Hatschek, B:

The Amphioxus and its development.

translated and edited by James Tuckey

London, Swan Sonnenschein Co.
1893

Introductory Science Text-Books

AMPHIOXUS


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THE AMPHIOXUS

THE AMPHIOXUS

AND

ITS DEVELOPMENT

BY

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TRANSLATED AND EDITED

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WITH NINE FULL PAGE ILLUSTRATIONS

London

SWAN SONNENSCHEIN & CO

NEW YORK: MACMILLAN & CO.

1893

Butler & Tanner,
The Selwood Printing Works,
Frome, and London.

TRANSLATOR'S PREFACE.

Dr. Hatschek's investigations respecting the *Amphioxus* have hitherto not been accessible to that portion of the scientific world which does not read German. The following Translation has been undertaken in response to a generally expressed wish that this want should be remedied.

My best thanks for valuable assistance are due to Dr. D'Amman, of Kew, and Professor Potter, of the College of Science in the University of Durham. The latter kindly undertook to revise the scientific terminology.

J. T.

University College,
Durham, *June*, 1893.

CONTENTS.

	PAGE
Translator's Preface	v
Introduction	1
Development of the Embryo	11
The Spawning, and the Duration of Development in the Embryo	26
First Period of Development	43
Second Period of Development	56
Third Period of Development	67
Fourth Period of Development	129
Fifth Period of Development	159
Explanation of the Figures	166
Signification of Letters in Plates.	181

INTRODUCTION.

PREFATORY REMARKS.

The researches of Kowalevsky with regard to the development of the *Amphioxus*, published in the year 1867, aroused the keenest interest in zoologists. This work may well be styled the beginning of the new epoch in comparative embryology, the way to which was thereby paved, and which received so great an impulse through Kowalevsky's further and more extensive investigations. Indeed, it may be said that it owes its foundation to his exertions alone.

By means of subsequent additions to, and corrections of, his earlier writings, Kowalevsky brought new facts to light, which strengthened the attention which had universally been given to a matter of such importance as the development of the *Amphioxus*.

It was very generally felt quite necessary to further

investigate and honestly test matters so important for many theoretical questions.

Several attempts which had been made to study again the development of the *Amphioxus* failed through the difficulty of procuring material for investigation. For, though the *Amphioxus* is in many places found in great numbers, yet there is difficulty in getting it to spawn in the aquarium, and generally in obtaining embryological material.

In the year 1879 I was able successfully to carry out my long-cherished plan of studying the development, and for this I was indebted to finding in Messina great abundance of the material which elsewhere it is so hard to procure.

Near the fishing village Faro, at the northern entrance of the Straits of Messina, there is a small lake of salt water, communicating with the sea by one narrow channel. This is called by the fishermen Pantano, and its sand is the home of the *Amphioxus* in innumerable quantity.

In this secluded pool are easily found in great abundance, not only the eggs which the animal has liberated, but also all stages of its development.

I spent ten weeks in this place,—namely, from

the beginning of April till the middle of June, 1879,—and there made a study of the development of the embryo, both on the living material, as well as with the assistance of reagents, and by sections. Further, any details which were then lacking I examined carefully in the course of the next year, by means of a large quantity of material which I had preserved.

In the main, the results at which I have arrived are merely a confirmation of Kowalevsky's discoveries; yet, in my opinion, our knowledge of this important development is largely increased, owing to the completeness of my investigation, the correction of several erroneous details, and the bringing to light of some important facts. There seems, therefore, sufficient reason for publishing these investigations in full.

SURVEY OF PREVIOUS TREATMENT OF THE QUESTION.

The knowledge which we have hitherto had regarding the development of the *Amphioxus* is derived chiefly from Kowalevsky's investigations. Before his time we had nothing but a few essays, written some

by Max Schultze,[1] some by Leuckart and Pagenstecher,[2] on the later stages of the larvæ. And in these, too, in view of the incompleteness of the investigation, there was no real understanding of these stages, nor was there shown any real knowledge of the development of the embryo, or of the transition to the fully developed animal. We may say that our knowledge of the larvæ was scarcely helped at all by Max Schultze. But we should not forget that through the writings of Leuckart and Pagenstecher a more intimate acquaintance was obtained with the remarkable want of symmetry of the larvæ, as well as with many other details, whose importance was first explained by Kowalevsky. The latter's explanation was however only partial, and there is now still need of further information on the matter.

Kowalevsky's first statements on the subject are to be found in a little-known and unimportant pamphlet, published in Russian, in 1865. This was afterwards

[1] *Zeitschr. f. Wiss. Zoologie*, 1852, pag. 416.

