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THE CELL-LINEAGE OF NEREIS.

A CONTRIBUTION TO THE CYTOGENY OF THE ANNELID BODY.

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THE following studies were undertaken, in the first instance, in the hope of clearing up certain perplexing problems involved in the origin of the germ-layers in annelids, especially those relating to the formation of the mesoblast in the polychætous forms; and, with this end in view, I sought to find a form in which the detailed history of the mesoblast might be followed in the cleavage-process, and its precise relation to the other layers thus determined. For a long time the search was fruitless. The eggs of most of the available American forms proved unfavorable for my purpose, on account of their opacity, the difficulty of orienting the early stages of development, and the lack of distinctive characters in the external features of the blastomeres. At length, however, my attention was directed to the eggs of two species of *Nereis* (*N. limbata*, Ehlers, and *N. megalops*, Verrill), which soon proved to be of unusual interest and importance, not only for the investigation of the mesoblast-formation, but also for the study of the cleavage of the ovum from a more general point of view. In both forms the mesoblast-bands could be traced back to a single cell and the relation of this cell to the other blastomeres accurately determined. The facts thus established, as I have endeavored to show in a preliminary paper (No. 30), go far to reconcile the various modes of mesoblast-formation in annelids, and, as I believe, throw a new light on the well-known researches of Salensky (No. 21) and Kleinenberg (No. 14) in this field. It soon appeared, moreover, that the early stages of *Nereis* possessed a more general interest. The ova are extraordinarily favorable for a detailed investigation of the history of the individual blastomeres in the cleavage-process. They are

transparent and of a convenient size; the differentiation of the blastomeres appears at a very early period; and from the beginning of its development the embryo possesses a number of peculiar features, by means of which it may at any stage be perfectly oriented, almost at a glance.[1] Furthermore, the eggs may easily be fixed and stained by a simple method, which shows with great clearness both the cell-outlines and the nuclear figures. This combination of favorable characteristics renders it possible to trace out the cellular genesis of various parts of the body, step by step from the beginning of development, with a completeness and precision that is rarely attainable. The results show, as I am convinced, that much is to be gained by considering the embryological development of animals from a point of view differing in some respects from that usually adopted, and this point of view I wish to make clear before proceeding to a detailed description of the facts to be set forth.

For many years (more especially since the publication of Haeckel's celebrated papers on the gastræa theory) embryological research has been dominated by certain general conceptions, usually designated as the "germ-layer theory," a term which need not be more precisely defined here. The germ-layer theory forms, in fact, the foundation on which the entire science of comparative embryology is built. And yet it does not require a very extensive search among the embryological writings of the last decade to discover that a surprising divergence of opinion exists among the best authorities in regard to some of the most fundamental propositions of this theory. This divergence need not here be reviewed *in extenso*, but I may be allowed to illustrate its character by quoting somewhat fully from two eminent embryologists. Ten years ago, Balfour wrote as follows:[2] —

"Since there are some Metozoa with only two germinal layers, and other Metozoa with three, and since . . . the third layer or mesoblast can only be regarded as a derivative of one or both the primary layers, it is clear that a complete homology between the two primary germinal layers does not exist.

[1] Dr. E. A. Andrews first called my attention to the favorable character of the *Nereis* eggs and the ease with which they may be procured in large numbers, and I am glad to acknowledge my great indebtedness to him.

[2] *Comparative Embryology*, Vol. II, 1881.

"That there is a general homology appears, on the other hand, hardly open to doubt" (p. 285).

"The fact of the triploblastic condition being later than the diploblastic proves in a conclusive way that the mesoblast is a derivative of one or both the primary layers. . . .

". . . The mesoblast did not at first originate as a mass of independent cells between the two primary layers, but . . . in the first instance it gradually arose as differentiations of the two layers, and . . . its condition in the embryo as an independent layer of undifferentiated cells is a secondary condition, brought about by the general tendency towards a simplification of development, and a retardation of histological differentiation" (p. 286).

Certain facts, clearly specified by Balfour, point, in his opinion, to the establishment of the two following propositions: —

"(1) That with the differentiation of the mesoblast as a distinct layer by the process already explained, the two primary layers lost for the most part the capacity they primitively possessed of giving rise to muscular and connective-tissue differentiations, to the epithelium of the excretory organs, and to generative cells. (2) That the mesoblast throughout the triploblastic Metozoa, in so far as these forms have sprung from a common triploblastic ancestor, is an homologous structure" (p. 287).

The point in this lucid and forcible statement to which I wish to call attention is that *Balfour did not regard the primary germ-layers as being strictly homologous with one another throughout the gastrulas of the triploblastica, or with the layers of the cœlenterate body.* The reasons given for this conclusion are so convincing as to amount almost to a demonstration.

We turn now to the views of Kleinenberg as developed five years later in the celebrated work on *Lopadorhynchus*. In certain respects, as Kleinenberg himself points out, his views coincide with those of Balfour. But when carefully examined as a whole they are found to lead logically to a conception that is diametrically opposed to that of Balfour, as may clearly be shown by a juxtaposition of the two views. It should be borne in mind, however, that it is difficult to give an adequate idea of Kleinenberg's views by the citation of a few passages, since they are set forth in a style which, though always entertaining

from a literary point of view, makes considerable demands upon the patience of the scientific reader. I believe, however, that the gist of the matter lies in the following passages, which follow directly after a very clear review of Balfour's views (the italics are mine): "Die ausgebildeten Cölenteraten besitzen kein Mesoderm und demgemäss erscheint auch das mittlere Keimblatt der Embryonen höherer Metazoen als ein bloss conventioneller, den Thatsachen nicht entsprechender, Begriff. Was man bisher so nannte ist entweder die Summe unabhängiger heterogener Anlagen, die im Bereich der primären Keimblätter entstehen, oder eine einzige Anlage eines bestimmten Gewebes oder Organs, die eventuell theilweiser Umbildung unterliegt. Am häufigsten sind mächtige ektodermale Muskelanlagen und paarige Anhänge des Urdarms zum mittleren Keimblatt gemacht worden. Die Frage nach der Homologie des sogenannten Mesoblasts in den verschiedenen Thierklassen beruht auf der Voraussetzung des Vorhandenseins eines Nichtvorhanden, und fällt daher von selbst weg. *Die Homologie der Organe muss in jedem Falle, mit Berücksichtigung etwaiger Substitutionem, durch die genetischen Beziehungen zu den beiden Blättern des Cölenteratenkörpers festgestellt werden. Ektoderm und Entoderm sind die ursprünglichen Grundlagen aller Gewebe und Organe — die Geschlechtszellen wahrscheinlich ausgenommen — bei den Cölenteraten: ebenso verhält sich das Ektoderm und das Entoderm der Entwicklungsformen.* Diese Blätter erzeugen besondere Gewebe ohne dadurch irgend wie die Fähigkeit neuer Gewebebildung einzubüssen (p. 18).

It would be manifestly unfair to take this passage as an adequate statement of Kleinenberg's views. But it leaves no doubt as to his conception of homologies. They are equivalent phylogenetic relationships to the layers of the cœlenterate body. It seems equally clear, furthermore, that he believes *these phylogenetic relationships to be repeated or expressed by corresponding equivalent ontogenetic relationships to the primary germ-layers of the gastrula.* Here is the key to Kleinenberg's whole position, for it obviously rests upon the assumption that the two primary germ-layers — the inner and outer layers of the gastrula — are respectively homologous with the layers of the cœlenterate body, and hence with each other throughout the Triploblastica. The two-layered gastrula is a strictly ancestral

larval form — and this, as I believe and shall endeavor to show further on, is an unwarrantable assumption which ultimately leads to the most contradictory and perplexing results. That this is really Kleinenberg's meaning is, I think, proved by his reasoning throughout the entire paper. It is strikingly illustrated, for example, in his treatment of the annelid trochophore (p. 176). After pointing out certain so-called homologies between the organs of the trochophore (ring-nerve, ring-muscle, ciliated belt) and those of a medusa (ring-nerve, ring-muscle, velum or umbrella-margin), he says :—

"Giebt man diese Homologien zu, so würde man ein fertiges geschlechtsreifes Thier von der Organisation der Lopadorhynchuslarve im System gewiss entweder in die Ordnung der Hydromedusen einstellen oder wenigstens am nächsten zu diesen setzen. Die Principien der Klassifikation müssen aber dieselben bleiben, ob es sich um die Endstadien oder um die Zwischenstadien eines Evolutionscyclus handelt, und wenn ich in der Entwicklung eines Annelids eine Form finde, die von der Annelidenorganisation gar nichts besitzt, dagegen in den wesentlichsten Theilen einer Meduse gleichartig ist, so nehme ich sie auch nicht für ein Annelid sondern für eine Medusa."

In passing I may remark that such "principles of classification" lead to a complete *reductio ad absurdum* when applied to the earlier stages of development (see p. 441). Here, however, I call especial attention to the fact that the passage is devoid of meaning, either in itself or in connection with those already quoted, except under the fundamental assumption that the inner and outer layers of the larva (and of the gastrula) are respectively homologous with those of the cœlenterate body.

It appears, therefore, broadly speaking, that Balfour and Kleinenberg reason in precisely opposite directions, the premises of either being the conclusions of the other. Balfour, assuming the homology of the mesoblast (with certain reservations that do not affect the general result) is logically compelled to deny the precise homology of the ectoblast and entoblast. Kleinenberg, on the other hand, assumes the homology of the primary layers, and is then led over the same path, but in the reverse direction, to the conclusion that the mesoblast is not homologous. Kleinenberg takes the gastrula as a primary or ancestral larval form; while Balfour, if I understand him cor-

rectly, regards it as *moulded* upon the Cœlenterate type, but modified by secondary changes involved in the differentiation of the mesoblast as a distinct germ-layer.

In what direction may we seek to break away from this deadlock of opinion? It appears to me that the only course open to embryological investigation is to examine more precisely the origin of the gastrula itself; *to take as a starting-point not the two-layered gastrula, but the ovum.* The "gastrula" cannot be taken as a starting-point for the investigation of comparative organogeny unless we are certain that the two layers are everywhere homologous. Simply to assume this homology is simply to beg the question. *The relationship of the inner and outer layers in the various forms of gastrulas must be investigated not only by determining their relationship to the adult body, but also by tracing out the cell-lineage or cytogeny of the individual blastomeres from the beginning of development;* and I am convinced that many apparent contradictions that appear under the ordinary germ-layer theory will disappear when thus examined.

It is from this point of view that I have considered the development of *Nereis.* It will be shown that many important organs and systems of the annelid body can be traced back to parent blastomeres — I propose to call them *protoblasts* ("Ur-zellen") — that are differentiated long before the completion of the "gastrula" stage, and whose relations to one another can be determined with all possible accuracy. The development in fact suggests a mosaic (to use a comparison of Roux's); the ontogeny may be resolved into a series of individual organogenies, each of which takes its beginning in a single protoblast or a small group of them. I shall endeavor, therefore, first to determine the origin and mutual relationships of the individual protoblasts in the cleavage process, and, second, to trace their later history. The relation of the protoblasts to the germ-layers of the gastrula becomes afterwards a simple question.

It remains to be seen whether this method of study will be found sufficiently practicable in other animals to afford a satisfactory basis of comparison, and to what conclusions it may lead, but some interesting results have already been attained in this direction. These researches need not be fully reviewed at this point, but I must refer briefly to Whitman's epoch-making researches on the Hirudinea,[1] which not only opened a new

[1] Nos. 27, 28.

field in the study of annelid embryology, but, as I believe, formed a new point of departure for a re-examination of the entire germ-layer theory. Whitman showed (in *Clepsine*) that the entire germ-bands of the trunk could be traced back to five pairs of cells (teloblasts), whose origin in the cleavage was accurately determined. One pair (primary mesoblasts) gave rise to the mesoblast-bands; one pair (neuroblasts) to the ventral nerve-cord; two pairs ("nephroblasts") to the trunk-nephridia, and one pair (lateral teloblasts), as he conjectured, were perhaps concerned in the origin of the muscles. That an entire system of organs, such as the ventral nerve-cord, or the trunk-nephridia could be traced back to a single blastomere was a fact so extraordinary that many morphologists, Balfour among them, at first refused to credit Whitman's statements, notwithstanding the fact that the origin of the entire mesoblast from a single cell had been established in a number of cases. Later investigation, however, not only confirmed Whitman's discoveries, but extended them to other Hirudinea and to the Oligochæta; and the fact that the special interpretation placed by him upon the "nephroblasts" has been disputed does not lessen the importance and significance of the work. Whitman's researches showed that the material for complicated adult organs might be so condensed and accelerated in development as to be set apart by a single stroke, as it were, in the early stages of cleavage, long before the establishment of the gastrula; and this fact opens up a long vista of possibilities regarding the secondary modification of the gastrula stage. It may be urged that these modifications can have little general interest for the very reason that they are secondary and take place only in a highly modified type of development. The reply to this is, how do we know what is the primitive type of gastrulation? The present state of embryology certainly does not enable us to give any positive answer to this question. Whether the primary form is the epibolic or the embolic gastrula, the plakula, the unipolar or multipolar delaminate planula, or a still different type, remains to be seen; and the very fact that the differentiation of the layers is effected in such a diversity of ways proves conclusively that these early stages of development are as susceptible to secondary modification as the later. I shall show, further on, that the history of the mesoblast in *Nereis* shows how a slight

change in the character of the segmentation in one direction or another would cause the mesoblast to take its apparent origin in the one case from the entoblast, in the other from the ectoblast. Supposing these divergent changes to take place in two originally similar forms — there is every reason to believe that exactly analogous changes have taken place — would the two resulting forms of "gastrulas" be equivalent? Is the two-layered larva of *Amphioxus* equivalent to that of *Lopadorhynchus?* I think not.[1]

The development of *Nereis* has been investigated, especially by Götte (No. 9), Salensky (No. 21), myself (No. 30), and v. Wistinghausen (No. 31). Götte describes, in *N. Dumerilii*, the cleavage, the general features of the gastrulation, the origin of the mesoblast from a single cell (which, however, has probably nothing to do with the mesoblast), and the general history of the free-swimming trochophore.

Salensky did not observe the early stages of cleavage, but accurately figured some of the later stages and made an admirable study of the early larva by means of actual sections. His results differ from those of Götte on two points of fundamental importance, of which the first relates to the mesoblast-formation, the second to the axial relations of the larva. (1) Götte describes the mesoblast as arising from a single cell (produced immediately after the eight-celled stage), which, after dividing into two, passes into the cleavage-cavity and gives rise to the mesoblast-bands, precisely as in the Oligochæta and Hirudinea. According to Salensky, on the other hand, the mesoblast is formed by a proliferation of the ventral ectoblast near the lip of the blastopore (*loc. cit.* p. 568). (2) As to the axial relations, Götte describes and figures the mesoblast-bands in their earlier stages as lying horizontally (*i.e.* parallel to the prototroch), so that the antero-posterior axis as thus determined is likewise horizontal. Salen-

[1] In a recent interesting paper (No. 26) Watase has called attention to the importance of the precise examination of the early stages of cleavage by tracing the cell-lineage. I am fully in agreement with the views there set forth, though I cannot fully accept his general conclusions (*cf.* p. 455), and it appears to me that the relation between this "cytological method" and the study of the germ-layers is not very clearly explained. The phrase "cytological method" is open to some objection, since in common usage the word *cytology* is applied to the study rather of the internal phenomena of the cell than of its external relations. The terms *cytogeny*, *cytogenetic* seem to me more suitable, though perhaps open to criticism on other grounds.

sky finds, on the other hand, that the mesoblast-bands are from the first perpendicular to the prototroch, — *i.e.* at right angles to the position described by Götte.

My own results, briefly set forth in the paper referred to, differ totally from those of Götte and agree essentially with the *facts* described by Salensky, though a study of the early stages leads to a very different interpretation. (1) I showed that the mesoblast arises from a single cell, the "second proteloblast" (by no means, however, the cell described by Götte), the offspring of which give rise to a V-shaped mass of cells, the two arms of which form the mesoblast-bands. These bands have in every respect the same position and relations as described by Salensky. The apex of the V extends to the surface at the posterior lip of the blastopore, so that the mesoblast-bands apparently take origin in the ectoblast, as Salensky states. The primary mesoblast-cell itself arises in a characteristic manner from the large, left posterior macromere, precisely as in *Clepsine*, *Rhynchelmis*, and in the gasteropod *Crepidula*, recently described by Conklin (No. 4).[1] (2) At the fourth cleavage a large cell, the "first proteloblast," separates from that macromere which afterwards gives rise to the mesoblast. From this cell (which is evidently identical with the primary mesoblast of Götte) arises the entire ventral plate of the trochophore, — *i.e.* the ventral neural plates, the seta-sacs, and probably a portion of the nephridia.

These results were published in a condensed form, in connection with the discussion of other questions, as I was still engaged in the investigation of other features of the ontogeny. The paper of v. Wistinghausen appeared soon afterwards, and his results were independently attained, although my paper had been received before their publication.[2] The species investigated was the "Nereis-form" of *N. Dumerilii* (the same as Götte's species), which lays its eggs in tubes and has a suppressed trochophore, whereas Götte's studies were based upon the "Heteronereis-form," which has pelagic eggs and a free-swimming trochophore. The results confirm my own on every essen-

[1] Through the kindness of Professor Conklin, I have been enabled to examine many of his drawings and preparations in advance of the publication of the full paper.

[2] It may be added that the investigations described in the present paper were practically finished before the appearance of v. Wistinghausen's paper.

tial point. The origin and fate of the two "proteloblasts" (called "somatoblasts" by v. Wistinghausen) are the same, and so are the orientation and general history of the germ-bands, though there are certain interesting differences of detail. His orientation of the first three cleavage-planes also agrees exactly with mine, though his terminology is different, owing to a different designation of the trochophore-axes. A more extended review of his results will be given further on.

A comparison of these investigations leaves no room to doubt that Götte's orientation of the mesoblast-bands and his account of the first origin of the mesoblast are both erroneous. It is barely possible that the free-swimming trochophores of *N. Dumerilii* differ from the suppressed trochophores in regard to the early development, but this is extremely improbable in view of the fact that the suppressed trochophores agree precisely with the free-swimming trochophores of three other species (*N. limbata*, *N. megalops*, and *N. cultrifera*). I venture to assert that a comparison of Götte's figures with those given in the present paper will leave no room to doubt that the cells described by him as "primary mesoblasts" are the posterior proteloblasts (*i.e.* the first progeny of the first somatoblast, p. 407), viewed obliquely in optical section, and that the real primary mesoblasts are figured by him as ordinary ectoblast-cells at the posterior lip of the blastopore. His figures of the horizontal mesoblast-bands I will not undertake to explain.

In the present paper I shall consider mainly the external aspects of the cleavage and the general relations of the larval and adult bodies. I have devoted my attention mainly to surface views, partly because this part of the subject has hitherto been least known and has proved most fruitful of new results, partly because this part of the work demanded so great an expenditure of time and labor that I have been forced to defer to a later paper an account of the internal phenomena of cleavage and a detailed study of the differentiation of the tissues and organs.

Egg-laying. — Methods.

The eggs of *N. limbata* and *N. megalops* are discharged at night while the animals are actively swimming at the surface of the water. At Wood's Holl, Mass., the season extends at least

from June to September, the most favorable time, as far as I have been able to determine, being in August and the earlier part of September. The animals appear in abundance only on warm, still nights, and even then are rarely found unless the water has been quiet for some days. When the conditions are favorable, they come forth soon after dark and swim rapidly about at the surface, sometimes in almost incredible numbers. There is something capricious about their occurrence, and I have never been able to find a satisfactory explanation of their vagaries. Sometimes both species occur together; at other times, under apparently similar conditions, only one species will be found, or one may be very abundant and the other rare. The sexes are, as a rule, readily distinguishable, since the males are bright red and swim very rapidly, while the females are paler in color (often nearly white) and swim slowly. The males are always more abundant than the females, and I have sometimes captured thousands of males without finding a single female. The females are often actively pursued by the males, and have a habit of swimming in a circle, closely pressed by numerous males, while the eggs and spermatozoa are being discharged into the water.

The best method of procedure is to capture the adults singly with a hand-net by the light of a lantern, which serves to attract the animals, and by means of which they can be distinctly seen. The sexes should be carefully separated, since otherwise the eggs are immediately fertilized and are so thickly covered with spermatozoa that it is afterwards difficult to study them. As soon as one or two ripe males are placed with the females, the eggs and spermatozoa are discharged. The animals should then be as quickly as possible removed (so as to allow the eggs to sink), and the water thereupon repeatedly changed until all superfluous spermatozoa are removed. If the sexes are kept apart, the eggs are as a rule not discharged, and I have often endeavored to keep the animals until morning, so as to study the development by daylight. Unfortunately, however, the animals usually die when thus treated, and even if the eggs are deposited and fertilized (either naturally or artificially) on the following day, they do not develop normally. Artificial fertilization is easily effected at night, and my best material has been obtained in this way, since a minimal quantity of spermatozoa is

thus ensured. Most of the eggs I have studied were deposited about 9 P.M., but in a few cases they were not laid until an hour or two later. The cleavage was repeatedly followed through the entire night on the living ova, and most of the cleavage-stages have been observed scores of times. Owing to the warmth of the lamp, the eggs develop more rapidly under the microscope than in the aquarium, so that, by keeping the aquarium cool and taking fresh material after the completion of each division, every step in the cleavage may be observed twice or more in the same lot of eggs.

I have found it best to examine the eggs on an ordinary glass slide under a very long narrow cover-glass, one end of which is supported by wax feet. The eggs are drawn up in a pipette and run under the cover-glass from the upper end, whereupon they arrange themselves in a single layer. By moving the cover-glass the eggs may be rolled, though with some difficulty owing to the presence of oil-drops which cause the eggs to lie with the animal pole downwards. In later stages, after the larvæ begin to swim, they may be paralyzed by adding to the water a few drops of a weak solution of cocaine in dilute methyl alcohol. The cilia may thus be brought to a standstill and their arrangement easily studied. The embryos may also be colored *intra vitam* to any desired extent by adding a one per cent aqueous solution of methyl-blue to the water. This method does not give a differential staining, but is very useful in certain stages by rendering the protoplasm and cell-outlines more distinctly visible.

For preserving the embryos various methods were employed. For sections the best hardening fluids are Flemming's fluid (Fol's weaker formula), Perenyi's fluid, sublimate, and chrom-acetic acid, especially the two former. Kleinenberg's picric acid, which gives beautiful results with many annelid larvæ, and which was successfully employed by v. Wistinghausen for *N. Dumerilii*, I have found unsatisfactory. It is, furthermore, a curious and instructive fact that Lang's sublimate acetic mixture, which v. Wistinghausen found useless, works very well with the American species. These reagents were employed in the usual manner, the eggs being left in them from ten to thirty minutes.

These methods are, however, of small value in comparison

with that employed for the surface-views and optical sections, and to it I owe many important results. This is simply strong acetic acid mixed in various proportions with glycerine and water. I have usually employed a mixture of glycerine, glacial acetic acid, and water in equal parts (a modification of "Haller's fluid," suggested to me by Dr. Watase). The eggs are placed directly in this fluid and kept there until needed, *i.e.* for an indefinite period. They are perfectly fixed, without change of form, and undergo no deterioration for several weeks except a gradually increasing vacuolation of the protoplasm. For examination they are stained as follows. A number of the eggs, *still lying in the fluid*, are transferred with a pipette to a watch-glass and a few drops of Schneider's acetic carmine (saturated solution of carmine in forty-five per cent acetic acid) are added. The proper degree of staining must be determined by examination; the time required depends upon the amount of carmine added. The color should be *light* red, and I have usually found three to five minutes sufficient with a rather weak carmine. The embryos are then washed *by repeatedly changing the glycerine-acetic* fluid until all superfluous color is removed; they are then mounted, still in the fluid, under a long cover-glass as usual. They may be examined immediately, but the embryos become far more transparent if the preparation be set aside for several hours until the water evaporates, *e.g.* over night. (After a day or two the color begins to alter and to darken, and the preparation soon becomes useless.) If, now, a favorable specimen be selected, slightly compressed and carefully rolled about from side to side by displacing the cover-glass with a needle, the cells may gradually be caused to separate from one another. If the process be stopped at precisely the right point, when the cells have barely begun to separate, preparations of the utmost beauty and clearness may be obtained. The protoplasm is colored pale red, the resting nuclei and the karyokinetic figures show with perfect distinctness, and especially the cell-outlines are shown with diagrammatic clearness. In good preparations, even of comparatively late stages, every cell in the embryo may be seen and the relations of the dividing cells studied with the utmost accuracy. Most of the figures have been drawn from specimens prepared by this method, and to it is owing the relative completeness with which I have been able to study the cleavage-

process. Unfortunately the preparations cannot be kept permanently. Fairly good permanent preparations may be made by mounting (in balsam or glycerine) specimens hardened in Flemming's or Perenyi's fluid and stained with hæmatoxylin or alum cochineal; but they are not to be compared with the others. Specimens preserved in Perenyi's fluid are apt to darken in time, possibly through the action of the tannin dissolved by the alcohol when cork stoppers are used. Such specimens often make very good preparations when mounted in balsam without staining.

I. The Unsegmented Egg.

The eggs are discharged separately into the water and soon sink to the bottom, where they lie with the animal pole turned downward. Each egg is surrounded by a transparent, thick, gelatinous envelope, which causes the eggs, when in masses, to be separated by considerable spaces. Occasionally the envelopes of a number of eggs adhere closely together, so that the eggs appear as if imbedded in a common jelly, as is regularly the case with *N. cultrifera*, and some other species; this, however, is exceptional, and, as a rule, the eggs move freely amongst one another. To the periphery of the gelatinous envelope the spermatozoa adhere in great numbers, so that the eggs appear to be surrounded by a kind of halo, which, if the water is not changed, soon becomes so dense as to obscure the vitellus within it.

The vitellus is transparent, finely granular, and contains (in *N. limbata*) at least three kinds of elements besides the protoplasm. These are (1) large oil-drops, (2) small oil-drops, and (3) deutoplasm-spheres. The latter are not present in the eggs of *N. megalops*, or, if present, are invisible, owing to their transparency. The large oil-drops, which vary in number from ten or twelve to twenty or more, and also vary greatly in size, are arranged in an irregular circle (Figs. 1, 2) near the periphery of the vitellus in the lower (vegetative) half of the egg, which is thereby caused to lie with this pole turned upwards. If the egg be turned over, it soon rotates back into its original position, so that it is difficult to follow continuously the history of the upper hemisphere in the living egg. The small oil-drops (Fig. 1) are very minute, highly refractive spheres

scattered at rather wide intervals through the entire vitellus. I am unable to say whether they are of the same nature as the large drops, but intermediate forms seem not to exist. The deutoplasm-spheres (Fig. 1) are intermediate in size between the two forms of oil-drops. They are at first equally distributed through the vitellus, and only after fertilization do they begin to disappear from the upper pole (as will be described further on). In appearance they are clear and homogeneous, approximately spherical, but with rather irregular outlines, more highly refractive than the protoplasm, but much less so than the oil-drops.

When first discharged, the eggs are somewhat irregular in form. Soon, however, they become perfectly spherical with a diameter of about 0.12 to 0.14 mm. in *N. limbata*, considerably less in *N. megalops* (the eggs of both species vary considerably in size). The vitellus is at first surrounded by two membranes, the outer of which is extremely thin and delicate, the inner (*zona radiata*) much thicker and with very distinct radial striations. I shall not attempt in this place to give any account of the internal phenomena of fertilization, but there are certain peculiar external phenomena to which it may be useful to call attention. From twenty to thirty minutes after fertilization the striæ of the zona suddenly become indistinct, and in the course of two or three minutes the zona itself entirely disappears, leaving only the outer membrane. The vitellus meanwhile becomes irregular in form, and after the disappearance of the zona, assumes an amœboid appearance, so as to be here and there widely separated from the membrane. For about ten minutes slow amœboid changes of form take place, after which the vitellus again becomes perfectly spherical, but is separated from the outer membrane by a slight space. Meanwhile certain changes, preparatory to the extrusion of the polar cells, take place at the upper pole.

