrants.*

The outer of the two rows of cells encircling the endoderm fundament will be called the *neuro-muscular ring*. (Fig. 71. The cells are stippled.) It is interrupted at three points by mesenchyme cells of the inner ring; in the middle line behind, by the small flattened cells, $C^{7.5}$, $D^{7.5}$; on the right side, by $A^{8.12}$; and on the left side, by $B^{8.12}$. It is thus divided into three portions, an anterior segment of eight cells, all descended from the anterior quadrants, and two latero-posterior segments, each composed of four cells, descended from one of the posterior quadrants. The anterior segment is composed purely of nerve mother-cells, which will form a considerable portion of the medullary plate. The other segments will form the entire longitudinal musculature of the larva, as well as a certain portion of the nervous system in the tail region.

In the two rings of cells just described are included all save two of the descendants of the cells forming the *equatorial band* of the 48-cell and later stages. These two cells are $D^{8.13}$ and $C^{8.18}$, situated at the posterior margin of the embryo (Fig. 71). They form, in my opinion, definitive ectoderm.

The remaining cells of the embryo number sixty-four, all descendants of the *ectodermal group* of the 48-cell stage. They will form definitive ectoderm, possibly also a portion of the medullary plate.

One again notices in this stage the striking difference in rate of division of the cells which he meets in passing from the vegetative toward the animal pole, a difference which made itself apparent as early as the

¹ Samassa ('94) identified the mesenchyme mother cells $D^{7.8}$ and $d^{7.12}$ (the cells 8 and 9 of his Fig. 10) as nerve cells. In my preliminary paper I expressed a different opinion, stating that they were mesoderm cells. Subsequent study has confirmed this view, but shown that I was wrong in stating, as I did, that they would contribute to the formation of "the longitudinal musculature of the tail." That organ has, as I shall show, an entirely different and hitherto unsuspected origin.

16-cell stage, and was foreshadowed still earlier by the internal constitution of the unsegmented ovum. The endoderm fundament is in the sixth generation¹ (Plate XI. Fig. 71, $d^{6.7}$, $d^{6.8}$, $d^{6.5}$, $a^{6.8}$, $a^{6.6}$, and the corresponding cells in the left half of the Figure); the chorda-mesenchyme ring is chiefly in the seventh generation, though a single pair of its cells has recently passed into the eighth ($D^{7.5}$, $D^{7.8}$, $d^{7.12}$, $d^{7.11}$, $A^{8.12}$, $A^{8.11}$, — the deep-lying sister cell of $A^{8.12}$, not shown in the Figure, — $a^{7.14}$, $a^{7.18}$, $a^{7.10}$, $a^{7.9}$, and the corresponding cells in quadrants *B* and *C*); the cells of the neuro-muscular ring are all in the eighth generation, except a single pair which lingers in the seventh ($D^{7.6}$, $D^{8.14}$, $D^{8.7}$, $D^{8.8}$, $A^{8.16}$, $A^{8.15}$, $A^{8.8}$, $A^{8.7}$, and the corresponding cells in quadrants *B* and *C*); the ectoderm cells are all in the ninth generation, those nearest the animal pole having been the first to divide and pass into that generation. We notice in this stage, as in the earlier stages, a region of delayed division in the equatorial band at the posterior end of the embryo.

B. HISTOLOGICAL.

Figures 63–70 (Plate X.) represent eight cross sections from a series through an embryo (not figured) a little more advanced in development than the one shown in Plate X. Figs. 61 and 62. The approximate position in the embryo of the sections figured is indicated by the horizontal lines on Figure 62.

The differing stainability of cells at this stage, together with other histological peculiarities, serves already to distinguish the fundaments of the various organs with considerable precision.

The endoderm cells ($d^{6.7}$, Fig. 64; $d^{6.8}$, Figs. 65 and 66; $a^{6.8}$, $d^{6.5}$, Fig. 67; $a^{6.6}$, Fig. 68; together with the corresponding cells in the left halves of these Figures) are, on account of their slow division, still very large. They are columnar in form, and contain large nuclei. Their cytoplasmic portion scarcely stains at all except in the region of the nuclei, being almost entirely taken up with close-packed yolk granules. A small amount of protoplasm staining a bright blue in hæmatoxylin extends out from either side of the nucleus in the long axis of the cell. In this small protoplasmic mass evidently lies an attraction sphere close up to the wall of the nucleus. The nuclei themselves contain numerous chromatic granules.

The mesenchyme cells ($D^{7.8}$, Fig. 64; $d^{7.12}$, Figs. 65 and 66; $A^{7.6}$, Fig. 67; together with the corresponding cells in the left halves of these Figures) are sharply distinguished from those of every other tissue by

¹ See the table on page 275.

the very intense blue color which they take upon treatment with a hæmatoxylin stain. Their cytoplasm is not homogeneous at this stage, but contains numerous large dark-looking granules. The granules are undoubtedly yolk granules, and their dark appearance can often be seen upon close inspection to be due to an enveloping film of deeply staining protoplasm, which often extends out in radial processes, giving the whole a star-like appearance. This I believe to be caused by the progressive assimilation of the yolk granules and their conversion into protoplasm. In the case of the mitotic cell $A^{7.6}$ (Fig. 67), and likewise of its mate in the left half of the same Figure, the characteristic mesenchyme staining appears only in the more superficial portion of the cell, its deeper portion being loaded with yolk granules, which are still almost unattacked by the protoplasm. Consequently, when the approaching division is accomplished, the sister cells formed will differ from each other in appearance, the more superficial one being deeply stained, the other being stained scarcely at all. Subsequently, however, the yolk-laden cell will come to resemble in appearance its sister cell, and will have the same ultimate fate. The nuclei of the mesenchyme cells resemble closely in appearance those of the endoderm cells. In the case of $c^{7.12}$ and $d^{7.12}$ (Figs. 65 and 66), the nuclei are relatively small on account of recent division.

The eight *anterior* chorda cells ($a^{7.14}$, Fig. 68; $a^{7.9}$, $a^{7.10}$, and $a^{7.18}$, Fig. 69; together with the corresponding cells in the left halves of these Figures) resemble closely in shape and stainability the endoderm cells. They are smaller, however, and contain nuclei, likewise smaller, with less conspicuous chromatic granules (omitted altogether in the Figures, as previously explained, to aid in readily distinguishing the chorda cells from those of other organs).

The two *posterior* chorda cells ($d^{7.11}$, Fig. 66; $C^{7.11}$ [by mistake of engraver for $c^{7.11}$], Fig. 67) stain more deeply than the anterior chorda cells, resembling to some extent their sister cells $d^{7.12}$ and $c^{7.12}$ (Figs. 65 and 66), from which they have recently been separated by division. However, they are many times smaller than their sister cells, and extend less deeply. This difference is connected with the oblique position of the spindles in the mother cells (see $d^{6.6}$, Fig. 60), a matter to which attention was called in the discussion of the 64-cell stage.

In the neuro-muscular ring the cells (stippled to distinguish them from those of other groups) have about the same histological character in both anterior ($A^{8.16}$, Fig. 68; $A^{8.15}$, Fig. 69; $A^{8.7}$, $A^{8.8}$, Fig. 70; together with the corresponding cells in the left halves of these Figures) and pos-

terior ($D^{8.8}$, Fig. 65; $D^{8.7}$, Fig. 64; $D^{7.6}$, $D^{8.14}$, Fig. 63; together with the corresponding cells in the left halves of these Figures) portions. The cytoplasm is finely granular and pretty homogeneous throughout, except in those portions of the cell most remote from the nucleus, where a certain amount of yolk is to be seen either unassimilated (Fig. 66, $O^{8.8}$) or in process of assimilation (Figs. 68-70, $A^{8.16}$, $A^{8.15}$, $A^{8.8}$, $A^{8.7}$, and their mates in quadrant *B*). In $O^{7.6}$ (the mate of $D^{7.6}$ in Fig. 63) both conditions are realized. Around the nucleus is the finely granular protoplasm, and in those portions of the cell most remote from the nucleus is the unattacked yolk. Forming a sharp line of boundary between the two is a zone in which assimilation is progressing, the yolk granules appearing here as large dark bodies. The color which the cells of the neuro-muscular ring assume is not so deep a blue as that of the mesenchyme cells; it is of a more grayish tint.

B. LATER STAGES OF GASTRULATION.

(a) *From the 112-cell to the 128-cell Stage.*

Figure 72 (Plate XI.) represents a dorsal view of a stage a little more advanced than the 112-cell stage shown in Figure 71. Sections (not figured) of this stage show (cf. sections of an older stage, Figs. 73-77) that the endoderm cells are in mitosis, the spindles being in all cases situated in an approximately horizontal position, so that after division the daughter cells will lie in a single layer forming a curved plate. The spindles are directed longitudinally in all the cells except two, viz. $c^{6.8}$ and $d^{6.8}$ (cf. Plate X. Fig. 62), in which they lie transverse to the long axis of the embryo.

Among the mesenchyme cells division has occurred in $D^{7.8}$, $O^{7.8}$ (cf. Figs. 71 and 74), the spindles standing vertically, as in the case of $A^{7.6}$, $B^{7.6}$ (Plate X. Figs. 62 and 67), which divided earlier. Vertical spindles are also present in $c^{7.12}$, $d^{7.12}$ (cf. Plate XI. Figs. 71 and 75).

The chorda cells are in the same generation as at the last stage, but the anterior ones are laterally compressed into a flattened or wedge shape, their thinner edges being directed backward. They are situated at the anterior margin of the blastopore (Fig. 72).

In the neuro-muscular band, two cells on each side of the blastopore ($D^{8.7}$, $D^{8.8}$, $O^{8.7}$, $O^{8.8}$) are seen to be in mitosis, their spindles being directed toward the centre of the blastopore. No evidence of division can be seen in any other cells of the embryo. It is therefore clear that the considerable advance in the process of gastrulation which is seen to

have taken place since the stage shown in Plate XI. Fig. 71, has come about chiefly by an invagination, independent of cell division on the aboral surface, which has carried inward the endoderm and mesenchyme cells, has left at the margin of the wide-open blastopore the anterior chorda cells and the muscle cells, and has brought plainly into view, outside the neuro-muscular band, another row of cells from the ectodermal surface extending round the entire margin of the embryo. A certain number of cells at the anterior end of this new ring is destined to serve the same purpose as the anterior segment of the neuro-muscular band; this fact is indicated in the figure by stippling.

A clearer idea of the changes just sketched in outline may perhaps be had from an examination of cross sections. In Figures 73-77 are represented five sections through the region of the blastopore of an embryo a little more advanced than the one shown in Figure 72. The approximate position of the sections in the embryo is indicated by horizontal lines at the margin of Figure 72. In the endoderm cells the long deferred division leading to the seventh generation has at last been accomplished (Figs. 73-77). The endoderm cells accordingly number twenty, and their nuclei are greatly reduced in size on account of the recent division (cf. $d^{8.8}$, Fig. 66, Plate X., with $d^{7.15}$, $d^{7.16}$, Fig. 75, Plate XI.). The columnar form of the mother cells is retained by their descendants.

It has been already stated that the spindles in the endoderm cells were at the recent division approximately horizontal in position. It is evident, therefore, that before the accomplishment of division the attraction spheres must have shifted from the position which they were seen to occupy in Plate X. Figs. 66-68, for otherwise the spindles would have stood vertically, and a two-layered arrangement of the cells would have resulted, such as we shall see does occur in the case of the mesenchyme cells. No mechanical explanation of this change in the position of the attraction spheres in the endoderm cells offers itself. The longest axis of the cells appears to be continuously the vertical axis, yet the spindles form in a direction transverse to this in every instance. Van Beneden et Julin's ('86) Figures 1 *c* and 2 *c* also show spindles occupying the short axis of the endoderm cells in the case of *Clavelina*.

Considering now the mesenchyme cells we see (Fig. 74) that $D^{.8}$, $C^{7.8}$ (Fig. 71) have divided in such a manner that a small superficial cell is separated in each case from a many times larger deep-lying sister cell (cf. Fig. 74, $D^{8.16}$, $D^{8.16}$, $C^{8.16}$, $C^{8.15}$). A division similar in direction and in the inequality of its products is foreshadowed for the next anterior pair of mesenchyme cells (Fig. 75, $c^{7.12}$, $d^{7.12}$), in which the spindles lie much

nearer to the superficial than to the deep ends of the cells. The most anterior mesenchyme cells appear in Figure 77 ($A^{8.12}$, $A^{8.11}$, $B^{8.12}$, $B^{8.11}$). They are descended from $A^{7.6}$, $B^{7.6}$ (Plate X. Fig. 62), in which cells the division leading to the eighth generation occurred at a stage considerably earlier than this. (See Plate X. Fig. 67.) In this case also ($A^{7.6}$, $B^{7.6}$) the spindles stood vertically and division was unequal, but the *more superficial* daughter cell was the larger, the deeper one being small and almost entirely filled with unassimilated yolk. (See Plate XI. Fig. 77, $A^{8.12}$, $A^{8.11}$, $B^{8.12}$, $B^{8.11}$.)

The posterior chorda cells ($d^{7.11}$, Fig. 77, and $c^{7.11}$ in the left half of Fig. 76, not lettered) show no essential change since the last stage figured (cf. Plate X. Figs. 66 and 67). The anterior chorda cells do not appear in the sections figured; they are still in the seventh generation.

Division has been completed in $D^{8.7}$, $D^{8.8}$, $C^{8.7}$, $C^{8.8}$ (Fig. 72), four of the neuro-muscular cells lying at the margin of the blastopore. (See Figs. 74-76, $D^{9.13}$, $D^{9.14}$, $D^{9.15}$, $D^{9.16}$, and the corresponding cells in quadrant C.) The other cells of the neuro-muscular ring and the entire ectodermal group have not been essentially modified since the last stage figured (Fig. 72).

If the foregoing account is correct, the embryo, sections of which are shown in Figures 73-77, contains one hundred and twenty-eight cells, distributed as follows.

Ventral hemisphere:—

Ectodermal group:

64 cells in the 9th generation $\left\{ \begin{array}{l} \text{ectoderm.} \\ \text{nerve cells.} \end{array} \right.$

Equatorial band:

2 ectoderm cells in the 9th generation.

8 neuro-muscular cells in the 9th generation.

10 " " " 8th "

2 " " " 7th "

8 mesenchyme " 8th "

2 mesenchyme " 7th "

32—

96

Dorsal hemisphere:—

2 mesenchyme cells in the 7th generation.

10 chorda " " "

20 endoderm " " "

32—

128

(b) *Closure of the Blastopore.*

In Figure 78 (Plate XI.) is shown a section parallel and slightly lateral to the median plane of an embryo a little more advanced than any thus far examined. It shows how the closure of the blastopore is coming about.

The ectoderm cells on the ventral surface are seen to be smaller toward the anterior (left in the figure) than toward the posterior end of the embryo. They have evidently divided and passed into a later generation than those more posteriorly situated. This has caused them to spread over a greater surface, and has shoved the cells anterior to them farther around on to the dorsal surface of the embryo. In the anterior chorda cells, one of which is seen — in mitosis — in Figure 78 ($a^{8.17}$, $a^{8.18}$), division is nearly completed, the spindles standing about vertically. By the continued overgrowth of the anterior lip of the blastopore, the more dorsally situated of the daughter cells in the chorda fundament, e. g. $a^{8.17}$, are carried posterior to their sister cells, e. g. $a^{8.18}$ (cf. Fig. 79), and are finally entirely covered from sight by the nerve cells. They then form a plate of eight cells lying in the dorsal wall of the archenteron anterior to the blastopore.

The endoderm cells of this embryo (Plate XI. Fig. 78) have undergone no new divisions since the 128-cell stage (Figs. 73–77), though their nuclei have considerably increased in size, as is invariably the case during the resting period. More lateral sections than the one shown in Figure 78 exhibit spindles directed longitudinally in the mesenchyme cells $A^{8.12}$, $B^{8.12}$ (cf. Figs. 71 and 77), and show that division has been completed in $d^{7.12}$, $e^{7.12}$ (cf. Fig. 75).

Sections through two other embryos, a little more advanced still in development, show in the muscle cells $C^{7.6}$, $D^{7.6}$ (cf. Figs. 71 and 72) spindles directed forwards, inwards, and downwards, i. e. about toward the centre of the gastral cavity, a condition which is realized in the same cells at a corresponding stage in *Clavelina*. (See Van Beneden et Julin's ('86) Figs. 1 *a* and 1 *b*.) These facts will aid us in interpreting sections of later stages.