[2] R. Leuckart und Alex. Pagenstecher: "Untersuchungen über niedere Seethiere, Amphioxus lanceolatus," *Arch. f. Anat. und Phys.*, herausgegeben von J. Müller, 1858, pag. 558–569.

followed by his famous and exhaustive treatise on the development of the *Amphioxus lanceolatus*.[1]

This treatise presents us with a picture of the procedure of development taken as a whole, together with many details of interest. More especially it makes us acquainted with the segmentation, and the formation of the gastrula in the *Amphioxus*, as well as with the remarkable organization of the unsymmetrical larvæ. Furthermore, in this work the meaning of the atrial cavity, hitherto indicated as the body cavity, was made plain to us. This means a considerable advance in the understanding of the comparative anatomy of the animal.

Some very important points were not stated accurately in this first treatise, and were corrected by the author in another of no less importance, which forms a valuable supplement to his first publications. This treatise was published among the writings of the Naturalist Society in Kiew.[2] Its contents, how-

[1] A. Kowalevsky, "Entwicklungsgeschichte des Amphioxus lanceolatus," *Mém. de l'acad. imp. desc. de St. Petersbourg*, 1867.

[2] A. Kowalevsky, "Zur Entwicklung des Amphioxus (neuere Studien)," Schriften der Naturforschergesellschaft in Kiew, Band 1, pag. 327. 1870.

ever, through its being written in Russian, are but little known to the zoologists of other countries. The author therefore published in German the results arrived at in this work, among the Archives of Microscopic Anatomy in 1876.[1] These later investigations make us acquainted with the formation of the mesoblast, the development of the notochord from the hypoblast, the details of the development of the neural canal, and the fate of the mouth of the gastrula (neurenteric canal).

This is not the place to give an exhaustive account of Kowalevsky's well-known results. I will however, in each separate section of my work, briefly refer to his observations, and allude to different results yielded by my own investigations.

REMARKS UPON THE SPECIES EXAMINED.

It is not my intention to give here any general answer to the question as to how far systematists are justified in their classification of the various species of the *Amphioxus*. I will merely show what

[1] A. Kowalevsky, "Weitere Studien über die Entwicklungsgeschichte des Amphioxus lanceolatus, nebst einem Beitrage zur Homologie des Nervensystems der Würmer und Wirbelthiere," *Arch. f. mikr. Anat.*, Bd. xiii. 1876.

is the relation of the species examined by me to that found at Naples, which furnished Kowalevsky with his material. My special reason for doing this is the hope that some differences in our investigations, minor though they be, may be found to have their origin in variety of species.

The *Amphioxus* of Faro is undoubtedly different from that of Naples. I have, it is true, not extended my investigation to the characteristic details of the full-grown animals; and therefore I can only give the general impressions received when occupied with them.

Dr. Eisig, who paid me a visit at Faro in June, 1879, examined the *Amphioxus* found there, and remarked that it differed from the Neapolitan type in its considerably larger size and the stronger development and protuberance of its sexual organs.

I have observed myself that the specimens of *Amphioxus* at Faro are, during spawning time, found almost always of equal size, and distended with sexual products. It is only seldom that any are to be found at all smaller and without the sexual parts fully developed, and these too are only shorter than the full-grown specimens by one-third.

The *Neapolitan* type is easily to be distinguished by its far inferior size, and through a somewhat clearer colouring; its form too is far slighter, this being specially due to the weaker development of the sexual organs. A circumstance moreover impressed me, which points to a total dissimilarity in the periods of development. This circumstance is that at Faro, with the exception of the pelagic living larvæ, specimens a trifle smaller than the rest were found among the sexually perfect, whereas I received from Naples, during the spawning time, every variety of size. Among these were some very small, measuring but a fraction of the length of the developed specimens, smaller than I had ever seen at Faro.

This points, at any rate, to a different variety. Possibly a closer investigation will yield specific differences.

SPECIAL PORTION.

In the special portion of this treatise, in which my observations are set down in a purely descriptive manner, I intend to avoid so far as possible all remarks of a theoretical character.

Any theoretical discussion is to be found in the

general portion, forming the conclusion of this work. The history of the development of the *Amphioxus* will be divided into two parts; namely, the development of the embryo and that of the larva. Under the former will be included all those stages in which, though the animal may have left its egg-membrane and swims about freely by means of cilia, it is still developed at the expense of the material stored up in the egg.

The processes of the development of the embryo take place in forty-eight hours, and thus differ owing to their speed from those of the development of the larva, which occupy months.

THE AMPHIOXUS.

I. DEVELOPMENT OF THE EMBRYO.

Methods of Investigation and Procuring of Material.