The freshly laid egg has a large germinal vesicle which lies excentrically, somewhat above the centre of the vitellus; a few minutes after fertilization the vesicle disappears. Towards the close of the amœboid phase the deutoplasm-spheres begin to migrate away from the upper pole, leaving a clear polar area composed of granular protoplasm. In it may be seen a few scattered oil-drops of the small form, but deutoplasm-spheres

are wholly absent. The polar area slowly increases in size until its diameter is about one-third that of the vitellus, and meanwhile a very distinct star appears in its centre. Thirty-five to forty minutes after fertilization the first polar cell is extruded, and the second follows ten or twelve minutes later. Viewed from the side at this period (Fig. 2) the vitellus appears somewhat flattened on the upper hemisphere and is separated from the membrane by a considerable space. I would call attention to the fact that the polar cells differ slightly both in size and in form, the first being pear-shaped or oval, while the second is spherical and somewhat smaller. The corresponding internal differences have not yet been investigated.

II. General Sketch of the Development.

The cleavage of the ovum takes place with a precision and regularity which oft-repeated examination only renders more striking and wonderful. Up to a stage when the foundations of all the more important organs have been established (sixty-two cells or more) the divisions take place with clock-like regularity, the only perceptible variations being slight differences in the time at which the individual blastomeres divide. Even these differences are so slight as to escape any but the closest scrutiny. As development proceeds the variations become more marked, and thus individual differences between the embryos gradually become apparent. Yet these differences, as before, are for the most part the result of slight time-variations in the development of individual blastomeres and their progeny, and, as far as can be determined, do not materially affect the end result. The entire ontogeny gives the impression of a strictly ordered and predetermined series of events, in which every cell-division plays a definite *rôle* and has a fixed relation to all that precedes and follows it.

The events of the cleavage fall into three very marked periods which I shall designate respectively as the (1) spiral, (2) transitional, and (3) bilateral periods. In the first period, which extends to the thirty-eight-celled stage, the germ-layers are completely differentiated. At the same time most of the individual blastomeres are differentiated into the parent-cells or proto-blasts from which the future organs arise. The embryological

material is, as it were, sifted out and arranged; but it is a very remarkable and interesting fact that the cell-divisions by which this is effected do not take place bilaterally in accordance with the adult structure, but show a peculiar modification of radial symmetry which is best characterized as spiral in character, and *which cannot be reduced to the bilateral type.*

The cleavage is total and unequal; no cleavage cavity is formed, the gastrulation is strictly epibolic, and the "blastopore" closes at a point that nearly coincides with the lower pole, *i.e.* 180° from the point at which the polar cells are formed. The first cleavage is transverse to the median plane of the trochophore and at right angles to the plane of the prototroch, hence horizontal with respect to the adult body, owing to a peculiar shifting of the axes, which will be described in the course of the paper. The second cleavage-plane ultimately coincides with the median plane of the trochophore and of the adult body, *though it does not divide the egg into equal halves.* The third cleavage-plane is as usual horizontal or equatorial (parallel to the prototroch) and separates four smaller micromeres above from four macromeres below.

Three sets of four "micromeres" each are successively separated from the macromeres (*A*, *B*, *C*, *D*, Diagram 1) by the following invariable law. The first four (a^1, b^1, c^1, d^1) are formed in a right-handed spiral, the second four (a^2, b^2, c^2, d^2) in a left-handed spiral, and the third set (a^3, b^3, c^3, d^3) in a right-handed spiral like the first set. (I follow Lang's terminology, as applied to the ovum of the polyclade, *Discocœlis*, with which the segmentation of *Nereis* accurately agrees up to a late stage.) From these twelve micromeres the entire ectoblast arises.

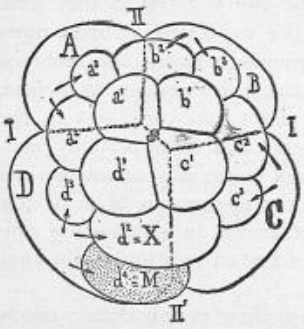


Diagram I.

Their formation is followed by the separation of the primary mesoblast, or "second somatoblast" (d^4 or *M*) from the left posterior macromere, with which event the differentiation of the germ-layers is completed and the strictly spiral period ends. The micromere d^2 or *X*, which is much larger than the

others, is the "first somatoblast," and from it arises the ventral plate.[1]

The spiral character of the cleavage is strictly maintained in the divisions of the micromeres which meanwhile take place. At the close of the period (Fig. 29) the embryo consists of thirty-eight cells, the relations of which to the germ-layers are as follows:—

4 Macromeres		= Entoblast.
34 Micromeres	33 Ectomeres	= Ectoblast.
	1 Mesomere	= Mesoblast.

Bilateral divisions now begin to appear among some of the ectomeres, but others still continue to divide spirally; *i.e.* the bilateral period is overlapped to some extent by the spiral. This period of overlapping, in which the number of cells increases from thirty-eight to fifty-eight (Figs. 30 to 38), I shall call the Transitional Period. Its principal event is the establishment of the prototrochal girdle and of a remarkable cross of cells on the upper hemisphere which gives rise in part to the cerebral ganglia.

In the third, or Bilateral Period, the divisions become essentially bilateral, and so remain as long as they can be followed. The embryo is still perfectly spherical, and its cells are not arranged according to a strict bilaterality, because they still show traces of their spiral mode of origin. Little by little, however, a complete bilaterality is established, which becomes very apparent as soon as the elongation of the body begins to take place.

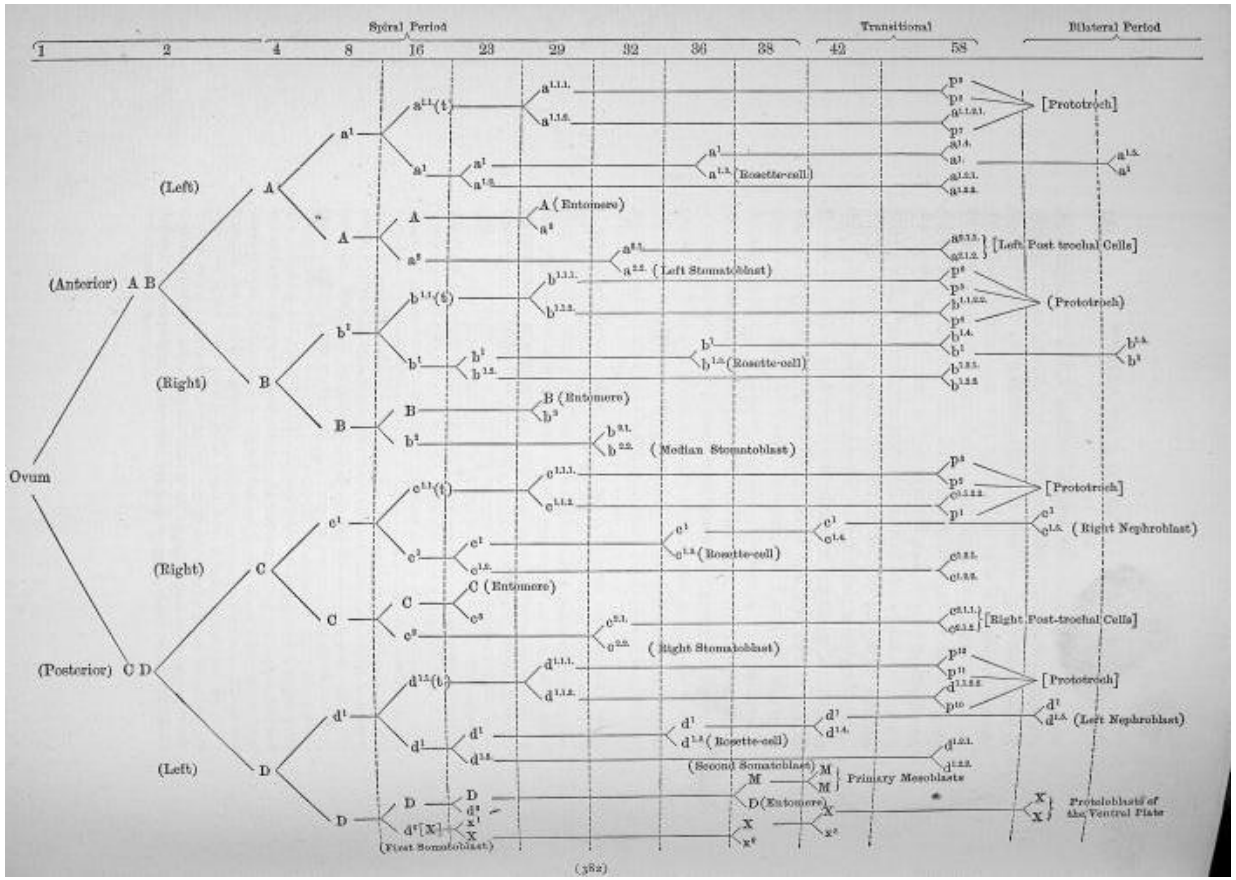
The young trochophore (Fig. 82) is nearly spherical in form. The prototroch occupies the equatorial plane, and is composed of a girdle of twelve principal cells (derived from products of the first set of micromeres). The girdle is interrupted in the median posterior (*i.e.* dorsal) line by a narrow space through which the small cells of the upper hemisphere are in continuity with those of the lower. In the centre of the upper hemisphere, at the point where the polar cells were formed, is an apical tuft of cilia. Two eye-spots are symmetrically placed on the upper hemisphere. The mouth, which opens into a large

[1] I shall adopt v. Wistinghausen's term "somatoblast" in place of the word "proteloblast" employed in my earlier paper, reserving the latter term for a different use (p. 407).

stomodæum, is in the median ventral line posterior to the prototroch. The anus is not yet formed, but its future position is indicated by a pigmented area that lies nearly in the centre of the lower hemisphere. At this point, as will be shown further on, the blastopore has closed, and here the mesoblast-bands reach the surface. The subsequent elongation of the body (Pl. XX) takes place in the vertical axis of the trochophore, which coincides with the principal axis of the ovum.

This preliminary statement will allow the following description to be made more condensed, and, I trust, more intelligible. It may be added that the spiral period of cleavage in *Nereis* is marvellously similar to the cleavage of the polyclade ovum as described especially by Götte, Selenka, and Lang, and shows an equally striking similarity to the cleavage of the molluscan ovum (Bobretzky, Blochmann, Conklin, etc.). It is important to notice, however, that although the *form* of cleavage in the spiral period of *Nereis* is identical with that of the polyclade, yet the corresponding blastomeres are only in part homologous. The significance of this very remarkable fact is discussed further on.[1]

[1] It is absolutely necessary to fix, at the outset, the terms employed in the orientation of the larva, since there is room for a wide difference of opinion which may easily lead to a confusion of terms, and the orientation I shall employ differs radically from that of v. Wistinghausen. I shall regard the principal or vertical axis of the trochophore as representing, *roughly speaking*, the dorso-ventral axis (antero-posterior, v. W.), the prototrochal plane as approximately longitudinal (dorso-ventral, v. W.), the gap in the prototroch as approximately posterior (median-dorsal, v. W.), and the opposite point as anterior (median ventral, v. W.). My orientation agrees essentially with that of Götte and Lang as applied to the polyclade larva, while v. Wistinghausen follows that of Selenka. The difference is essentially this: v. Wistinghausen orients the ovum and the trochophore directly with reference to the adult body, and thus identifies the principal or vertical axis of the ovum with the antero-posterior axis of the adult. I believe, on the other hand, that a shifting of the axes takes place, so that, *broadly speaking*, the larval axes are at right angles to those of the adult. Thus, (1) the posterior extremity, (2) the ventral region, and (3) the anterior region of the embryo and early larva become in v. Wistinghausen's terminology respectively (1) dorsal, (2) posterior, and (3) ventral. I regret this confusion of terminology, since I am perfectly in accord with v. Wistinghausen in regard to the facts. I wish, however, expressly to disclaim any intention of attaching a profound morphological significance to the terminology employed; for, as will be shown hereafter, the axes of the young embryo cannot be very accurately defined. The general axial shifting that takes place in the metamorphosis is, however, not ideal, but real—it is an actual transference of material; and if this shifting is not recognized, the terminology of the early stages becomes in the highest degree confusing and contradictory. My orientation is, therefore, adopted for purely practical reasons, *i.e.* for convenience of description.


Spiral Period
Transitional
Bilateral Period
1
2
4
8
16
23
29
32
36
38
42
58
Ovum
(Anterior)
(Posterior)
(Left)
(Right)
(Right)
(Left)
A
B
C
D
(Entomere)
(Rosette-cell)
(Left Stomatoblast)
(Median Stomatoblast)
(Right Stomatoblast)
(Second Somatoblast)
(First Somatoblast)
[Prototroch]
(Prototroch)
[Left Post-trochal Cells]
[Right Post-trochal Cells]
(Left Nephroblast)
(Right Nephroblast)
Primary Mesoblasts
Protoblasts of the Ventral Plate
(382)

A general view of the cleavage may be obtained from the accompanying diagram or cytogenetic tree (p. 382), which represents accurately the genetic relations of the blastomeres up to the complete establishment of bilaterality. The number of blastomeres in the successive stages is given in the upper line; the succession of the divisions is shown by the vertical columns. The record is without a gap up to the fifty-eight-celled stage. Beyond this point the development of the embryo as a whole cannot be fully represented in the diagram, on account of increasing variations in the order of division of the individual cells. Special diagrams showing the history of some of the individual protoblasts will be given further on.

III. Spiral Period of Cleavage

(1 to 38 blastomeres).

1. *The First Cleavage* (Figs. 2^a to 3).

The first cleavage takes place about seventy-five minutes after fertilization. The plane of division passes slightly to one side of the polar cells, and divides the egg into two unequal parts (*AB* and *CD*). The subsequent history shows that the first cleavage plane is vertical and at right angles to the median plane of the larval body, and at right angles to the prototroch. The smaller of the two cells (*AB*) is anterior ("ventral," v. Wistinghausen), the larger (*CD*) is posterior ("dorsal," v. Wistinghausen).

The division is initiated by the appearance of a shallow, transverse furrow on the upper side of the ovum, which gradually deepens and ultimately cuts through the entire vitellus from above downwards. The furrow is so placed as to divide the clear polar area into unequal parts, about one-third of it passing into the smaller cell and two-thirds into the larger. As the furrow travels downwards, a number of interesting phenomena may be observed. The furrow is at first very broad, with widely separated margins, which only approach and come into contact when the furrow has cut about one-third through the vitellus. As they approach, the margins become irregular and put forth amœboid processes, consisting mainly of clear protoplasm, though

occasionally containing a few deutoplasm-spheres. The processes of the opposing margins first come into contact near the middle of the furrow (*i.e.* near the upper pole), and are gradually flattened out against one another, so that the central portion of the furrow becomes a straight, even line. From this initial point of union, the closure of the furrow proceeds in like manner in each direction, around to the opposite side of the vitellus, after which the two blastomeres become pressed together, and the egg enters upon a resting period of fifteen to twenty minutes. The entire cleavage occupies about five minutes.

The behavior of the vitellus during the division gives the impression that the protoplasm is a viscid, tenacious mass, like pitch, which is pulled apart into two masses, against a considerable resistance. When, for example, the furrow has cut nearly through the vitellus, a narrow bridge of protoplasm is often left at the lower pole. As this bridge is cut through, the deutoplasm-spheres within its substance are drawn out into an oval shape, as if the viscid mass were being dragged out by the ends. The formation of the pseudopodial processes shows, however, that the protoplasm is actively at work, and that it is not passively sundered by attractive forces, emanating from a central point within each incipient blastomere. It is worthy of note, furthermore, that the edges of the furrow appear to be pressed together with considerable force, since the pseudopodial processes may be seen to flatten out against each other, as if urged from the rear. While this operation is taking place on the upper portion of the furrow, however, the lower portion is still cutting down through the vitellus, — a fact which likewise has some bearing on the theory of attractive centres. As the cleavage-furrow passes downward, it seems to carry with it the anterior third of the polar area which disappears between the two blastomeres as they flatten together. The remaining two-thirds still persists in the larger blastomere as an irregular, clear space. In this space I have been able to see, in some specimens, but not in all, an irregular, coarsely granular mass, which shows a marked contrast to the surrounding protoplasm, although it has no definite boundary. This mass lies somewhat to the left of the middle line, and at the next cleavage passes into the left posterior macromere (*cf.* Figs. 3 and 5). Its later history I have not followed with sufficient care, but I am tolerably certain that

it increases in bulk and ultimately passes, in part into the first somatoblast, in part into the second. It would be interesting to investigate carefully the nature and history of this peculiar structure, but for the present I must pass it by.

2. *The Second Cleavage* (Figs. 4ª, 4, 5).

The second cleavage-plane coincides with the median plane of the adult body. It divides the smaller blastomere (*AB*) into approximately equal right and left halves (*B* and *A* respectively), and the larger (*CD*) into unequal parts. Of these, the larger (*D*), on the left side, is much larger than any of the other three; the smaller (*C*), on the right, is intermediate in size between *D* and *A* or *B*. The precise mode of formation and ultimate relation of these four cells must be accurately understood, since they determine the orientation of the embryo throughout the early development, and suggest some interesting comparisons with various other animals.

As in the case of many other eggs, the division of the smaller cell takes place slightly in advance of the other, and the respective cleavage-lines of the two cells are not precisely in the same position. The second cleavage may, in fact, best be treated, not as a single cleavage of the egg as a whole, but as consisting of two rapidly succeeding divisions of the first two blastomeres. Both divisions are of the same type as the first cleavage, the furrow first appearing on the upper side and cutting thence downwards. The smaller cell begins to divide about eighty-five minutes after the fertilization. Starting from the upper side, the furrow cuts downwards somewhat obliquely towards the left side, and finally meets the first cleavage-line to the left of the lower pole. The smaller blastomere is thus divided into two parts (*A* and *B*), of which the left (*A*) appears to be slightly the smaller when seen from above.

During the division of *AB*, the larger blastomere (*CD*) begins to divide in the same manner. At first, the furrow on the upper side is nearly or quite a continuation of the cleavage-line between *A* and *B*. As it travels downwards, however (*cf.* Figs. 4, 4ª, 5), it passes slightly to the right so as to meet the first furrow at a considerable distance to the right of the cleavage-line between *A* and *B*. Thus the first cleavage-line on the lower pole is divided into

three parts, the middle of which assumes an oblique position (*f*), and constitutes the well-known "cross-furrow" ("Brechungslinie" of Rauber), which occurs in so many eggs at this stage. Meanwhile, through a slight displacement of the four blastomeres, a cross-furrow appears on the upper pole, much shorter than the lower one and *at right angles to it*. At the completion of the division, therefore, the four blastomeres have the arrangement shown in Fig. 5.

This arrangement is of great interest with reference to the law of cleavage, and is of the utmost practical importance in the orientation. At the upper pole *B* and *D* are separated, while *A* and *C* are in contact along the short cross-furrow, *which passes from left to right*. On the lower hemisphere, this arrangement is reversed, *B* and *D* being in contact along the cross-furrow, *which passes from right to left* (viewed from the upper pole), while *A* and *C* are separated. The significance of this arrangement will be discussed in Part X, and I will here only point out its importance as a means of orientation. Owing to the fact that the four primary entomeres do not divide, the lower cross-furrow remains unaltered up to a stage when the prototroch is in full activity, the mouth, eyes, and mesoblast-bands have been formed, and the segmentation of the body has become apparent (see Pl. XX, Figs. 85, 86, and preceding figures). Thus the relations of the four-celled stage to the trochophore are, I believe, unmistakable, and all the intermediate stages may be oriented with perfect certainty by means of the position of the furrow, taken in connection with the size and arrangement of the cells. Furthermore, the slight acceleration in the division of the smaller of the first two blastomeres is a safeguard against error in determining the relations of the four-celled stage to the first two cleavage-planes. I believe, therefore, that I can state with absolute certainty that the second furrow coincides with the median plane of the larval and adult bodies.

Side views of the four-celled stage (Fig. 6) show that the difference in size between the large blastomere and the others is much greater than appears from the polar view, since its vertical diameter is greater than that of the others. The oil-drops lie far down in the blastomeres, the upper portions of which still have the same structure as in the unsegmented egg.

3. *The Third Cleavage.*

In the third cleavage, which takes place about one and three-quarters hours after fertilization, the spiral character first comes clearly into view. Each of the blastomeres divides somewhat obliquely (Fig. 7^e) into unequal parts, a smaller upper micromere, which contains none of the large oil-drops, and a larger macromere below. The four micromeres thus formed (a^1, b^1, c^1, d^1), I shall call the first group of micromeres. From them arise the entire upper hemisphere of the trochophore, the head-kidneys, and the cells of the prototroch.[1]

Side views at the moment of division (Fig. 7^e) show that each micromere is displaced somewhat towards the left. This displacement increases during the division, and reaches a maximum during the succeeding resting stage (Figs. 10, 11, 12), when the four micromeres alternate with the macromeres. [This is only approximately true, since the left posterior micromere (d^1) only slightly overlaps the anterior macromere, A.] The displacement may be described as a rotation of the four micromeres through an angle of 45° to the right [the embryo being viewed from above], and the cleavage as a whole may be characterized as a right-handed spiral — *i.e.* following the hands of a watch.

I have examined the transition from the four-celled to the eight-celled stage with especial care, in order to determine if possible whether the rotation of the micromeres (which is a very common phenomenon in other animals) is a purely mechanical process caused by pressure, etc., or is rather a result of the internal phenomena of division. The result leaves little doubt that external mechanical causes cannot be regarded as the only cause of the rotation, since *the nuclear spindles show the spiral arrangement before there is any external sign of division.* Fig. 7 (from a hardened and stained specimen) shows a four-celled stage from the upper pole, immediately before the division. The upper poles of the spindles are represented by the centres of the stars, the lower by the small circular outlines. Of the four

[1] Von Wistinghausen asserts that they give rise only to the cerebral ganglia with their appendages, and hence terms them "encephaloblasts." If this be true, the development of *N. Dumerilii* must differ to an extraordinary degree from that of the American species; but it appears to me that v. Wistinghausen gives very little evidence in support of his conclusion. (See p. 435.)

spindles only that in *C* has a radial position. The other three are oblique, and already show clearly the direction of the future divisions. Figs. 8 and 9 represent in two positions an embryo in the closing stages of the division, and show the increased obliquity of the spindles. Figs. 10, 11, 12 are the completed eight-celled stage. *The primary cause of the rotation, therefore, lies within the cells*, though it may be heightened by external causes operating at the time of division, or subsequent to it.

It is an interesting fact that the two posterior micromeres, c^1 and d^1, are usually (but I believe not always) formed slightly earlier than the others. This is the first indication of a tendency, henceforward more and more marked, towards an acceleration of development in the products of the two posterior macromeres, which may be followed up to a late stage. This fact, which has been observed in embryos of several mollusks and polyclades, is especially noteworthy in the case of *Nereis*, since the *anterior* of the two primary blastomeres is always the first to divide (again as in some mollusks and polyclades); and, moreover, it is the posterior region of the annelid that in later stages longest retains the embryonic character.

4. *The Fourth Cleavage* (Figs. 13 to 18).

The fourth cleavage, which takes place about two and one-half hours after fertilization, is of extreme importance, since of the eight new blastomeres five can be identified as definite protoblasts, each of which form a new point of departure. The essential features of the cleavage are as follows: The four micromeres divide unequally in a right-handed spiral, while the four macromeres divide unequally in a left-handed spiral. An examination of Figs. 13 to 17 will obviate the need of a detailed description. The four smaller cells derived from the micromeres ($a^{1.1}$, $b^{1.1}$ $c^{1.1}$ $d^{1.1}$) (blue) *are the parent-cells of the prototroch, and hence may be termed trochoblasts.*

The four new cells derived from the macromeres (a^2, b^2, c^2, d^2 or X) may be called the second group of micromeres. Three of them (a^2, b^2, c^2) are of nearly equal size; the fourth (d^2 or X, colored brown throughout the plates) is much larger, and will henceforward be termed the *first somatoblast.* The three smaller micromeres give rise to the stomodæum and to a portion of the

ectoblast of the lower hemisphere. *The large posterior micromere or first somatoblast gives rise to the entire ventral plate of the larva, and hence to its products, the ventral nerve-cord, the seta-sacs, and probably a portion of the nephridia.*

The position of these cells should be clearly understood (*cf.* Figs. 14 and 17). The lower hemisphere is occupied by the four macromeres, *A*, *B*, *C*, *D*, which have the same arrangement as in the four-celled stage, the lower cross-furrow remaining quite unchanged. The nucleus of each lies in its upper portion (Fig. 17), towards the left side, as seen in side view. Around the upper pole lie the four primary micromeres (a^1, b^1, c^1, d^1) with the four trochoblasts adjoining them. Of the three smaller micromeres of the second group (a^2, b^2, c^2), b^2 lies in the median line in front, opposite the cleavage-line between *A* and *B*, while a^2 and c^2 lie symmetrically, one on either side, a^2 opposite the cleavage-line between *A* and *D*, c^2 opposite the corresponding line between *B* and *C*. The first somatoblast, *X*, which has assumed an oval form, occupies the posterior region of the embryo, opposite the cleavage-line between *C* and *D*, but *somewhat to the left of the middle line.* This slightly asymmetrical position of *X* is not only perfectly constant in this stage, but it may be traced for a long time in the progeny of *X*. Ultimately, however, it is rectified, and the numerous descendants of *X* are bilaterally arranged with respect to the median line of the body.

It is clear, however, that *in the sixteen-celled stage the embryo is not bilaterally symmetrical.* The arrangement of the cells is radial, but in such wise that the radii do not pass straight outward from the principal axis, but are bent to one side. This arrangement, which may be termed a spiral symmetry, is retained up to a late stage.

Extremely clear optical sections are afforded by the acetic-glycerine specimens after staining with Schneider's carmine. They show (Fig. 18) that there is no trace of a segmentation-cavity, all the cells being accurately fitted together. The protoplasm, in such preparation, shows marked differentiations. That of the primary and secondary micromeres and of the trochoblasts is granular and reddish in color. In the somatoblast the granulation of the protoplasm is coarser and the color somewhat deeper, so that there is a marked contrast between it and the others. In unstained specimens this contrast is still greater,

since the protoplasm of the somatoblast assumes a brownish tint. In either case the somatoblast can always be recognized at a glance. In the macromeres the protoplasm is now differentiated into two distinct portions, separated by a very definite boundary, — an upper granular portion, in which the nucleus lies, and a lower clear portion containing the fat-drops. The boundary-line is very irregular, since the granular protoplasm sends numerous pseudopodia-like processes into the clear substance. In living specimens deutoplasm-spheres can still be seen in all of the cells, but they have diminished in number and are less conspicuous than in earlier stages.

5. *The Fifth Cleavage* (Figs. 19 to 26).

Up to this point all of the blastomeres divide nearly simultaneously at each cleavage. Henceforward the divisions are no longer synchronous throughout the embryo. The mosaic character of the development comes more and more plainly into view, and it soon becomes necessary to consider the cleavage not as a whole, but as a series of parallel cytogenies, each of which takes its origin in a group of blastomeres as in a single protoblast. A definite thirty-two-celled stage is, nevertheless, attained, and we may therefore speak of a "fifth cleavage."

The thirty-two-celled stage is attained by a series of steps which follow a definite but not invariable order. *First*, the four central micromeres (a^1, b^1, c^1, d^1) divide unequally in a left-handed spiral (Fig. 19), thus giving rise to four smaller cells ($a^{1.2}$, $b^{1.2}$, $c^{1.2}$, $d^{1.2}$) that alternate with the four trochoblasts ($a^{1.1}$, $b^{1.1}$, $c^{1.1}$, $d^{1.1}$) and form with them an eight-celled girdle that completely surrounds the central micromeres (Fig. 20). Every stage of this division may be clearly observed in preparation by means of the nuclear figures, which are very large and distinct. The spindles have from the first the spiral arrangement shown in Fig. 19, and the position of the newly formed cells is thus predetermined before the parent-cell shows any external sign of constriction.

Second, immediately following the last division (Fig. 20), or sometimes simultaneously with it (Figs. 21, 22), the somatoblast [X] buds off a small cell (x^1) at its right-hand apex, and at the same time the two posterior micromeres of the third

group (c^3, d^3) are budded forth from C and D, respectively as shown in Figs. 20, 21, 22. The embryo now consists of twenty-three cells, of which two are median (X, b^2), ten are on the left side, and eleven on the right side. A study of the position of the spindles shows that the two new micromeres are budded off in a right-handed spiral like the first set. (The nuclear stars and spindles lie, of course, in the granular portion of the macromeres.)

Third, the two anterior micromeres of the third group (a^3, b^3) are budded forth, in a right-handed spiral, from A and B respectively. At the same time the four trochoblasts divide in a meridional plane (Fig. 23), so that the girdle (colored blue) now consists of twelve cells (twenty-nine-celled stage).