Figure 80 (Plate XI.) exhibits a dorsal view of a stage more advanced than any yet examined. The blastopore has greatly contracted (cf. Fig. 72) and now lies in the posterior half of the embryo. As gastrulation has progressed, there has taken place an ingrowth of cells round the margin of the blastopore into the inner layer of the embryo. We have already seen how by this process the anterior chorda cells attain a

position in the dorsal wall of the archenteron; we may now observe that certain cells of the latero-posterior segments of the neuro-muscular ring are also involved in this ingrowth or invagination. At the stage shown in Figures 74-76 there was a double row of neuro-muscular cells on each side of the blastopore, now (see Fig. 80) the inner row is nearly covered from sight by the row of cells outside it.

Already at the stage shown in Figure 72 the most posterior neuro-muscular cells ($D^{7.6}$, $C^{7.6}$) had moved from their originally lateral position toward the median plane (cf. Fig. 71). There they were destined presently to meet each other, covering over the small mesenchyme cells $D^{7.5}$, $C^{7.5}$ (cf. Figs. 71 and 72 with Fig. 80); now (Fig. 80) they or their descendants lie at the posterior angle of the blastopore, and are in turn being covered over by the more laterally and anteriorly situated neuro-muscular cells.

The nerve cells anterior to the blastopore have increased considerably in number, perhaps through additions from the ectodermal group (cf. Figs. 72 and 80, also Figs. 78 and 79).

Three sections from a horizontal series through an embryo of about the stage shown in Figure 80 are represented in Plate XI. Figs. 81-83. The series consists of thirteen sections 6.67μ thick, of which Figure 81 represents the third, Figure 82 the fifth, and Figure 83 the seventh. The sections are a little oblique, and consequently strike the right and left halves of the embryo at slightly different levels. The left side of Figure 82 shows best the history of the mesenchyme cells since the last stage examined in detail (Figs. 73-77). Lateral to the small gastral cavity we find the sister cells $B^{9.23}$, $B^{9.24}$, descendants of the common mother cell $B^{8.12}$ (Fig. 77). Evidence of the derivation of these two cells has been cited in the observation of a spindle longitudinally directed in the cell $B^{8.12}$ in two different embryos less advanced than this.

Lateral to $B^{9.23}$ and $B^{9.24}$ are the sister cells $B^{9.21}$, $B^{9.22}$, descendants of $B^{8.11}$ (Fig. 71; cf. $A^{8.11}$, Fig. 77). They stain more faintly than $B^{9.23}$ and $B^{9.24}$, a distinction which, it will be remembered, existed between the respective mother cells $B^{8.11}$ and $B^{8.12}$ (Fig. 77). Though a spindle has in no embryo been directly observed in $B^{8.11}$, evidence of the sistership of $B^{9.21}$ and $B^{9.22}$ (Fig. 82) exists in the still persistent interzonal filaments which stretch between their nuclei. This evidence is supported by the similarity of the cells in size and stainability. Posterior to the quartette of cells just discussed, the common descendants of $B^{7.6}$ (Plate X. Figs. 62, 67), are the two daughter cells derived from $c^{7.12}$ (Fig. 71), which was seen to be mitotic at an earlier stage (Fig. 75).

It was predicted from the position of the spindle in this cell that the division would be unequal, the more centrally and dorsally situated daughter cell being the smaller. This smaller cell is represented by $c^{8.24}$ (Fig. 82), but only the upper end of its large sister cell, $c^{8.28}$, appears in this section. In Figure 83 (Plate XI.) we see the deeper portion of $c^{8.28}$, which contains a nearly horizontal spindle.

Posterior to the pair of cells just described are the descendants of $C^{7.8}$ (Fig. 71), the next to the hindmost of the mesenchyme cells in the left half of the embryo. In Figure 74 they were in the eighth generation ($C^{8.16}$, $C^{8.15}$). One, the smaller, still remains in that generation ($C^{8.16}$, Fig. 82), but its larger, more deeply situated sister cell has passed into the ninth generation, and is now represented by $C^{9.30}$, $C^{9.29}$, Figure 83. The direct evidence of mitosis has not been observed for the division here assumed, but very strong indirect evidence for it exists in the fact that at the last cell division in the mesenchyme cells, $C^{7.8}$ divided earlier than $c^{7.12}$ (cf. Figs. 74 and 75). If the same order of division is followed in case of the daughter cells, division ought to occur earlier in $C^{8.15}$ than in $c^{8.28}$. But the latter cell is seen in Figure 83 to be in process of division; therefore it is reasonable to suppose that at the same stage the former cell has already divided.

The small posterior mesenchyme cells, $C^{7.5}$, $D^{7.5}$, lie one *behind* the other deep down in the floor of the gastrula (Fig. 83), just posterior to the endoderm cells and overlaid by muscle cells, — for such the invaginated cells of the neuro-muscular ring become.

These muscle cells have been crowded inward and downward at the posterior margin of the blastopore in consequence of the rapid contraction of that opening.

In the most posterior pair of muscle cells, viz. $C^{7.6}$, $D^{7.6}$ (Fig. 71), mitosis was observed to occur, as already stated, at a stage earlier than this. The daughter cells arising from that division are readily recognized in $C^{8.11}$, $C^{8.12}$, and $D^{8.11}$, $D^{8.12}$ (Fig. 83). The nuclei of $C^{8.11}$ and $D^{8.11}$ lie in the section intermediate between those represented in Figures 82 and 83.

I am not able to declare with certainty the lineage of each of the other muscle cells in this series of sections, so I shall not attempt to point them out one by one. As a group, however, they are clearly distinguished from the ectoderm cells on the one hand, and from the mesenchyme cells on the other, by their large nuclei, their considerable size, and the peculiar stainability of their protoplasm. They resemble very closely in stainability the nerve cells lying anterior to the blasto-

pore, but they are much larger than the nerve cells. They now lie lateral and posterior to the blastopore. (See Figs. 81-83; cf. Figs. 79 and 80.)

The backward growth of the anterior lip of the blastopore has carried the crescent-shaped anterior chorda fundament (Fig. 71) from its original position to about the middle of the embryo's dorsal surface (Fig. 81). It was seen in Figure 71 to consist of eight cells, which have now increased (Figure 78) to sixteen, and lie crowded together in two rows, one superposed above and overhanging the other (cf. Figs. 79 and 81).

In Figures 62 (Plate X.) and 71 (Plate XI.) we saw that the two posterior chorda cells, viz. $c^{7.11}$, $d^{7.11}$, were separated from the anterior chorda cells by the mesenchyme cells, $B^{7.6}$, $A^{7.6}$, or their descendants. In Figure 82 we see that the descendants of $B^{7.6}$, $A^{7.6}$ (viz. $B^{9.21}$, $B^{9.22}$, $B^{9.23}$, $B^{9.24}$, and the corresponding cells in quadrant A) during the process of invagination have been pushed down to the level of the other mesenchyme cells, allowing the anterior chorda cells to come into contact with the isolated posterior chorda cells (Fig. 81, $c^{8.21}$, $c^{8.22}$) above them. The posterior chorda cells were seen to be in the seventh generation in Figure 71 ($c^{7.11}$, $d^{7.11}$). At the stage represented in Figure 81, there is good reason to believe that they have divided and passed into the eighth generation, since every other cell of the dorsal hemisphere is known to have done so previous to that stage; they are therefore represented by the cells $c^{8.21}$, $c^{8.22}$, $d^{8.21}$, and $d^{8.22}$, the last named cell being hidden from view in Figure 81 by the overlying muscle cell.

The endoderm cells still remain in the eighth generation, and number twenty. Their arrangement is made clear by an examination of Figures 81-83, in comparison with Figure 79, which represents a section near and parallel to the median plane of a slightly earlier stage. Fourteen of the twenty endoderm cells abut on the median plane, and six are placed laterally toward the anterior end of the embryo. The median double row of cells consists of $b^{7.11}$ (Fig. 81), $b^{7.12}$ (Fig. 82), $b^{7.15}$, $b^{7.16}$, $c^{7.16}$, $c^{7.14}$, $c^{7.13}$ (Fig. 83), and the corresponding cells in the right half of the embryo. The nuclei do not appear in the centrally and posteriorly situated endoderm cells of Figure 83 because they lie in later (deeper) sections of the series, not figured (cf. Fig. 79). Only the narrow upper ends of the cells in question appear in Figure 83, which therefore gives no adequate idea of their size, but a correct idea of this may be had by an examination of Figure 79. The laterally situated endoderm cells are $c^{7.9}$ (Fig. 82), $c^{7.10}$, $c^{7.15}$ (Fig. 83), and the corresponding cells in the right half of the embryo.

From a series of cross sections through an embryo in about the same

stage as is represented in Figure 80, four sections have been selected to make more clear the relations of the fundamentals of the various organs. (See Plate XII. Figs. 84–87.) Figure 84 represents a section just behind the blastopore (compare with it Plate XI. Fig. 73); in it the ectoderm is seen to have slightly overgrown from behind the most posterior muscle cells. (Compare Fig. 79, Plate XII.) Only one of the pair of small posterior mesenchyme cells ($D^{7.5}$, $C^{7.5}$) appears in the section; the other lies in the section just posterior to this.

Mitosis is again setting in among the endoderm cells, as is shown by the spindle in $d^{7.13}$; the next section anterior to this likewise shows spindles in the endoderm cells that are cut, $c^{7.14}$ and $d^{7.14}$. The spindles in each case ($^{7.13}$ and $^{7.14}$) are directed longitudinally, and in such a manner that the eight resulting cells will all lie in a single slightly concave layer. The consequence of these divisions will be a considerable elongation of the double row of endoderm cells at the posterior end of the embryo.

It is worthy of note, though not shown in this series of sections, that at this division, as in the preceding and in subsequent ones, the spindles of the endoderm cells do not lie in the longest axis of the cells, which is the vertical.

Figure 85 shows a section through the still open blastopore at its posterior margin. A comparison of this figure with Figures 72, 74, and 75 (Plate XI.) shows that the ectoderm has grown rapidly in superficial extent through cell multiplication, and shoved the neuro-muscular cells $C^{9.13}$, $D^{9.13}$, inward to a position overlying their sister cells, $C^{9.14}$, $D^{9.14}$. The small mesenchyme cell, $C^{8.16}$ (cf. Plate XI. Fig. 74, $D^{8.16}$), is in process of division, following the lead of its large sister cell, $C^{8.15}$ (cf. Figs. 74, $D^{8.15}$, and 84, $D^{9.29}$, $D^{9.30}$). The mate of $C^{8.16}$, viz. $D^{8.16}$, has already divided. One of its daughter cells is seen in this section ($D^{9.32}$, Fig. 85), the other lies in the next posterior section. The large mesenchyme cells, $c^{8.23}$, $d^{8.23}$ (Fig. 85), are in mitosis (cf. Plate XI. Fig. 83).

The section represented in Figure 86 encounters the blastopore farther forward than the one last described (Fig. 85), in its broader portion (cf. Plate XI. Figs. 72, 76, and 77). Here, too, the muscle cells have been crowded inward and partially invaginated; $C^{9.16}$ and $D^{9.16}$ overlie their sister cells, $C^{9.16}$ and $D^{9.16}$, respectively. Of the posterior chorda cells only $c^{8.22}$ and $d^{8.22}$ appear in this section. Their more laterally placed sister cells, $c^{8.21}$ and $d^{8.21}$, lie in the next posterior section (not figured), and at a slightly higher level (cf. Fig. 81, Plate XI.).

Figure 87 (Plate XII.) represents the first section anterior to the

blastopore (cf. Plate XI. Fig. 80). On its depressed dorsal surface are seen six cells of the anterior chorda fundament, which is being rapidly covered over from the sides and anterior end by the ectoderm. Extending deep down on either side of the chorda appears a deeply stained cell (stippled in the drawing) with large nucleus. These two are the most posterior cells of the medullary plate, which now lies at the dorsal surface of the embryo anterior to the blastopore, having been formed chiefly by the anterior segment of the neuro-muscular ring (cf. Plate XI. Fig. 80).

The mesenchyme cells, $A^{9.24}$, $B^{9.24}$ (Fig. 87), are seen to lie on each side of the gastrula cavity (cf. Plate XI. Fig. 82); lateral to them lie the relatively small and faintly stained mesenchyme cells, $A^{9.22}$, $B^{9.22}$. The respective sister cells of those just mentioned, viz. $A^{9.23}$, $B^{9.23}$, $A^{9.21}$, and $B^{9.21}$ (cf. Plate XI. Fig. 82), lie in the next two posterior sections (not figured).

The stage next to be discussed differs in external appearance from that shown in Figure 80 chiefly, first, in the further contraction of its blastopore to a small aperture in the dorsal surface somewhat posterior to its centre; secondly, in a slight elongation of the embryo and narrowing of its posterior end, foreshadowing the formation of the tail; and thirdly, in a slight depression of the medullary plate to form a neural or medullary groove (cf. Fig. 98).

From a series of transverse sections through an embryo in this stage, five are represented in Plate XII. Figs. 88–92. Figure 88 (Plate XII.) shows a section posterior to the blastopore (cf. Fig. 98). It passes through the region of the small posterior mesenchyme cells, $C^{7.5}$, $D^{7.5}$. Lateral or dorsal to them are seen four pairs of muscle cells containing large nuclei. The finely granular cytoplasm of these muscle cells takes a deep grayish blue stain in hæmatoxylin. Bounding the whole section is the uninterrupted ectoderm.

The next anterior section, which has nearly twice the area of this, is likewise completely surrounded by ectoderm.

The second section anterior to the one shown in Figure 88 is represented in Figure 89 (Plate XII.). Two endoderm cells, the most posterior ones, appear in it. The small size of their nuclei indicates that they belong to a later generation than the endoderm cells seen in Figure 84. Unquestionably they are in the eighth generation. To right and left of them appear two muscle cells, probably descendants of $C^{7.5}$, $D^{7.5}$, Plate XI. Fig. 73 (cf. Plate XI. Fig. 83, $C^{8.11}$, $C^{8.12}$, $D^{8.11}$, and $D^{8.12}$). Lateral to the muscle cells mentioned are seen in Figure 89

(Plate XII.) mesenchyme cells, three on each side. Two of them are undoubtedly descendants of the mesenchyme cells $C^{8.15}$, $D^{8.15}$, shown in Plate XI. Figs. 73 and 74, and represented in Plate XI. Fig. 83, and Plate XII. Fig. 84, by the cells $C^{9.20}$, $C^{9.80}$, $D^{9.20}$, and $D^{9.80}$. Their nuclear condition shows that they have arisen from a recent division. Dorsal to the groups of cells already mentioned are seen in Figure 89 muscle cells extending up in a solid mass to the dorsal surface of the embryo. In the mid-dorsal surface of the section is a pair of cells, probably nerve cells, between which at an earlier stage lay the open blastopore. The periphery of the section is elsewhere bounded by ectoderm.

Figure 90 (Plate XII.) represents the second section anterior to that shown in Figure 89. It passes through the widest portion of the blastopore. The only other section of the series which passes through the blastopore is the next preceding one, in which the blastoporic opening is extremely narrow, in fact, scarcely more than a slit. Figure 91 shows the first section anterior to the blastopore. In it we see a plate of seven cells ($cd.$) belonging to the anterior chorda fundament and forming the roof of the archenteron (cf. Fig. 81). In Figure 90 we find the posterior chorda cells ($cd.$) lateral to the blastopore (cf. Plate XI. Fig. 81, $c^{8.22}$ and $c^{8.21}$). Ventral to the chorda cells in Figures 90 and 91 are the mesenchyme cells descended from $c^{8.23}$, $c^{8.24}$, $d^{8.23}$, and $d^{8.24}$ (cf. Plate XI. Figs. 82 and 83).

Dorsal to the chorda cells in Figure 91 are four cells unquestionably nervous, the two lateral ones being large and in mitosis, the other two small, evidently produced by recent divisions. In the next anterior section (not figured) the two lateral mitotic nerve cells again appear; completely filling the space between them are four small nerve cells similar to the two seen in Figure 91. A medullary groove is thus clearly formed anterior to the blastopore, and the four cells dorsal to the chorda fundament in Figure 91 evidently are only lateral backward prolongations of the medullary plate. The two large cells at the margins of the blastopore in Figure 90 are probably $C^{9.15}$ and $D^{9.15}$ (cf. Fig. 86); their deeper lying sister cells $C^{9.16}$ and $D^{9.16}$ have been carried into the more posterior sections by the crowding backward of the chorda cells and the elongation of the embryo.