It is my purpose to describe here somewhat fully the methods of investigation which I employed, my aim being that any succeeding investigator may thereby find his labour much lightened and his time saved. It is principally with a view thereto that this chapter is written, and it may therefore be omitted by any who do not wish personally to make a study of the *Amphioxus*. We will suppose, then, that the reader would wish from the methods to form a judgment as to the reliability of the investigation, or perhaps to discover therein methods which may be of general service.

Before everything else, it was my endeavour to

study as completely as I possibly could all that is to be seen in the living animal.

Now it was very evident that the use of reagents was absolutely necessary, and that for several reasons. The constant movement of the ciliated embryos makes the application of reagents which kill them desirable for the purpose of observing the object from every point of view quietly and at one's leisure. This can easily be done by carefully moving the cover-slips resting on wax feet, and so turning the dead object without pressing it. In this way one and the same embryo may be looked at from various sides, and various optical sections of the same can be got, this being of great importance in all stages. Further, through the use of reagents many peculiarities of structure stand out more sharply, especially the cellular formation in certain stages; and thus it is made possible to inspect more satisfactorily the formation of the embryo.

A third method, and one of great importance, is to cut the embryos into transverse sections. I was not contented with getting single transverse sections, but as far as possible made unbroken series of them. The great difficulties in my way, when dealing with so

small an object, did not deter me; for I regarded the cutting into an unbroken series of sections as absolutely essential to gaining an accurate knowledge of the formation.

The reagents and methods which I employed were not the same for all the stages.

I next made a thorough study of the segmentation in the living object. For this part of the development such method would be quite sufficient were it not for the unfortunate circumstance that the segmentation takes place at night, and therefore can only be studied by artificial light. Some control should be had over such observation through investigation of preserved material to be undertaken by day.

My drawings of the segmentation stages have been all taken from the living object by means of the Camera lucida. This, I must say, caused me some difficulty on account of the double illumination required for the microscope and for the drawing; it was too trying for the eye. My results I merely tested on material that I had preserved.

In studying the segmentation, it is most important to gain a view of the objects from different sides by a careful movement of the cover-slip, and especially to

get a clear understanding with regard to the chief axis reaching from the upper (animal) to the lower (vegetable) pole.

Kleinberg's picric sulphuric acid proved itself extremely useful for the preservation of the segmentation stages, since the form and direction of the segmentation elements undergo no change through its employment.

I set a row of little watch-glasses which were almost filled with picric sulphuric acid. Into these, so soon as a new characteristic stage began, a little pipette was emptied full of individuals of exactly the same ages. This row of little watch-glasses which, without any break, contained the successive segmentation stages, was subjected to a further process the next morning. In accordance with Kleinberg's well-known instructions, the objects were washed in alcohol to remove the picric sulphuric acid, and were finally preserved in the same somewhat diluted. From this row of segmentation stages which I had preserved, I was able, after the lapse of a year, to make preparations of a suitable kind. It is possible from these glasses, by means of a pipette, to bring up a sufficient quantity of segmentation stages; and these, freshened with glycerine, yield preparations which very closely represent the living object. I am acquainted with no object from which can be obtained so suitable demonstrative preparations.

The employment of stains I consider superfluous. In these early stages, when the granules of the yolk are still very numerous, osmic acid turns the elements far

too black, and prevents them from ever being transparent.

The stages of the invagination of the blastula and of the closing of the mouth of the gastrula can, I am convinced, best be studied in the living object. The reason is that the latter is sufficiently transparent, and so in it the individual cells and cell nuclei can be quite plainly distinguished.

The use of reagents is again of importance for all stages subsequent to, and inclusive of, that in which the formation of the mesoblastic somites begins. In these stages the histological elements, as well as the other details, cannot be distinguished with sufficient clearness in the living object. With the picric sulphuric acid, which proved so useful for the segmentation stages, I could here obtain no satisfactory results. On the other hand the use of osmic acid was here fully justified.

The treatment of the embryos was various, depending upon the end in view; namely, whether the entire object was to be studied, or the procuring of sections was intended.

I proceed now to discuss the treatment requisite for the former of these purposes. The stages from the

beginning of the formation of the mesoblastic somites up to the separation of three mesoblastic somites were handled in a manner somewhat different from the later ones, since they bear a different relation to the reagents, owing to their greater richness in yolk granules. The embryos in sea-water under the cover-slip (as far as the stage with three mesoblastic somites) I killed by letting fall upon them a drop of half per cent. perosmic acid, and on the appearance of a faint brown colour I carefully added diluted glycerine. This treatment should be regulated in such a manner that first of all the brown colour does not become too deep, which would cause the objects to be less transparent than they should be. Secondly, care must be taken that the form of the living object be fully preserved without any shrinking taking place. This end can be secured by keeping control over the alterations under the microscope. Round those preparations whose cover-slips were supported by wax feet, I put no varnish, so that I might be able to turn the objects as I wished by shifting the cover-slip. Here, too, the preparations kept for a year or more. Carmin staining I found unnecessary, since, without its aid, the clear granules are quite plainly to be distinguished.