Fourth, the three smaller micromeres of the second group (a^2, b^2, c^2) divide approximately in a meridional plane (somewhat oblique), thus completing the thirty-two-celled stage (Figs. 24, 25, 26; the last two, however, are already in transition to the thirty-six-celled stage).

A careful study of the embryo through these changes shows that all of the cell-divisions conform to the spiral type. This is at once apparent in the divisions of the four macromeres and of the four primary micromeres. It is also easily seen in the divisions of the secondary micromeres (a^2, b^2, c^2, X). Each of them divides somewhat obliquely (*cf.* Figs. 25, 26, 33), so that one of the cells lies somewhat lower than the other, and in most cases the lower cell is obviously smaller than the upper. The difference in size is very great in the case of X and x^1, but is much less in the case of the others ($a^{2.1}$, $a^{2.2}$, Fig. 33). [In the specimen shown in Figs. 25, 26, on the other hand, there is no appreciable difference in size, but I have never seen a case in which the upper cell is the smaller.] If this group of cells be followed around the embryo from right to left (against the hands of a watch), the upper (larger) cell always comes first; *i.e.* the first division of the second group of micromeres takes place in a left-handed spiral, *like the second division of the first set of micromeres.*

The division of the trochoblasts, as far as I can determine, is meridional and equal; *i.e.* of a true radial type, of which the spiral type is a modification.

6. *Third Spiral Division of the Primary Micromeres; Formation of the Apical Rosette; Origin of the Second Somatoblast; Second Division of the First Somatoblast.*

The strictly spiral period of the cleavage is brought to a close by three events.

(1) The four primary micromeres (a^1, b^1, c^1, d^1) bud forth four small cells at their inner angles (at the upper pole), which arrange themselves in a very regular apical rosette, the cells of which alternate with the central micromeres (Figs. 27, 28, etc.). The position of the spindles is the same as in the first division of a^1, b^1, c^1, d^1; *i.e.* the division follows a right-handed spiral, but the character of the division is very different, since the smaller cells are formed at the central instead of the peripheral angles of the cells (*i.e.* towards instead of away from the vertical axis of the embryo). In this case the spiral character is accentuated by the fact that the cells divide in the following regular order: c^1, d^1, a^1, b^1. I have observed this several times in the living embryo, and I have also obtained several preparations like Fig. 27, in which the order of division is completely shown by the nuclear figures. (*Cf.* also Figs. 25, 26.) At a later period the apical tuft of cilia appears exactly at the point where the rosette is formed, and there can be no doubt that it arises, in part at least, from the rosette-cells. I have not been able, however, to trace the connection in detail. An almost exactly similar rosette is formed in the polyclade *Eurylepta* (Selenka, No. 22), but its fate is doubtful.

(2) During the formation of the rosette (or sometimes somewhat later) the somatoblast buds forth at its *left* apex a small cell (x^2) which lies symmetrically with x^1 (Figs. 25, 29, 31).

(3) After a short pause, a large granular cell (*M*, Figs. 29, 31, 33, 34), somewhat smaller than *X*, is budded off obliquely from the left posterior macromere (*D*). *This cell is the second somatoblast or primary mesoblast, from which the mesoblast-bands are formed.* It lies below *X* (*i.e.* anterior to it), and considerably to the left of the median line (*i.e.* the cleavage-line between *C* and *D*). Its exact position and mode of formation may be seen by a comparison of Figs. 29, 31, 33, 47, and 48.

The formation of the second somatoblast ends the spiral period of development, and it is a very significant fact that the

close of this period marks also the complete differentiation, not only of the germ-layers, but also of many of the protoblasts from which the adult organs arise. The segregation of the embryonic material is in fact so nearly completed, that this last spiral stage may be taken as a new point of departure. The embryo (Fig. 29) now consists of thirty-eight blastomeres, as follows (*cf.* also the diagram, p. 440):

4.	The macromeres A, B, C, D, or entomeres	= Entoblast.
4.	The first group of micromeres, a^1, b^1, c^1, d^1,	Ectomeres = Ectoblast.
8.	The products of the trochoblasts, $a^{1\cdot1\cdot1}$, $a^{1\cdot1\cdot2}$, $b^{1\cdot1\cdot1}$, $b^{1\cdot1\cdot2}$, $c^{1\cdot1\cdot1}$, $c^{1\cdot1\cdot2}$, $d^{1\cdot1\cdot1}$, $d^{1\cdot1\cdot2}$,	
4.	The four intermediate girdle-cells, $a^{1\cdot2}$, $b^{1\cdot2}$, $c^{1\cdot2}$, $d^{1\cdot2}$,	
4.	The rosette-cells, $a^{1\cdot3}$, $b^{1\cdot3}$, $c^{1\cdot3}$, $d^{1\cdot3}$,	
3.	The three smaller secondary micromeres, $a^{2\cdot1}$, $b^{2\cdot1}$, c^{21},	
3.	The stomatoblasts, $a^{2\cdot2}$, $b^{2\cdot2}$, $c^{2\cdot2}$,	
3.	The first somatoblast (X) and its progeny (x^1, x^2),	
4.	The four tertiary micromeres (a^3, b^3, c^3, d^3),	
1.	The second somatoblast or mesomere	= Mesoblast.
38		

Each of these ten categories of blastomeres might be taken as the starting-point for a separate description. Practically, however, it will be useful to pursue the general development somewhat further before turning to the history of the individual cytogenies. The embryo may now be termed a "gastrula," in so far as it consists of an "inner" and an "outer" layer. If I employ this term, it is, however, solely for the sake of convenience. The embryo is "two-layered" only in a conventional sense. The "outer layer" is a mes-ectoblast in which the two constituents are completely separate. The origin of the mesoblast in *Nereis* is ectoblastic (because it forms a part of the "outer layer"), or entoblastic (because it arises from one of the four entomeres), or neither (because it forms the lip of the "blastopore"), according to the reader's preference.

IV. Transition to the Bilateral Period.

As far as the development of the permanent organs is concerned, the transition from the spiral to the bilateral type of development is remarkably abrupt. It is only in the peculiar changes involved in the formation of a larval organ, the prototroch, that the spiral form of division overlaps the bilateral

period and necessitates the recognition of an intermediate stage. The formation of the prototroch is, however, so intimately connected with the first bilateral cleavages of the upper hemisphere that they must be described together.

1. (*a*) *Bilateral Division of* c^1 *and* d^1; (*b*) *Third division of the First Somatoblast;* (*c*) *Fission of the Second Somatoblast* (Figs. 30–32).

These three events occur nearly at the same time (about six hours after fertilization), though there is a certain amount of variation. (*a*) The first bilateral division always takes place in the two posterior primary micromeres (c^1 and d^1), which divide transversely into unequal parts, the anterior being the smaller. The spindles are exactly radial in position (*cf.* the spindles in the same cells in the last spiral cleavage, Fig. 27), and the anterior cells ($c^{1.4}$, $d^{1.4}$, Figs. 31, 32, 35) are perfectly symmetrical with respect to the second cleavage-plane, which now forms the median plane of the body. Since the posterior cells (c^1, d^1) long retain their superiority in size, we may continue to call them the posterior primary micromeres. The four cells thus produced form, as it were, an arch (Diagram II, p. 396), the ends of which rest upon the girdle-cells, while the keystone is formed by the posterior rosette-cell ($d^{1.3}$).

(*b*) Meanwhile a curious event happens, the exact nature of which I only made out after repeated examination, both of living specimens and of preparations. The first somatoblast (X), namely, buds forth a small cell (x^3) from its posterior or dorsal border in the median line. *This cell pushes before it the posterior intermediate girdle-cell* ($d^{1.2}$), *which is thus carried up into the arch formed by* d^1, $d^{1.4}$, $d^{1.3}$, $c^{1.4}$, c^1, *and loses its connection with the girdle* (Fig. 31). The girdle is thus reduced from twelve to eleven cells, and is interrupted in the median posterior (dorsal) line. We shall see hereafter that this interesting occurrence *is the cause of the median interruption of the prototroch* that appears to be a common feature of the trochophore larva. As a rule, this division of X takes place simultaneously with the bilateral division of c^1, d^1, and the sudden transformation of the postero-dorsal region is, in the living embryo, an interesting spectacle. Sometimes, however, the division of X follows the others, as in Fig. 31.

(*c*) In the third place, soon after the events just described, the second somatoblast or mesomere divides into equal parts (*M.M.*, Figs. 32, 48, 49, 50). These are the primary mesoblasts, which give rise to the mesoblast-bands.

The embryo now consists of forty-two cells. Though a distinctly bilateral arrangement is now apparent, the peculiar displacement of the somatoblasts towards the left is still apparent, and it is only gradually overcome as the development progresses.

2. *History of the First Group of Micromeres. Origin of the Cross and of the Prototroch.*

It will be useful at this point to recapitulate the history of the girdle, since it is immediately connected with the origin of the prototroch. The four primary micromeres first give rise (at the fourth cleavage) to four smaller cells, the trochoblasts ($a^{1.1}$, $b^{1.1}$, $c^{1.1}$, $d^{1.1}$) in a right-handed spiral division (Figs. 13, 14), and, then (at the fifth cleavage), in a left-handed spiral division, to four similar cells alternating with the trochoblasts, which may be called the intermediate girdle-cells ($a^{1.2}$, $b^{1.2}$, $c^{1.2}$, $d^{1.2}$). Thus a girdle of eight cells is formed that surrounds the central micromeres (Fig. 20). [In the figures the trochoblasts are colored a deeper blue than the others.] Later, in the course of the fifth cleavage, the trochoblasts divide in a meridional plane (Fig. 23), so that the number of cells in the girdle increases from eight to twelve.

When fully formed the prototroch is likewise found to consist of twelve cells, and I at first supposed that they were identical with the twelve girdle-cells. Further investigation proved, however, that *the four intermediate girdle-cells are drawn out of the girdle, and the prototrochal cells are formed from the eight remaining cells, derived from the trochoblasts.*

In the eight-celled girdle (Fig. 20) the cells are very regularly arranged, two girdle-cells lying opposite each micromere. After the division of the trochoblasts, however, the intermediate girdle-cells are gradually displaced, so that they first come to lie opposite the intervals between the micromeres, and then begin to extend up between them (*cf.* Figs. 23 to 30, and Diagram II). The posterior median girdle-cell is the first to be drawn out of the girdle, being pushed up between the products

of c^1 and d^1 by the median division of X, as already described (Figs. 31, 32).

Shortly after the bilateral division of the posterior micromeres, c^1 and d^1, a similar bilateral unequal division takes place in the anterior pair a^1 and b^1 (Figs. 35 to 38, Diagram II). As this takes place, the micromeres move apart both from each other and from the posterior micromeres so as to form an extremely regular and symmetrical cross, the centre of which is occupied by the apical rosette. The three remaining intermediate girdle-cells ($a^{1.2}$, $b^{1.2}$, $c^{1.2}$) are at the same time drawn up into the angles between the arms of the cross, and thus recede from the girdle, though they do not leave it entirely until somewhat later. This will be rendered clear by the accompanying diagram (No. II).

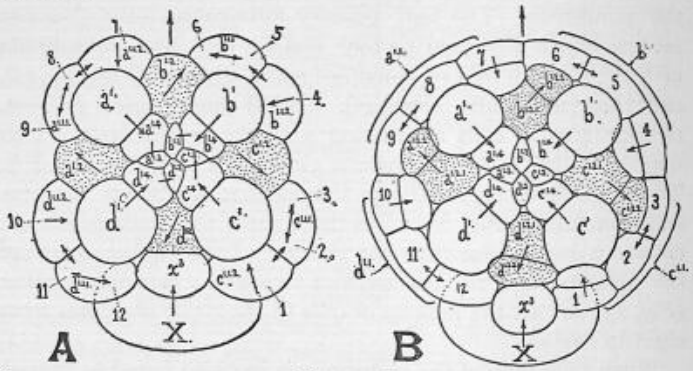


DIAGRAM II.

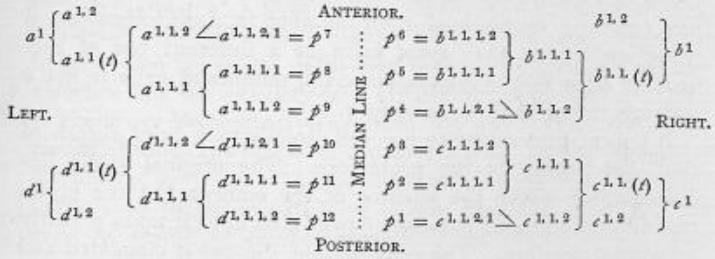
The shaded cells represent the intermediate girdle-cells and their progeny.

Nearly at the same time, the entire set of girdle-cells divide in a very interesting symmetrical manner, which is illustrated by the diagram and is shown in Figs. 35, 36, 39. In Diagram II, *A* (*cf.* Fig. 35) the direction of the division-spindles is shown by the arrows. Diagram II, *B* (*cf.* Fig. 39), shows the result. It will be seen that each intermediate girdle-cell divides in a somewhat oblique plane, and is at the same time completely drawn out of the girdle. The products of the trochoblasts meanwhile divide in such wise that in each pair of cells one divides horizontally and one vertically, in regular succession in a left-handed spiral around the girdle. Thus each of the origi-

nal trochoblasts gives rise to four cells, of which three remain in the prototroch, and the fourth lies above it in the upper hemisphere of the larva. The twelve prototrochal cells thus fall into four groups of three each, which are at first distinctly separated from each other (*cf.* Figs. 38, 41). The gaps soon close up, however, *excepting the posterior, which persists as the well-known interruption in the median dorsal (posterior) line.*

The examination of a large number of embryos has led me to believe that this law of division is perfectly constant. The order of division varies considerably, as is shown in the figures. Thus in Fig. 32 some of the posterior girdle-cells have already divided, while the anterior micromeres and girdle-cells are still quiescent, and only occasionally is a specimen found that shows the divisions as completely as in Figs. 35, 36. The division-planes, however, appear to vary only within very narrow limits.

The following scheme shows the derivation of the prototrochal cells and brings out certain curious points in their mode of origin (see also the general cytogenetic tree, p. 382).



An inspection of this scheme brings out the fact that although the prototrochal cells are bilaterally symmetrical in position, they are not so in origin, since the brackets on the two sides do not correspond. The unlettered lines lead to the cells expelled from the prototroch in the horizontal divisions (*cf.* Figs. 27, 35, 39).

The significance of the prototroch-formation remains to be seen, since nothing is known of the phenomena in other forms. The number of prototrochal cells has not hitherto been determined with certainty in a single form. Hatschek (No. 10), confirmed by Fraipont (No. 8), describes the prototroch of *Polygordius* as consisting of numerous cells. Von Drasche (No. 5)

gives several figures of the larva of *Pomatoceros*, in which the numbers of the prototrochal cells are respectively 19, 18, 17, and 15, but he does not appear to have given special attention to the point. In *Eupomatus* Hatschek figures specimens with 10, 9, and 8 prototrochal cells, but makes no mention of their number in the text. Kleinenberg states that in *Lopadorhynchus* there are usually fourteen nuclei in the prototroch (the cell-outlines could not be distinguished). The only form that I have carefully examined, besides *Polygordius* and *Nereis*, is *Hydroides* (*Serpula*) *dianthus*, Verrill, in which the prototrochal cells of the young free-swimming trochophore are eight in number.

It is easy to suggest comparisons between the eight-celled prototroch of *Hydroides*, the eight-celled girdle of *Nereis*, the eight series of vibratile plates in the Ctenophore, etc., but such comparisons have no value on account of the paucity of data. It would, however, be very interesting to investigate the comparative development of the prototroch, for it is certain that the cells vary in number in different annelids. If Kleinenberg has correctly determined their number in *Lopadorhynchus*, it would seem that they must arise by a different law of cell-division from that of *Nereis*, since the number of cells is not a multiple either of three or of four.

It will be convenient to proceed directly to a brief sketch of the later history of the prototroch. The original girdle lies considerably above the equator of the embryo, but the prototrochal girdle gradually passes downwards until it takes a nearly equatorial position. The cells become somewhat elongated and numerous vacuoles appear in them. About the tenth or eleventh hour the cilia appear and the larva begins to rotate slowly (Fig. 60). As in the case of many other annelids, the cilia are put forth through pores in the egg-membrane, which appears to persist as the cuticle of the adult worm. The development of the cilia is very sudden, the operation requiring only a few minutes. At the same time the apical cilia are put forth in the following curious manner. The egg-membrane is at this period separated from the upper side of the embryo by a considerable space. A narrow process is now rather suddenly put forth from the middle of the upper hemisphere (Fig. 60). This process is extended until it comes into contact with the mem-

brane, over a small area from which the cilia are immediately put forth. At this stage the embryo recalls the larva of *Sipunculus* at the time of the amnion-formation (see Hatschek, No. 11). The space surrounding the apical tuft is, however, soon obliterated, and the larva again becomes spherical and closely surrounded by the membrane. It seems possible, nevertheless, that this peculiar process may give the key to an explanation of the origin of the amnion in other forms.

The prototrochal cilia are put forth from the large cells alone and not from the adjoining smaller cells. Nevertheless, the girdle of large cells is accompanied by a second girdle of smaller cells lying just above it. It is only in later stages that this auxiliary row becomes clearly defined (see Figs. 77, 78, 82), when the cells are found to correspond accurately in number and position with the large cells. I much regret my failure to determine their origin, and this is one of the greatest gaps in my work. Their precise correspondence in position with the large cells makes it seem probable that they are derived from the latter by horizontal divisions; but, except in the case of $a^{1.1.2}$, $b^{1.1.2}$, $c^{1.1.2}$, and $d^{1.1.2}$, I have never seen the least evidence of such a division, although a large number of embryos were examined to determine the point. This may perhaps be taken as indirect evidence that the auxiliary cells are differentiated from the cells of the upper hemisphere — *i.e.* from the products of the intermediate girdle-cells and from the four arising by the horizontal divisions of $a^{1.1.2}$, etc. This conclusion is, however, of too great importance to be accepted on purely negative evidence and must be tested by future research. The origin of the ring-nerve and of the ring-muscle I have not yet studied.

V. Bilateral Period of Cleavage.

With the completion of the prototroch, the cleavage loses every trace of the spiral symmetry of earlier stages, and becomes strictly bilateral. The median plane, with respect to which the bilaterality manifests itself, is the plane of the second cleavage, which finally persists as the median plane of the adult body. Hence the appearance of the bilateral divisions is the first definite step in the long series of changes by which the spirally symmetrical embryo is converted into the bilateral adult.

Nevertheless, although the *form of cell-division* is strictly spiral up to the thirty-eight-celled stage, an adult bilaterality is foreshadowed, long before the bilateral divisions begin, in the arrangement of the cells. The anterior macromeres are symmetrically placed with reference to the future median plane, and so in a certain sense are the primary micromeres, though this is somewhat obscured by their rotation to the right. I may call especial attention, however, to the fact that they gradually move back into their original position (*cf.* Figs. 20, 23, 28), and are thus symmetrically placed, with reference to the median plane, before the close of the spiral period. This proves that *the rotation is but a temporary effect of mechanical conditions, and hence has no morphological meaning.*

Up to the close of the spiral period, however, the embryological material is not equally distributed on either side the future median line, mainly on account of the storage of the substance of the somatoblasts in the left posterior macromere. *Upon the separation and transference of this substance to the middle line, the asymmetry disappears, the posterior pair of entomeres being equal, like the anterior pair* (*cf.* Figs. 47, 48, 51, 55–57, 86). *Immediately afterwards the bilateral divisions begin.* This I believe to be one of the most significant and important points in the entire ontogeny.

1. *General History of the Upper Hemisphere. Origin of the Head-Kidney.*

The *first bilateral division* of the four primary micromeres and the origin of the cross have been described at p. 395. Attention may be again called to the fact that the division of the posterior micromeres (c^1, d^1) takes place some time before that of the anterior pair (a^1, b^1). *The second bilateral division of c^1 and d^1 gives rise to a pair of cells, from which arise the head-kidneys;* I shall therefore term them the *cephalic nephroblasts.* These cells (Figs. 37, 38, $c^{1.5}$, $d^{1.5}$, colored orange) are smaller than c^1 and d^1, and from the first differ from them in structure, the protoplasm being clear and watery, and showing a marked tendency to vacuolation. Shortly after this division of c^1, d^1, the anterior pair of micromeres (a^1, b^1) divide in a similar manner (Figs. 39, 40), giving rise to $a^{1.5}$ and $b^{1.5}$ respectively. At this time the

upper hemisphere contains a beautiful and symmetrical cross of cells, the four arms of which, inclined at an angle of 45° to the median plane, are the products of the four primary micromeres, a^1, b^1, c^1, d^1 respectively. The centre of the cross is occupied by the apical rosette, and each arm consists of three cells, the terminal of which is larger than the others, like a teloblast, and may still be regarded as the parent micromere. For the sake of brevity, I shall henceforward call them the *pole-cells* of the cross. The middle cell of each posterior arm is the *nephroblast* (henceforward designated as *n*).

The pole-cells still lie in contact with the prototroch; the interspaces between the arms of the cross and the prototroch are occupied by the descendants of the intermediate girdle-cells, which I have not succeeded in following in detail beyond the stage shown in Fig. 41.

The later history of the cross may be briefly treated. Both the pole-cells and their derivation continue to divide bilaterally as long as the individual cells can be followed; but, owing to the appearance of longitudinal divisions (*i.e.* parallel to the arms of the cross) in the cross-cells (*cf.* Figs. 40, 41), and to divisions in the descendants of the intermediate girdle-cells, the outlines of the cross become less distinct and finally quite indistinguishable. The pole-cells likewise ultimately disappear; the anterior pair are lost first, while the posterior pair persist, and continue their symmetrical divisions up to a late period (Fig. 59).

The second bilateral division of a^1, b^1 is accompanied by an oblique division of $c^{1.4}$, $d^{1.4}$ (Fig. 39). This is followed by *the third bilateral division* of c^1, d^1 (giving rise to $c^{1.6}$, $d^{1.6}$, Fig. 40), which is nearly longitudinal (with respect to the arms of the cross). At the same time, $a^{1.4}$ and $b^{1.4}$ divide longitudinally (Fig. 40). Next, $a^{1.5}$ and $b^{1.5}$ divide in the same plane (Fig. 41), and a little later the anterior pole-cells divide (Fig. 41) in an approximately transverse plane.

At this stage each of the original primary micromeres has undergone three spiral and three bilateral cleavages, in the last four of which the posterior pair (c^1, d^1) invariably preceded the anterior pair. Beyond this point I have not been able to follow the anterior pair (a^1, b^1), since they become lost amongst the surrounding cells. *The fourth bilateral cleavage* of c^1, d^1 (giving rise to $c^{1.7}$, $d^{1.7}$, Figs. 42, 43, 44) is nearly at right angles to

the last, as shown in the figures. Later bilateral divisions of c^1, d^1 are shown in Figs. 45 and 59, but I have not followed them in detail beyond the seventh division. There can be no doubt that the cross gives rise in large part to the cerebral ganglion. For a further account of it see p. 421.

The descendants of the intermediate girdle-cells have meanwhile continued to divide so as to fill the interspaces of the cross, but I have thus far not attempted to follow this history in detail.

2. *History of the Head-Kidney.*

We may now return to the history of the cephalic nephroblasts ($c^{1.5}$, $d^{1.5}$). From their first formation these cells are overlapped by the adjoining cells, and as the development proceeds they soon sink below the surface, and are finally entirely covered in (Figs. 38 to 45). The protoplasm meanwhile becomes clear and vacuolated and shows so marked a contrast to that of the adjoining cells that after a little practice the nephroblasts may, in good preparations, be distinguished at the first glance. In the Schneider-acetic preparations every stage of the enclosure may be clearly followed, especially in the side-views, where the exact relation of the nephroblast to the adjoining cells may be seen in optical sections by rolling the embryo from side to side. The nephroblasts meanwhile become more elongated and somewhat pointed at the ends. After sinking below the surface they slowly migrate outwards and downwards towards the prototroch, forcing their way between the outer layer of cells and the four entomeres. In this way they pass downwards until they lie quite in the lower hemisphere below the prototroch (Figs. 74–76), where the remainder of their development is accomplished.

After taking up its position below the prototroch (Fig. 75) the nephroblast rapidly elongates, extending itself forwards and backwards between the outer cells and the entomeres. It is thus converted into an elongated organ which extends about half-way around the body (Figs. 62, 63, 75–77, 79, 80–84).

Mention has already been made of the vacuolation of the protoplasm of the nephroblasts. As far as I have observed, the vacuoles always appear at one side of the cell (Figs. 74, 75), the nucleus being crowded to one side. As the nephroblast

elongates (Fig. 76), the vacuoles coalesce so as to form a sinuous canal in the protoplasm. As the elongation proceeds, the canal becomes narrower and more distinct, and the head-kidney is thus converted into a slender tube (Figs. 77, 81, 82). Up to the stage shown in Fig. 76 the head-kidney is certainly unicellular; whether the nucleus divides in later stages I cannot say.

When fully formed, the head-kidneys entirely surround the body and again lie partly inside the prototroch, as in Fig. 82. Near their anterior ends (which appear always to overlap, as shown in Fig. 79), they expand somewhat and then taper to a blunt point. Their posterior ends (Figs. 67 to 70) are pointed and terminate near the gap in the prototroch. In the latest stages in which I have observed it (Fig. 89) the head-kidney lies completely inside the prototroch, considerably flattened against the cells of the latter. Its ultimate fate remains undetermined.[1]

[1] I have termed this organ the head-kidney only with a certain reservation, since I have not succeeded in observing any evidence of cilia in the cavity, or any sign of an external opening. It is, however, very difficult to explore the interior of the organ in the living embryo on account of the confusion produced by the active vibrations of the prototrochal cilia; and the external opening of the head-kidney in other forms is notoriously difficult to see. The only other structures with which it might be confounded are the ring-nerve and the ring-muscle, with the appearance of which in other annelid trochophores I am familiar. Neither the effect of methyl-blue nor of osmic acid gives decisive results. The protoplasm shows no longitudinal striation, and the canal always distinctly appears.

It should be added that I have not been able to see either ring-nerve or ring-muscle in the *Nereis* trochophore. That the muscle is really absent is indicated by the fact that the prototrochal region is never contracted either in preserved specimens or in living larvæ, however the latter may be stimulated.

In view of the peculiar mode of origin of the "head-kidneys," I may be permitted a few words of explanation. I had observed various stages of the enclosure and migration of the nephroblasts in scores of specimens before realizing the true nature of the process, for such a migration seems from an *a priori* point of view so improbable that the possibility never occurred to me, especially as I had looked for a wholly different origin for the head-kidney. In the earlier stages I had often observed the nephroblasts projecting below the ectoblast, and in later stages the developing head-kidneys lying below the prototroch. The disappearance of the nephroblasts from their original position I supposed to be caused simply by their withdrawal into the ectoblast. I was led to the discovery of what actually occurs first, by my failure to account for the nephroblasts in the surface-views, and second, by finding stages like Fig. 74, where the nephroblast lies directly inside of the prototroch. A careful examination of the preceding stages soon showed every transition, and I believe that whatever doubt may exist as to the nature of these organs, there can be none as to their mode of origin.

Before leaving the head-kidney I may add that the vacuolation and granulation of the prototrochal cells suggest that they, too, may possess an excretory function, as has been pointed out by Hatschek in the case of *Polygordius* (No. 10). The marked tendency to vacuolation in the post-trochal cells (unfortunately I am not able to state whether the vacuoles exist in the living embryo) suggests a comparison with the so-called external or post-velar excretory cells of the molluscan veliger, with which they agree in position. In the Mollusca the cells of the velum itself are often extensively vacuolated, and it has been suggested (for a review of the literature see *McMurrich*, No. 17) that the velar cells, the post-velar cells, and the head-kidneys originally formed part of one system concerned especially with the excretion of waste matters produced by the intense activity of the velar cilia. In the Mollusca the post-velar excretory cells and the head-kidney replace one another (*i.e.* do not occur together). In *Nereis*, if the comparison has any value, the three coexist side by side, and possibly represent the primitive condition of the trochophore larva.

VI. HISTORY OF THE SOMATOBLASTS. ORIGIN OF THE VENTRAL PLATE AND OF THE MESOBLAST-BANDS. CLOSURE OF THE BLASTOPORE. DEVELOPMENT OF THE STOMODÆUM. (Plates XVII and XVIII.)

We turn now to the history of the lower hemisphere, in the course of which the most complicated and interesting events of the ontogeny take place. These events are so intimately bound together that it will be necessary to give first a brief *resumé*.