Figure 92 (Plate XII.) represents the third section anterior to the one shown in Figure 91. The medullary plate and chorda are here represented each by four cells. The mesenchyme cells visible on each side of the archenteron are $A^{9.23}$, $B^{9.23}$, $A^{9.21}$, and $B^{9.21}$ (cf. Plate XI. Fig. 82). The cells $A^{9.24}$, $B^{9.24}$, $A^{9.22}$, and $B^{9.22}$ (cf. Fig. 82) lie in the

next two anterior sections. Of the eight cells mentioned, $A^{9,23}$, $A^{9,24}$, $B^{9,23}$, and $B^{9,24}$ (cf. Fig. 82) are all in mitosis, but the four more laterally situated and smaller ones are still quiescent.

Considering as a whole the mesenchyme of this embryo, we see that it consists of two lateral bands which have elongated with the elongation of the embryo. They now extend through ten different sections from near the anterior end of the embryo to a region posterior to the blastopore (Fig. 89). The muscle cells, on the other hand, are gathered into a pretty compact mass at the sides of and posterior to the blastopore (Figs. 88-90).

In the subsequent stages of development the portion of the embryo lateral and posterior to the blastopore will be rapidly drawn out to form the tail of the larva, while the portion anterior to the blastopore will form the trunk. This will not come about, however, without a considerable shifting of cells from one portion into the other, for the chorda cells, which now lie anterior or lateral to the blastopore, must in large part pass into the tail, while the mesenchyme cells, which are more ventrally located, and some of which now extend behind the blastopore, will all pass forward into the trunk region.

An examination of Figure 98 (Plate XII.) may help to give a clearer idea of the stage just described. This figure shows a section made nearly parallel to the sagittal plane, but a little to one side of it, through an embryo slightly older than the one last under discussion (Figs. 88-92). The anterior chorda fundament, it is seen, has been carried back beyond the middle of the embryo's dorsal surface. The muscle cells have been forced backward and downward into a nearly vertical position behind the blastopore, and are nearly covered over with ectoderm (cf. Fig. 93).

Numerous cell divisions have recently occurred in the ectoderm, and the number of endoderm cells has also plainly increased. A very marked elongation of the embryo has attended these divisions. Several cells in the medullary plate are also dividing. On account of the slight obliquity of the plane of sectioning, the small posterior cells $C^{7,5}$, $D^{7,5}$ (*ms'chy.*), do not actually appear in this section as represented, but have been projected there from the adjacent section. In that section the endoderm extends back in a double row of cells into contact with $C^{7,5}$, $D^{7,5}$, as at the stages shown in Plate XI. Figs. 78 and 79.

In Figures 93-97 (Plate XII.) are represented five cross sections through an embryo in about the same stage as is shown in Figure 98. The approximate position of the sections in the embryo is indicated on

Figure 98 by the five vertical lines 93-97. Figure 93 shows a section posterior to the blastopore. It passes through one (*ms'chy.*) of the small posterior mesenchyme cells, $D^{7.5}$, $C^{7.5}$ (cf. Fig. 88), the other lying in the next section posterior to this. The interior of the section is filled with a solid mass of muscle cells, or more properly nerve cells and muscle cells; for it is highly probable that the four most dorsally situated of these cells, which form a group not quite covered in by the ectoderm, are to become part of the nerve cord of the tail (cf. Plate XIII. Figs. 99-101, *n.*). However, they are not distinguishable in histological characters from the more laterally and ventrally situated cells of the section. Cell division has recently occurred in the ectoderm, which plainly is soon to cover in completely the nerve cells in this region of the embryo. The muscle cells have evidently been reduced in size by division since the stage shown in Figures 88 and 89.

The second section anterior to this is shown in Figure 94. It is the only section of the series which passes through the blastopore, now reduced almost to a slit.

The blastopore is bordered on each side dorsally by a large nerve (!) cell, *n.* (cf. Fig. 90). Ventral to the nerve cells lie the posterior chorda cells, *cd.*, lateral and still ventral to which are muscle cells. The most posterior pair of endoderm cells lies underneath the open blastopore, and a single small mesenchyme cell lies deep down in each half of the section.

The second section anterior to the blastopore is shown in Figure 95; the second section anterior to that, in Figure 96; and one situated still two sections farther forward, in Figure 97.

In Figure 96 the medullary plate is not at all depressed at its centre; it consists of four large cells closely packed together and columnar in form. In Figure 97 the medullary plate is not even flattened, but conforms to the evenly rounded contour of the embryo in that region. It consists of six cells sharply distinguished from the cells of the ectoderm in stainability, though the size of the more lateral ones is not materially different from that of the ectoderm cells. The chorda plate has diminished to a breadth of only three cells in Figure 96, and is entirely wanting in Figure 97, where endoderm cells occupy the space dorsal to the archenteron underneath the medullary plate. The mesenchyme bands cover considerable area in Figure 96, but are reduced to a single cell on each side of the body in Figure 97, from which it is seen that in this region the interior is nearly filled with a solid mass of endoderm. The section represented by Figure 97 lies well toward the anterior end of the embryo, as is indicated by the rapidly diminishing size of the sections.

To summarize our observations on the series of sections just examined (Figs. 93-97):—

(1) The fundament of the *nervous system* consists of a medullary plate extending from near the anterior end of the embryo to the blastopore, and continued backward by cells lying on each side of the blastopore and along the line where the lips of the blastopore have fused. The transformation of the medullary plate into a medullary groove proceeds from the blastopore forward.

(2) The *chorda* fundament consists of a plate of cells immediately underneath the medullary plate, but extending neither so far forward nor so far backward in the embryo. A part of it lies on each side of the blastopore, but the larger part is anterior to the blastopore.

(3) The *mesenchyme* extends in two lateral bands from the region of the blastopore forward through about two thirds of the extent of the embryo anterior to the blastopore.

(4) The *muscle cells* lie principally posterior to the blastopore in a pretty compact mass. They extend no farther forward than the first section anterior to the blastopore.

(5) The *endoderm* consists of a double row of large cells ventrally situated extending from the first section behind the blastopore through the next five anterior sections; it then broadens out and occupies nearly the whole inner layer of the embryo, both dorsally and ventrally, anterior to the *chorda* fundament.

C. SUMMARY ON GASTRULATION.

1. In the gastrulation of *Ciona* two processes can be distinguished: (a) a progressive invagination of the cells on the dorsal surface of the embryo, beginning at its centre; (b) a concomitant overgrowth of cells from the ventral side of the embryo, caused by more rapid cell division in that region. The overgrowth is greater at the anterior than at the posterior end of the embryo, because cell division proceeds more rapidly at the anterior end.

2. Early in the process of gastrulation one can recognize a ring of cells encircling the blastopore peculiar in their stainability, forming the common fundament of the nervous system and the longitudinal musculature of the larva.¹ Anterior to the blastopore the ring broadens out

¹ The existence of this peculiar ring of cells was first pointed out by Van Beneden et Julien ('86) in the case of *Clavelina*; but these authors made the mistake of regarding it as exclusively nervous.

to form the medullary plate. Those cells of this *neuro-muscular* ring which lie on each side of and posterior to the blastopore are for the most part invaginated, and form the entire longitudinal musculature of the tail. Some of them, however, form the most posterior portion of the nerve cord.

3. Lying just within the margin of the blastopore, and encircled by the neuro-muscular ring, is another ring of cells, interrupted at the posterior end of the embryo only. Its anterior portion gives rise to the greater part of the chorda; its remaining (lateral) portions produce the mesenchyme or trunk mesoderm, besides contributing to the chorda a single cell at each lateral margin of the blastopore. The descendants of these two chorda cells meet in the median plane at the closure of the blastopore. They form the most posterior portion of the chorda.

We may regard the chorda-mesenchyme ring as being completed morphologically by the two small sub-chordal mesoderm cells $C^{7.5}$, $D^{7.5}$, which have been wedged in between the most posterior cells of the neuro-muscular ring. Like the other cells of the chorda-mesenchyme ring, they lie in contact with the endoderm cells on one side, and with cells of the neuro-muscular ring on the other. Ultimately they probably form mesenchyme in the tail region. Possibly by a cœnogenetic reduction in size to their present minute dimensions, a gap has been left on each side of the embryo between them and the lateral portions of the chorda-mesenchyme ring. This change may have attended a cœnogenetic lengthening of the posterior end of the organism to subserve locomotion. There is evidence from other sources that the trunk of Ascidians formerly extended farther back into what is now the tail region of the larva. At that time the mesenchyme also probably extended farther back, and the chorda-mesenchyme fundament was in ontogeny, as we suppose it to have been in phylogeny, an uninterrupted ring.

4. The blastopore, at first widely open, closes more rapidly from the anterior margin and from the sides than from behind. Consequently it comes to lie in the posterior portion of the dorsal surface of the embryo, and is triangular in form. The right and left sides of the triangular blastopore, however, fuse from behind forward, beginning in the region of the pair of small, flattened mesoderm cells, $C^{7.5}$, $D^{7.5}$. Along the line of union of the lateral lips of the blastopore lies superficially on each side of the median plane a row of nerve cells. These are subsequently covered in by ectoderm from the sides and from behind, and form the posterior portion of the nerve cord. Underneath them, and at first not distinguishable from them in histological characters, are other cells, likewise derived from

the posterior portion of the neuro-muscular ring; these are destined to form the longitudinal musculature of the tail. The medullary plate, which produces the entire nervous system of the *trunk* region, lies wholly anterior to the region of "concrecence" of the lips of the blastopore.

5. The posterior margin of the blastopore does not grow forward over the blastopore covering in the medullary canal as described by Van Beneden et Julin ('86) in the case of *Clavelina*.¹

6. I heartily concur in Samassa's ('94) conclusion that there is no rotation of axes during the gastrulation of *Ciona*, such as Korschelt u. Heider ('93), on theoretical grounds, conjectured might occur in Ascidians. Their hypothesis is, so far as I know, entirely unsupported by observation.

4. Formation of the Larva.

The further changes which the embryo undergoes in its transformation into the larval tadpole will be understood from an examination of Figures 99-105 (Plate XIII.), which represent seven sections through an embryo with completely closed blastopore. Figure 99 shows the third section (in passing from behind forward) of the series; it contains about half a dozen muscle cells and four nerve cells, surrounded by an epithelial layer of ectoderm. The first section of the series shows merely the ectoderm cut tangentially; the second contains six muscle cells surrounded by the ectoderm, but no nerve cells or chorda. The four nerve cells in Figure 99 show precisely the same arrangement as is found later in a cross section of the tail of the larva. (See the four cells at the right of *cd.* in the right portion of Fig. 106.)

In Figure 100 (Plate XIII.) the number of nerve cells (seven) is seen to be increased, and the chorda makes its appearance as a group of seven cells ventral to the nerve cells.

In Figure 101 (Plate XIII.) the nervous and chorda fundaments appear about as in the section shown in Figure 100, but underneath the chorda is seen a group of four small mesoderm cells, the descendants of *D^{7.5}*, *C^{7.5}* (Plate XII. Fig. 88), which have at last divided. Just anterior to them in the embryo (Figs. 102 and 103) extends the double row of caudal endoderm cells. As I have already suggested (page 262), the subchordal mesoderm cells (Fig. 101) probably have the same fate as

¹ The authors mentioned were doubtless led into this mistaken interpretation by identifying as nerve cells the muscle cells which lie behind the blastopore at the time of its closure. (See their Figs. 1 a, 1 c, 2 c, 3 a, Pl. VII. These figures are reproduced in Korschelt u. Heider's ('93) Figs. 741 A, 741 B, 742 B, and 745 B, respectively.)

the caudal endoderm cells, i. e. are resolved into mesenchyme at a later stage.

Figure 102 (Plate XIII.) represents a section through what probably was the region of the blastopore. In it is seen the most posterior pair of endoderm cells (cf. Plate XII. Fig. 94). Since the stage last examined (Plate XII. Figs. 93-97) the chorda cells have closed together into a single plate in this region, and the chorda fundament has grown farther back in the embryo. The nerve cells which lay at each side of the blastopore (Plate XII. Fig. 94) have also met in the median plane to form a single plate, which is now closing into a canal. A real canal is never formed posterior to the blastopore, though the nerve cells in that region potentially form one.

Figure 103 (Plate XIII.) represents the second section anterior to the one shown in Figure 102; Figure 104, the second anterior to that; and Figure 105, the fourth anterior to that. It will be seen that the muscle cells which in the series last examined (Figs. 93-97) were aggregated chiefly behind the blastopore, have now extended themselves not only posterior, but also anterior, to the blastopore. They extend as far forward as the next section in front of the one represented by Figure 103, i. e. through three sections anterior to the blastopore. They have pushed before them the mesenchyme, which in this series first appears in the section shown in Figure 103. The chorda fundament has meanwhile moved toward the posterior end of the embryo. It now extends two sections behind the blastopore and overlies the small posterior mesenchyme cells (Fig. 101, cf. Plate XII. Fig. 93). Accompanying the changes just mentioned, has come a diminution of the diameter of the embryo at its posterior end, which is already elongating to form the tail region.

The mesenchyme extends forward of the section shown in Figure 103 through six sections. The medullary plate extends forward two or three sections farther still. The endoderm consists of a double row of cells extending forward underneath the chorda as far as the section seen in Figure 104, in which four endoderm cells are found; the arrangement there shown has been derived from that shown in Plate XII. Fig. 96, and still earlier in Plate XII. Fig. 91, by the meeting in the median plane underneath the chorda of the more laterally placed endoderm cells. Later, these four cells, or their descendants, will move apart so as to enclose between them the lumen of the posterior portion of the digestive tract. Anterior to the section shown in Figure 104 the endoderm rapidly increases in amount, while the chorda and mesenchyme diminish.

In the region shown in Figure 105, it fills the entire interior of the section.

In Figure 106 (Plate XIII.) is shown a section through an embryo in which the tail is already recognizable as a distinct portion of the embryo, though it has not yet reached anything like its maximum length.

It is curved ventrad under the trunk, so that the section passes transversely through both trunk and tail. The section passes through the trunk in the brain region, but intersects only one of the mesenchyme bands, the other one not extending so far forward in the embryo. The endoderm cells are seen to have arranged themselves round a potential lumen in the form of an epithelium. However, they still lie two deep in places. Their shape is clearly becoming columnar.

In the tail region appears the chorda, now transformed into a single row of flattened, disk-shaped cells, rapidly becoming vacuolated. They form an axial rod extending through the entire tail region and the posterior portion of the trunk. Dorsal (right in the Figure) to the chorda lies the nerve cord of the tail, composed in cross section of about four small cells.

Ventral to the chorda is the sub-chordal endoderm strand consisting of a double row of cells (*en'drm.*). On each side of the chorda are seen in the section about three muscle cells.

SUMMARY ON FORMATION OF THE LARVA.

1. The nerve cord in the limited region of concrescence of the lips of the blastopore is covered over by the ectoderm first at its posterior end and then successively in its more anterior regions, following the course of concrescence. The nerve cells in that portion of the embryo never form a *real* canal, but only a *potential* one. They are arranged in a solid strand, which usually shows in cross section four cells placed round a common centre, the potential canal.

The medullary plate arises wholly anterior to the blastopore. At the time when the blastopore is about to close, the medullary plate has come to extend over a great part of the length of the embryo, and has sunk down in the form of a shallow groove deepest at its posterior end, the anterior margin of the blastopore. When the blastopore closes, it begins to form a canal. This process, like the fusion of the lateral margins of the blastopore, advances from behind forward.

2. Beginning shortly before the closure of the blastopore, a rapid elongation of the embryo takes place, accompanied by a considerable change in its form and a rearrangement of the cells composing some of

its organs. The posterior end of the embryo, which toward the completion of gastrulation was broader than the anterior end, becomes narrower and narrower, and ultimately forms the tail, which is curved ventrad around the trunk of the embryo within the egg membranes.

Before the closure of the blastopore the chorda is a plate of cells lying in the dorsal wall of the archenteron, anterior and lateral to the blastopore. The portions lateral to the blastopore meet in the median plane when the blastopore closes. The chorda fundament then elongates, owing to a shoving together of its cells from each side, "like a pack of cards in shuffling" (Van Beneden et Julin), until they form, instead of a plate, a single median row of disk-shaped cells arranged one behind another like a row of coins and reaching backward underneath the nerve cord to the extreme posterior end of the embryo. Anteriorly it terminates not far from the middle of the trunk region.