The boundary between the upper and lower hemispheres is formed by the prototroch which, as has been shown, is a product of the first group of micromeres. The superficial cells of the lower hemisphere are therefore wholly derived from the second and third sets of micromeres and from the products of the second somatoblast.

After the formation of the second somatoblast (*i.e.* at the end of the spiral period, Figs. 29, 31, 33, 49) there are in all fourteen of these cells, viz. :—

The three smaller secondary micromeres and their progeny ($a^{2.1}$, $a^{2.2}$, $b^{2.1}$, $b^{2.2}$, $c^{2.1}$, $c^{2.2}$).

The first somatoblast (X) and its progeny (x^1, x^2).
The third group of micromeres (a^3, b^3, c^3, d^3).
The second somatoblast (M).

The arrangement of these cells with respect to the blastopore is shown in Diagram III. The entomeres are considerably more than half enclosed by the other cells (Figs. 33, 34), and the open space may be called the blastopore. It will be seen from the diagram and from a comparison of the figures, that the blastopore is somewhat asymmetrical, and that its hinder lip (which is formed by the second proteloblast) lies somewhat posterior to the lower pole (which lies in the middle of the cross-furrow).

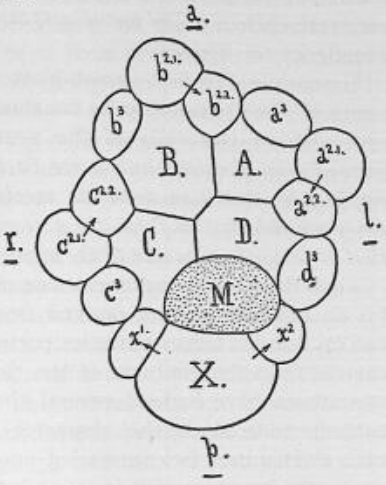


DIAGRAM III.

The closure of the blastopore takes place precisely at the lower pole (Figs. 55–57) *by convergence of the cells from all sides*, though the shape and position of the blastopore are such that the principal growth of the cells during the closure takes place from in front backwards (*i.e.* as in *Rhynchelmis* or *Clepsine*, and not as in *Lumbricus*).

The margin of the blastopore is formed at first by all of the cells of the lower hemisphere excepting x^2 (*cf.* Diagram III and Figs. 25, 26, 31, 33). As it narrows (Fig. 56), its anterior and lateral margins are formed in part by the descendants of a^3, b^3, c^3, and d^3, and in part by three large cells (*st*) *which give rise in large part at any rate to the stomodæum, and may, therefore, be called stomatoblasts.* The stomatoblasts, for reasons to be hereafter set forth, I believe to be the cells $a^{2.2}$, $b^{2.2}$, and $c^{2.2}$, or their derivatives.

The posterior lip is formed by a group of small cells that are budded forth from the primary mesoblasts derived by the bilateral division of the first somatoblast (Figs. 56, 57). *These cells*

afterwards become pigmented (see figures of later stages) and are finally pushed into the interior to form a part of the mesoblast, the remaining portion being formed from the residue of the primary mesoblasts. The mesoblast, therefore, arises from the posterior lip of the blastopore.

Meanwhile the first somatoblast undergoes an extraordinary series of the divisions, quite constant in form and order, which give rise to the cells of the ventral plate. *The cells thus formed grow forward and cover in the primary mesoblasts, coming into juxtaposition with the small cells (budded from the primary mesoblast) that form the posterior lip of the blastopore, and that afterwards give rise to the pigment-area.*

Thus the ventral region consists of two distinct portions, viz. (1) an anterior portion derived from the primary mesoblasts, and (2) a much larger posterior portion, the ventral plate proper, derived from the products of the first somatoblast. After the mesoblasts have budded several times at the surface and are entirely covered in, the character of their division changes, each divides into two somewhat unequal parts (Figs. 61, 63), of which the lower (mesial) is somewhat the larger. By continued divisions (Figs. 79–82) the two cells on each side give rise to the two mesoblast-bands, which are continuous below with the superficial cells of the pigment-area (Fig. 70). *Ultimately the pigment-cells themselves migrate inwards, and give rise, in part at least, to the splanchnic mesoblast* (Figs. 89–91). *The longitudinal muscles arise from the mesoblast-bands;* i.e. *from the products of the primary mesoblasts after the superficial cells (pigment-cells) have been budded forth.*[1]

[1] This account of the mesoblast differs somewhat from that contained in my preliminary paper, since I at first failed to observe the superficial budding of the primary mesoblasts, and described the latter as only sinking below the surface after their first division into two. This error of detail does not, however, affect the general significance of the phenomena. I may add, at this point, that the essential point of difference between v. Wistinghausen's account and my own is that he believes some of the small cells budded forth from the primary mesoblasts to be true ectoblast-cells which remain at the surface, whereas I believe that all migrate inwards, and are, therefore, mesoblast *sensu stricto*. It appears to me that v. Wistinghausen has been unduly influenced by Kleinenberg's views on this point, and I believe that *N. Dumerilii* is a far less favorable object for the investigation of the question, on account of the lack of the pigment. My own studies are, however, not yet concluded, and I hope to study other forms before publishing further accounts of the facts in *Nereis*.

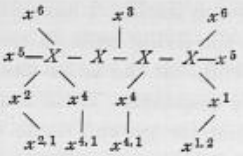
1. *The First Somatoblast.*

Nothing in the development of *Nereis* has excited my interest in a higher degree than the history of the first somatoblast, which undergoes a long series of divisions, continually changing in their character, comparable in regularity and precision with the cleavage of the ovum itself. I have followed these changes many times, both in the living embryo and in preserved specimens, and am convinced that the order and form of the divisions are almost absolutely constant. Their character is such, moreover, as to bring to the foreground some of the most interesting problems in the mechanics of cell-division.

The first three divisions have already been described, but may briefly be reviewed. The somatoblast *first* buds off a small cell (x^1) at the right side (Fig. 20), *second*, a similar cell (x^2) at the left side (Fig. 29), and *third*, a third cell (x^3) in the median postero-dorsal line (Fig. 31). These three cells are approximately 120° apart, and are nearly, but not quite, symmetrical with respect to the median line (*cf.* Figs. 32 and 48). The *fourth* division cuts the somatoblast into equal parts (Figs. 37, 38, Pl. XVI; 50, 51, Pl. XVII), which are nearly symmetrical with respect to the median plane, but always show a slight displacement towards the left. These two cells I shall call the *posterior proteloblasts* (*XX*). At the *fifth* division (Figs. 52–54) each of the proteloblasts buds forth a smaller cell (x^4, x^4) at its anterior extremity. At the *sixth* division (Figs. 42, 43, Pl. XVI) each buds forth a small cell (x^5, x^5) at its outer margin. At the *seventh* division (Fig. 54) two small cells (x^6, x^6) are budded forth at the latero-posterior angles.[1] At the *eighth* division (Figs. 46, 56–58) each proteloblast divides longitudinally into two equal parts (*XXXX*). The four cells thus formed I shall call the *posterior teloblasts*. Meanwhile x^1, x^2, x^3, and x^4, x^4 divide transversely, the result being shown in Fig. 46, Pl. XVI. The order of division varies somewhat. As a rule, x^2 (Figs. 50, 52) divides first (not x^1, as

[1] In regard to this division, there is unfortunately some uncertainty, owing to the fact, as I believe, that I overlooked the sixth division in the living embryos, and only subsequently observed it in the prepared specimens. That such a division takes place at this period in many specimens, there is no doubt. It is possible, however, that this is only a variation of the fifth division, though, in view of the precision that in general characterizes the behavior of the proteloblasts, I must regard this possibility as rather remote.

we might have expected); but sometimes the reverse is the case (Fig. 53). These are followed by x^4, x^4 (Fig. 56). At this period, therefore, the progeny of the somatoblast are approximately seventeen in number, as shown in the accompanying diagram. I say approximately, because in some cases, at any rate,



the progeny of x^2 and x^1 begin to divide before the equal division of the two X's (see Fig. 55), and it now becomes extremely difficult to distinguish certainly between them and the cells at the side of the embryo derived from the second and third sets of micromeres (*i.e.* c^3, d^3, $a^{2.2}$, $c^{2.1}$). A comparison of the figures (43, 46, 54, 55, etc.) will show the anterior x-cells passing forwards and growing over the primary mesoblasts. This is shown with perfect clearness from the side in optical section (Figs. 71 to 73, Pl. XIX). Actual sections through these stages confirm the optical sections in every respect.

During these stages the primary mesoblasts have budded several times, giving rise to the group of small secondary mesoblast-cells (Figs. 55 to 57) that form the posterior lip of the blastopore. They may be distinguished from the x-cells by their nuclei, which are smaller and appear coarsely granular on account of the character of the chromatic network. (In the figures, these nuclei are made granular, while the others are left clear.)

The *ninth* division is shown in Fig. 59. Each of the teloblasts divides transversely into unequal parts, of which the smaller is anterior. The inequality in size is much less marked than in the preceding unequal division, but it is always distinctly recognizable. This stage is usually attained from the tenth to the eleventh hour, but the specimen figured is thirteen hours old. Soon afterwards (Fig. 60) the cilia are put forth in the manner already described, and the embryo begins to rotate.

Up to this point I have repeatedly observed every division of the X-cells in the living embryos (with the exception of the

sixth, as already explained). The movements of the embryo now render this impossible, and hence the later changes have been studied mainly from preparations, though most of the stages have also been observed in the living state. At the *tenth* division (the result of which is shown in Fig. 61) each of the teloblasts again divides transversely into two unequal parts, the smaller, as before, lying in front. I do not feel certain, however, that this division really takes place (*i.e.* I may have confounded it with the last), as the cells of the ventral plate are now so numerous that it is impossible to trace the exact genetic relationships among them. The occurrence of such a division is an inference from the fact that the teloblasts are found dividing in this manner a considerable time (*i.e.* from one to two hours) after the stage shown in Fig. 59, and the cells of the ventral plate are more numerous than before. The point (which is mainly of importance in a theoretical consideration of the history of the nuclear figures) could easily be determined by a re-examination of these stages.

The *eleventh* division (the result shown in Figs. 63, 64) is one of great importance, because by it the outer pair of teloblasts disappears. Each divides lengthwise into nearly equal parts, which can scarcely be distinguished from the remaining cells of the ventral plate. I have never succeeded in finding a specimen to show the spindles of this division, and hence only infer its occurrence from the position of the cells, but I have no doubt of its occurrence. The two remaining teloblasts will henceforward be called the *residual teloblasts.*

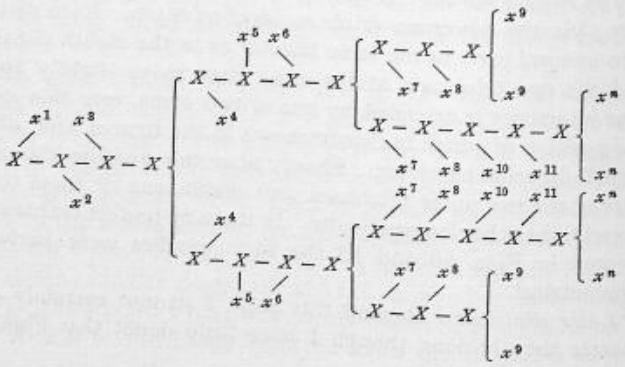
The *twelfth* division (Figs. 65, 66) marks another important step; viz. the *divergence of the residual teloblasts.* Each divides into unequal parts in the same manner as in the eighth division, and the two teloblasts at the same time move slightly apart. The interspace is occupied by one or two clear, very thin cells, the position of which is clearly shown in the figures, and which I shall describe hereafter. Shortly after this division the products of the two outer teloblasts also divide, one of them transversely, the other longitudinally. In the very perfect preparation figured in Figs. 66 and 76 the four spindles were perfectly symmetrical.

Later divisions. Beyond this point I cannot certainly enumerate the divisions, though I have little doubt that Figs. 67,

68, represent the thirteenth. The residual teloblasts continue to divide in the same manner for some time, but finally break up into small cells indistinguishable from the remaining cells of the ventral plate. The latest stage in which I could certainly distinguish them is shown in Fig. 70, but in considerably later stages (Figs. 81, 82) a group of larger cells can still be seen on either side, which is probably the product of the teloblast, and marks the site of its disappearance. *During these divisions the residual teloblasts move apart so as to leave a triangular space between them covered with small transparent cells (dor), and at the same time they gradually recede from the prototroch towards the lower pole.* This change of position in the teloblasts is of fundamental importance, since I believe the triangular area to represent the middle dorsal region of the adult body, and the residual teloblasts to mark the posterior limit of the ventral plate. *Under this interpretation, therefore, the recession of the teloblasts towards the lower pole represents the shifting or bending of the antero-posterior axis of the larva towards its definite position in the adult body.* This view is fully discussed at p. 431.

With the disappearance of the teloblasts the history of the first somatoblast may be said to close, and I will here only call attention to the side and ventral views of the various stages shown on Pl. XIX. The later history of the ventral plate will be briefly touched upon further on, but I shall defer to a future paper an account of the histological differentiation.

The following diagram gives a general view of the divisions up to the disappearance of the residual teloblasts :—



2. *The Second Somatoblast. Origin of the Mesoblast-bands.*

We may now return to the end of the spiral period of cleavage (Fig. 29) and follow the history of the second somatoblast (*M*). Its mode of formation is shown in Fig. 47 (Pl. XVII). The spindle, which lies in the granular portion of the macromere *D*, is inclined at an angle of 45° to the second cleavage (median line), and as the somatoblast forms, it is pushed over towards the right so as to lie nearly in the middle line in front of the first somatoblast. As a rule the division is completed before the formation of x^2, but I have seen some specimens in which the two cells were simultaneously formed, and one (Fig. 33) in which the somatoblast was completely separated before the appearance of the x^2-amphiaster. The *first* division of the somatoblast cuts it into two equal parts. These are the primary mesoblasts (*M. M.*, Figs. 49, 50). They are invariably displaced towards the left — probably, as I conjecture, because on the right side (Fig. 48) the second somatoblast abuts against the first and there is less resistance towards the other side.

At the *second* division each of the primary mesoblasts buds forth a small cell at the surface, near its anterior margin (Figs. 53, 72. The lessened diameter of the cells in the former figure is owing to their rounding out; in their quiescent state they are considerably flattened against the entomeres). Examination of many specimens shows that there is a good deal of variation in the direction of the spindles, and I found two specimens in which one of the spindles was longitudinal, the other exactly transverse. The budding of the mesoblasts is continued in the same way for a considerable period, until a group of small cells is formed, which lie superficially and form the posterior lip of the blastopore (Figs. 54–57, 73). They become continuous with the anterior cells of the ventral plate, but, as has been already mentioned, they can usually be distinguished from the x-cells, even before the appearance of the pigment, by the granular appearance of their nuclei.

Through this process and by the forward growth of the x-cells, the primary mesoblasts are soon covered in, and lie quite beneath the surface. At the same time the blastopore closes in completely (by a process to be described later). A longitudinal (actual) section through this stage (passing slightly to one side

of the median line and showing two of the entoblast nuclei) is shown in Fig. 88, XX. There is no segmentation cavity, and the entomeres are everywhere closely surrounded by the one-layered ectoblast. The section shows the exact relations of the primary mesoblast (*M*) to the cells of the ventral plate (*v.p.*). Just anterior to the primary mesoblast is a crowded group of cells, some of which lie below the surface and *some of which contain bluish-black pigment-granules.* This group of cells are certainly in part the offspring of the primary mesoblast and form the beginning of the pigment-area (*p.a.*) which has so great a significance in the later history of the larva. These cells I shall call the *secondary mesoblast.* Close examination shows a distinct line of demarcation between the secondary mesoblast cells and those of the ventral plate, but this boundary is not so distinct as it afterwards becomes.

The primary mesoblasts continue to bud forth secondary mesoblast cells for some time after their enclosure. At length, however, the budding ceases and the mesoblasts divide longitudinally into two somewhat unequal parts (Figs. 61, 63), of which the two mesial cells are the larger. By subsequent divisions of the same character (Figs. 77–82) *each primary mesoblast gives rise to a group of cells that extends upwards on each side along the cleavage-line between the anterior and posterior pairs of entomeres,* i.e. *along the line of the first cleavage and at right angles to the prototroch. These two groups of cells are the foundations of the mesoblast-bands, which have, therefore, from the beginning a position at right angles to the margins of the blastopore; i.e.* at right angles to their direction in *Lumbricus, Clepsine,* and *Rhynchelmis.*

Meanwhile the pigment-area increases in size and becomes very distinctly marked off from the surrounding cells (Figs. 79–82, XIX). The pigment is at first arranged in two somewhat V-shaped masses (Fig. 79), but in later stages these become more or less completely connected together and surrounded by scattered granules that occupy a fairly-well defined circular area at the lower pole of the trochophore. *The paratroch is subsequently developed around the margins of this area* (Figs. 85, 90), *and still later the anus appears in the centre of the paratrochal ring. Hence the pigment-area represents the posterior extremity of the adult body.*

I have made many actual sections through the pigment-area which clearly show the structure of the mesoblast-bands, but I figure an optical section (Fig. 70), since it shows much better the relations of the parts. The sections show that the cells of the mesoblast-bands (*m.b.*) are perfectly continuous with those of the pigment-area, from which they are no longer to be distinguished in size. The pigment-cells extend to the surface and might be described with Salensky or Kleinenberg as forming a part of the ectoblast. They are, however, sharply differentiated from the cells of the ventral plate (*v.p.*), not only by the presence of the pigment-granules, but also by a distinct line of demarcation, and the cells do not take the color (Schneider's acetic carmine) in quite the same degree.

In this paper I shall treat very briefly of the later history of the mesoblast, since I wish to leave this to a second paper. I will therefore give only such a brief sketch of the leading facts as will serve to connect the embryonic topography with the larval and adult bodies. The pigment-area remains distinct until the elongation of the body has made considerable progress (Figs. 85–89) and the mesenteron has acquired a small cavity. The pigment-cells then migrate inwards (Figs. 90, 91) and spread out upon the wall of the mesenteron, where their nuclei and outlines can easily be seen in entire specimens mounted either in glycerine or in balsam. Every stage in this progressive migration may easily be seen, and as the cells pass inwards they disappear correspondingly from the surface, though one or two small pigment-spots remain until a rather late period. The anus is finally formed in the middle of the area formerly occupied by the pigment-cells.

I have not yet fully investigated this process by means of section, and hence must speak of it with some reserve. It is possible to suppose that no actual inward migration takes place, and that the appearance is caused by the successive development of pigment in the inner cells and its concomitant disappearance from the outer. All the appearances, however, speak for an actual migration of the cells, and actual sections of the earlier stages show the cells of the pigment-area much elongated, often with the bodies of the cells lying below the surface, with long pointed ends directed outwards.

The longitudinal muscles (*l.m.*, Fig. 85) are differentiated at

a relatively early period, before the inward migration of the pigment-cells. For this and other reasons it is nearly certain that they arise from the mesoblast-bands, *i.e.* from the progeny of the primary mesoblasts after the pigment-cells have been budded forth. They form, at first, a small loose bundle on either side dorsal to the seta-sacs, whence they extend themselves over the dorsal region. The remaining differentiations of the mesoblast I shall consider hereafter.

3. *History of the Second and Third Groups of Micromeres.*

The last points to be considered in the embryonic development relate to the general history of the second and third groups of micromeres, including the formation of the stomodæum and the general relations of the blastopore. It will be convenient to begin with one of the middle stages, and to consider the earlier history afterwards.

At a stage shortly before the closure of the blastopore, three cells are always found, symmetrically disposed about the lips of the blastopore; these cells I have called the *stomatoblasts* (*st.*, Fig. 56, Pl. XVII), since from them the stomodæum is in large part, perhaps wholly, derived. One of them (*st.m.*) lies in the middle line in front, wedged into the cleavage-line between *A* and *B*. The other two (*st.r.*, *st.l.*) lie, one on either side, opposite the cleavage-lines between *B* and *C*, and *A* and *D* respectively. These cells are easily distinguishable from the surrounding cells, not only on account of their size, but also because they appear (perhaps on account of their thickness) somewhat darker and more granular than the others.

These cells have certain constant and characteristic relations to the surrounding cells, which are shown in Diagram No. IV. The space between the median and the left stomatoblasts is occupied by a group of smaller cells, most of which, at any rate, are the products of a^3 (the left anterior micromere of the third group). The corresponding space between the median and right stomatoblasts is in like manner occupied by the progeny of b^3 (the right anterior micromere of the third generation). The posterior lip of the blastopore, as has been shown, is formed by the secondary mesoblast-cells, budded forth from the primary mesoblasts. Wedged in between them and the left stomatoblast

is invariably found a larger cell (Figs. 55 to 57), that I for a long time mistook for a product of the primary mesoblast, but which is unquestionably d^3 (the left posterior micromere of the third group or one of its progeny), as will be shown later. Corresponding to it on the other side, adjacent to the right stomatoblast, is c^3 (the right posterior micromere of the third group), and between this and the secondary mesoblast-cells is a cell which I believe to be one of the progeny of x^1. It will be

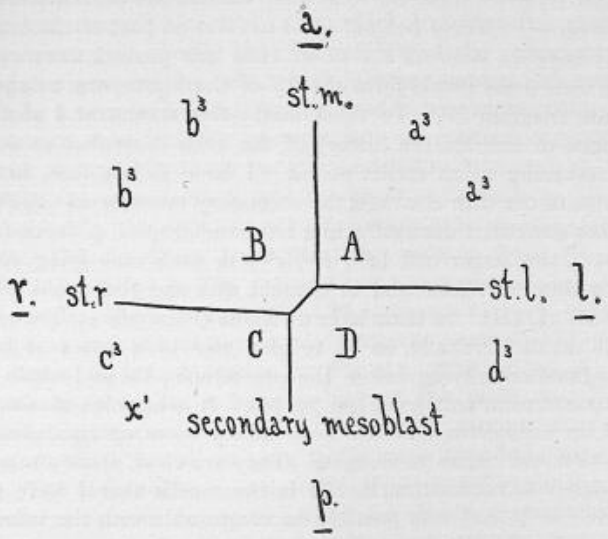


DIAGRAM IV.

observed that the margin of the blastopore is not quite symmetrical, since it includes one of the x-cells on the right side. This curious asymmetry is, I believe, quite constant, and appears to be caused simply by the displacement of the second somatoblast towards the left, so as to come into juxtaposition with d^3 and *st.l.* (Fig. 53), and thus separate x^2 from the blastopore. It is by the convergence of this circle of cells that the blastopore closes; and, before taking up the rather complicated phenomena involved in the closure, I will endeavor to trace the origin of the cells in the cleavage. The condition of the blastopore at the end of the spiral period has been described

at p. 404, and I refer again to Diagram III, which shows its marginal cells (*cf.* Figs. 25, 26, 31, 33, 36, 47 to 48: it will of course be understood that in such a Mercator's projection some distortion is inevitable, which appears mainly in the excessive size of a^3, b^3, c^3, d^3). It will be observed that $a^{2.2}$, $b^{2.2}$, and $c^{2.2}$ lie opposite the respective cleavage-lines between the macromeres, — a point of importance in the subsequent orientation. As the closure of the blastopore proceeds, $a^{2.1}$, $b^{2.1}$, and $c^{2.1}$, which from the first lie somewhat outside the others, are, as I believe, left entirely behind, so as to form no part of the blastopore-margin; whereas the other cells are pushed downwards, and their descendants form the lip of the blastopore, as shown in the Diagram IV. To substantiate this statement I shall be obliged to describe the history of the cells somewhat in detail by returning to an earlier period. I have shown that, in the course of the fifth cleavage, the secondary micromeres (a^2, b^2, c^2) divide somewhat unequally, in a left-handed spiral (as seen from above), the larger cell ($a^{2.1}$, $b^{2.1}$, $c^{2.1}$) in each case lying somewhat above the other and in contact with the girdle-cells (Figs. 25, 26, 33, III). In their later divisions (Figs. 36, 45) the upper cells divide vertically, so as to give rise to a series of large polygonal cells, lying below the prototroch; these I shall call the *post-trochal cells* (*cf.* Figs. 75, 76). It is between these cells and the entomeres that the head-kidney takes up its definitive position and begins to elongate. They are clear, show a marked tendency to vacuolation, and it is these cells that I have suggested at p. 403 may possibly be comparable with the velar or external excretory organ of the molluscan veliger.

Meanwhile the smaller or lower cells ($a^{2.2}$, $b^{2.2}$, $c^{2.2}$) also divide, but in a different manner. The first division is horizontal (more or less oblique) and also unequal, a smaller cell being budded off above, next the prototroch. That this takes place in all of three of the cells in the same manner, I only infer from their later arrangement; but I have several times observed the actual division, in the manner described, in $c^{2.2}$, and it seems fair to conclude that it holds for the others also. The first divisions of the third group of micromeres (a^3 to d^3), which meanwhile take place, are also horizontal (*cf.* Figs. 49, 50, 53).

We have now arrived at a stage, however (Figs. 52 to 55), where *$c^{2.2}$ and $a^{2.2}$ can with certainty be recognized as the right and*

left stomatoblasts respectively. I cannot state as positively that $b^{2.2}$ is the median stomatoblast, but I have no doubt that such is the case. The first division of c^3 and d^3 is shown in Fig. 50, and the connection between this and our starting-point (Fig. 55) is given by Figs. 53 and 54.

4. *Closure of the Blastopore. Formation of the Stomodæum.*

The blastopore now rapidly closes by a convergence of all these cells towards a central point, which lies slightly anterior to the lower pole, usually about the middle of the cross-furrow. The convergence of the cells, which is rather sudden, is brought about by a very interesting process, which has superficially the appearance of an embolic gastrulation. A marked depression appears around the lower pole, bounded in front and at the sides by the stomatoblasts and the small cells between them, and behind by the cells immediately covering the primary mesoblasts. This is shown from the lower pole in Figs. 62, 63, XVII, in side-view in Fig. 77; and the same embryo is shown in optical section in Fig. 78. The depression now rapidly disappears and the embryo again becomes perfectly spherical (as shown in actual section in Fig. 88, XX). This is not caused, however, by a bending out of the walls, but by a *drawing together or convergence of the cells towards a central point*, at which a distinct pore is for some time left (see Fig. 79). The stomatoblasts meanwhile divide radially, and by the curious process just described they are drawn together so as to form an arc of cells, just behind which appears the pore (Fig. 79). The small cells that occupied the floor of the depression are meanwhile crowded together and entirely change their form. Hitherto they have formed a thin pavement-epithelium over the floor of the depression. Now they are squeezed together until they assume a narrow, prismatic form, and form a kind of plug (*cf.* Figs. 78, 88) between the stomodæal arc and the primary mesoblast-cells (immediately internal to which lie the entoblast-nuclei). *The pigment appears in the cells of this plug on either side the pore as the convergence takes place, and a comparison of the figures shows from their position that they must be, in large part at any rate, the secondary mesoblast-cells.*

It is an extremely important question, but one well-nigh

impossible to decide with certainty, what is the fate of the small cells (the products of a^8 and b^3) that lie between the stomatoblasts, before their convergence. Do they pass into the pigment plug with the secondary mesoblast-cells, and if so, do they migrate inwards with them to form a part of the mesoblast? Here we have, as I believe, the only possibility in the development of *Nereis* that the mesoblast may arise from the ectoblast in Salensky's and Kleinenberg's sense. Unfortunately I cannot give a definite answer to this question. But after studying a large number of favorable surface-views (in which the history of the cells can be traced much better than in section), it is my belief that they do not pass inwards, for the following reasons: First, some of these cells certainly become wedged in between the large stomodæal cells as they converge, while others remain as a group of unpigmented cells that lie between the pore and the stomodæal arc (Figs. 79, 80, which accurately represent the cells as far as they are visible). This group of cells (as shown in Fig. 80) is enclosed by the stomodæal arc in later stages, and thus still more definitely separated from the pigment-area, and it is certain that if any contribution is made by these cells to the mesoblast, it must be of minimal amount.

The stomodæal arc rapidly enlarges by radial divisions of its cells (Fig. 80) and is soon converted into a circle with a small opening in the middle which forms the mouth (Fig. 81). The cells at the same time assume a columnar or wedge-shaped form and are partially overgrown by the neighboring small cells, so as to reach the surface only at the lips of the mouth-opening (*cf.* Figs. 82, 84, 88). Meanwhile a group of cells (*s.g.*) arises on either side the stomodæum (undoubtedly derived from it), which in later stages forms a pair of glandular masses (?) lying at the junction of the stomodæum (pharynx) and the mesenteron (Fig. 92). These may be called the *stomodæal glands.*

I will finally call especial attention to the fact that upon the demarcation of the pigment-area, which takes place at the same time as the completion of the stomodæum, it lies in immediate juxtaposition to the latter (Figs. 81, 82). The pigment-area, however, marks the posterior end of the adult body, *and hence*

the median ventral line of the adult body does not yet exist. Mouth and anal area arise side by side in the region where the blastopore closes, though the anus does not break through until they have been widely separated.

In the meantime the remaining descendants of a^3, b^3 extend themselves over the anterior and lateral regions, between the stomodæum and the prototroch. The smaller cells budded off from the median stomatoblast, and also the descendants of $b^{2.1}$ become indistinguishably intermingled with them. From this group of cells arises the ectoblast surrounding the stomodæum and forming the superficial part of the body-wall of the antero-lateral region. The postero-lateral region on each side (between the margins of the ventral plate and the prototroch) is occupied by the descendants of the post-trochal cells (offspring of $a^{2.1}$ on the left side and of $c^{2.1}$ on the right) and of c^3 and d^3. These cells are continuous behind with the small cells that separate the posterior teloblasts from the prototroch; *i.e.* the descendants of x^3 and (?) of x^5, x^6 (see p. 410). *From the latter, as I believe, arise the cells that occupy the triangular area between the two residual teloblasts after their divergence. This area afterwards forms the middle dorsal region of the trunk.*

The line of parapodia may be taken to represent the lateral region of the adult body on each side. Now, the seta-sacs (which mark the position of the parapodia) are developed from the ventral plate. *Hence the cells lying above the ventral plate on each side* (i.e. *the descendants of the post-trochal cells*) *form the lateral portion of the dorsal region of the trunk.* (This conclusion will be rendered clear by referring to the discussion of the axial relations, with the accompanying diagrams, at p. 426.)

With this my account of the cleavage closes. The general relation of the primary blastomeres to the larval and adult bodies are shown in the following table:—

Entomeres.	Mesomere. Second Somatoblast.	First Group of Micromeres.	Second Group of Micromeres.	Third Group of Micromeres.
Mesenteron.	Mesoblast-bands.	1. Prototroch. 2. Apical organ. 3. Head-kidneys. 4. General ectoblast of the upper hemisphere. 5. The cross and the cerebral ganglion.	*a*. The first somatoblast. Ventral Plate. 1. Ventral nerve-cord. 2. Seta-sacs. 3. General ectoblast of post-oral ventral, and lateral regions. 4. Portions of the nephridia(?) 5. Ectoblast of the middle dorsal trunk-region. *b*. The three smaller micromeres (a^2, b^2, c^2). 6. The stomodæum. 7. Portions of the circum-oral general ectoblast. 8. The post-trochal cells. Ectoblast of the lateral dorsal trunk-region.	Portions of the general ectoblast of the circum-oral and circum-anal regions.

VII. General History of the Metamorphosis.

As a considerable time may elapse before the publication of the second part of this paper, it seems desirable to give a sketch of the structure and metamorphosis of the larva in order to make clear its rather peculiar relations to the adult body and to justify my orientation of the early stages.

After the disappearance of the ventral depression the larva becomes perfectly spherical, and this form is retained up to the time when the stomodæal arc closes (a stage slightly later than Fig. 80, 18 hrs.). After the occurrence of this event the lower hemisphere begins to change its shape, becoming slightly flattened in front (Fig. 82), so that the most prominent point is somewhat behind the lower pole, which lies about in the centre of the pigment-area. As this marks the first step in the metamorphosis, the general structure of the larva at this stage may be briefly described. The entomeres are still undivided and

retain their original arrangement (*cf.* Fig. 86). They are everywhere closely surrounded by the ectoblast except at the sides, where the mesoblast-bands intervene (Fig. 81). The prototroch encircles the equator of the embryo; the mouth lies in the median ventral line about half-way between the prototroch and the margin of the pigment-area. There is no post-oral band of cilia, and no ventral ciliated area. The prototroch shows the usual interruption in the median dorsal line. On the upper hemisphere a pair of eye-spots have appeared, the position of which may be seen by a comparison of Figs. 82, 85, 86. On the anterior half of the upper hemisphere are five spherical bodies arranged in a regular arc, one of them lying in the median line, the others symmetrically placed on either side of it. These bodies, which I at first mistook for sense-organs, I shall call the *frontal bodies*. Each of them appears to be developed out of a single cell, in which appears a clear space like a vacuole surrounded by a layer of granular protoplasm. The clear space stains intensely with hæmatoxylin precisely like the contents of the gland-cells that occur so commonly in the larvæ of other annelids (*e.g.* in *Spirorbis* or *Terebella*); and from this fact and from their later history I am led to regard them as glands. It will be shown further on that these bodies are of great importance in the orientation of the larva, since *they mark the anterior extremity*.

The ventral plate (Fig. 81) is composed of crowded prismatic cells. Posteriorly it ends in two distinct groups of cells (*X*, Figs. 81, 82), which I believe to be the last traces of the residual teloblasts. These groups of cells are separated from the prototroch on each side by a lateral area and from each other by a median triangular area, over which the cells are very thin and delicate and show a marked contrast to those of the ventral plate. The triangular area represents the median portion of the dorsal region, the lateral areas the lateral portions of the dorsal region of the adult body. The relations of these regions to the cleavage-blastomeres have been already given.

There is still no sign of metamerism or of a body-cavity. On the upper hemisphere the ectoblast shows distinctly the beginning of the supra-œsophageal ganglia in the form of a broad transverse thickening of the ectoblast (neural plate), narrower in the middle, and extending down on either side to the prototroch. The central part bears the apical tuft of cilia, still in

full activity. The approximate outlines of the neural plate are shown by the dotted lines in Fig. 86, but there is no distinct boundary, the neural plate being indicated only by the greater depth of the cells and by their smaller size.

The head-kidneys now completely surround the body in the position shown in Fig. 82.

At this period the larvæ swim very actively, in the usual manner, rotating about the vertical axis and exhibiting a very marked heliotropism.

From this time forward I shall employ a different set of terms in the orientation (see p. 426 for the grounds on which this procedure is based). I shall designate the site of the frontal bodies as the anterior extremity, the centre of the pigment-area as the posterior, the flattened (anterior) side of the lower hemisphere as ventral, and the opposite side as the dorsal.

The general character of the metamorphosis is shown in Pl. XX. The body gradually elongates in an axis that coincides with the original vertical axis (*i.e.* at right angles to the prototroch), the pigment-area being carried away from the mouth and remaining at the posterior end of the body. Meanwhile the cells of the ventral plate grow inwards from each side towards the middle line between the pigment-area and the stomodæum, which are thus forced apart; and in the space thus formed the two halves of the ventral plate undergo a regular process of concrescence along the median ventral line, where a distinct seam may for some time be seen in the surface-views (Figs. 83, 87). The origin of the median ventral region may thus be followed step by step — almost cell by cell — from the beginning. On either side the line of concrescence appears a distinct thickening, the ventral neural plate, from which arises the ventral nerve-cord. Thus the ventral cord arises from two separate halves which have at first no connection with the cephalic neural plate.

As the elongation begins, the seta-sacs make their appearance, and with them is given the first indication of metamerism. Those of the first two somites appear first (Figs. 83, 84), the dorsal and ventral rami being represented each by a somewhat vague rounded mass of cells. Those of the third somite appear somewhat later (Fig. 85), about the same time with the differentiation of the longitudinal muscles. At the same time the

setæ appear in the two anterior pairs, and the paratroch (*par.*) appears around the margin of the pigment-area.

The setæ are protruded from the two anterior pairs of seta-sacs about the fortieth hour (Fig. 89), the others not until a considerably later period. This is followed by the appearance of the parapodial cirrhi in the second and third somites (they are never developed in the first), and at the same time two incomplete mesotrochal bands of cilia appear (Figs. 90, 91) at the posterior margin of the first and second somites respectively.

From this time forwards the body rapidly develops into the adult condition. About the sixtieth hour (Fig. 91) the anal cirrhi (*a.c.*) make their appearance inside the paratrochal ring; at the same time the cephalic tentacular cirrhi (*t.c.*) appear, *posterior to the prototroch*, two new pairs of eye-spots are developed, and the setæ of the third somite are put forth. The somites become distinctly marked off, the parapodia become prominent, and the larva now possesses a distinct head and a trunk consisting of three somites.

Certain other details in the larval development demand a brief mention. Towards the fortieth hour (or earlier) a granular reddish-brown pigment appears in the cells adjoining the large prototrochal cells, and to some extent in the peripheral portions of the ciliated cells themselves. The large ciliated cells are thus rendered extremely conspicuous (Figs. 89, 90), and may easily be counted in the living larvæ. The pigment is very irregularly distributed, and varies extremely in different individuals. It is moreover changeable in the same individual, for a sudden disturbance seems to cause a contraction either of the pigment-cells or of the pigment-granules (chromatophores?). I have often observed finely pigmented specimens swimming in a watch-glass, which when brought under a cover-glass (still actively swimming) at once became nearly colorless. As the animal grows older, the girdle of pigment becomes broken up into irregular patches, two of which, one on either side the head, usually persist until a late stage (*cf.* Fig. 92). Ultimately, however (fifteen days), the pigment disappears.

About the sixtieth hour (Fig. 90) a number of peculiar sense-hairs appear at the anterior and posterior extremities. Anteriorly there is a group of about seven short, stiff, knobbed hairs on the apical region (Fig. 90) (the apical cilia have disappeared).

In the perianal area a long, stiff flagellum (*a.f.*) appears in the middle line, and on either side of this one or two knobbed hairs, like those of the apical region. As the anal cirrhi grow forth, these knobbed hairs are borne at their tip (Fig. 91), where they remain up to the latest stages I have observed. In connection with these sense-organs, mention may also be made of a series of pigment spots that appear on either side of the latero-dorsal surface, about the middle of each somite.

In the three-segmented stage, the larva remains for a relatively long period (about twelve days), during which the only important external changes concern the development of the head (Fig. 92).

In the first place, the head changes its form, becoming at first flattened (Fig. 91) and afterwards elongated, *the frontal bodies being borne at its anterior extremity*, while the prototroch becomes interrupted above and below, so that only two lateral tufts of cilia remain. At the same time the head becomes distinctly marked off from the trunk-region by *a constriction that lies immediately anterior to the first pair of parapodia, and posterior to the tentacular cirrhi, and therefore in the lower hemisphere of the trochophore.* At a later period, a distinct segment-line encircles the body at this point (Fig. 92), and the head is completely marked off. The mouth lies far anterior to this and in front of the tentacular cirrhi, and there is no trace of a division between the head and a mouth-segment ("mund-segment"). The mouth-segment, therefore, belongs to the unsegmented part of the body, and arises morphologically as a differentiation of the head, — a result in entire accordance with my studies of *Lumbricus*. It is to be observed, further, that to the head-region belongs, not only the upper hemisphere, but also the prototroch and a portion of the lower hemisphere.

Meanwhile the frontal bodies undergo certain changes of form and finally disappear. They are at first spherical, but afterwards assume a pear-shape, the clear space extending out into the narrower portion, which is perhaps to be regarded as a kind of duct. At first separated from each other, they are later brought into contact, crowded closely together, and, after diminishing in size and becoming distorted in form, they disappear in the surrounding ectoblast.

Meanwhile the tentacular cirrhi grow out into long, slender

appendages (Fig. 92). A pair of frontal antennæ (*f.a.*) grow forth from the apical region, and a third pair of appendages (*pl.*) make their appearance just in front of the mouth. The latter differ in form in the two species, being slender and pointed in *N. megalops*, but blunt and rounded in *N. limbata*. These appendages I take to be the "palpi." They, like the antennæ and cirrhi, bear a number of knobbed sense-hairs, some of which are also found on the surface of the head itself. A pair of pigment-spots also appear on the lower side of the head.

On the upper side of the head, the supra-œsophageal or cerebral ganglia appear as an enormous bilobed thickening of the ectoblast. They are distinctly posterior to the frontal antennæ, which extend straight forwards from the anterior extremity of the body. Now, the "frontal bodies" are last seen in the interval between the bases of the developing frontal antennæ; *i.e.* they also are at the anterior extremity. In the spherical trochophore, however (Figs. 84 to 86), *they lie in front of the cephalic neural plate, and in front of the upper pole. The upper pole of the trochophore, therefore, does not coincide with the anterior extremity, but lies in the dorsal region.*

The history of the alimentary canal may be briefly dismissed. As the body elongates, the stomodæum changes its form, becoming finally drawn out until it reaches the middle of the first somite (Figs. 91, 92). The jaws appear within its cavity about the fourth day. Meanwhile the mesenteron acquires a cavity, which appears as a central space between the entomeres. I need not describe the origin of the alimentary epithelium, as this has been carefully studied by Salensky and v. Wistinghausen; but I may call attention to the history of the oil-drops. In the undivided egg there are from ten to twenty of the large oil-drops. As the segmentation proceeds, the drops in each macromere gradually fuse, until only one drop is finally left in each. The four oil-drops are arranged in two pairs, the anterior of which, contained in the anterior entomeres (*A* and *B*), are much smaller than the others. Since the drops do not change their position with the movements of the embryo, they form an admirable means of preliminary orientation, both in living and in preserved specimens. The oil-drops persist long after the appearance of the mesenteric cavity (Figs. 90, 91), but are finally absorbed. The development of the proctodæum I have not yet fully investigated (see Salensky).

I may call attention finally to the frequency with which the three-segmented larva occurs among annelids, — a fact which almost tempts one to compare it with the Crustacean *Nauplius* or the three-segmented Pycnogonid larva. In *Nereis* the three segments are well developed by the middle of the third day, while the fourth segment does not appear until about the twelfth day. During the whole of this period the larva continues to swim, though latterly much less actively than at first. The same pause after the appearance of the first three segments takes place in many other forms, and it is not easy to avoid the conclusion that this curious analogy with the *Nauplius* is more than accidental.

VIII. Axial Relations of the Nereis Trochophore.

The axial relations of the *Nereis* larva are of great interest and seem to afford new evidence on a number of important questions. At first sight they appear to present a number of features which are irreconcilable with those of the typical trochophore (*e.g.* of *Polygordius*). When carefully studied, however, these peculiarities are found not only to be easily explicable, but even to afford new data for a consideration of the axial relations of the trochophore in general. In the fully established trochopore (Diagram V, *A*, *B*) the mesoblast-bands (dotted) extend straight forwards from the pigment-area (*p.a.*), at the centre of the lower hemisphere, at right angles to the plane of the prototroch. The subsequent elongation of the body takes place in the direction of the mesoblast-axis, *m*; *i.e.* parallel to the mesoblast-bands; and the anus is formed in the centre of the space originally occupied by the pigment-area. *The pigment-area, therefore, represents the posterior extremity of the adult body.*

Thus far the larva agrees precisely with the typical trochophore of *Polygordius*, *Eupomatus*, or *Hydroides*. As soon, however, as the ventral plate is considered, we encounter an apparent contradiction. For the neural axis, *n* (which coincides with the long axis of the ventral plate, of which the neural plates form a part), is *parallel* to the prototroch, and hence *at right angles to the mesoblast-axis.* For reasons given at p. 436, it is impossible to doubt that the posterior teloblasts of *Nereis*

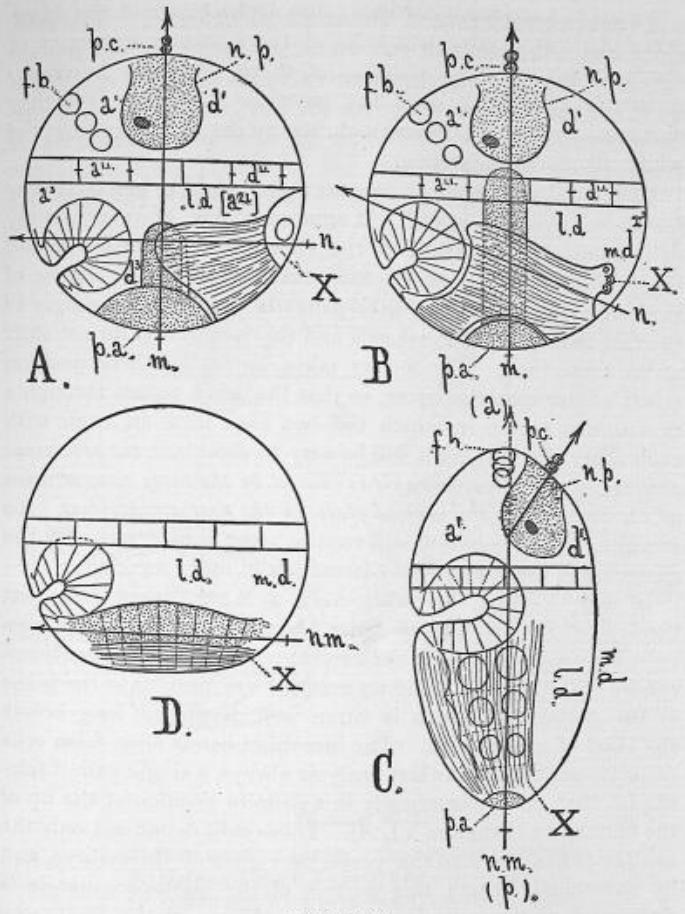


DIAGRAM V.

A. Early trochophore of *Nereis* from the left side; a^1, d^1, a^2, $a^{2\cdot1}$, $a^{1\cdot1}$, $d^{1\cdot1}$, x^2, regions derived from the corresponding micromeres; *f.b*, frontal bodies; *l.d.*, latero-dorsal region; *m.d.*, median dorsal region; *m.*, mesoblast axis; *n.*, neural axis; *n.p.*, cephalic neural plate; *p.c.*, polar cells; *p.a.*, pigment-area. The cephalic neural plates, the pigment-area, and the mesoblast bands are stippled; the ventral plate (product of *X*) shaded with lines. The dark body is the eye-spot.

B. Corresponding view of later stage. Beginning of the shifting of the neural axis. Concrescence of the ventral neural plates.

C. Diagram of the three-segmented larva (adult condition). Completion of the axial shifting, coincidence of the neural and mesoblast axes. Shifting of the frontal bodies and of the cephalic neural plates.

D. Young larva of *Lopadorhynchus* from the right side, before the shifting of the axes.

(*X*) are the homologues of the neuro-nephroblasts of the Hirudinea and Oligochæta, which lie at the posterior extremity of the germ-bands. The position of these teloblasts in *Nereis* must, therefore, also mark the posterior extremity; yet they lie in contact with the prototroch, ninety degrees away from the centre of the pigment-area.

This curious discrepancy appears at first sight to be a fatal blow for the "Achsenzoologen." It appears to me, however, simply to demonstrate the truth of the view urged by Hatschek and others that a shifting of the axes takes place in the course of the metamorphosis. The discrepancy is due in *Nereis* simply to the fact that the mesoblast axis and the neural axis do not shift at the same time. The former takes up its definitive position much earlier than the latter, so that the larva passes through a transitional period in which the two axes form an angle with each other (*B*). I think it will be easy to show that *the precocious shifting of the mesoblast-axis is caused by the early concentration of the material of the ventral plate in the first somatoblast.* To establish this proposition will require some consideration of the gastrulation and trochophore-formation in other annelids.

It may, I think, be taken as a well-established fact that throughout the trochopore series (Annelida, Mollusca) the blastopore occupies primarily the central portion of the lower hemisphere ("Gegenfeld"), and its margins are parallel to the plane of the prototroch, which is often well developed long before the close of gastrulation. The mesoblast-bands arise from cells (usually, perhaps in the last analysis always, a single pair of teloblasts) that lie symmetrically in a definite position at the lip of the blastopore (Diagram VI, *A*). These cells define not only the median plane, but also the posterior region of the embryo, and by almost all writers this portion of the blastopore-margin is designated as the posterior lip. The closure of the blastopore typically proceeds forwards from the posterior lip, in the direction of the arrow, its anterior portion usually persisting as the mouth, or marking its position. During, or sometimes before, the process of closure the mesoblast-cells pass into the cleavage-cavity, where they ultimately give rise to the mesoblast-bands.

Among the annelids the axial relations of the mesoblast-bands (and also of the neural bands) with reference to the blastopore are of two types. (1) *In the first type, typically represented by*

Lumbricus (Diagram VI, *B*) *and in a modified form by Rhynchelmis and the Hirudinea, the primary mesoblasts remain in their original position, the mesoblast-bands are parallel to the original lips of the blastopore, and the embryonic mesoblast-axis persists in the adult.* The same is true of the neural axis; for the neural bands are parallel to the mesoblast-bands, forming with the latter the principal basis of the so-called germ-bands.

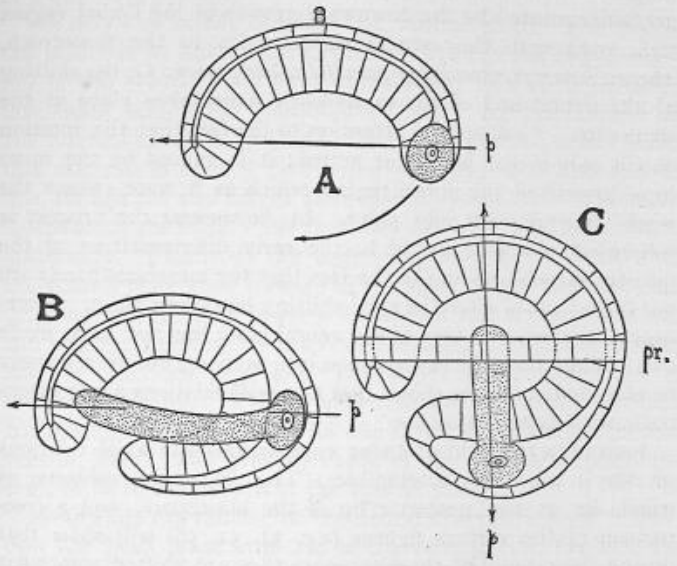


DIAGRAM VI.

A. Early annelidan gastrula. The mesoblast is shaded.
B. Horizontal type of the fœtal form (*Lumbricus*).
C. Vertical type of the larval form (*Polygordius*, etc.).

For the evidence on which this statement rests I refer to my paper on *Lumbricus* (No. 29) and to the well-known researches of Whitman and Vejdovsky.

(2) *In the second type, represented by the Polychæta in general* (Diagram VI, *C*), *the primary mesoblasts or their equivalents are sooner or later carried downwards until they lie approximately at the centre of the lower hemisphere. The mesoblast-bands are therefore at right angles to the lips of the blastopore;* i.e. *the*

mesoblast axis is rotated through 90°. The same is true of the neural axis.

The truth of this statement becomes at once apparent on considering the development of *Lopadorhynchus*, where the rotation of the axis is effected very late in the development. In the young lava (Diagram V, *D*) the mesoblastic and neural bands are horizontal and parallel to the prototroch (*cf.* Kleinenberg, No. 14). In the course of the metamorphosis both are gradually rotated by the downward growth of the dorsal region (*l.d.*, *m.d.*) until they are at right angles to the prototroch, *always, however, remaining parallel to each other; i.e.* the shifting of the neural and of the mesoblastic axes takes place at the same rate. I call special attention to the fact that the rotation of the axis is not ideal, but actual; it is caused by the more rapid growth of the dorsal region, which as it were swings the whole ventral plate into place. In *Eupomatus* the process is somewhat obscured owing to the early differentiation of the primary mesoblasts and to the fact that the mesoblast-bands are not formed until after the axial shifting has taken place. Moreover, the precise history of the neural plate has not been made out. When these facts are taken into account, however, a study of Hatschek's figures shows that the axial relations agree essentially with *Lopadorhynchus*.

Viewed in the light of these comparisons the axial relations of *Nereis* are easily intelligible. The primary mesoblasts, as usual, lie at the posterior lip of the blastopore, and a comparison of the various figures (*e.g.* 34, 51, 56) will show that during the closure of the blastopore they are shifted somewhat downwards and forwards, as is proved by their position with reference to the cross-furrow. The actual change of position is, however, very slight, since, as the figures show, *the mesoblast (second proteloblast) is from the first prevented from occupying its usual position by the huge mass of the first somatoblast.* It is accordingly segmented off from the macromere in nearly its definitive position.[1]

The shifting of the mesoblast-axis is in fact brought to pass primarily by the rotation of the cleavage-spindle (*i.e.* the plane of division), by which the somatoblast is separated from the

[1] The justice of this statement will, I think, be realized after an examination of Figs. 47 to 50. *XVII.*

entoblast, and only secondarily by the subsequent displacement of its products. This represents the opposite extreme to *Lumbricus*, where the mesoblast retains its original position. *Lopadorhynchus* occupies a middle ground. The series seems to me an interesting example of the reaction of external mechanical conditions on the form of cell-division.

The problem of inheritance which it involves is touched on in a following passage.

We may now briefly consider the ventral plate. The essential points in its history are as follows: At first the entire material of the ventral plate (first somatoblast) lies in the median line, quite behind the mesoblast (V, *A*). By the division of the somatoblast into two, and ultimately into four, equal parts, this material is equally distributed on either side the middle line, though the two halves subsequently become so intimately united along the middle line that they can only be distinguished posteriorly. Finally, as the body elongates, the two halves of the ventral plate grow forward at the sides of the pigment-area (*cf.* Diagram V, *A*, *B*), inwards between it and the stomodæum, and undergo a process of concrescence along the median ventral line, as described at p. 422. The pigment-area meanwhile is carried backwards. (The pigment-area might be said to migrate backwards, virtually cutting its way through the middle of the ventral plate. The primary cause of the change seems, however, to lie in the growth of the ventral plate.)

Meanwhile the shifting of the neural axis takes place. Mention has been made at p. 410 of the recession of the residual teloblasts from the prototroch. This, I conceive, is caused by the increasing development of the dorsal region, which has hitherto remained in a rudimentary state, and it marks the beginning of the rotation of the neural axis, as shown in Diagram V, *B*. I have not been able to follow the recession further than the stage indicated in the diagram; but when the facts of *Lopadorhynchus* are recalled, it is impossible to doubt that the region of the residual teloblasts is ultimately carried down to the region of the pigment-area.[1] The final condition

[1] This view of the axial shifting is essentially in accordance with the earlier view of Hatschek, which he now seems inclined to abandon in favor of the view that it is only the mouth and stomodæum that undergo a change of position (*Zoölogie*, III, pp. 319, 320).

is shown in Diagram V, *C*, where the neural and mesoblast axes practically coincide. It is probable that the actual space between the residual teloblasts and the pigment-area is never obliterated — *i.e.* that the rotation is completed simply by the form of growth as the body elongates. I may point out that in *Lumbricus* also the posterior extremities of the neural and mesoblastic bands are separated by a considerable space. This shows simply that the posterior extremity is not a geometrical point.

We may finally review in a few words the axial history of the upper hemisphere. The facts stated at p. 425 in regard to the frontal bodies and the cerebral ganglia render it nearly certain that the centre of the upper hemisphere lies in the dorsal surface and does not represent the anterior extremity. The polar cells are extruded at this point, where they persist up to a late stage (Fig. 41). There is no doubt that this point coincides with the centre of the cross, and von Wistinghausen's observations on *N. Dumerilii*, in connection with my own, leave little doubt that the centre of the cross coincides with the centre of the cephalic neural plate, from which the cerebral ganglia develop. These ganglia, however, lie behind the anterior extremity (p. 425, and Diagram V, *C*), the position of which is nearly indicated by the position of the frontal bodies. The anterior and posterior extremities are, however, but vaguely defined in the early larva. They, like all the other features of the body, are only gradually marked out as the development goes forward.

IX. Comparison with Other Forms.

1. *Comparison with Other Species of Nereis.*

The works of Götte and Salensky, which have already been briefly reviewed at p. 369, are too fragmentary to afford a satisfactory basis for a comparison of the early stages with the American species, and I shall therefore depend mainly upon v. Wistinghausen. Still, both the former authors observed certain important points which it is desirable to consider.

Götte (*N. Dumerilii*, free-swimming trochophore) figures correctly the first three cleavages, and gives also the true orientation of the embryo. He then describes accurately the formation

of the first somatoblast, from the left posterior macromere. With this, according to his statements, the separation of the germ-layers is completed. The first four micromeres give rise to the entire ectoblast, the somatoblast contains the entire mesoblast, while the four macromeres form the entoblast. I have given, at p. 371, reasons for regarding this account as erroneous. A careful comparison of Götte's figures with those of Salensky, v. Wistinghausen, and my own, renders it practically certain that Götte entirely overlooked the formation of the second and third sets of micromeres (the first somatoblast of course excepted), and of the second somatoblast or primary mesoblast, for which he mistook the first somatoblast. It is impossible to believe that the discrepancy is owing to the difference of species, for reasons already given.