The muscle cells, which originally lay on each side of and behind the blastopore, extend themselves a single cell deep in two bands, one on each side of the chorda throughout its entire length.

The mesenchyme cells originally formed the lateral portions of the chorda-mesenchyme ring. As the blastopore gradually closed, they were thrust down to a deeper level than the muscle cells, and forward. Ultimately they come to lie wholly in the trunk region, chiefly in its posterior portion, in two pretty compact lateral masses of small deeply stained cells, two or more layers deep. At a still later period, these lateral masses are resolved into migratory cells, i. e. blood corpuscles, mantle cells, etc.

Before the closure of the blastopore the endoderm forms the entire lining of the archenteron in its most anterior portion, where its lumen is almost obliterated. Farther back the chorda forms the dorsal wall of the archenteron, the mesenchyme cells forming its sides, the floor only being occupied by the endoderm cells. In the region where the blastopore closes, the endoderm cells occur only as a double row ventrally situated along the median line.

This double row is extended back in the larva underneath the chorda throughout almost the entire length of the tail, forming a "subchordal endoderm strand," which is ultimately resolved into wandering cells, or perhaps utilized as food material by the mesenchyme cells of the trunk region. At the posterior end of this caudal endoderm strand lie the small mesoderm cells which Van Beneden et Julin mistakenly included in the nervous fundament. These cells are to be regarded as the most posterior constituents of the original chorda-mesenchyme ring.

Like the cells of the endoderm strand just anterior to them, they probably become wandering cells.

In the posterior portion of the trunk region, where before the closure of the blastopore the endoderm strand broadens out into a plate of four or more cells, the more laterally placed endoderm cells move dorsad at the closure of the blastopore, and meet in the median plane underneath the chorda. In this way the endoderm of the trunk region is converted into a closed vesicle, pear-shaped and broadest in its anterior portions; at its posterior end it is overlaid by the chorda and flanked on each side by the mesenchyme.

VII. DISCUSSION OF SOME THEORETICAL QUESTIONS.

The facts presented in the foregoing pages have a certain bearing on several questions of general interest. Of these I shall make brief reference to, — 1. The origin of the germ layers of Chordates; 2. The Cœlom theory; and 3. The ancestry of Chordates.

A. Origin of the Germ Layers of Chordates.

According to the generally accepted doctrine of Haeckel, all the higher metazoa are ultimately derived from a simple cup-shaped or sac-like ancestor composed of two cell layers, an inner and an outer, continuous with each other at the margin of the cup or sac. The two cell layers are called the primary germ layers. The outer layer is known as the primary ectoderm; the inner, as the primary endoderm. Among the Chordates, this supposed ancestral condition is most nearly realized in ontogeny in the case of *Amphioxus*. The homologues of its inner and outer germ layers are traced by embryologists through all the groups of the chordate phylum. A third or middle layer, derived from one or both of the others, makes its appearance between the two primary germ layers in all the higher Metazoa. Whether this middle layer, or mesoderm, is homologous throughout the different groups of Metazoa is one of the most difficult and disputed questions in the whole realm of comparative embryology. Into this question I do not propose to go in this paper; I shall confine my attention to the mesoderm of Chordates.

It is commonly believed that the mesoderm of Chordates is derived entirely from the inner germ layer, which is accordingly often referred to as mes-endoderm. With this view, however, my observations on *Ciona*

force me to take issue. In an earlier part of this paper it has been shown that during the process of gastrulation in *Ciona* there is a progressive ingrowth of cells around the blastopore from a position in the outer to a position in the inner layer of the gastrula. Whether, therefore, we shall include a particular cell in the primary ectoderm or primary endoderm depends on whether we consider the embryo at an earlier or a later period in the process of gastrulation. Lwoff ('94) has recently stated, and it seems to me on excellent evidence, that in *Amphioxus* and all Vertebrates there occurs in the formation of the germ layers an ingrowth of cells from the outer to the inner layer of the embryo, very similar to that which I have observed in *Ciona*. He accordingly distinguishes what we may call a *primary invagination* of the cells destined to form chiefly the alimentary tract from a *secondary invagination* involving the cells destined to form the chorda and a portion of the mesoderm, viz. the musculature. The matter seems to me of sufficient importance to warrant the quotation of the author's own words. Speaking of *Amphioxus* he says:—

“Ich bin also zum Schluss gekommen, dass *die dorsale Wand der Gastrulhöhle, die ich als dorsale Platte bezeichnen will, von den Ektodermzellen gebildet ist*. Dieses Ergebniss ist von sehr grosser Bedeutung. Wie die weiteren Entwicklungsstadien lehren, stellt diese dorsale Wand die Anlage der Chorda und des Mesoderms dar, indem aus der mittleren Zellenpartie derselben die Chorda, aus zwei seitlichen Theilen das der Chorda anliegende Mesoderm entsteht, aus welchem, wie bekannt, die Muskelelemente entstehen. Dies zeigt, dass *die Chorda und das anliegende Mesoderm aus einer ektoblastogenen Anlage entstehen*, die ursprünglich als eine zusammenhängende Platte (dorsale Platte) erscheint. Was die eigentlichen Entodermzellen betrifft, welche die übrige Wandung der Höhle bilden, so will ich hier in Kürze vorbemerken, dass sie jederseits einige an die dorsale Platte angrenzende Zellen als ihren Beitrag zur Bildung des Mesoderms abgeben; die Ränder des übriggebliebenen Entoderms wachsen unter den seitlichen Mesodermanlagen nach der Mittellinie zu, vereinigen sich unter der Chorda und bilden auf solche Weise den Darm.

“*Das Hauptergebniss dieser Untersuchungen ist, dass die Einstülpung bei Amphioxus keineswegs als eine einfache Gastrulation zu betrachten ist, wie es bisher angenommen. Es sind vielmehr hier zwei verschiedene Prozesse zu unterscheiden: erstens die Einstülpung der Entodermzellen, aus denen der Darm entsteht; zweitens die Einstülpung der Ektodermzellen vom dorsalen Umschlagsrande aus, welche die ektoblastogene Anlage der Chorda*

und des Mesoderms bildet. Die Einstülpung der Entodermzellen ist als Gastrulation zu betrachten. Es ist ein palingenetischer Process, den die Chordaten von ihren Vorfahren ererbt zu haben scheinen, wo dieser Process gleichmässig und radial symmetrisch vor sich ging, wie es sich bei einigen wirbellosen Thieren beobachten lässt. Die Einstülpung der Ektodermzellen ist dagegen als ein coenogenetischer Process zu betrachten, der mit der Bildung des Darmes nichts zu thun hat und durch den die Bildung der ektoblastogenen Anlage der Chorda und des Mesoderms eingeleitet wird. Wie in den folgenden Abschnitten gezeigt werden soll, lassen sich diese zwei Processe — die Bildung des Darmes und die Bildung der ektoblastogenen Anlage der Chorda und des Mesoderms — auch in der Entwicklung aller Wirbelthiere von einander unterscheiden." (Separate, pp. 11, 12.)

Whether Lwoff is right in including the chorda and the greater portion of the mesoderm of Amphioxus and the Vertebrates in the secondarily invaginated part of the inner germ layer, I do not attempt to say. That question must be decided by an examination of the forms on which he made his observations. However, in *Ciona*, at least, the cells which are destined to form chorda and mesenchyme (chorda-mesenchyme ring) must be regarded as taking part in a *primary invagination* along with the definitive endoderm. But plainly a very important part of the mesoderm, viz. the cells which form the longitudinal musculature, is carried into the inner layer by a *secondary invagination*. The secondarily invaginated cells are derived from the posterior segments of the neuro-muscular ring. At the beginning of gastrulation they clearly lie in the outer layer of the gastrula, but at the conclusion of gastrulation they lie within the margin of the blastopore.

Accordingly, I regard the definitive endoderm fundament and the encircling chorda-mesenchyme ring as constituting the *primary endoderm* in *Ciona*. In the *primary ectoderm*, I would include the neuro-muscular ring and the "ectodermal group" of cells, both of which lie entirely in the outer layer of the gastrula when the closure of the blastopore begins. If this view is correct, the mesoderm or middle germ layer of Ascidians must be regarded as derived in part from the primary endoderm and in part from the primary ectoderm. Lwoff reached a similar conclusion concerning the origin of the mesoderm in *Amphioxus* and the Vertebrates. In *Ciona* that part of the mesoderm which is derived from the outer germ layer produces the longitudinal musculature of the larva. It forms the whole of this tissue, and nothing else. Similarly in *Amphioxus* and the Vertebrates, Lwoff concluded that the ectodermal mesoderm

formed the longitudinal musculature. It would seem, therefore, that the *muscle plate* of the mesodermal somite of *Amphioxus* is homologous with the muscle cells of the *Ciona* tadpole. Both in *Amphioxus* and in *Ciona* the muscle fundament arises from cells lying lateral to the chorda and derived from the primary ectoderm. In *Amphioxus* the musculature, like the chorda with which it is intimately associated, becomes (œnogenetically?) extended far forward to the anterior end of the trunk region; whereas in *Ciona* neither musculature nor chorda extends farther forward than about the middle of the trunk region.

The mesoderm lateral to the muscle plates of *Amphioxus* seems to be the homologue of the mesenchyme of *Ciona*. Both are derived from the *endodermal* portion of the mesoderm. (Cf. the quotation from Lwoff, pages 268, 269.)

My conclusions differ from those of Lwoff chiefly regarding the origin of the chorda. He considers this organ to be derived from the primary ectoderm in *Amphioxus* and the Vertebrates, whereas I regard it as formed in *Ciona* exclusively by the primary endoderm. I think that Lwoff has been led to include the chorda cells in the primary ectoderm chiefly because they are in *Amphioxus* (as in *Ciona*) smaller and clearer than the less rapidly cleaving endoderm cells. These criteria I regard as insufficient. Only a study of the cell lineage can give in any case a *positive* answer to the question whether the chorda cells at the beginning of gastrulation lie in the outer or the inner layer of the embryo.

That a distinction is rightly made in the case of Ascidians between the two kinds of mesoderm which I have recognized, viz. musculature and mesenchyme, is unanimously agreed to by embryologists; *but the fact has been heretofore overlooked that these two kinds of mesoderm are derived from different fundaments early distinguishable both histologically and topographically, and that these fundaments should be regarded as derived from different primary germ layers.*

A minor point of theoretical importance is whether or not the chorda shall be regarded as a *mesodermal* organ. Lwoff does not so consider it, though he recognizes two facts which, it seems to me, would naturally lead one to that conclusion: the first, that in *Amphioxus* and the lower groups of Vertebrates the chorda is derived from a common fundament with what is universally regarded as mesoderm; the second, that the chorda, like the undoubted mesoderm, comes to occupy a position between the inner and outer layers of the embryo. For these two reasons, which I have shown to exist also in the case of Ascidians, we must, to be consistent, regard the chorda as a *mesodermal* organ.

Seeliger ('85), Davidoff ('89), and Samassa ('94) all state that the first equatorial plane of cleavage in the ascidian egg separates the two primary germ layers. According to my definition of the primary germ layers in *Ciona*, this is not true, for several of the cells composing the chorda-mesenchyme ring (included by me in the primary endoderm) are derived from the four ventral cells, which according to their view are exclusively ectodermal. The statement that the first equatorial plane of cleavage separates the two primary germ layers is equally untenable, if tested by the definition of primary germ layers accepted by the authors mentioned; for they include in the primary endoderm the *entire mesoderm*, which I have shown to be derived *chiefly* from the four ventral cells, which produce the definitive *ectoderm*.

B. The Cœlom Theory.

The brothers Hertwig ('81) divided the higher Metazoa into two groups according as the body cavity arises by a pair of outpocketings of the primary endoderm enclosing an *enterocœl* between visceral and parietal mesoderm layers, or by a simple splitting or moving apart of cells in a solid mass of mesoderm, which is then said to enclose a *schizocœl*. The Chordates were unhesitatingly placed by them among the Enterocœlians, and *Amphioxus* was cited as a typical example. The Tunicates were thus classed as Enterocœlians, though no one had ever observed in their ontogeny the formation of an enterocœl. Van Beneden et Julin ('86) supplied the lack by their studies on *Clavelina*; but considerable doubt has been thrown on the accuracy of their observations by the independent researches of Seeliger ('85) upon an undetermined species of the same genus, and by those of Davidoff ('91) upon the identical species studied by Van Beneden et Julin. Neither Seeliger nor Davidoff could detect a trace of enterocœl formation in the ontogeny of *Clavelina*, and Davidoff was equally unsuccessful in finding an enterocœl in *Distaplia*. My own observations on *Ciona* are entirely in agreement with those of Seeliger and Davidoff on this point. Van Beneden et Julin, notwithstanding their belief that an enterocœl is formed in *Ascidians*, as well as in *Amphioxus*, rejected the classification of the brothers Hertwig on other grounds.

Lwoff ('94) has recently shown that in *Amphioxus* the cavities enclosed by outpocketings of the wall of the gastral cavity are evanescent structures, and have nothing to do with the subsequently formed body cavity, which, as in all Vertebrates, arises by a wandering apart of mesoderm

cells. He therefore concludes "*dass ein wahrer Enterocoelie unter allen Chordaten nicht existirt.*"

I am not able to criticise Lwoff's conclusion from the vantage ground of personal investigation of *Amphioxus*, but his account bears internal evidence of careful and exact observation. He calls attention to a fact, shown by his figures, that, when the mesodermal pouches arise, spindles, if any are present in the mesoderm cells, invariably stand vertically to the evaginated layer of cells, foreshadowing an arrangement of the daughter cells in two layers. This is exactly the position which the spindles take during gastrulation in the mesenchyme cells of *Ciona*, but in no other cells of the embryo. The form of division in the mesoderm cells of *Amphioxus* at the period mentioned tends to obliterate the lumen of the mesodermal pouches, a result which, as Lwoff's figures show, actually comes about. A body cavity is formed only secondarily by the moving apart of the mesoderm cells which are arranged in solid masses, the protovertebræ.

Davidoff ('91) likewise observed in the case of the compound Ascidian, *Distaplia*, that spindles stand vertically in the cells which give rise to the mesoderm at the time of the separation of the middle germ layer. He believes that the Tunicates can in no sense be regarded as Enterocoelians, and, further, that the distinction made by the brothers Hertwig between those Metazoa which possess a "mesoderm" and those which possess "mesenchyme" is an artificial and unsound one. With these conclusions I entirely agree.

Regarding Rabl's ('89) distinction between "gastral" and "peristomal" mesoderm, my observations lead me to the same conclusion as has been expressed by Davidoff, "*dass das peristomale Mesoderm der Ascidien sich im weiteren Verlauf der Entwicklung zum gastralen herausbildet, oder dass das gastrale Mesoderm ursprünglich peristomales Mesoderm ist.*" O. Hertwig ('92) draws a similar conclusion regarding Rabl's distinction as applied to the Vertebrates.

I should also state that both Lwoff and Wilson ('94) find that the pole mesoderm cells described by Hatschek in the case of *Amphioxus* do not exist. Certainly nothing of the kind is found among Ascidians. Hence we may conclude that such cells are entirely wanting among Chordates.

C. Ancestry of the Chordates.

To determine the phylogenetic relationship of the Chordates to the other groups of Metazoa is a very difficult problem. Various solutions

of it have been offered, but none is very generally accepted among zoölogists. The group is sharply marked off from all others by the possession of certain peculiar characters, such as the chorda, gill slits, and hypophysis. Among the higher Metazoa the Chordates seem to have no *near* relatives.

An ingenious suggestion, which has gained considerable currency, is that a chordate is homologous with an annelid whose dorsal and ventral surfaces are reversed. This "annelid hypothesis" has been ably advocated by Dohrn ('75 and '82-'91) and Eisig ('87). An extensive adverse criticism of the hypothesis has been made by Brooks ('93). Is any light thrown on the question by the ontogenetic history of Tunicates? The evidence from that source seems to me chiefly negative. Recent studies of the embryology of Annelids and Mollusks show a truly marvellous correspondence between the developmental processes in these two groups; it is even possible to refer back particular organs in both to homologous blastomeres, and to trace their differentiation through unmistakably similar processes. No doubt is left in the mind as to the close phylogenetic relationship of Annelids and Mollusks. The embryology of Chordates, however, follows an altogether different course, and is as unlike that of Annelids as the adult forms are different.

It is possible that we must go as far down in the animal scale as the Coelenterates to find an ancestor *common* to the Chordates and any other group of the higher Metazoa. The embryology of Tunicates seems to me to support this view.