As regards the later stages, I believe Götte's interpretation of the axial relations to be essentially correct. His account of the shifting of the axes, of the origin of the dorsal trunk-region, and of the relations of the upper hemisphere of the larva to the adult head, agree nearly with my own. The one important difference relates to the mesoblast-bands, which Götte describes and figures as horizontal, the mesoblast-axis coinciding with the neural axis and shifting with it. On this point, again, Götte is almost certainly in error, as shown by the united testimony of Salensky, v. Wistinghausen, and myself.

Salensky (*N. cultrifera*, suppressed trochophore) failed to observe the early stages of cleavage. He showed, however, that the ectoblast does not arise simply from the first four micromeres, but is increased by the separation of additional micromeres from the macromeres. He figures with perfect accuracy the two somatoblasts (Fig. 3 *n.*, Pl. XXIII, "*e*" = *X*, "*b*" = *M*) in their relations to each other and to the blastopore, and they agree precisely with the American species. In the succeeding stage (Fig. 3 *n.c*), the fission of the somatoblasts takes place exactly as in our species, the second somatoblast dividing first. The agreement is so precise and detailed that even the cell x^2 is shown ("*c*" in Salensky's figures) in the same position and dividing at the same time as in *N. limbata* and *N. megalops*. Nevertheless, Salensky did not succeed in establishing the connection between these stages and the later ones, and hence, misled by Götte's statements, he is curiously wide of the

mark in his conclusions. He correctly describes the mesoblast as arising by a proliferation of the ectoblast near the posterior lip of the blastopore. This region of the ectoblast, however, he supposed to be derived from two large *superficial* cells (*me.*, Figs. 4 *n.*, 4 *n.a.*, Pl. XXIII; their relations to the cells "*e*" and "*b*" of the last stage were not determined), which he correctly regarded as homologous to the "primary mesoblasts" of Götte. His figures leave no doubt that these cells are the two posterior proteloblasts (*X*, *X*). His conclusions, therefore, differ from Götte's mainly in the form of statement, since he regarded the "primary mesoblasts" as forming part of the "ectoderm," while Götte regarded them as of entoblastic origin.

In my preliminary paper I first showed the true relations of the somatoblasts to the germ-layers, and v. Wistinghausen's paper (suppressed trochophore of *N. Dumerilii*), which appeared soon afterwards, contained a welcome confirmation of my general results. According to this author (whose description of the first three cleavages coincides with Götte's and my own), three sets of micromeres are separated from the macromeres, and the origin of the somatoblasts is the same as in the American species. He has, however, overlooked the left posterior micromere of the third group (d^3), the place of which, as he supposes, is taken by the second somatoblast. I am persuaded that this is an error, for two reasons; first, because I also for a long time overlooked this micromere, which is very hard to see on account of its transparency and its position, wedged in as it is beside the somatoblast; and second, because a precisely corresponding micromere is found in the embryo of the gasteropod *Crepidula* (as described by Conklin), the cleavage of which agrees with that of *Nereis*, step by step, up to the close of the spiral cleavage.[1]

Regarding the fate of the somatoblasts, v. Wistinghausen's general conclusions agree closely with my own. They differ, however, in the interpretation of the "secondary mesoblast," which v. Wistinghausen calls the "untere Urzellen des Rumpfes," and which, as he asserts, give rise to the anterior portion of the ventral ectoblasteal. The primary mesoblasts (*i.e.* after separation of the secondary mesoblast) are called "myoblasts." Since the second somatoblast contains ectoblastic as well as

[1] I have since found this micromere in four other genera of Polychæta.

mesoblastic material, he denies that the two cells arising by its fission are "primary mesoblasts," homologous with those of *Lumbricus* or *Clepsine*.

A careful examination of v. Wistinghausen's descriptions and figures has not enabled me to discover the evidence on which this conclusion is based. His figures prove simply that the "myoblasts" (*i.e.* the primary mesoblasts) sink below the surface, as I have described. There is not the slightest indication of the fate of the "untere Urzellen des Rumpfes." It appears to me that v. Wistinghausen's conclusions have been influenced more by the supposed theoretical demands of the views of Kleinenberg than by actual observation. I readily grant that my own conclusions are not yet fully established. They rest, however, on definite observations, which appear to be more complete and satisfactory than those of v. Wistinghausen.

It is difficult to take as serious v. Wistinghausen's criticism on my comparison of the "posterior teloblasts" of *Nereis* to those of the Hirudinea and Oligochæta. It resolves itself simply into this: that this comparison is inadmissible because of the lack of distinct cell-rows in the ventral plate (!). It is not necessary to waste space by replying to such a criticism.

The divergence between v. Wistinghausen's results and my own becomes more serious when the fate of the micromeres is considered; but it is clear that this is owing largely to the wide and obvious gaps in his observations. The first set of micromeres ("encephaloblasts") give rise, as he asserts, only to the cephalic lobes (Kopflappen), from which are produced the cerebral ganglia and the cephalic sense-organs. The second and third sets (the somatoblasts of course excepted) "are not concerned in the formation of the body, inasmuch as they afford no essential material for the formation of individual organs or their parts." They give rise only to "the epidermis of the annelid and an embryonic structure, namely, the præ-oral ciliated belt" (*l.c.*, p. 54). These conclusions so obviously rest upon incomplete observations that they scarcely demand examination. It is clear from v. Wistinghausen's own figures that he entirely missed the three spiral cleavages of the primary micromeres, and not one of the divisions of the other micromeres is described or figured. His statements regarding the fate of the micromeres, the origin of the prototroch, stomodæum, etc., rest

merely on sections and on general views of embryos prepared by unsuitable methods; and the errors into which he has fallen are a striking illustration of the necessity of employing a method which will give clear and definite surface-views.

2. *Comparison with Other Annelida.*

Many embryologists have made observations on the early stages of cleavage among the Polychæta, but almost without exception these stages were not brought into sufficiently definite connection with the larvæ and adult structure to admit of any detailed comparison with *Nereis*. The most complete of these studies we owe to Hatschek, whose account of *Eupomatus uncinatus* gives a clear view of the general relations of the cleavage stages to the gastrula and the larva. Even in this case, however, the history of the individual blastomeres is imperfectly known, and neither the relation of the mesoblast to the four-celled stage nor the origin of the ventral plate was determined.

I turn, therefore, to the Oligochæta and Hirudinea, the early stages of which have been far more thoroughly studied than those of the Polychæta. As a basis, I shall take Whitman's work on *Clepsine* (Nos. 27, 28) and Vejdovsky's on *Rhynchelmis* (No. 25), and my task is much simplified by the fact that Whitman has himself given a full comparison between these two forms, and between *Clepsine* and the other Hirudinea.

The eight-celled stage has the same structure, and in all probability arises in the same manner, in the three forms, the only difference being the much greater relative size of the micromeres in *Nereis*. (I agree with Whitman that Vejdovsky is probably in error in his account of the second cleavage. There is no doubt of the exact agreement of *Clepsine*, *Nereis*, and many other annelids on this point.) There is, however, a remarkable difference in the axial relations, since in both *Clepsine* and *Rhynchelmis* the large macromere marks the posterior region, as in the polyclades, and the first two cleavage-planes are inclined approximately at an angle of 45° to the median plane (see p. 454).

In *Clepsine* the large macromere first gives rise to a large cell ("neuro-nephroblast") and then divides into two "primary

mesoblasts" from which the mesoblast-bands arise. The neuro-nephroblast divides into two, four, and finally eight cells (teloblasts), from which the outer strata of the germ-bands arise. The eight teloblasts arrange themselves in two groups of four each; each of these gives rise to the germ-band of the corresponding side, which is composed of four corresponding rows of cells. Of these rows the lowermost (*i.e.* the one that ultimately lies nearest the median ventral line) gives rise to the corresponding half of the ventral nerve-cord, and is, therefore, called the neural row, the corresponding teloblast being accordingly a *neuroblast*. The adjoining two rows were believed by Whitman to be concerned in the origin of the nephridia, and were hence termed nephric rows, their teloblasts *nephroblasts*. The fourth, whose history is doubtful, is the outer row, produced by the outer or lateral teloblasts. Inside these four rows is the mesoblast-band, the whole structure constituting the "germ-band." The structure and origin of the germ-band, as thus described, has been fully confirmed in the case of *Clepsine*, and I have found an almost precisely similar structure in the germ-bands of *Lumbricus* (No. 29). Bergh (No. 1) has confirmed both Whitman's account and my own, but asserts that the "nephroblasts" and lateral teloblasts give rise to the circular muscles, and hence are to be regarded as *myoblasts*.

In *Lumbricus*, unfortunately, the origin of the neuro-nephroblasts in the cleavage has not yet been determined. In *Rhynchelmis*, however, Vejdovsky describes the origin of a group of cells that, as Whitman points out, are almost certainly to be identified with them, although Vejdovsky did not determine their later history. Of these cells there are at first two ("first and second mesomeres") successively budded forth from the large macromere. This is followed by the separation from the same macromere of a "third mesomere," which divides longitudinally to form the two primary mesoblasts. The remainder of the large macromere enters into the formation of the mesenteron, as in *Nereis*. The first and second mesomeres divide so as to form *four* large cells lying in the ectoblast at the posterior extremity of the incipient germ-bands, and there is scarcely room to doubt that these four cells represent the four posterior teloblasts of *Nereis* and the eight (at one time four) of *Clepsine* and *Lumbricus*.

Now there can be no question that the ectoblastic portion of the germ-bands in *Lumbricus* and *Rhynchelmis* is the homologue, on the one hand, of the corresponding portion in the Hirudinea, and on the other hand, of that structure in the Polychæta that I have called the ventral plate. In the Hirudinea and Oligochæta this structure is from the beginning separated into two halves, which subsequently grow together along the median ventral line. In the Polychæta the two halves are always distinguishable, but sometimes (as in *Lopadorhynchus* or in *Polygordius*) they develop in their definitive position side by side; sometimes (as in *Nereis*) they are primarily in contact, but secondarily separate and again come together along the median ventral line, as in the Oligochæta.

It follows that *the first somatoblast of Nereis is the homologue of the primary neuro-nephroblast of Clepsine*, with which it precisely agrees both in mode of origin and in ultimate fate. It differs only in the fact that it gives rise on each side to two instead of four teloblasts. In *Rhynchelmis* it is represented by the first and second "mesomeres" taken together, and it would be interesting to determine whether these two are already differentiated from one another (*e.g.* one a neuroblast, the other a nephroblast or myoblast). In *Nereis* I have been unable to distinguish between the products of the inner and outer pairs of the four teloblasts; this may, however, be owing simply to the fact that the cell-rows become indistinguishably fused together.

It is clear without further discussion that *the second somatoblast of Nereis is the homologue of the "third mesomere" of Rhynchelmis and of the primary mesoblast of Clepsine before its division into two.*

Further than this I shall not carry the comparison. It has, I think, been shown that representatives of the three leading groups of annelids show an extraordinarily precise correspondence in the cleavage of the ovum, the origin of the germ-layers, and the differentiation of the principal elements of the germ-bands; and this correspondence warrants the confident expectation that the apparently aberrant mode of development represented by *Lopadorhynchus* will be found to conform to the ordinary type when a detailed study of the early stages shall have been made.

3. *General Comparisons with Other Animals.*

It is not my purpose to undertake an extended comparison of the cleavage with that of other animals, but there are certain resemblances so striking in themselves, and so interesting when carefully analyzed, that it is impossible to pass them by. These resemblances relate especially to the cleavage of the molluscan and the polyclade ovum, and they are of such importance that I give a number of diagrams to set them clearly forth. For the sake of condensation I shall pass by most of the earlier literature and select as types the development of the Polyclade, *Discocœlis*, as described by Lang (No. 15), of the gasteropod *Neritina* (Blochmann, No. 2), and of the gasteropod *Crepidula* (Conklin, No. 4).[1]

Up to a late stage in the spiral period (twenty-eight cells) every individual blastomere and every cell-division is represented by a corresponding blastomere and a corresponding cell-division in the embryo of the polyclade, and in that of the gasteropod. In all three the first two cleavages and the upper and lower cross-furrows have the same relations. In all, three groups of four micromeres each are successively separated from the macromeres, — the first group in a right-handed spiral, the second in a left-handed spiral, and the third in a right-handed spiral, like the first. The micromeres of the second and third groups alternate with one another so as to form an outer belt of eight cells that surrounds the four primary micromeres (Diagram VII, *A*, *B*).

In all, the primary or central micromeres likewise undergo three spiral cleavages, the first right-handed, the second left-

[1] I much regret that I cannot make full use of Conklin's beautiful studies of the gasteropod cleavage, since they have not yet been published in full. The work has been carried on at Wood's Holl at the same time with my own studies on *Nereis;* and to Professor Conklin's courtesy I owe the opportunity to examine his entire set of unpublished drawings, and to verify their accuracy in respect to some of the most important stages by the study of the original preparations. This is by far the most accurate and complete study of the molluscan cleavage that has thus far been made. The resemblance to the *Nereis* cleavage is so close and extends over so long a period as to be most extraordinary. Since, however, the figures may not be published for some time, I am compelled for the present to take Blochmann's *Neritina* as my principal basis of comparison. I may add that, in the course of the past summer, Conklin has ascertained that the first cleavage-plane in *Crepidula* is not longitudinal, as he at first described it, but transverse, precisely as in *Nereis*. The preparations he has shown me seem to leave no room for doubt on this point.

handed, and the third right-handed again. The first two sets of cells thus produced alternate with each other, and thus form a second or inner belt of eight cells (*C, D. E*), interposed between

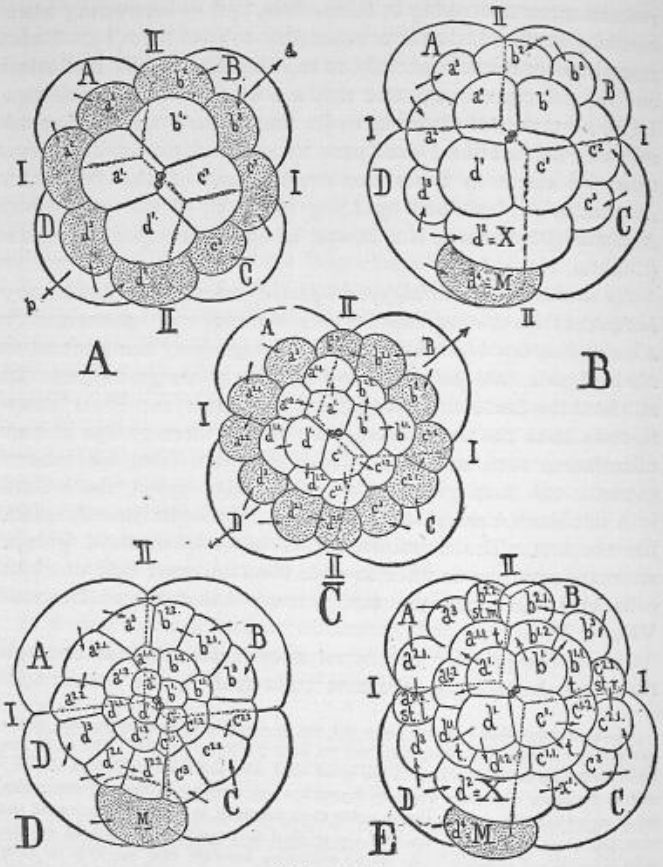


DIAGRAM VII.

A. Diagram of the polyclade embryo, showing the relations of the micromeres to the macromeres. Mesoblast shaded. The Roman numerals designate the cleavage-plans.

B. Corresponding diagram of the *Nereis* embryo.

C. Twenty-eight-celled stage of polyclade.

D. Molluscan embryo after the differentiation of the germ-layers.

E. Corresponding diagram of *Nereis*.

the central (primary) micromeres and the outer belt. Finally, the second set of micromeres divide meridionally, and in *Nereis* and *Crepidula* this can be distinctly recognized as a left-handed spiral. Thus arise four pairs of cells, with which alternate the four micromeres of the third set; and the outer belt now consists of twelve cells, which form the lips of the blastopore (*C*, *D*, *E*).

All three agree, furthermore, in the meridional division of the first-formed progeny of the primary micromeres ($a^{1.1}$, $b^{1.1}$, $c^{1.1}$, $d^{1.1}$), so that the inner belt is likewise composed of twelve cells. The third spiral cleavage of the primary micromeres gives rise to four apical cells (the "rosette-cells" of *Nereis*, the "Scheitelzellen" of *Eurylepta* and *Discocœlis*), which strikingly recall the four apical cells of the *Echinus* embryo (Selenke, No. 23). At this point the polyclade diverges from the annelid and mollusk. In the former, the differentiation of the germ-layers is complete. In the latter two, one further step is taken, by the separation of the primary mesoblast-cell from the left posterior macromere.

The general later history of the blastomeres thus formed is as follows: *In the polyclade the first group of micromeres gives rise to the entire ectoblast, the second and third groups to the mesoblast, the macromeres to the entoblast. In the mollusk and annelid, on the other hand, the second and third groups of micromeres give rise to ectoblast, like the first set, and the mesoblast arises subsequently.* This remarkable divergence between the polyclade on the one hand and the mollusk and annelid on the other is a fact of capital importance, for it proves that cells having precisely the same origin in the cleavage, occupying the same position in the embryo, and placed under the same mechanical conditions, may nevertheless differ fundamentally in morphological significance. We cannot escape the conclusion that the cell possesses a definite hereditary tendency upon which primarily its nature depends, however much its outward form or mode of division may be affected by the mechanical conditions of its environment in the body; and full weight must be given to this heredity in every attempt to interpret the origin and meaning of cleavage-forms.

Let us now pursue the comparison between the annelid and the mollusk. Blochmann describes and figures on the upper hemisphere of the *Neritina* embryo a perfectly symmetrical cross

of cells, which Conklin has also found in *Crepidula*. The arms of the cross meet at a right angle, in the centre of the upper hemisphere, and lie directly above the four respective macromeres, as in *Nereis* — *i.e.* they are inclined at an angle of 45° to the first two cleavage-planes (see Blochmann's Fig. 53). The principal portion of each arm likewise consists primarily of three cells, precisely as in *Nereis*. The resemblance is in fact so precise that at first I had no doubt the two were perfectly homologous. An exact comparison shows, however, that *the cross-cells have a totally different origin in the two cases*. In *Nereis*, the four arms arise respectively from the four primary micromeres, after the completion of their three spiral divisions, and the remains of the four micromeres (a^1, b^1, c^1, d^1) persist as the pole-cells at the extremities of the cross. The composition of the arms is therefore, a^1, $a^{1.5}$, $a^{1.4}$; b^1, $b^{1.5}$, $b^{1.4}$, etc., the centre being occupied by the rosette-cells, $a^{1.3}$, $b^{1.3}$, etc. (*cf.* Figs. 37, 38). In *Neritina*, on the other hand (accepting Blochmann's conclusions), the arms are derived from the *second set of micromeres*, the centre being occupied by the four primary micromeres. The arms of the cross in the two forms have in fact the following composition as compared with one another (I use throughout the nomenclature employed for *Nereis*, which differs somewhat from Blochmann's):—

Nereis	a^1,	$a^{1.5}$,	$a^{1.4}$, — b^1,	$b^{1.5}$,	$b^{1.4}$ — etc.
Neritina	$a^{2.2.1}$,	$a^{2.2.1.1}$,	$a^{2.2.1.1.1}$, — $b^{2.2.1}$,	$b^{2.2.1.1}$,	$b^{2.2.1.1.1}$ — etc.

In other words, the cross-cells of *Neritina* correspond, in mode of origin, to derivatives of the stomatoblasts and of the first somatoblast in *Nereis*!

Professor Conklin informs me that his observations on *Crepidula* do not quite agree with Blochmann's, as the outer portion of each arm arises from the second set of micromeres, the inner portion from the first group (*i.e.* from $a^{1.2}$, $b^{1.2}$, etc.). This, however, agrees no better with *Nereis* than Blochmann's account.

It is impossible to explain the differences between the annelidan and the molluscan cross by assuming inaccuracy of observation on Blochmann's part, since the pole-cells of the lateral arms show a peculiar granulation that may be seen in the parent-cells ($a^{2.2}$, $c^{2.2}$) from which they arise. It is certain that,

although the two crosses have exactly the same structure, they have a completely different origin. Are they to be regarded as homologous or not? The different origin of the cells does not in itself necessarily give a negative answer to this question. Aside from this, however, there is good reason for believing that they are not homologous. Conklin and Blochmann agree that, in the molluscan cross, two of the arms fall ultimately in the median line, while the other two are transverse to it. The annelidan cross, on the other hand, is inclined at an angle of 45° to the median and transverse planes. They must, therefore, give rise in the two cases to different regions of the upper hemisphere. Taking this in connection with the different history of the cells, there is every reason to believe that the annelidan and the molluscan crosses are analogous, but not homologous, structures, whose origin is in some way connected with the mechanical conditions of cleavage. What these conditions are I am unable to conjecture.

We come in the last place to the relation of the molluscan velum to the annelidan prototroch. According to *Blochmann* the velum arises from the terminal cells of the lateral arms of the cross ("Ur-velarzellen") which travel forwards, give rise to a transverse series of cells on either side, and from these arise the corresponding halves of the velum. If this account be correct, the velum has a totally different origin from the annelidan prototroch (in the annelid from $a^{1.1.1}$, $b^{1.1.1}$, $c^{1.1.1}$, $d^{1.1.1}$; in the mollusk from $a^{2.2.1}$, $c^{2.2.1}$). Does this indicate that the two structures are not homologous? I do not think that this conclusion necessarily follows.

X. General Interpretation of the Cleavage.

It is impossible to reflect upon the complicated yet perfectly ordered events of the cleavage in *Nereis* without attempting to discover the nature of the causes by which their course is determined. The completeness with which the history of the individual blastomeres can be followed and their mutual relations determined, the definite periods into which the ontogeny falls, the sudden transitions from one phase of activity to another — these and many other features of the development bring to the foreground some of the most interesting and fundamental problems of cytology. The solution of many, perhaps all, of

these problems may lie far in the future. Yet an attempt to consider them may, at least, serve one useful purpose by defining more clearly their outlines; and it is possible that the embryo of *Nereis* may prove in some respects an unusually favorable object for their investigation.

What is the significance of the spiral and bilateral forms of cleavage, and where lie the causes that determine the transformation of the one into the other? What determines the form and succession of the divisions of the individual blastomeres, which, as in the case of the first somatoblast, may have so complicated and yet so definite a history? Is the blastomere, like the ovum, a self-regulating mechanism that contains within itself the causes of its own transformations, that is wound up like a clock, as it were, and must of necessity run the course predetermined in its own structure? Or are its successive phases of activity determined or guided by influences proceeding from without — by the interaction of the cell with its fellows in the cell-complex? I scarcely need to point out that this latter question bears immediately upon that most pressing of all current general questions, the relation between the somatic cells and the germ-cells; and it is a problem upon which a flood of light is being thrown by recent investigations. It must be said at the outset that the development of *Nereis* gives no certain answer to this question. It does, however, as I believe, give strong indirect evidence which is worthy of attentive consideration.

The most striking feature in the cleavage, and the one on which the entire discussion may be made to turn, is the sudden appearance of bilateral symmetry in the cleavage. The meaning of the bilateral cleavages in themselves is perfectly obvious. They are the forerunners of the bilateral arrangement of parts in the adult; and, as such, their explanation belongs to the general problem of bilateral symmetry, which need not be considered here. The all-important point is that *the bilaterality does not appear at the beginning of development.* It appears only at a comparatively late stage, and by a change so abrupt and striking as to possess an absolutely dramatic interest. I lay especial stress on this point because it is wholly opposed to the opinion, which has of late rapidly gained ground among embryologists, that (1) the bilaterality of the adult is predeter-

mined in the ovum and therefore (2) manifests itself throughout the cleavage. The first of these propositions is a barren truism, for every adult characteristic is in one sense predetermined in the ovum. The second, however, is a premature generalization, and it does not follow from the first. *In some cases*, it is true, the cleavage does show a bilaterality from the beginning; and sometimes it is marvellously perfect, as shown, for instance, by the brilliant studies of van Beneden and Julin on the ascidian cleavage (No. 24) and of Watase on that of the cephalopod (No. 26). But this is by no means invariably the case. The earlier stages of many annelids, mollusks, polyclades, and other bilateral forms are bilateral neither in structure nor in the form of cell-division. It is true that the adult bilaterality may be in a certain way foreshadowed in the spiral period (*cf.* p. 400). But the following facts are indisputable: (1) that at no time during the spiral period is it possible to pass a plane so as to divide the body into equivalent right and left halves; and (2) that the planes of cleavage throughout this period are symmetrically distributed with respect, not to the future median plane, but to the vertical axis of the ovum.

What determines the period at which the first bilateral divisions appear? To this question a very simple and obvious answer can be given which, as I believe, gives a key to the entire development. *The bilateral asymmetry of the early stages depends mainly upon the fact that the substance of the somatoblasts* (i.e. *the mesoblast and the material of the ventral plate*) *is stored in the left posterior macromere. Bilateral symmetry is established upon the reduction of this macromere* (*D*) *to the size of its fellow* (*C*) *by the separation of the somatoblasts and their transportation to the median line. Immediately upon this event follows the appearance of bilateral cleavages throughout the embryo, except in the cells which give rise to the prototroch, a purely larval organ.* In other words, the bilateral cleavages appear as soon as the embryonic material becomes equally distributed with respect to a plane, which ultimately becomes the adult plane of symmetry. Is the correspondence between these two events a mere coincidence, or do they stand in the relation of cause and effect? Under the former alternative we must suppose that the coincidence is the result of a kind of pre-established harmony, such that the cells of the upper and lower hemi-

spheres, different as their past history has been and independent of external conditions, arrive at the period of bilateral divisions practically at the same moment. When we reflect upon the history of the nuclear spindles, the diverse forms and mechanical relations of the cells, such an assumption makes, under the theory of probabilities, a demand upon our credulity which I for my part am not prepared to meet. To accept the second alternative, however, is to admit that the form of the individual cell-divisions is in some degree controlled by influences emanating from the entire cell-complex.

That such influences exist is, however, certain in view of recent researches upon embryological mechanics. Roux, in one of his justly celebrated contributions to this subject (No. 20) showed, in the first place, that if one of the first two blastomeres of the frog-embryo be killed (by puncture with a heated needle), the remaining blastomere develops into a half-blastula, followed by a half-gastrula. He succeeded also in producing three-quarter embryos by killing one of the blastomeres of the four-celled stage. From this he drew the following conclusion (*Separat-abdruck*, p. 30): "Die Entwicklung der Froschgastrula und des zunächst daraus hervorgehenden Embryo ist von der zweiten Furchung an eine Mosaikarbeit und zwar aus mindestens vier verticalen, sich selbständig entwickelnden Stücken." In the second place, however, he showed that ultimately the missing half or fourth is perfectly restored by a process of "post-generation," which begins about the time of the formation of the medullary folds. As far, therefore, as the later stages are concerned, Roux's own results contradict the mosaic hypothesis; for the normal course of events in the uninjured blastomere, or its products, is radically altered by the changes *on the other side of the embryo.*

This significant fact is rendered still more striking by the recent experiments of Driesch (No. 6) and Fiedler (No. 7) on the embryos of sea-urchins, and by those of Chabry (No. 3) on ascidians. Driesch shows, in his extremely interesting paper, that if the two primary blastomeres of the *Echinus* embryo be completely, or even partially, separated (by shaking), each blastomere may give rise to a perfect embryo of half the normal size. The cleavage of each blastomere (whether isolated or still in partial contact with its fellow) at first follows the same course

as if it formed a part of a normal embryo, giving rise to stages of two, four, eight cells, etc., which are practically identical with the corresponding *halves* of the four-, eight-, and sixteen-celled stages, etc. As in the frog, a perfect half-blastula is produced. The opening then closes, and a blastula is then formed which differs from a normal blastula only in being half the normal size. The gastrulation and later stages follow the normal course.

This result agrees essentially with Roux's, with the interesting difference that the regeneration of the missing half takes place much earlier. It proves conclusively that in the normal development each of the blastomeres is profoundly influenced by the other; that the cell is not an isolated mechanism whose mode of action is wholly predetermined in its molecular structure. It proves in fact that the form of cell-division is determined by two factors. The first factor is the inherited tendency of the cell to pursue a definite course, a tendency which we may assume exists by virtue of a corresponding molecular or protoplasmic structure (*cf.* p. 441). The second factor is the influence upon the cell of other cells in the colony. When the second factor is removed or modified, the first is correspondingly modified, and a complete readjustment takes place. I can see no logical halting-point in the application of this principle between the embryonic and the adult stages. If the experimental results be well founded, — and it is impossible to question the validity of the combined evidence, — then no cell in the embryo or in the adult is isolated, *not even the germ-cells.*

In view of these facts there seems to be very strong reason to accept the conclusion that there is a causal connection in the *Nereis* embryo between the bilateral distribution of material and the ensuing bilateral divisions. I do not mean to assert that this connection is independent of the inherited tendency of the cells. It is probable, on the contrary, that the action has produced an hereditary effect. This result, however, only extends the causal connection over a longer period, and in nowise diminishes its significance.

We pass now to a discussion of the spiral period itself, which has many interesting and significant features. The principal datum on which the entire discussion rests is the fact that *the spiral form of cleavage has no necessary relation to the homology*

of the blastomeres, and hence is without phylogenetic significance. The truth of this proposition is at once apparent upon comparing the spiral cleavage of the annelid with that of the polyclade. The form of cleavage is identical in the two, the products extremely different, as has been pointed out at p. 441. Unless, therefore, we are prepared to maintain the absurd proposition that the mesoblast of the polyclade is homologous, not with the mesoblast of the annelid, but with the ectoblast of the lower hemisphere (including, of course, the ventral plate with the ventral nerve-cord), we cannot escape the conclusion that *exact equivalence of embryological origin is not a proof of homology*, as far, at least, as the cleavage-stages are concerned. Balfour, long ago, pointed out the fact that similarities in the general form of cleavage have no necessary relation to adult relationships, but it is a very surprising fact that a resemblance so close as that between the polyclade and annelidan ovum should be without morphological meaning. This is the more surprising because the comparative study of the annelids shows that within the limits of this group adult homologies are represented by accurate cell-homologies in the cleavage-stages (p. 436). We must conclude, however, that precisely similar modes of cleavage may arise quite independently of the nature of the materials, upon which the cleavage operates.

If, then, the spiral form of cleavage has no phylogenetic meaning, and at the same time has no direct relation to the adult form (as has been sufficiently pointed out), it must be due to mechanical conditions peculiar to the earlier stages of embryonic life. Before attempting to consider the nature of these conditions, I must call attention to a fact, which has been fully and clearly set forth in a profoundly interesting paper of Rauber's (No. 19), namely, that the mechanical laws of cell-division have been far more thoroughly investigated in plants than in animals; and it is to botany that we must look for a clue to the significance of cleavage-forms among animals. I need not review *in extenso* the brilliant researches of Sachs and others in this field, since their principal results may be found in his well-known *Pflanzenphysiologie* (Vorlesung XXIV) as well as in Rauber's paper. The essential points may be thus briefly summarized. *The form of cell-division is the result and not the cause of the form of the dividing mass;* the form of the

mass is caused by its growth as a whole, and is not a resultant of the growth of the cells individually considered. This is proved in plants by many facts, of which, perhaps, the most obvious is the existence of multicellular forms, in which the characteristic form of growth is fully determined *before the mass divides into cells*. Sachs concludes that the fundamental law of cell-division is such that the cleavage-planes primarily are vertical to one another and either vertical or parallel to the surface of the dividing mass; and he shows by a masterly analysis that the cleavage-planes in growing structures of various forms conform very nearly to the theoretical mathematical demands of this law.

From an *a priori* point of view there is every reason to believe that a like relation between growth and cell-division exists in animals, although its demonstration is difficult, especially for the reason that the two processes so generally go hand in hand. As Rauber points out, however, the segmentation of the ovum is a case in which, very often at least, there is no appreciable growth or change of form throughout a long series of cell-divisions, the ovum having assumed its form and size before the beginning of cleavage.

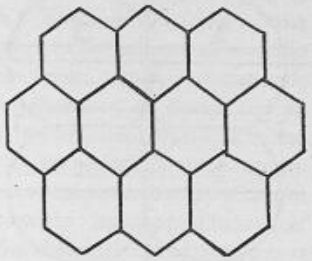
I am but expressing a commonplace of embryology in stating that the more nearly the ovum approaches its ideal form, the sphere of uniform protoplasm, the more nearly do the first three cleavages divide it into equal octants by division-planes cutting each other at right angles; in other words, the more nearly do they conform to the theoretical requirements of Sachs's law. It is interesting to find that the first three cleavages of such an ovum as that of *Amphioxus* or *Echinus* are almost exactly duplicated by the first three divisions of the spherical embryos of ferns and phanerogams. These facts in themselves create a strong presumption that plants and animals agree in the fundamental relations of growth and cell-division. If we examine the first three cleavages more narrowly we find that two types of division (apart from the bilateral) may be distinguished. The first is what may be called the true radial type (represented typically by *Amphioxus* and *Echinus*), in which the first two cleavages are perfectly vertical and the third perfectly horizontal, so that the four upper cells lie exactly above the four lower cells respectively. The second is the spiral type, represented

by *Eupomatus*, *Discocœlis*, *Nereis*, etc., in which the third cleavage is more or less inclined, so that the upper cells are displaced (apparently in all cases towards the right — *i.e.* following the hands of a watch), and in extreme cases are thus caused to alternate with the lower four.

I wish to make the point that the foregoing considerations render it probable that *the spiral type is a secondary derivative of the true radial type*, for the latter conforms more nearly to Sachs's law, and agrees with the cleavage of spherical plant-structures. Both are primarily owing to the spherical form of the ovum, but the spiral modification must be caused by some additional secondary factor. The nature of this factor seems to be very obvious, although when closely examined it is found to lead to some conclusions so important that they cannot be accepted without very strong evidence. The factor in question is *the effect of mutual pressure* among the cells. It is well known that free cells tend to assume a spherical form, whereas in masses they tend primarily to assume the same geometrical forms as soap-bubbles or other regular elastic bodies, — *i.e.* regular dodecahedrons when in bulk, regular hexagonal prisms when arranged in a single layer upon a plane surface. In the case of lifeless bodies (*e.g.* soap-bubbles) it is certain that these forms are the result of mutual pressure, and it is mathematically demonstrable that they are the forms which afford the greatest economy of space. The approximate conformity of cell-forms to these ideal mathematical forms makes it impossible to doubt that they are due to the same mechanical laws.

All these are elementary commonplaces. But the remarkable fact, and one which does not seem to be very clearly recognized, is that the effect of these mechanical conditions on the dividing cells *has become hereditary*. This I think is clearly proved by a comparison of the true radial and the spiral types of cleavage; the comparison at the same time explains a number of interesting features in the early stages, among others the cross-furrows of the four-celled stage. In the segmenting ovum we have to deal essentially with cells arranged in a single layer, either surrounding a central cavity or (as is especially clear in the *Nereis*-embryo) spread out on the spherical surface of a group of larger cells. They tend, therefore, towards the hexagonal form, though probably they never actually assume the ideal form, since they

are in a curved and not a plane layer, and differ more or less in size. Still the hexagonal form may be taken as the type without serious error. The arrangement of regular hexagons (as for instance in the honeycomb) may be roughly characterized as *alternate*, since, as shown in the accompanying diagram, the cells of each row alternate with those of the adjoining rows. Sooner or later this arrangement is assumed by the blastomeres of all segmenting ova, and the difference between the radial and the spiral types is caused simply by the earlier appearance of the alternation in the latter, as a result, undoubtedly, of different mechanical conditions. In the true radial type, beautifully shown in the cleavage of *Echinus* and *Synapta* (Selenka, No. 23), it does not appear until a late stage. In the spiral type it appears more or less distinctly from the beginning of development. This is obvious as far back as the eight-celled stage (Diagram VIII, *E*), but it is easy to show that the peculiarities of the four-celled stage are due to the same cause. The cross-furrows of this stage do not exist in the true radial type (Diagram VIII, *A*). In the spiral type they are owing to an actual or virtual displacement of the cells, so that they assume the cross-form shown in Diagram VIII, *B*, *C*, *D*, the diagonally opposing cells, *A* and *C*, lying above, and the others, *B* and *D*, below. This displacement may be characterized as a rotation, which is primarily in the vertical transverse plane, as may be seen from a comparison of Diagram VIII, *A*, *B*, *C*. In typical cases, of which *Sagitta* (Hertwig, No. 13) and *Asterina* (Ludwig, No. 16) are examples, the rotation is complete and symmetrical, the upper and lower cross-furrows being equal and at right angles to each other. Many gradations exist between this and the true radial type, one of the most interesting being the embryo of *Hydroides*, in which the two cross-furrows are equal and at right angles to one another, but so short as to escape any but the closest examination. It appears to be invariably the case that in telotethical ova, with unequal cleavage, the cross-furrows are unequal, the upper being more or less reduced, as in the embryos of many



mollusks and annelids. This reduction stands in obvious relation to the different size of the cells produced at the two poles, and requires no further discussion.

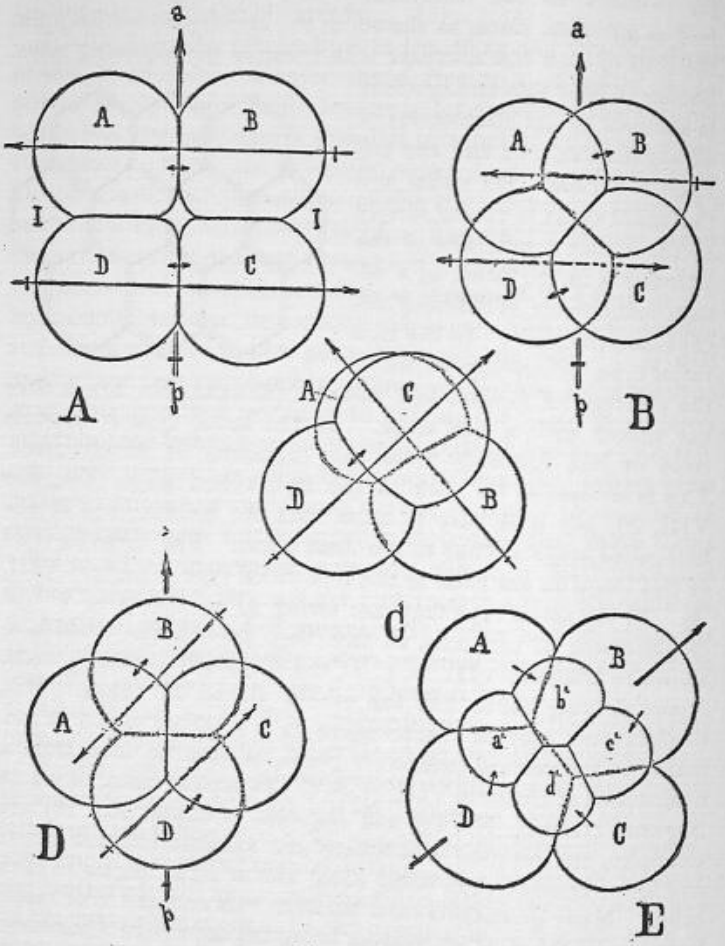


Diagram VIII.

A. Four-celled stage of true radial type (*Amphioxus, Synapta*).
B. Spiral type (*Nereis, Crepidula*).
C. Side-view of the last.
D. Spiral type (*Discocœlis*).
E. Eight-celled stage (*Discocœlis*).

We come now to the main point, which is that *the rotation of the cells is in the spiral type in many cases predetermined in the parent-cells, as is proved by the position of the spindles and by the form of division.* It is, in other words, to a greater or less extent, a virtual instead of an actual rotation. This is the case, for example, in *Asterina*, as described by Ludwig, where the division-planes of the two primary blastomeres are at right angles to each other at the second cleavage. The same is true of the polyclade embryo (Lang). It remains to be seen how far the virtual rotation is accompanied by an actual rotation of the cells after their formation. In the later cleavages of *Nereis* (*e.g.* in the formation of the rosette-cells at the fifth cleavage and of the intermediate girdle-cells at the fourth), the spindles lie in the undivided parent-blastomeres, exactly vertical to the future plane of division and the rotation is purely virtual. In the third cleavage, however, this is not the case, since the obliquity of the spindles is at first very slight and constantly increases during the division, until they form an angle of nearly 45° with the vertical axis. There is also in this case a certain amount of actual rotation after the complete separation of the cells, as may be seen by a comparison of Figs. 11 and 12. Regarding the second cleavage, the available data are very scanty, since this stage has not been accurately examined with reference to the point. I can state positively, however, that in *Ascaris megalocephala* (which, through the courtesy of Dr. Boveri, I have been enabled to study in the living state as well as in preparations) there is a very extensive actual rotation in the four-celled stage, after the full completion of the division. The value of this case as an illustration is, however, lessened by the fact that the second cleavage differs radically from the usual form.

To sum up, I conclude that the spiral form of cleavage is owing to a precocious appearance of the alternation of the cells, which, in its turn, is a result of mutual pressure. The "true radial" type differs from the spiral only in the fact that the alternation appears at a later period; in other words, the cleavage longer adheres to the primary type. The primary type owes its characteristics to the form of the ovum, in accordance with the general laws of cell-division. Thus the characteristics of the spiral period are, in their broadest outlines, the result of mechanical conditions which have no relation to the adult structure.

What, then, is the origin of bilateral forms of cleavage? It appears to me that *they must be the result of a throwing back or reflection of the adult bilaterality upon the early stages.* In some cases this influence has extended to the very beginning, as in the Cephalopod or in the ascidian, or even to the unsegmented ovum itself, as in some insects and other forms. In some cases, of which *Nereis* is a beautiful example, it has not extended so far; the early stages are still dominated by the mechanical conditions peculiar to them, and the bilateral form only appears when these conditions have been in a measure overcome.

I may call attention to the fact that there is a definite relation between the law of spiral cleavage and the fact that among annelids and mollusks it is invariably the *left* posterior macromere that is the largest. In all cases that have been accurately investigated, the first spiral cleavage of the four macromeres is right-handed, the second left-handed, and the third right-handed again. The subsequent origin of the mesoblast (as in *Nereis*, *Crepidula*, etc.) may be regarded as belonging to another left-handed spiral cleavage. Now the superior size of the left posterior macromere, as has been pointed out, is owing to the storage in it of the material for the somatoblasts, which, by the second and fourth left-handed spiral divisions of the macromeres, is pushed over into the middle line, where it belongs. There is, therefore, a necessary relation between the law of spiral cleavage and the storage of material in the left macromere. The exact nature of this relation, however, cannot be stated, for there is no positive evidence to show which is cause and which effect. The comparison with the polyclade, although at first sight promising, yields no result, on account of the lack of necessary data.

One other point may briefly be considered, namely, the remarkable differences in the fundamental axial relations between forms closely allied in the form of cleavage. The annelids and the mollusks show exactly parallel differences in this regard. In *Planorbis* (Rabl), *Neritina* (Blochmann), and some other gasteropods, the first two cleavage-planes are inclined at an angle of 45° to the median plane, and the representative of the "large macromere" is in the median line, precisely as in the polyclade (Diagram VIII, *D*, *E*). In *Crepidula*, on the other hand, the first two cleavage-planes coincide with the transverse

and median planes respectively. Among the annelids, *Nereis* agrees with *Crepidula*, while *Clepsine* (Whitman) and *Rhynchelmis* (Vejdovsky) agree with *Planorbis*, *Neritina*, and the polyclade. It is perfectly clear that this difference can have no morphological significance, but is a result of mechanical causes of some kind. Either the ectoblastic cap or the group of macromeres has been rotated 45° from its primary position, but I am at present unable to suggest an explanation that will satisfy all of the conditions of the problem. These differences certainly afford a forcible illustration of the fact that the relations of parts in the early stages of development may readily be modified by secondary conditions.

In conclusion, I may call attention to the bearing of my general interpretation of the cleavage upon the significance of the protoblasts and the study of cell-lineage. The general results show that it is necessary to be very cautious in drawing morphological conclusions from the comparative study of early cleavage-stages. I cannot entirely agree with Watase that "the earlier cleavage-processes are more fundamental, and, from the morphological standpoint, more significant than the later ones." Blastomeres having precisely the same mode of origin and precisely the same spatial relations to the rest of the embryo are by no means necessarily equivalent, either physiologically or morphologically, and the early cleavage-stages *in themselves* have little morphological value. The respective values of the blastomeres must be determined by their ultimate fate; and this is an indispensable datum for the study of comparative embryonic anatomy. *The fundamental forms of cleavage are primarily due to mechanical conditions, and are only significant morphologically in so far as they have been secondarily remodelled by processes of precocious segregation.* To this precocious segregation we must ascribe the early differentiation of the protoblasts. The facts show, however, that this process has very generally taken place in a greater or less degree; and that from the study of cell-lineage, if rightly applied, we may hope ultimately to attain a firm basis for an estimate of the different forms of gastrulas and a comparison of the germ-layers.

MUNICH, December, 1891.

APPENDIX.

Further Observations on the Cleavage and Gastrulation of Polychæta.

Since the foregoing paper was sent to press I have made at Naples an extensive series of observations on the early development of *Polymnia nebulosa*, Mont. (*Terebella Meckelii*, D. Ch.), *Spio fuliginosus*, Clp., and a form that is almost certainly the *Aricia fœtida* of Claparède. As the results cannot be published in full for some time, and are an important confirmation and extension of the work on *Nereis*, I may briefly review them here.

These three forms not only belong to three widely divergent families of Polychæta, and represent both Errantia and Sedentaria, but in addition to this differ from *Nereis* in all the conditions of embryonic development. In all three the eggs are opaque, are deposited in a jelly-mass, and in *Polymnia* and *Aricia fœtida* the trochophore is partially suppressed, the free-swimming life being of very brief duration. In all, the four macromeres continue to divide (as in *Rhynchelmis*) after the separation of the ectoblast and mesoblast; and in *Polymnia* and *Aricia fœtida* (probably also in *Spio*) there is a well-marked *embolic* gastrulation, which leads to the formation of a large blastopore.

It might, therefore, have been expected that the cleavage would differ widely from that of *Nereis*. As a matter of fact, on the contrary, it is not only quite of the same type in the three forms, but shows step by step an extraordinarily detailed and striking likeness to the *Nereis* cleavage, which may, therefore, probably be taken as typical, in all of its leading features, of an extensive series of annelids. The differences are in fact quite insignificant up to a period when the germ-layers have become fully differentiated and the somatoblasts have assumed their typical position.

I.

I will first briefly describe the facts common to the three forms and to *Nereis*, and afterwards point out some of the specific peculiarities.

The first five cleavages (to the thirty-two-celled stage) are nearly identical in all, excepting slight differences in the relative size of the blastomeres. Three sets of ectomeres are formed in the typical manner, and the somatoblasts, *X* and *M*, have essentially the same origin, position, and relation to the blastopore-margin. In all, the apical rosette and the cross arise in precisely the same manner, and at nearly the same period; and in all the first bilateral divisions first take place after the reduction of the left posterior macromere, *D*, to the size of its fellow, *C*. In all, furthermore, the primary girdle, consisting at first of eight and afterwards of twelve cells, arises exactly as in *Nereis*. The prototroch is developed in the region of the girdle-cells, but I have not yet succeeded in tracing its origin in detail. In all, finally, the first somatoblast, *X*, undergoes three unequal divisions, as in *Nereis*, thus giving rise successively to x^1 on the right side, x^2 on the left, and x^3 in the middle dorsal line. It then divides into equal parts, *X*, *X*, the posterior proteloblasts.

II.

I pass now to the leading individual peculiarities, some of which are very interesting from a comparative point of view.

(1) *Spio* differs from the others in the immense relative size of the left posterior macromere, *D*, and of the somatoblasts, *X* and *M*, derived from it.

(2) In *Spio*, *Aricia fœtida* and *Polymnia* x^1 and x^2 are very much larger than in *Nereis*, so that after their formation *X* becomes considerably reduced in size, and the posterior proteloblasts are relatively small.

(3) The later development of the cross in the same three forms differs somewhat from that of *Nereis*, the cells $c^{1.5}$, $d^{1.5}$ ("nephroblasts" in *Nereis*) being much smaller and formed in a slightly different position.

(4) *Polymnia* differs from the other forms in the fact that the second somatoblast, *M*, is from the first partially covered by the macromere, *D*, and at the time of its fission extends to the

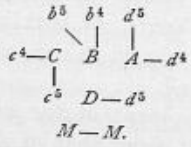
surface over only a small area. The primary mesoblasts pass into the cleavage-cavity before the formation of the blastopore; *i.e.* at a much earlier period than in the other forms, so as to approach the condition found in *Lumbricus*.

(5) In *Spio* and *Aricia fœtida* the primary mesoblasts first bud forth small superficial cells, from their anterior margin, at the posterior lip of the blastopore ("secondary mesoblast" in *Nereis*), a process which takes place at a relatively earlier period than in *Nereis; i.e.* before the fission of *X*. In *Aricia fœtida* (probably also in *Spio*) *only a single pair* of such cells are found, which are very minute and appear to be rudimentary. After their formation the mesoblasts bud forth into the cleavage-cavity (as in the "blastula" of *Lumbricus*) two rows of cells that form the mesoblast-bands.

In *Polymnia* the preliminary superficial budding seems not to take place, and the mesoblasts only divide after their complete enclosure (as in *Eupomatus*, *t.* Hatschek).

(6) In *Polymnia* and *Aricia fœtida*, (probably also in *Spio*) the macromeres undergo two additional spiral divisions after the separation of the twelve ectomeres. The first of these (fourth spiral division) is left-handed, and of the four resulting cells one ($d^4 = M$), formed somewhat earlier than the others, becomes the second somatoblast, while the other three (a^4, b^4, c^4) form part of the entoblast-plate. The second (fifth spiral division) gives rise to four entomeres (a^5, b^5, c^5, d^5), formed in a right-handed spiral, after which no further divisions take place in the entoblast-plate until after the completion of gastrulation.

The entoblast-plate now consists of eleven cells, which (in *Aricia fœtida*) assume the bilateral arrangement shown in the following diagram:—



(7) In *Polymnia* and *Aricia fœtida* (*Spio* not observed) an embolic invagination now rapidly takes place. The blastopore is at first elongated, its floor being formed (in *Aricia fœtida*) by *D*, *B*, and b^4, which lie in the median line, and its posterior limit by the primary mesoblasts, *M*, *M*. Thus (in *Aricia*

fœtida) *the first two cleavage-planes are oblique to the plane of symmetry*, as in *Clepsine* or *Rhynchelmis*, and differ from their position in *Nereis*. This difference is, however, caused simply by late displacements of the entomeres, and does not affect the earlier stages; it is, therefore, without morphological significance.

(8) The blastopore assumes a triangular form by the narrowing of its posterior portion. It appears to close completely from behind forwards, and the mouth afterwards appears at the point of final closure, *i.e.* in front.

(9) In *Aricia fœtida* the primary mesoblasts pass into the interior as the blastopore closes.

I have followed up to the thirty-two-celled stage the cleavage of a series of *Hydroides*[1] which agrees closely in development with the "Eupomatus uncinatus" studied by Hatschek and is of special interest as a representative of the so-called "equal cleavage" among annelids. The order and direction-planes of the divisions are nearly the same as in the other forms, but the spiral symmetry is complete up to the latest stage observed. The first four blastomeres, *A*, *B*, *C*, *D*, are exactly equal. At the third cleavage four slightly smaller upper cells, a^1, b^1, c^1, d^1 ("micromeres") are separated, in a right-handed spiral, from four lower "macromeres." The fourth cleavage agrees closely with that of *Polymnia*, *Nereis*, etc., but the micromeres of the second group, a^2, b^2, c^2, d^2, are of exactly equal size, and are but little smaller than the macromeres. The fifth cleavage is likewise essentially like that of the other forms, and the third set of ectomeres, a^3, b^3, c^3, d^3, are much smaller than the second set (a^2, b^2, etc.).

The embryo now (thirty-two-celled stage) agrees essentially in the arrangement of the blastomeres with the corresponding stage of the "unequal type" (*Nereis*, etc.), but differs from it (*a*) in the equal size of the four macromeres, and (*b*) in the lack of a differentiated first somatoblast (*i.e.* d^2 is no larger than a^2, b^2, c^2). It is at this period that the second somatoblast, *M*, is formed in the unequal type, where, as I have shown above (*Polymnia*, *Aricia fœtida*), it forms one of the four cells (a^4, b^4,

[1] The species is related to *Hydroides* ("*Eupomatus*") *uncinatus*, but differs from it both in the looser and more delicate growth of the tube, and in the more numerous divisions in the crown of the operculum.

etc.) produced at the fourth spiral division of the macromeres. In *Hydroides* these four cells now arise in a left-handed spiral, as in the other forms, but they are equal in size and are formed simultaneously. If the analogy holds, one of these cells (d^4) should be the equivalent of the second somatoblast, *M*, from which the mesoblast arises, the other three (a^4, b^4, c^4) forming part of the entoblast-plate. I have not yet succeeded, however, in tracing the cytogeny with certainty beyond this point, and the origin of the mesoblast therefore still remains undetermined. Should my surmise prove well founded, we would have a very simple explanation of the difference between the equal and unequal types of cleavage in annelids — a difference which has hitherto seemed to involve a difficult problem with regard to the mesoblast.

Naples, April, 1892.

POSTSCRIPT.

While correcting the last pages of the proofs of this paper I received Oscar Hertwig's extremely interesting work entitled "Urmund und Spina bifida," etc. [*Arch. f. Mic. Anat.*, Bd. 93, Heft III., 1892], in which the author, after a critical review of the work, especially of Roux, Driesch, and Chabry, throws the immense weight of his authority on the side of the same conclusions that I have adopted at p. 447 regarding the dependence of the development of individual blastomeres on that of the whole embryo. In opposition to Roux, Hertwig maintains that, (1) "Die Entwicklung eines Organismus ist keine Mosaikarbeit," and, (2) "die Theile eines Organismus entwickeln sich in Beziehung zu einander oder die Entwicklung eines Theiles ist abhängig von der Entwicklung des Ganzen" (p. 480).

It seems to me, however, if I may venture the suggestion, that Hertwig underestimates the importance of early differentiation among the blastomeres (*i.e.* the hereditary element in the development of individual parts), and that the mosaic view may still, *in a modified form*, be of value. In the echinoderm embryo the individual blastomeres show very little morphological differentiation until a late period. In the annelid such differentiation exists from the very beginning of the cleavage, and the mosaic appearance of the development cannot be overlooked. The facts seem to accord best with the hypothesis that the blastomeres are capable within certain limits of pursuing their individual development, yet at the same time depend *in a greater or less degree* on that of the whole. How far this dependence goes, and how far the various blastomeres may be capable of replacing one another, is a question to be determined not by analogy, but by direct experiment.

Naples, May 3, 1892.

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LETTERING OF THE PLATES.

The blastomeres are lettered according to the following system, for an illustration of which see Diagram, p. 378: —

A, B, C, D. The four primary blastomeres and the corresponding macromeres or entomeres.

a^1, b^1, c^1, d^1. First group of micromeres.

a^2, b^2, c^2, d^2 (X). Second group of micromeres.

a^3, b^3, c^3, d^3. Third group of micromeres.

Their products are in general lettered according to the principle illustrated in the diagram, except in the case of the somatoblasts, the four macromeres, and the four primary micromeres, which retain their original lettering throughout (*cf.* Diagram, p. 396).

$$a^2 \begin{cases} a^{2.1} \begin{cases} a^{2.1.1} \\ a^{2.1.2} \end{cases} \\ a^{2.2} \begin{cases} a^{2.2.1} \\ a^{2.2.2} \end{cases} \end{cases} \text{ etc.}$$

Additional letters as follows: —

a.c. Anal cirrhi.
a.f. Anal sensory flagellum.
an. Anterior.
a.t. Apical tuft of cilia.
bp. Blastopore.
dor. Dorsal region.
e. Eye-spot.
ec. Ectoblast.
e.n. Entoblast-nuclei.
f. Cross-furrow.
f.a. Frontal antennæ.
f.b. Frontal bodies.
h.k. Head-kidney.
inf. Inferior (post-trochal) region.
i. Jaws.
l. Left side.
l.d. Latero-dorsal region.
l.m. Longitudinal muscles.
M. Second somatoblast, mesomere, primary mesoblast.
m. Secondary mesoblast.
m.b. Mesoblast-band.
mes. Mesenteron.
mh. Mouth.
ms. Mesotrochal ciliated belt.
n. Nephroblast.
n.p. Neural plate.
o.d. Oil-drop.
p. Prototrochal cell.
p.a. Pigment-area.
par. Paratroch.
p.c. Polar cell.
pig. Pigment.
p.l. Palpi.
pr. Prototroch.
p.p. Parapodia.
ps. Posterior.
p.t. Post-trochal cells.
r. Right side.
s. Setæ.
s.g. Stomodæal gland.
s.h. Sense-hairs.
s.s. Seta-sac.
st. Stomodæum.
st.l. Left stomatoblast.
st.m. Median stomatoblast.
st.r. Right stomatoblast.
sup. Upper (supra-trochal) region.
t. Trochoblast.
t.c. Tentacular cirrhi.
v. Ventral.
v.p. Ventral plate.
X. First somatoblast, and the resulting teloblasts.
x. Derivatives of *X*.
z. Zona radiata.