Brooks ('93) has shown good reason for believing that all the principal groups of Metazoa arose as small, permanently pelagic forms, such as we find represented to-day, in a somewhat modified form, by Appendicularia in the case of Chordates.

Amphioxus, because of its adaptation to life on the bottom, has probably undergone considerable modification from the ancestral type. For example, the chorda has been extended forward to the extreme anterior end of the body to admit of the animal's burrowing in the sand; a marked asymmetry of the body has also arisen, and its size has doubtless greatly increased, calling for a metameric arrangement of its organs.

The ascidian tadpole, too, has probably been somewhat modified by a great shortening of the free-swimming (ancestral) period of its existence; but here the changes have probably been restricted to a *suppression* of certain processes or organs, so that those which remain are more certainly ancestral than those which occur even in Amphioxus. The *post-larval* history of Ascidians clearly exhibits a process of degeneration, which of course is wholly cœnogenetic.

VIII. CONCLUSIONS.

1. In the maturation of the ascidian egg the polar globules arise at the *vegetative* pole, i. e. in the future *endodermal* portion of the egg.

2. The archoplasms (attraction spheres) of the first cleavage spindle, and consequently of all subsequent spindles of the fertilized ascidian egg, are derived exclusively from the spermatazoön.

3. The archoplasm (attraction sphere) is not an organ of heredity, since in sexual reproduction it is frequently derived from one parent only.

4. Cleavage in the ascidian egg is bilateral from the very beginning. The course of cleavage is less variable in the egg of *Ciona* than in that of *Amphioxus* or the Vertebrates, and is predetermined by the internal constitution of the unsegmented egg.

5. The first equatorial plane of cleavage does not separate completely the two primary germ layers, though it does separate definitive endoderm from definitive ectoderm.

6. The fundaments of the principal organs are arranged in zones around the chief axis of the egg.

7. The nervous system and the longitudinal musculature of the larva are derived from a *common fundament*, which is a (neuro-muscular) ring of cells encircling the margin of the blastopore. This ring of cells must be regarded as a part of the primary ectoderm.

8. The chorda and mesenchyme (or trunk mesoderm) are derived from another ring of cells lying just within the margin of the blastopore. This ring of cells is to be regarded as a part of the primary endoderm.

9. The mesoderm of Ascidians is therefore derived in part from the primary ectoderm, and in part from the primary endoderm. It is formed exclusively by cells of the two rings already mentioned, one of which belongs to each of the two primary germ layers. Recent careful observations indicate that likewise in *Amphioxus* and the Vertebrates the mesoderm is derived from both primary germ layers.

10. The longitudinal musculature of the Ascidian tadpole is homologous with that of *Amphioxus*; the mesenchyme of the Ascidian, with the mesoderm lateral to the muscle plates in *Amphioxus*.

11. The chorda should be regarded as a mesodermal organ.

IX. TABLE OF CELL LINEAGE OF CIONA.

2-cell.	4-cell.	8-cell.	16-cell.	24-cell.	32-cell.	48-cell.	64-cell.
			$A^{5.1}$	$A^{6.1} = \text{Ectoderm}$			
		$A^{4.1}$		$A^{6.2}$			$A^{7.3} = \text{Ectoderm (+ Nervous ?)}$
			$A^{5.2}$	$A^{6.3}$			$A^{7.4} = \text{Nervous}$
				$A^{6.4}$			$A^{7.5} = \text{Ectoderm}$
			$a^{5.8}$				$A^{7.6} = \text{Mesenchyme}$
		$a^{4.2}$	$a^{5.4}$		$a^{6.5} = \text{Chorda}$		$A^{7.7} = \text{Ectoderm (+ Nervous ?)}$
					$a^{6.6} = \text{Endoderm}$		$A^{7.8} = \text{Nervous}$
			$D^{6.1}$	$D^{6.1} = \text{Ectoderm}$			
		$D^{4.1}$		$D^{6.2}$		$D^{7.3} = \text{Ectoderm}$	
			$D^{5.2}$	$D^{6.3}$		$D^{7.4} = \text{Neuro-muscular}$	
				$D^{6.4}$		$D^{7.5} = \text{Mesenchyme}$	
			$i^{5.3}$			$D^{7.6} = \text{Neuro-muscular}$	
						$D^{7.7} = \text{Ectoderm + Neuro-muscular}$	
						$D^{7.8} = \text{Mesenchyme}$	
			$i^{4.2}$		$i^{6.5} = \text{Endoderm}$		$d^{7.11} = \text{Chorda}$
					$d^{6.6}$		$d^{7.12} = \text{Mesenchyme}$
				$i^{5.4} = \text{Endoderm}$			

Note.—In this Table the lineage is given for only the right half of the egg in the 4-cell and subsequent stages. The complete lineage for the left half of the egg may be expressed by substituting *B* for *A*, and *C* for *D* in the Table.

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- '93. Amphioxus and the Mosaic Theory of Development. Journ. Morph., Vol. VIII. pp. 579-638, 10 Plates.

Wilson, E. B., and Mathews, A. P.

- '95. Maturation, Fertilization, and Polarity in the Echinoderm Egg. New Light on the "Quadrille of the Centers." Journ. Morph., Vol. X. pp. 319-342, 7 Figs.

EXPLANATION OF PLATES.

All Figures were drawn with the aid of the Abbé camera lucida. The magnification is stated for each plate separately. The egg membranes have not been represented in any of the Figures. Arrows in the Figures connect sister cells, i. e. cells which have arisen by division of a common mother cell. For an explanation of the system of nomenclature employed, see page 226.

In many of the Figures, only the cells of one half of the embryo (usually the right, that is, quadrants *A* and *D*) have been lettered. The reader will be able readily to supply the deficiencies for the cells of the other half, since they are almost perfectly symmetrical in position with those that are lettered. Corresponding to each cell on one side of the median plane (in quadrant *A* or *D*) will be found a cell similarly situated on the *other* side of the median plane (in quadrant *B* or *C*); this cell should receive the same exponents as its mate, and either the letter *B* or *C* according as that mate is lettered *A* or *D*. Thus, the mate of $A^{7.2}$ in Figure 58 (Plate X.) is the cell immediately to the right of it, which would be called $B^{7.2}$; the respective mates of $D^{7.5}$ and $D^{7.6}$ in Figure 57 would be called $C^{7.5}$ and $C^{7.6}$.

In Figure 62 (Plate X.) and all following Figures, the endoderm cells are indicated by *granular nuclei* in a cell body that is left without tint or stippling; the chorda cells without tint or stippling, and with the outlines only of nuclei; the mesenchyme cells by a flat tint; the ring of neuro-muscular cells by stippling of the body of the cell. The ectoderm cells, since they are easily distinguishable from the endoderm cells, have been left, like the latter, without stippling or tint; their nuclei are sometimes drawn true to nature, i. e. with granulations, sometimes in outline only.

ABBREVIATIONS.

<i>ast.</i> ♀	= female astral sphere.	<i>ms'chy.</i>	= mesenchyme.
<i>ast.</i> ♂	= male astral sphere.	<i>mu.</i>	= musculature.
<i>cd.</i>	= chorda.	<i>n.</i>	= nervous system.
<i>cl. pol.</i>	= polar globule.	<i>pr'nl.</i>	= pronucleus.
<i>cl. pol.^{1.}</i>	= first polar globule.	<i>pr'nl.</i> ♀	= female pronucleus.
<i>ec'drm.</i>	= ectoderm.	<i>pr'nl.</i> ♂	= male pronucleus.
<i>en'drm.</i>	= endoderm.		

PLATE I.

All Figures represent sections ; magnification, 560 diameters. Yolk granules are not represented, except in Fig. 2.

- Fig. 1. First maturation spindle, and formation of first polar globule.
- Fig. 2. Portion of section through recently impregnated egg. The spermatozoön lies at the centre of a region free from yolk granules.
- Fig. 3. Second maturation spindle.
- Fig. 4. Section through the same egg in region of male pronucleus.
- Fig. 5. Impregnated egg ; male and female pronuclei visible in the same section.
- Fig. 6. Conjugation of pronuclei, viewed in the direction of the axis of the first cleavage spindle, which is in process of formation. (Cf. Plate III, Fig. 13.)

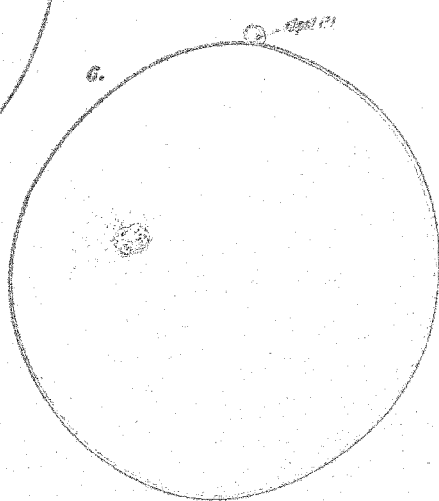
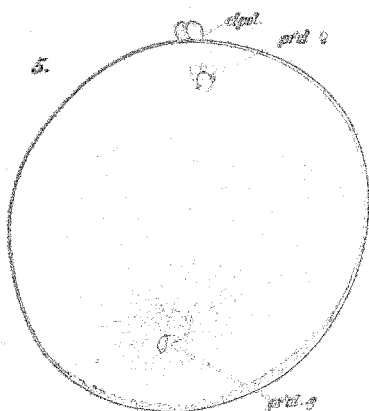
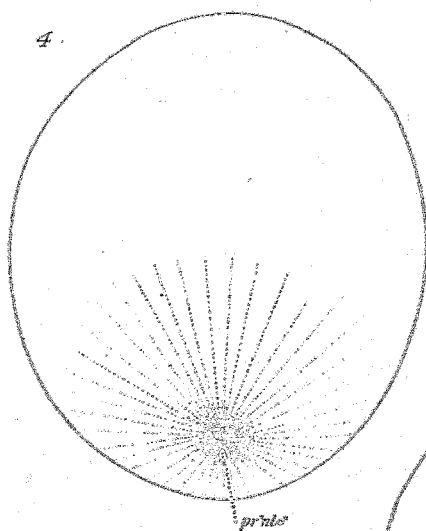
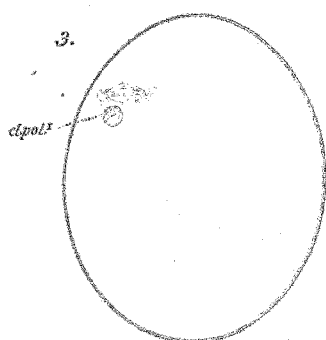
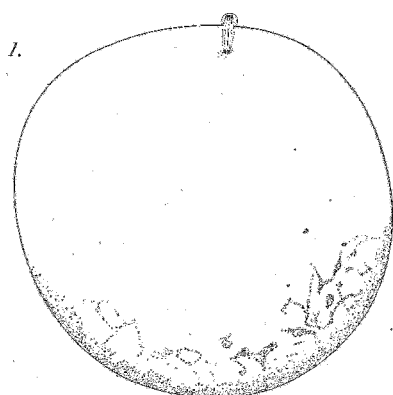


PLATE II.

Figs. 7-10. Four sections through an impregnated egg; magnification, 560 diameters.

Fig. 7. Fourth section of series, showing the male pronucleus and archoplasmic spheres. Compare Figs. 11 and 12.

Fig. 8. Seventh section of series, showing the female pronucleus.

Fig. 9. Tenth section of series, showing the female archoplasm.

Fig. 10. Twelfth section of series, showing polar globules.

Fig. 11. Graphic reconstruction of the series on a plane, perpendicular to that of Fig. 7, indicated by the line ab , Fig. 7.

Fig. 12. A similar reconstruction on a perpendicular plane, the projection of which is the line $a'b'$, Fig. 7.

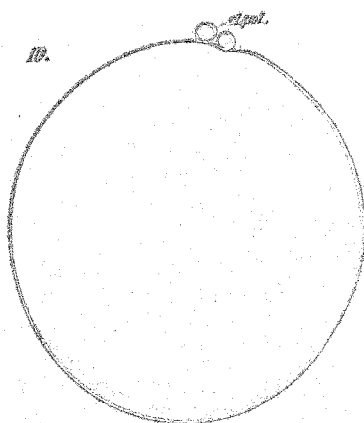
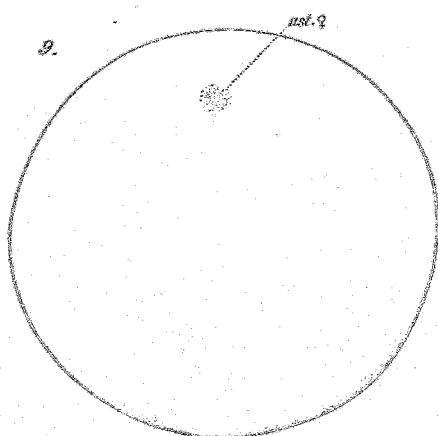
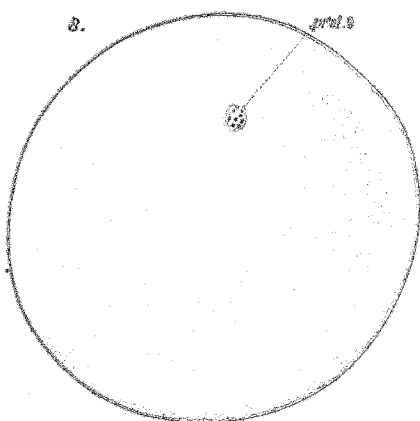
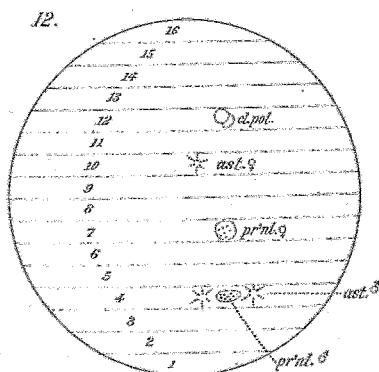
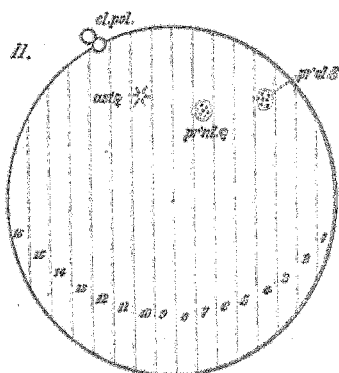
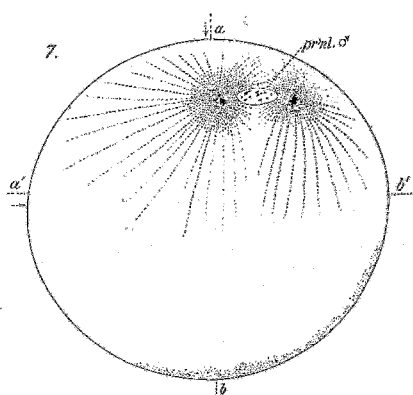
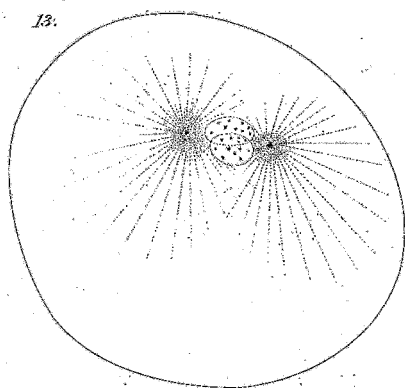


PLATE III.

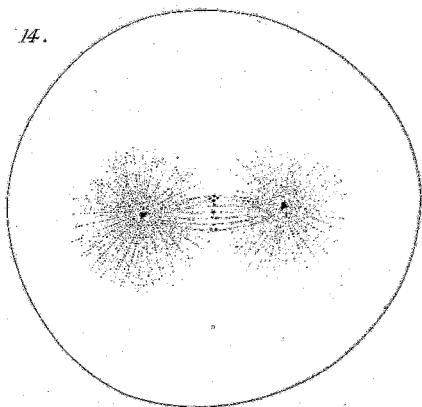
All Figures represent sections ; magnification, 560 diameters.

- Fig. 13. Conjugation of pronuclei, viewed in the direction of the equator of the first cleavage spindle. (Cf. Plate I. Fig. 6.)
- Fig. 14. First cleavage spindle.
- Fig. 15. First cleavage nearly completed. Each of the newly formed nuclei is made up of two vesicles as yet incompletely fused.
- Fig. 16. Section through one of the cells of a 2-cell stage, parallel to median plane of the embryo.
- Fig. 17. Section from the same series as Fig. 16 through one of the two cells, near the median plane of the embryo. *z*, finely granular protoplasm, which marks the posterior-ventral side of embryo (cf. Plate VIII. Fig. 45, *z*), and is traceable up to the larval stage.
- Fig. 18. Section through an 8-cell stage parallel to median plane of the embryo.

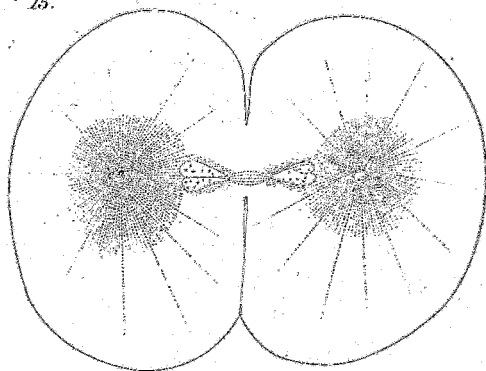
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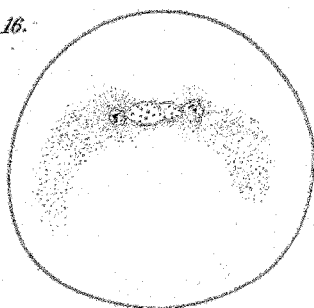
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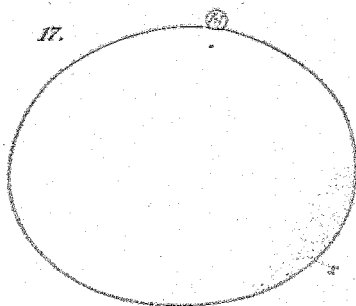
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18.

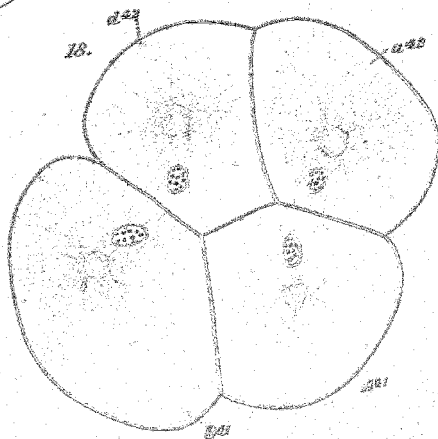


PLATE IV.

Eight successive views of a living egg, seen from the left side; magnification, 315 diameters. Arrows indicate the direction of spindles.

- Fig. 19. Matured but unsegmented egg.
- Fig. 20. 2-cell stage.
- Fig. 21. 4-cell stage approaching.
- Fig. 22. 4-cell stage, "resting" condition.
- Fig. 23. 8-cell stage, just formed.
- Fig. 24. 8-cell stage, nine minutes later.
- Fig. 25. 16-cell stage, just formed.
- Fig. 26. 16-cell stage, some minutes later.

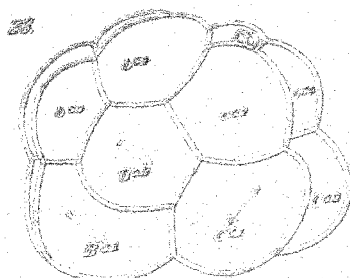
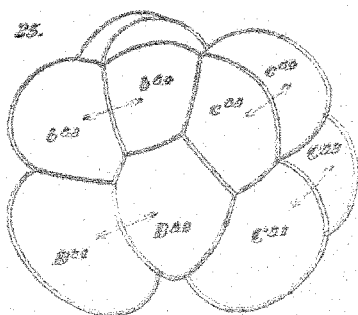
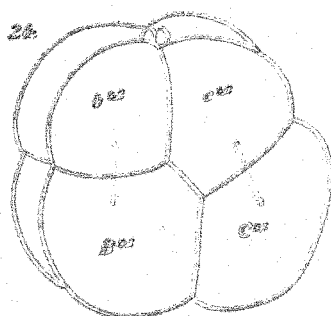
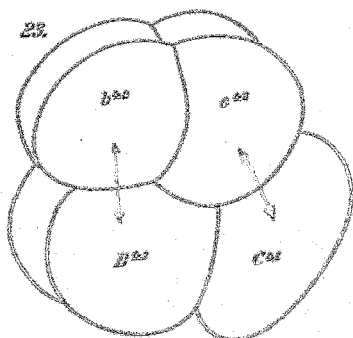
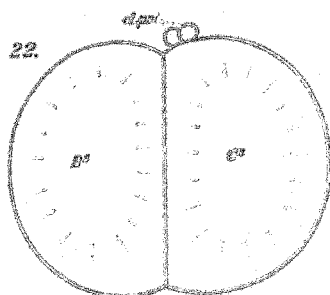
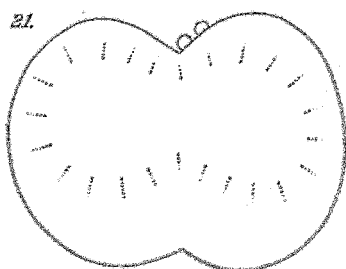
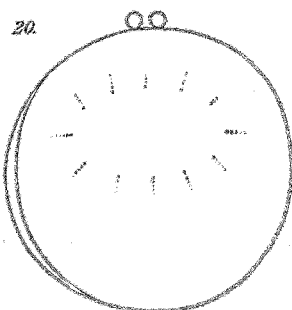
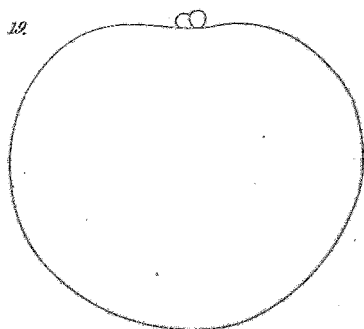
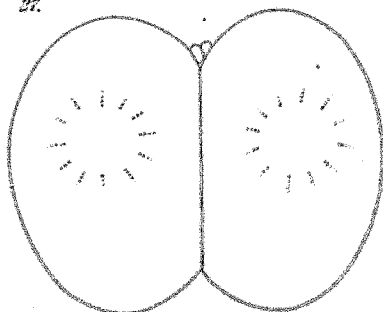


PLATE V.

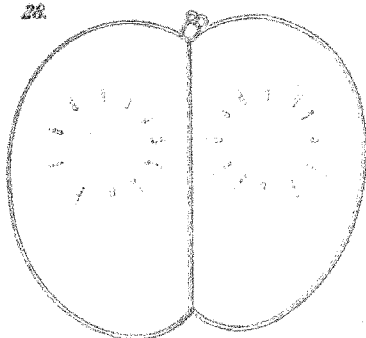
Six successive views of a living egg, seen from the anterior end; magnification, 315 diameters. Figs. 27-31 show the egg viewed as a transparent object. Fig. 32 is a surface view.

- Fig. 27. 2-cell stage, newly formed.
- Fig. 28. 2-cell stage, a few minutes later.
- Fig. 29. 2-cell stage, a few minutes later still.
- Fig. 30. 4-cell stage.
- Fig. 31. 8-cell stage, just formed.
- Fig. 32. 8-cell stage, a few minutes later.

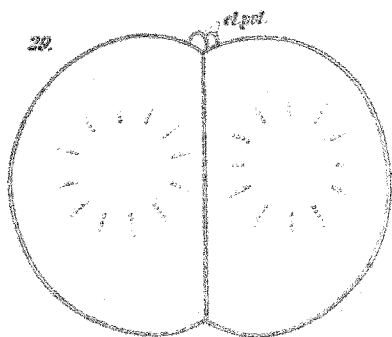
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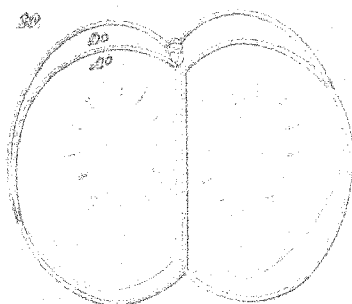
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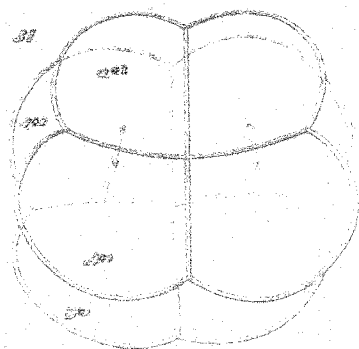
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31.



32.

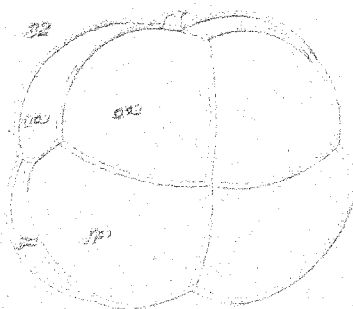


PLATE VI.

Magnification of all Figures, 315 diameters. Arrows indicate the direction of spindles.

- Fig. 33. ⁰ Later stage of the same egg as that shown in Fig. 32, Plate V. 16-cell stage, just formed.
- Fig. 34. The same egg, passing into 24-cell stage.
- Figs. 35-38. Ventral aspect of four successive stages of a living egg.
- Fig. 35. 4-cell stage.
- Fig. 36. 8-cell stage, just formed.
- Fig. 37. 8-cell stage, a few minutes later.
- Fig. 38. 12-cell stage, just formed.

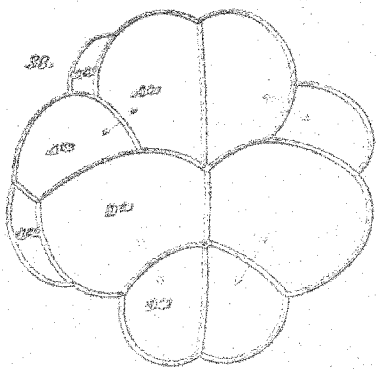
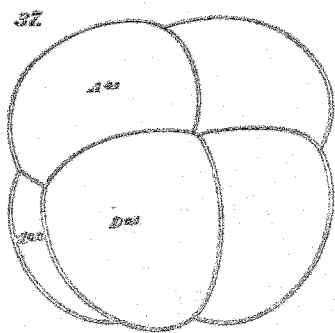
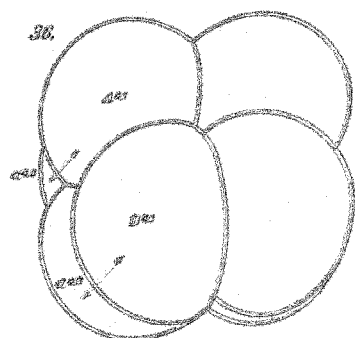
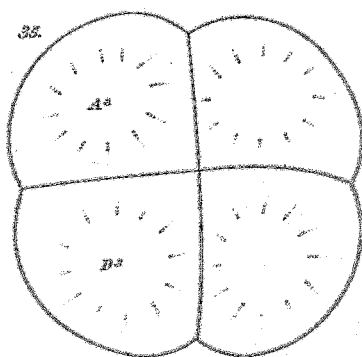
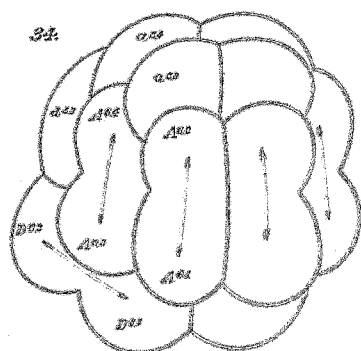
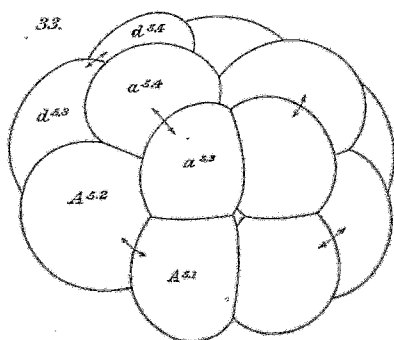


PLATE VII.

Magnification of all Figures, except 43 and 44, 315 diameters; magnification of Figs. 43 and 44, about 300 diameters.

Figs. 39-42. Four later views of the egg shown in Figs. 35-38, Plate VI.; same (ventral) aspect.

Fig. 39. 16-cell stage, just formed. This view five minutes later than that in Fig. 38.

Fig. 40. 16-cell stage, five minutes later.

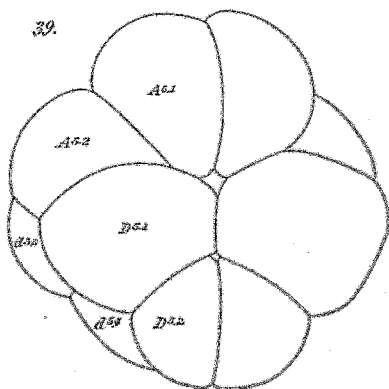
Fig. 41. 16-cell stage, ten minutes later still. Arrows indicate the direction of spindles.

Fig. 42. 24-cell stage, just formed, some minutes later than the last view.

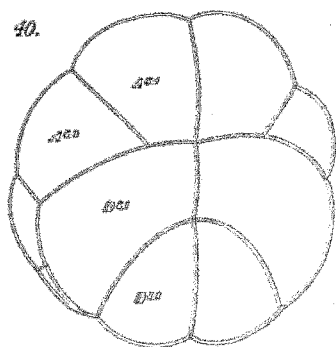
Fig. 43. A late 24-cell stage, viewed from the right side.

Fig. 44. Optical section of the same egg, near the median plane. The cells of the dorsal hemisphere are in mitosis.

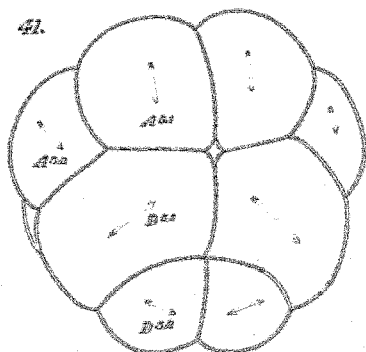
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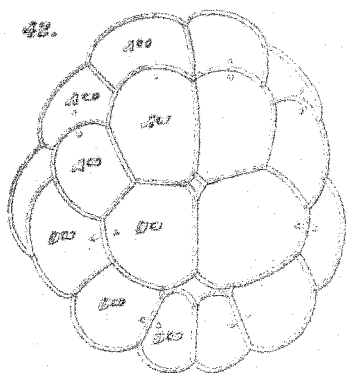
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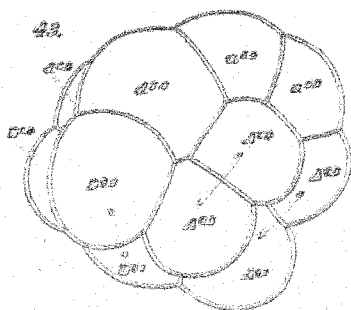
41.



42.



43.



44.

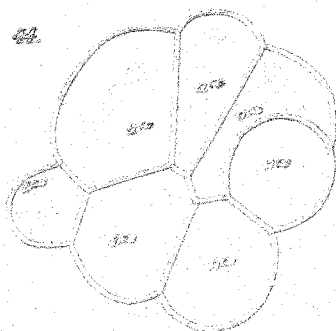
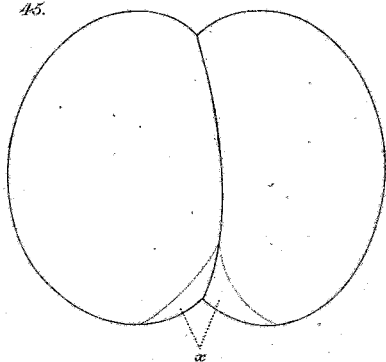


PLATE VIII.

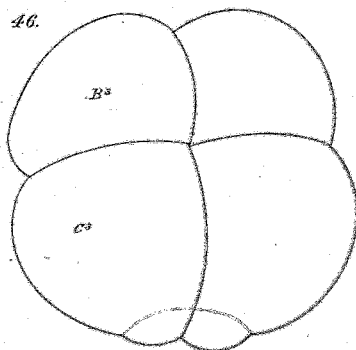
Six successive views (obliquely from the left, above, and behind) of a living egg; magnification, 315 diameters. Arrows indicate the direction of spindles.

- Fig. 45. 2-cell stage. *x*, region of finely granular protoplasm (cf. description of Plate III. Fig. 17).
- Fig. 46. 4-cell stage, just formed.
- Fig. 47. 8-cell stage, approaching.
- Fig. 48. 8-cell stage, fully formed (viewed as a transparent object).
- Fig. 49. 16-cell stage.
- Fig. 50. 24-cell stage. During the formation of the 24-cell stage, the egg has rotated so that the view is almost exactly dorsal.