EXPLANATION OF PLATES.

All of the figures, unless otherwise stated, were drawn with the aid of the camera, but in many cases the finer details have been added free-hand to the camera sketch. Most of the figures were drawn from a single specimen, but in a few cases, in order to economize space, a single figure combines the sketches from more than one specimen. In the later stages, for the most part, no attempt has been made to reproduce the texture of the protoplasm, but it is of such glass-like transparency that it is fairly well represented by the shading of the figures. The coloring of the figures was an afterthought, and was adopted for purely practical reasons. Although the effect of the drawings is injured by it, so much is gained in clearness and facility of comparison that I feel sure of the approval of every reader who has labored through with a long series of imperfectly described plates.

All of the figures, unless otherwise stated, are from *N. limbata*, Ehlers. The enlargement is in most cases about 400 diameters.

PLATE XIII.

[Figs. 1 to 6, 7*a*, and 10 from living specimens; the others from preparations.]

FIG. 1. Unsegmented egg, from the upper pole, before the extrusion of the polar cells. Zona radiata still present.

FIG. 2. Side-view after the formation of the polar cells and disappearance of the zona.

FIG. 2*a* to 2*d*. Four stages in the first cleavage; 2*a* and 2*b* are side-views of successive stages of the same egg; 2*c* is from the upper pole in the earlier part of the division, and 2*d* from the lower pole at a later stage, showing the still undivided bridge of protoplasm on the lower side.

FIG. 3. Completed two-celled stage from the upper pole; *AB* the anterior, *CD* the posterior blastomere (1 h. 55 m.).

FIG. 4. The same egg from the upper pole; second cleavage in progress; the anterior blastomere divided into *A* and *B*, the posterior incompletely divided (2 h. 10 m.).

FIG. 4*a*. View from the lower pole of slightly later stage, showing the cross-furrow *f*, although *CD* is still incompletely divided (2 h.).

FIG. 5. Four-celled stage from the upper pole. The fainter lines represent the cleavage-lines of the lower pole, seen through the transparent vitellus. The deutoplasm-spheres omitted (2 h. 19 m.).

FIG. 6. Four-celled stage, from the right side.

FIG. 7. View from the upper pole of an egg (preparation) about to divide into eight cells, showing the position of the spindles. The stars represent the upper asters; the small circles, the centres of the lower asters.

FIG. 7*a*. Side-view of third cleavage (2 h. 20 m.).

FIG. 8. View from the upper pole of an egg later in the third cleavage, to show the position of the spindles.

FIG. 9. Side-view of the last.

FIG. 10. Side-view of completed eight-celled stage.

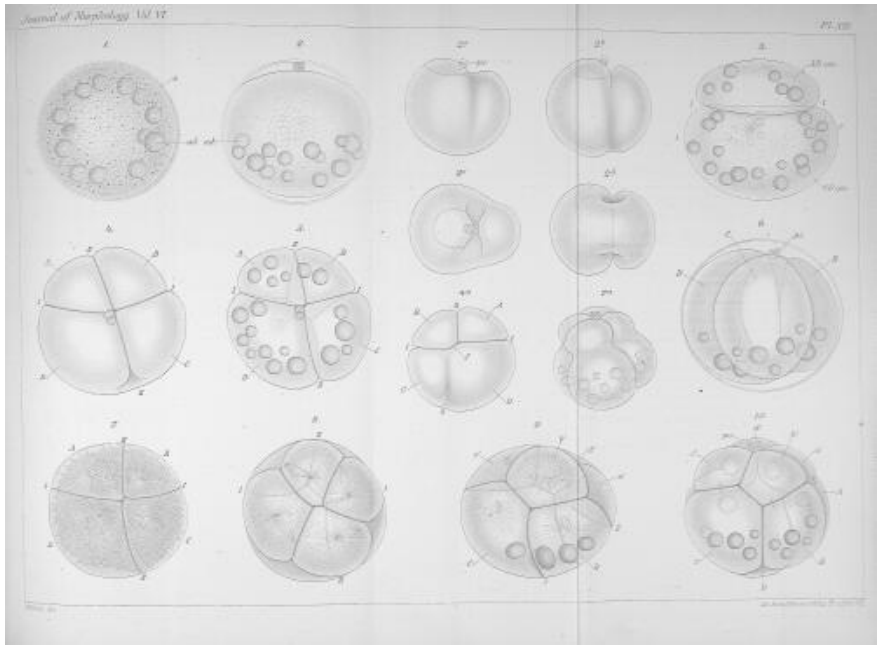

PLATE XIV.

[Figs. 11–17, 20 from life; 18, 19, 21, 22 from preparations.]

FIG. 11. The same egg shown in Figs. 3, 4, 5, immediately after completion of the third cleavage (2 h. 33 m.).

FIG. 12. The same seven minutes later.

FIG. 13. Fourth cleavage, from the upper pole (3 h. 21 m.).

FIG. 14. The same egg; end of the fourth cleavage (3 h. 30 m.).

FIG. 15. Fourth cleavage; posterior view, showing origin of the first somatoblast (*N. megalops*).

FIG. 16. Fourth cleavage, from the right side (2 h. 40 m.).

FIG. 17. The same egg, nearly in the same position, in the sixteen-celled stage (2 h. 55 m.).

FIG. 18. Optical longitudinal section of sixteen-celled stage.

FIG. 19. Beginning of the fifth cleavage, from the upper pole; primary micromeres preparing for their second spiral division (*cf.* Fig. 14).

FIG. 20. Twenty-two-celled stage, immediately following the last, from the upper pole. First division of *X*, formation of the two posterior tertiary micromeres c^3, d^3 (3 h. 58 m.).

FIG. 21. Rear view of same stage. Division of *X*, spindles of c^3, d^3.

FIG. 22. The same embryo, from the left side.

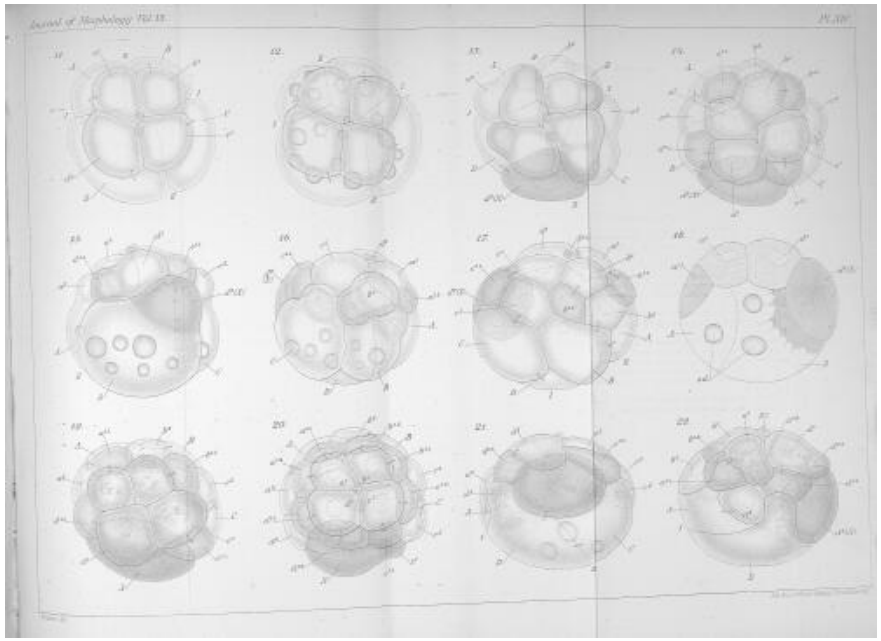

PLATE XV.

[Figs. 23, 28, 29, 32 from life; the others from preparations.]

FIG. 23. Twenty-nine-celled stage, from upper pole. The trochoblasts have divided radially (completing twelve-celled girdle); the four tertiary micromeres formed, secondary micromeres still undivided (3 h. 43 m.).

FIG. 24. Succeeding stage, from right side. Second group of micromeres in division.

FIG. 25. Thirty-two- (four-) celled stage, right side-view. Third spiral cleavage of a^1, b^1, c^1, d^1 in progress ($4\frac{1}{2}$ h.).

FIG. 26. Anterior view of the same embryo.

FIG. 27. From the upper pole. Third spiral cleavage of a^1, b^1, c^1, d^1 (formation of apical rosette). Transition from thirty-two- to thirty-six- celled stage.

FIG. 28. Thirty-six-celled stage, from the upper pole (4 h. 7 m.).

FIG. 29. Thirty-eight-celled stage, rear view. Second division of *X*. Origin of second somatoblast (4 h. 55 m.).

FIG. 30. Stage immediately following the last. First stage of transitional period. The two posterior primary micromeres, c^1, d^1, preparing for their first bilateral division (5 h. 15 m.).

FIG. 31. Forty-celled stage. Third (median dorsal) division of *X*. Bilateral division of c^1, d^1 completed (forming $c^{1.4}$, $d^{1.4}$) (6 h. 30 m.).

FIG. 32. Forty-three- (typically forty-two-) celled stage. Posterior view of a living specimen followed from a stage precisely like Fig. 29. Third division of *X* completed, interruption of the girdle by the displacement of $d^{1.2}$. Fission of the second somatoblast to form the primary mesoblasts, *M.M.* (5 h. 25 m.).

FIG. 33. Left side-view of peculiar thirty-celled stage in which the apical rosette is not yet present, though the second somatoblast is formed. Second group of micromeres just dividing. Second division of *X* not yet effected (7 h.).

FIG. 34. Optical longitudinal section, thirty-eight-celled stage; position of the left stomatoblast ($a^{2.2}$) indicated by the dotted line.

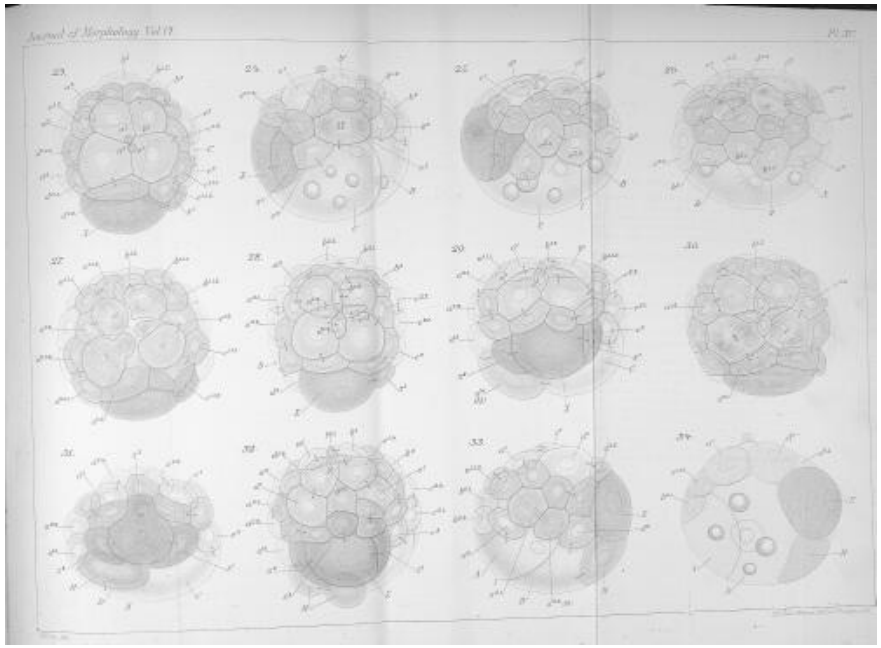

PLATE XVI.

[All of the figures from preparations.]

FIG. 35. Transition from forty-two- to fifty-eight- celled stage. View from the upper pole, showing first bilateral division of a^1, b^1, establishment of the cross, transformation of the girdle into the prototroch (5 h. 30 m.).

FIG. 36. Anterior view of the same specimen.

FIG. 37. Posterior view, about fifty-eight cells. Second bilateral division of c^1, d^1 to form the nephroblasts, $c^{1\cdot5}$, $d^{1\cdot5}$. Spindle of fourth division (fission) of X (5 h. 30 m.).

FIG. 38. Complete establishment of bilaterality. Immediately following the last. Formation of the nephroblasts and fission of X completed. Delayed division of $c^{1\cdot2}$ (5 h. 30 m.).

FIG. 39. Second bilateral division of a^1, b^1, forming $a^{1\cdot5}$, $b^{1\cdot5}$. Division of $c^{1\cdot4}$, $d^{1\cdot4}$. Beginning of the enclosure of the nephroblasts (6 h. 30 m.).

FIG. 40. Third bilateral division of c^1, d^1 to form $c^{1\cdot6}$, $d^{1\cdot6}$; division of $a^{1\cdot4}$, $b^{1\cdot4}$ (7 h.).

FIG. 41. Third bilateral division of a^1, b^1 to form $a^{1\cdot6}$, $b^{1\cdot6}$; division of $a^{1\cdot5}$, $b^{1\cdot5}$. The nephroblasts nearly enclosed.

FIG. 42. Fourth bilateral division of c^1, d^1 to form $c^{1\cdot7}$, $d^{1\cdot7}$; fifth division of X (7 h. 30 m.)

FIG. 43. Posterior view of the same.

FIG. 44. Right side-view, somewhat from above, after the fourth bilateral division of c^1, d^1. At the left upper side the nephroblast is shown in optical section, below $d^{1\cdot6}$ (7 h. 30 m.).

FIG. 45. Right side-view after enclosure of the nephroblast and the beginning of its migration. Fifth bilateral division of c^1, d^1. The proteloblasts (XX) have divided (eighth division of X) to form the four posterior teloblasts (X, X, X, X) (8 h. 30 m.).

FIG. 46. Rear view of slightly earlier stage (8 h. 30 m.).

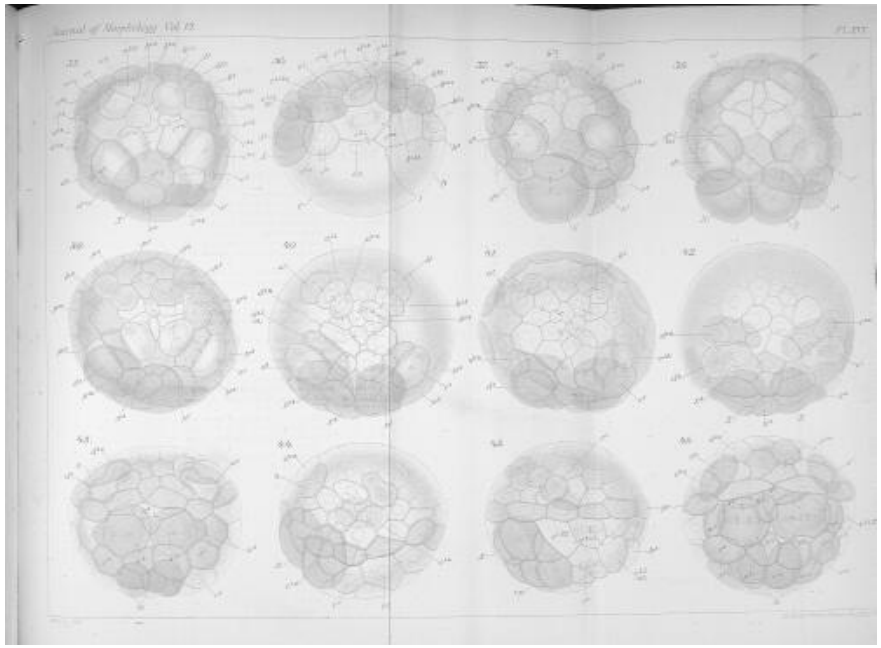

PLATE XVII.

[All of the figures from preparations.]

FIG. 47. Postero-ventral view, thirty-six-celled stage. Origin of the second somatoblast (6 h. 15 m.).

FIG. 48. Succeeding stage, thirty-eight cells (*cf.* Fig. 30) (5 h. 15 m.).

FIG. 49. Postero-ventral view. Fission of the second somatoblast to form the primary mesoblasts. Transition from Figs. 31 to 32 (6 h. 15 m.).

FIG. 50. Rear view of same stage as Fig. 37. Fission of *X* (fourth division), division of d^3, c^3, x^2; second bilateral division of c^1, d^1 (6 h. 15 m.).

FIG. 51. Ventral view immediately after the fission of *X* (*cf.* Fig. 38) (7 h.).

FIG. 52. Rear view. Fifth division of *X*, x^1 dividing, x^2 divided into two (6 h.).

FIG. 53. The same stage, seen more from the ventral side; x^2 undivided, x^1 dividing. Fifth division of *X*. First gemmation of primary mesoblasts (6 h.).

FIG. 54. Postero-ventral view. Sixth division of *X* (*cf.* Figs. 42, 43) (7 h. 30 m.).

FIG. 55. Ventral view of later stage. Four secondary mesoblast cells have been formed, the primary mesoblasts are nearly covered in, and the blastopore is nearly closed (9 h.).

FIG. 56. Slightly later stage. Eighth division of *X* (fission of the proteloblasts), gemmation of the primary mesoblasts (probably the third), approximation of the stomatoblasts, division of d^3 (*cf.* Figs. 45, 46) (9 h. 45 m.).

FIG. 57. Similar view of later stage (the same specimen shown in Fig. 74) (9 h. 30 m.).

FIG. 58. Rear view of still later stage, showing the four posterior teloblasts (8 h. 30 m.).

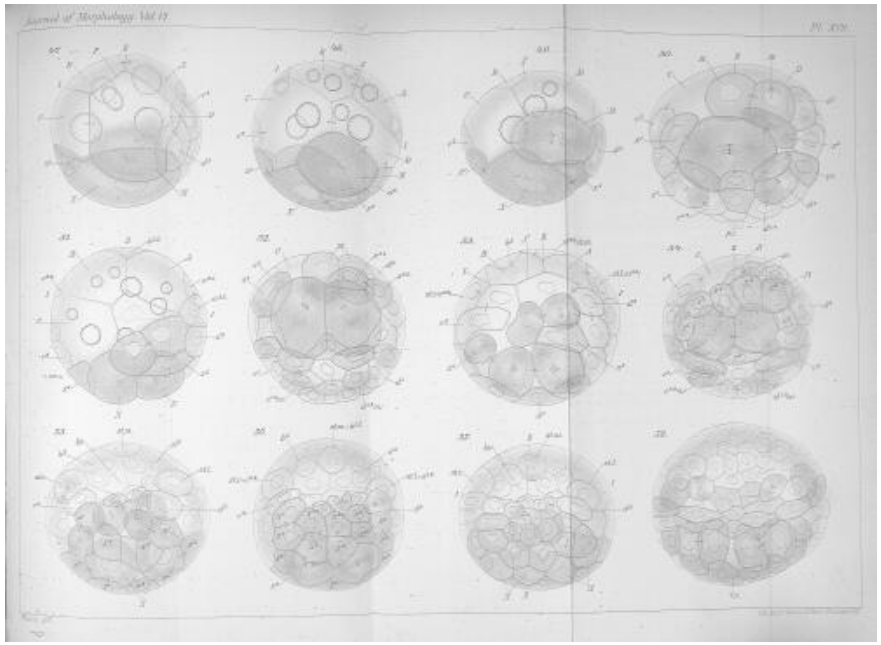

PLATE XVIII.

[All excepting Fig. 60 from preparations.]

FIG. 59. Rear view of first ciliated stage. Ninth division of *X* (13 h.).

FIG. 60. Living embryo in the same stage (10 h.).

FIG. 61. Postero-ventral view, after completion of the tenth division of *X* (11 h.).

FIG. 62. Ventral view of nearly the same stage, showing the relations of the stomatoblasts, blastopore, mesoblasts, head-kidneys, prototroch, and the posterior teloblasts (13 h.).

FIG. 63. Similar view of later stage, after the eleventh division of *X* (disappearance of the outer teloblasts), and closure of the blastopore. Extension of the head-kidneys, infolding of the blastopore-region, convergence of the stomatoblasts (13 h.).

FIG. 64. Rear view of nearly the same stage (11 h. 30 m.).

FIG. 65. Succeeding stage, showing division, displacement, and initial separation of the residual teloblasts (twelfth division of *X*).

FIG. 66. Slightly later stage with symmetrical division of the products of the outer teloblasts (11 h. 30 m.).

FIG. 67. Similar view of still later stage. Separation of the residual teloblasts, appearance of the rudimentary dorsal region, extension of the head-kidneys (14 h.).

FIG. 68. Rear view of still later stage (14 h.).

FIG. 69. Similar view of later stage (18 h.).

FIG. 70. Postero-ventral view of the latest stage in which the residual teloblasts can be distinguished. The upper part of the figure in surface-view (prototroch, residual teloblasts), the lower part in optical section (entoblast, mesoblast, ventral plate, pigment-plug) (14 h. 30 m.).

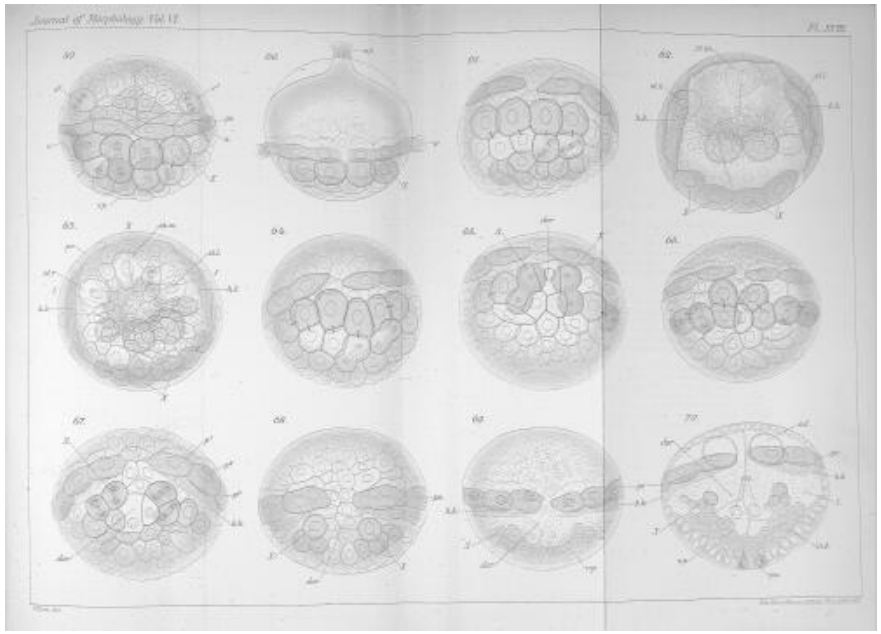

PLATE XIX.

[All of the figures from preparations.]

FIG. 71. Longitudinal optical section about the time the blastopore closes. Position of the prototroch indicated by the faint lines (9 h. 45 m.).

FIG. 72. Similar view after the first gemmation of the primary mesoblasts (the same specimen shown in Fig. 41) (7 h. 30 m.).

FIG. 73. Slightly later stage, showing gemmation of one of the mesoblasts. The section is somewhat oblique and shows the hinder part of the other mesoblast wedged in between the dividing cell and the posterior teloblast (8 h. 30 m.).

FIG. 74. Right view of the same specimen shown in Fig. 57. Nephroblast passing beneath the prototroch. Derivatives of c^1 in division (9 h. 30 m.).

FIG. 75. Later stage in similar view. Head-kidney extending itself beneath the post-trochal cells (offspring of c^2) (12 h. 30 m.).

FIG. 76. Similar view of the same embryo shown in Fig. 66. Extension and canalization of the head-kidney (11 h. 30 m.).

FIG. 77. Left side-view of about the same stage shown in Fig. 67. The head-kidneys nearly surround the body, the prototroch is double; prostomial depression still present; first appearance of the pigment (14 h.).

FIG. 78. Left side-view of same stage as Fig. 68. Prototrochal region in surface-view (also the left residual teloblast), the remainder in optical section (*cf.* Fig. 88). Formation of the pigment-area; its relations to the stomatoblasts (13 h. 30 m.).

FIG. 79. Ventral view of the same stage. The position of the residual teloblasts indicated in optical section (15 h. 45 m.).

FIG. 80. Similar view of later stage. Stomodæal arc, pigment-area, mesoblast-bands (18 h.).

FIG. 81. Completion of the stomodæum, demarcation of the pigment-plug, position of the mesoblast-bands. Last traces of the residual teloblasts (?) (20 h.).

FIG. 82. Left side-view of the same specimen. Prototroch, eye-spot, and frontal bodies in surface-view. The stomodæum and pigment-area in juxtaposition (20 h.).

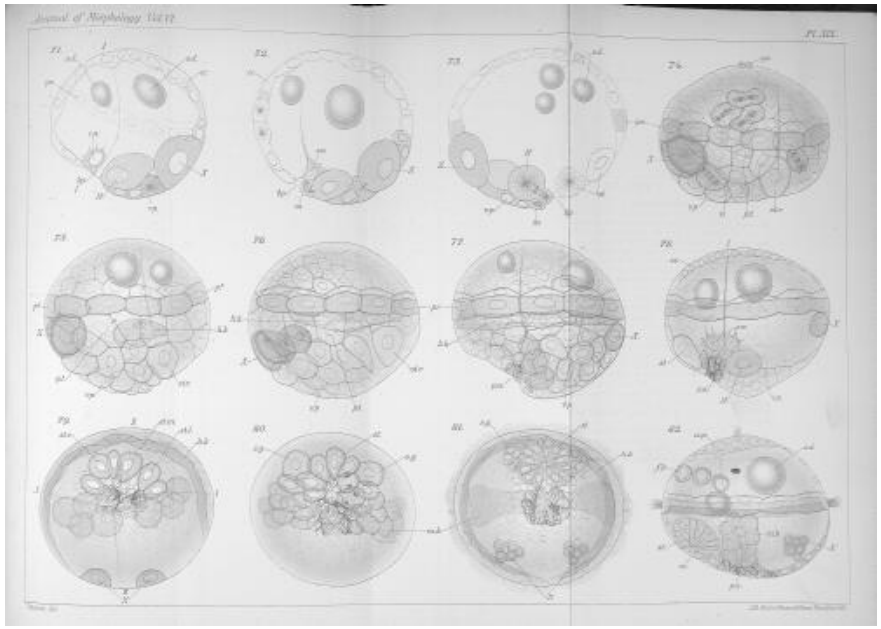

PLATE XX.

[All of the figures from preparations. Prototrochal pigment, and the cilia, from living specimens.]

FIG. 83. Ventral view. Ingrowth and concrescence of the neural plates, separation of the stomodæum and pigment-area; first appearance of metamerism (24 h.).

FIG. 84. Right side-view of the same specimen (slightly distorted by pressure).

FIG. 85. Later stage, right side-view. Formation of the setæ, longitudinal muscles, parapodial cirrhi (40 h.).

FIG. 86. The same specimen from the upper pole. First and second cleavage-lines, cross-furrow, eye-spots, frontal bodies. *Cf.* the cleavage-stages.

FIG. 87. The same specimen, ventral view. Concrescence of the neural plates, growth of the ventral region, overlapping of the head-kidneys (40 h.).

FIG. 88. Actual longitudinal section of a stage like Fig. 78. Relations of the ventral plate, stomodæal cells, primary mesoblasts, entoblast-nuclei, and the inward migrating cells of the pigment-area with their pigment (14 h.).

FIG. 89. Oral view of early stage in the elongation. Protrusion of the setæ (43 h.).

FIG. 90. Protrusion of the parapodial cirrhi. Apical and anal sense organs, *migration of the pigment-cells* (60 h.).

FIG. 91. Typical three-segmented larva, dorsal view. Completed migration of the pigment-cells, appearance of the mesotrochal ciliated belts, circular muscles, three pairs of eye-spots, first pair of tentacular cirrhi (post-trochal), and anal cirrhi (60 h.).

FIG. 92. Head of later stage (4½ d.) in ventral view. Demarcation of the head, appearance of the jaws, growth of the frontal antennæ, tentacular cirrhi, palpi, etc. Interruption of the prototroch.