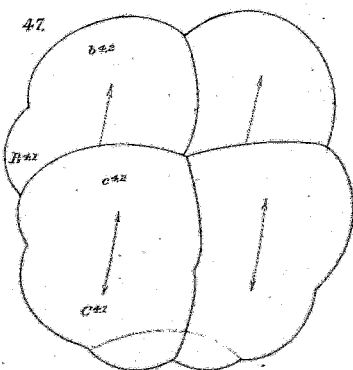
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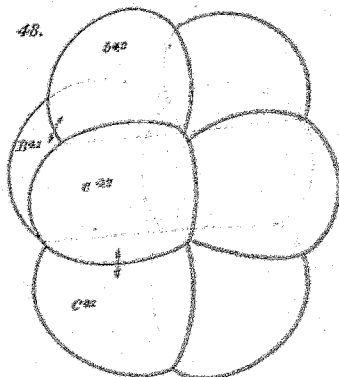
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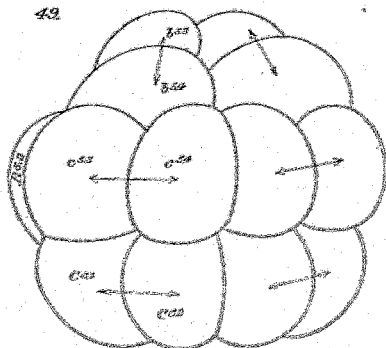
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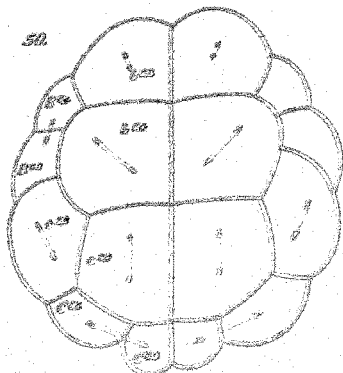


PLATE IX.

Surface views of preparations; magnification, 400 diameters.

- Fig. 51. 24-cell stage, ventral view.
- Fig. 52. The same egg, in the same stage, dorsal view (cf. Plate VII Figs. 43 and 44).
- Fig. 53. 32-cell stage, ventral view.
- Fig. 54. The same egg and stage, dorsal view.
- Fig. 55. 46-cell stage, ventral view.
- Fig. 56. The same egg and stage, dorsal view.

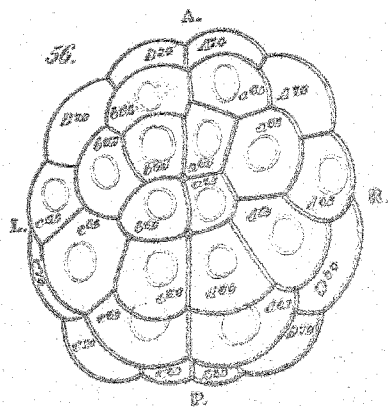
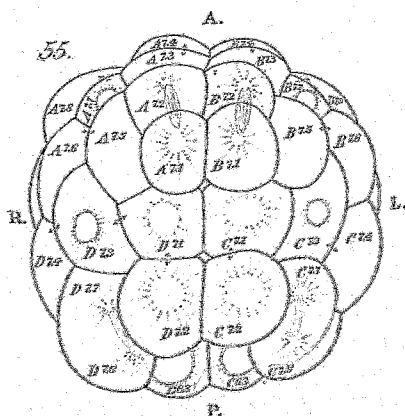
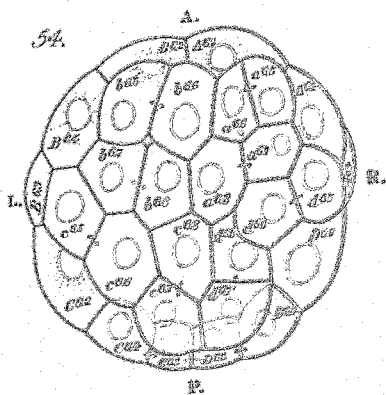
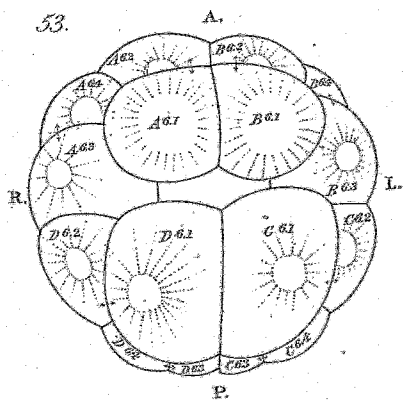
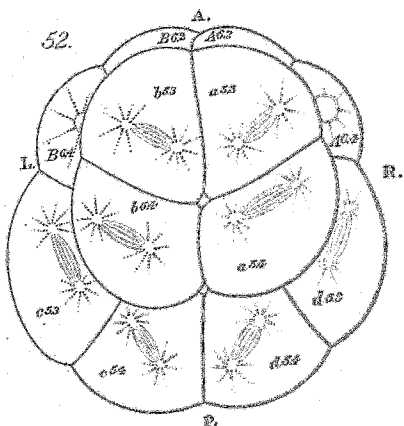
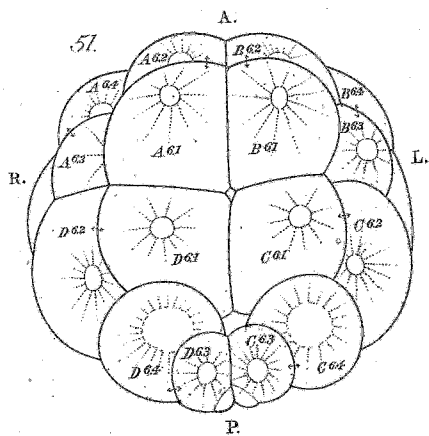


PLATE X.

Figs. 57-62. Surface views of preparations; magnification, 560 diameters.

Fig. 57. 48-cell stage, viewed from behind.

Fig. 58. The same egg and stage, viewed from in front.

Fig. 59. 64-cell stage, ventral view.

Fig. 60. The same egg and stage, dorsal view.

Fig. 61. 76-cell stage, ventral view.

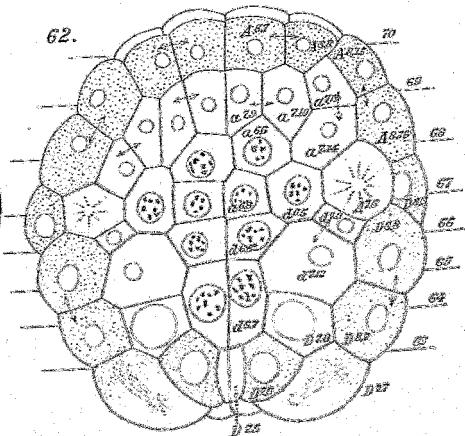
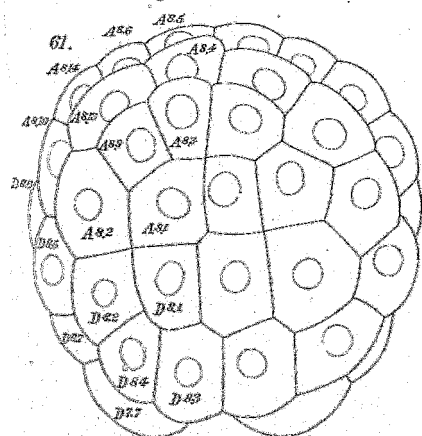
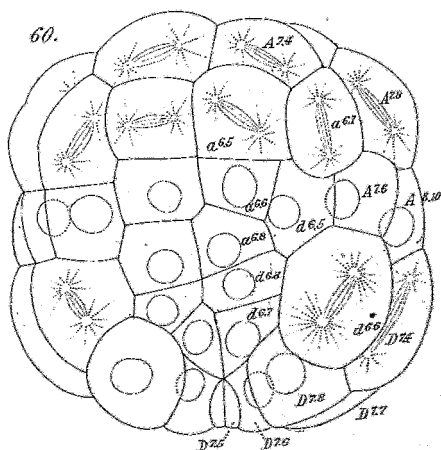
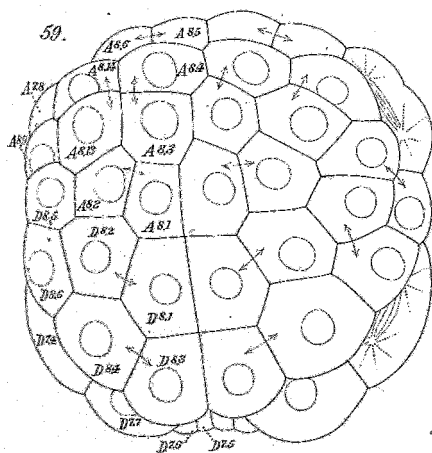
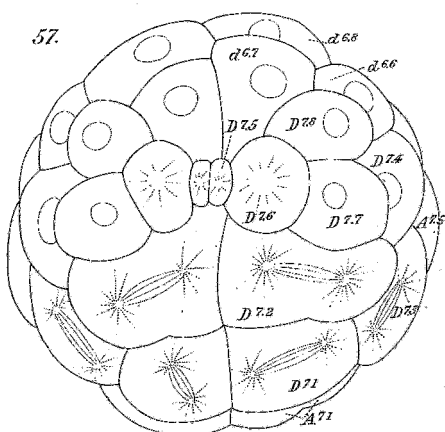
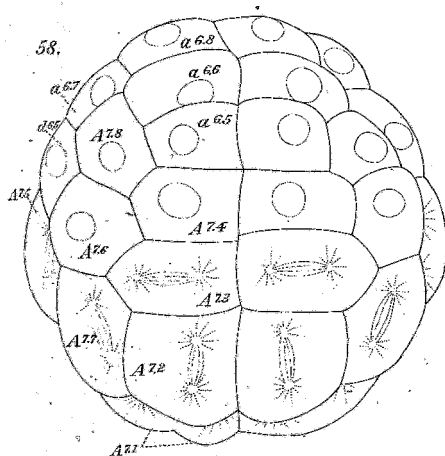
Fig. 62. The same egg and stage, dorsal view.

Note. — Consult general statement under Explanation of Plates. The cell $D^{8.6}$ and its mate in the left half of the Figure were stippled by mistake.

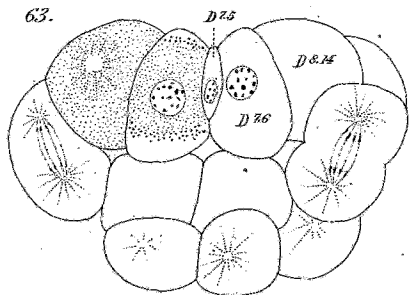
Figs. 63-70. Eight cross-sections from a series through an embryo in late 76-cell stage; magnification, 560 diameters. For position of sections in embryo, see horizontal lines 63-70 in Fig. 62.

Note. — In Fig. 63, the cells $D^{7.6}$ and $D^{8.14}$ should be stippled like their mates. In Fig. 67, the cell $C^{7.11}$ should be $c^{7.11}$.

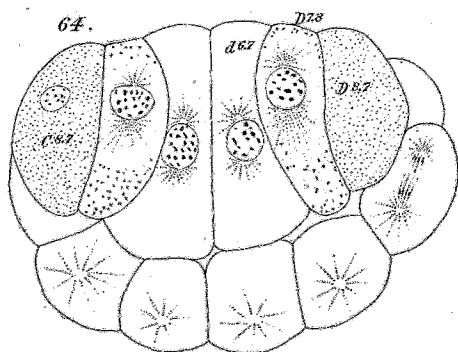
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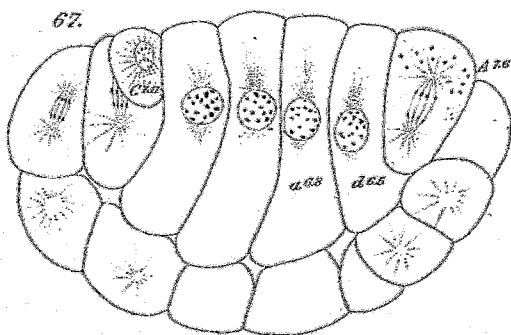
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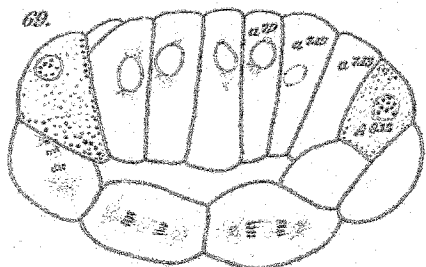
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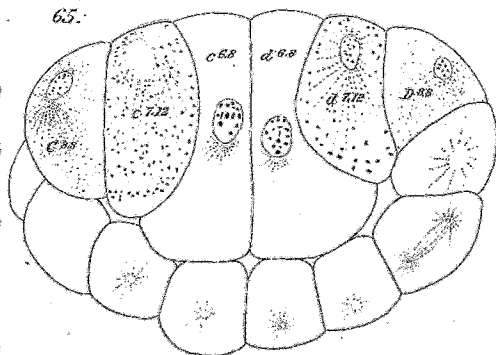
67.



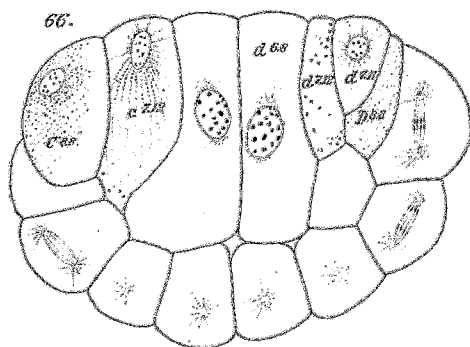
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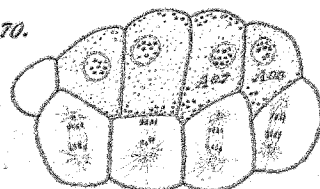
65.



66.



70.



68.

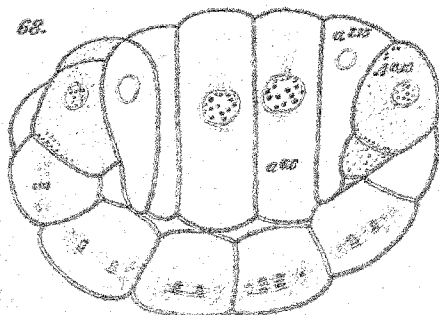


PLATE XI.

Magnification of all Figures, 560 diameters.

Fig. 71. 112-cell stage, dorsal view.

Fig. 72. Early gastrula, dorsal view.

Figs. 73-77. Five cross sections through an early gastrula (128-cell stage). For position of sections in embryo, see horizontal lines 73-77 in Fig. 72.

Note. — Consult general statement regarding lettering under Explanation of Plates.

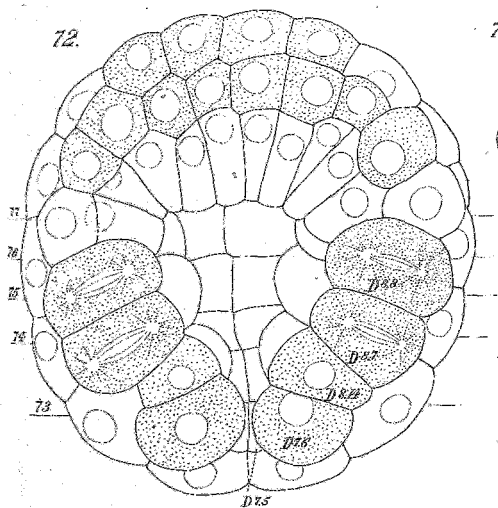
Fig. 78. Sagittal section through an early gastrula (older than that shown in Fig. 72).

Fig. 79. Similar section through a slightly older stage.

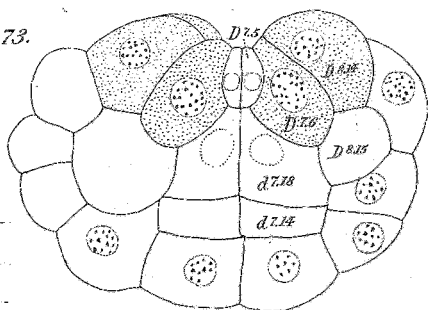
Fig. 80. Surface (dorsal) view of late gastrula.

Figs. 81-83. Three horizontal sections from a series through a late gastrula.

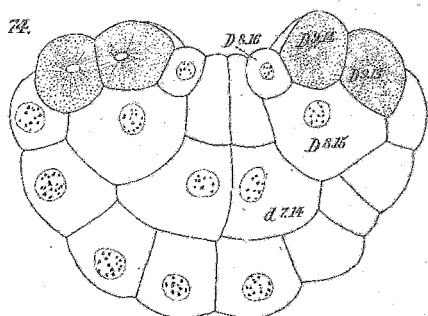
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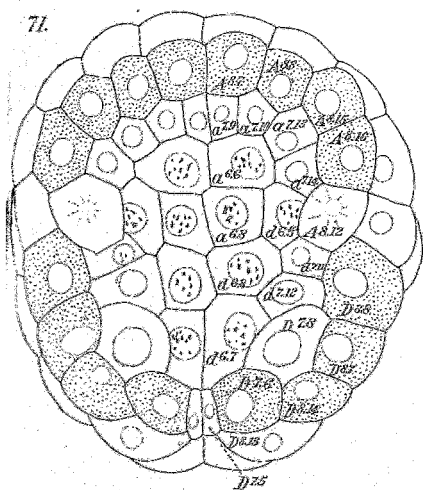
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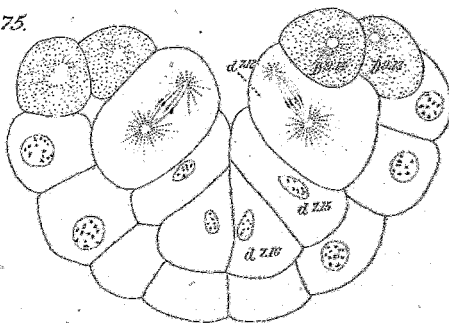
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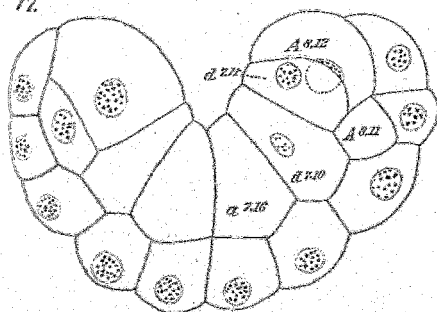
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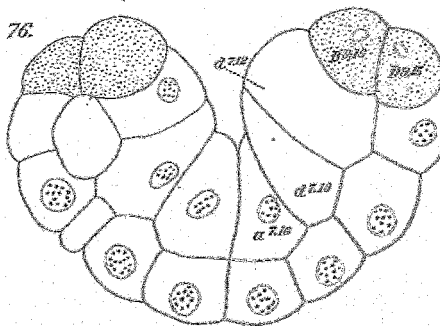
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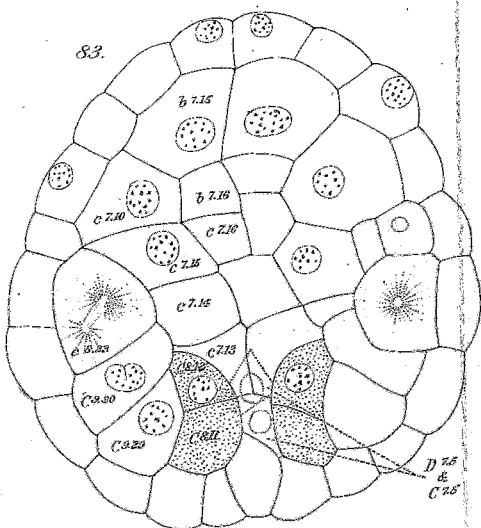
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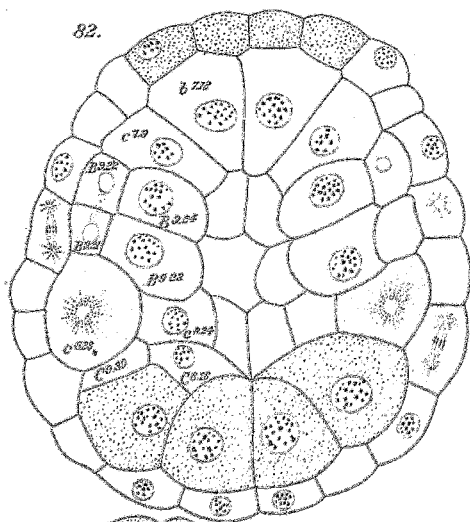
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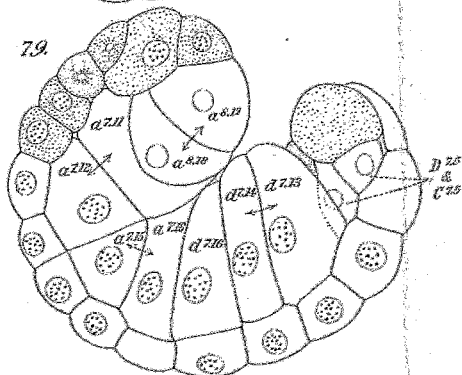
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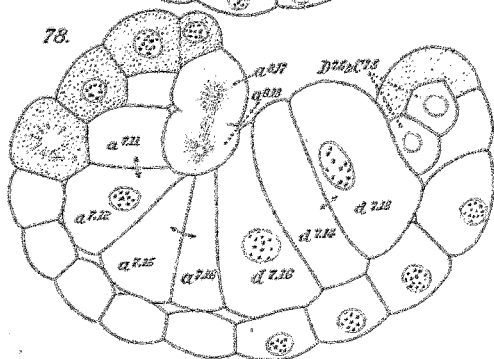
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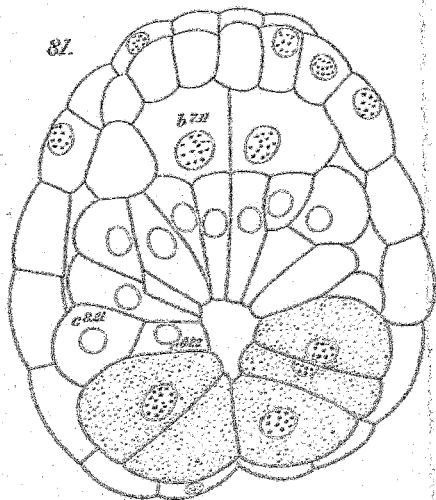
79.



78.



81.



80.

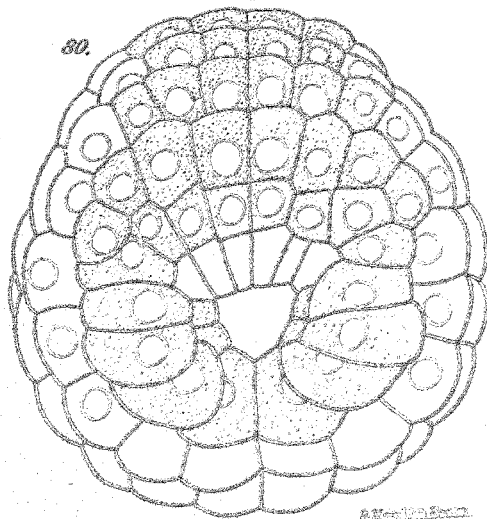


PLATE XII.

Magnification of all Figures, 560 diameters.

Figs. 84-87. Four cross sections from a series through a gastrula with wide-open blastopore.

Fig. 84. Section posterior to the blastopore.

Fig. 85. Section through the posterior portion of the blastopore.

Fig. 86. Section through the anterior portion of the blastopore.

Fig. 87. Section anterior to the blastopore.

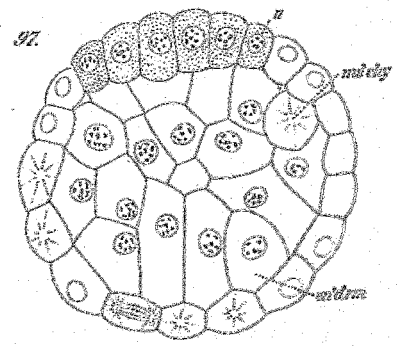
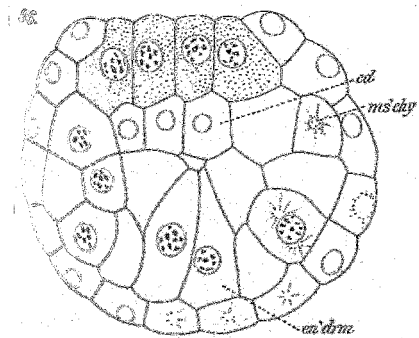
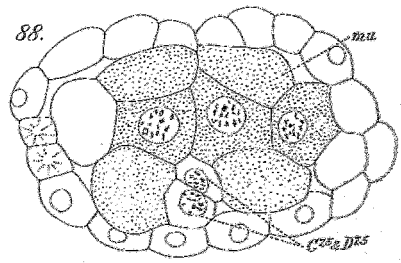
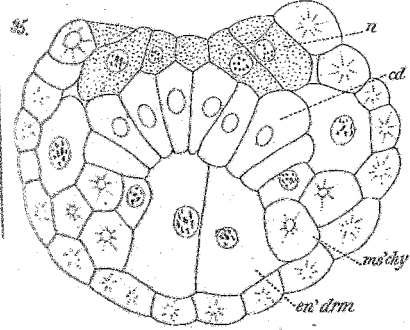
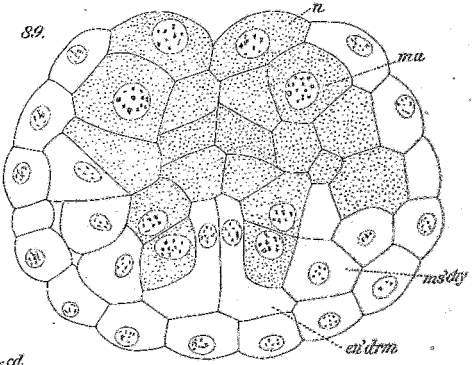
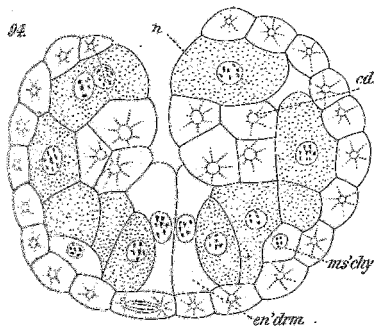
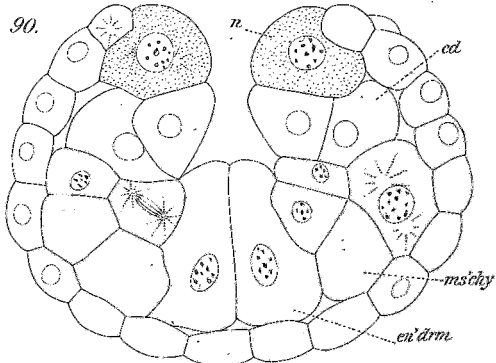
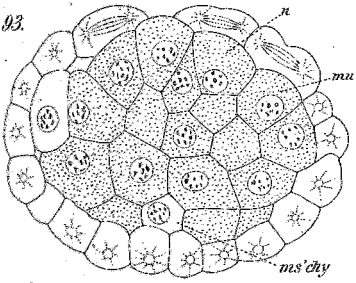
Figs. 88-92. Five cross-sections through an embryo with greatly contracted blastopore; Figs. 88 and 89 posterior to blastopore; Figs. 91 and 92 anterior to blastopore.

Note. — The large unstippled cell in the left half of Fig. 88 should have been stippled.

Figs. 93-97. Five cross sections through a slightly older embryo. Compare vertical lines 93-97 in Fig. 98. Fig. 93, section posterior to blastopore; Figs. 95-97, sections anterior to blastopore.

Note. — The large unstippled cell in the left half of Fig. 93 should have been stippled, likewise two large cells situated laterally in Fig. 95, one in either half of the Figure.

Fig. 98. Sagittal section through an embryo with nearly closed blastopore.



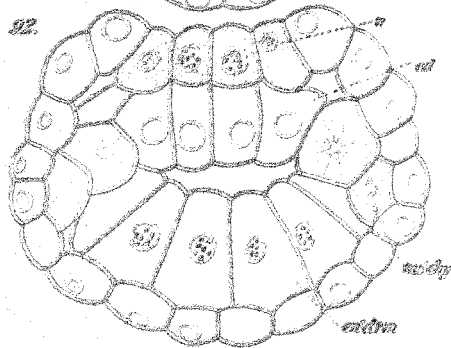
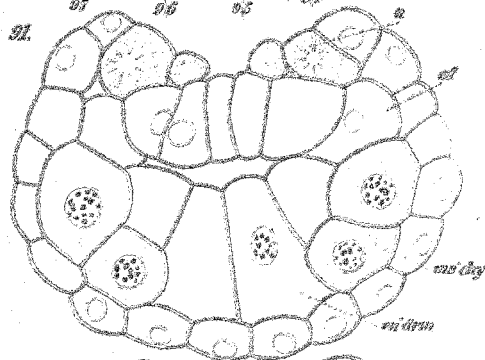
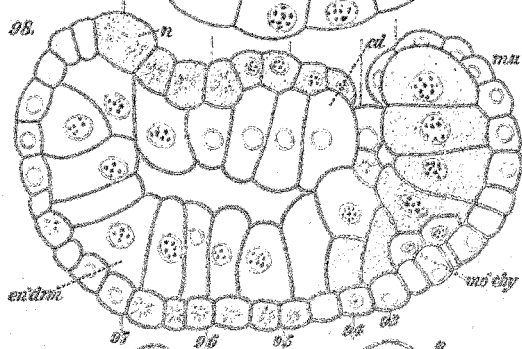
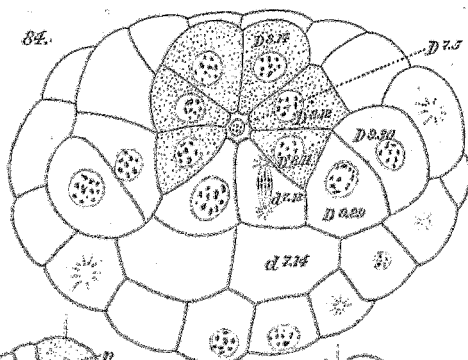
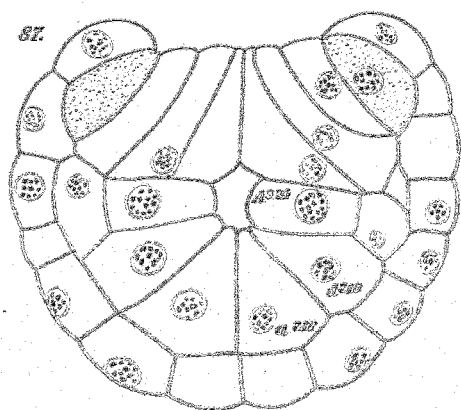
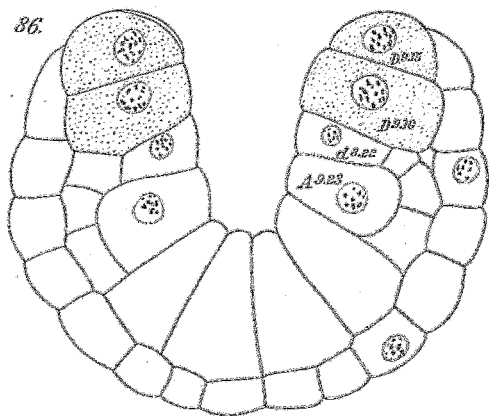
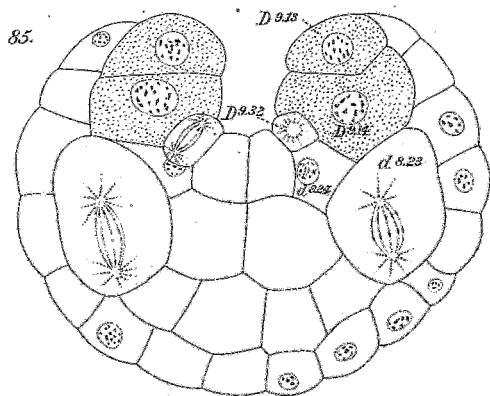


PLATE XIII.

Magnification of all Figures, 560 diameters.

- Figs. 99-105. Seven sections from a series through an embryo with completely closed blastopore. Figs. 99-101, posterior to region of final closure of blastopore; Figs. 103-105, anterior to region of closure of blastopore.
- Fig. 106. Cross section through an early larval stage (unhatched). The trunk region is shown in the left portion of the Figure, the tail region in the right portion.