Moreover, staining, by making the objects less transparent than they should be, was seen to be only a hindrance. For the later stages, however, I found very useful a treatment with Beal's carmin, or picrocarmin, following after the osmic acid, the reason being that the distinctness of the forms was considerably increased by the carmin staining. Indeed, in the stages subsequent to, and including, those with eight mesoblastic somites, it may be regarded as absolutely necessary. The objects stained with carmin were in like manner cleared in glycerine. I procured at the same time preparations preserved in Canada balsam.

I must now describe the treatment of the embryos, supposing that the procuring of sections is the end in view. I procured a number of serial sections, beginning with that stage in which the mouth of the gastrula becomes considerably smaller and the dorsal surface flattened. From the early spherical stages, optical sections of the living object can be obtained in all directions, while the procuring of artificial transverse sections is seen to be superfluous, yielding as it does no new results. With a view to the procuring of the sections, I first of all applied perosmic

acid to all the embryos, and afterwards carmin (either Beal's carmin or picrocarmin), then I hardened them in successive strengths of alcohol. Special care should be taken that the application of perosmic acid be not too weak, as otherwise there is an alteration of the forms of the embryo, the epiblast being raised like a bladder from the inner cells. Neither should it be too strong, since in this case the embryos become later on so dark in the alcohol that they are practically useless for sectional methods. It is always as well, when dealing with a considerable number of embryos, to test under the microscope some individual specimens after the osmic acid and after the carmin staining.

Bearing in mind the great speed of development, it is as well to preserve a very considerable number of stages. In the early stages up to that of the ninth mesoblastic somite, I preserved specimens at very short intervals, every half-hour in fact. Later on the intervals may be greater, as the embryo itself, in a space of several hours, undergoes only trifling alterations.

From the stage with mouth and first gill-slit, the embryos were no longer reared in glasses, but collected in the sea.

I will here describe somewhat more fully my manner of procedure. By means of a little pipette the embryos are conveyed into little watch-glasses. The embryos I mention are those which in the earliest stages lie inside the egg membranes at the bottom of the glass, but as soon as they leave this membrane rise up and swarm on the side of the glass which is turned away from the light, and immediately beneath the surface of the water. In this manner it is possible to have several hundred embryos at once in a little watch-glass full of sea-water. In the watch-glass are now added a few drops (about ten) of a half per cent. solution of perosmic acid. The quantity of the latter should be determined by the results of experience, as also the duration of its action. Then the whole contents of the watch-glass must be quickly poured into a little cylindrical glass. We allow the embryos to settle at the bottom of the latter, and then carefully pour away the sea-water as far as possible. Then a sufficient quantity of carmin must be poured in, and the embryo well covered with it. The embryos which were to be used for cutting sections were subjected to a very strong staining, for which about five minutes are sufficient. The washing out of the carmin is done in the glass itself. Water is poured in until the glass is full. When the embryos have sunk to the bottom, and the water is coloured by the carmin, as much as possible of it must be thrown away; and this process should be several times repeated, namely till the water shows no more sign of the carmin. Then we must gradually add alcohol, first weak and then stronger; at last this too must be removed, and the embryos kept in absolute alcohol. The effect of the perosmic acid is especially to be seen in the course of time in a considerable darkening of the embryos, and this often to an extent that is inconvenient.

Before being preserved the object was always examined in

a living state for the purpose of exactly determining the stage. The glass too was marked with a number, because in the case of the embryos which had been subjected to a dark staining and treated with reagents, an exact determination of the stage was not so readily possible as before. At the same time, a record was made in which the stage, as determined by examination of the living object, was accurately marked with reference to the number. In this way I collected in unbroken succession complete series of embryos ready prepared to be cut into sections.

In cutting the sections the main point is that the embryos should be examined and placed in the embedding material accurately with reference to the direction of the sections. The embryo is taken out of absolute alcohol, placed upon a slide, and cleared with a drop of oil of cloves. The oil of cloves is spread over the whole slide, so that the embryo lies there just wetted. Then a few drops of a slightly warmed mixture of wax and oil are carefully poured upon it. The slide is now turned over, and the dark embryo can be seen through it clearly separating itself from the white mass of wax. It is easy, by turning the readily moved wax surface, to get the embryo parallel with the length of the slide. This I have always done with as much accuracy as possible under the lens. The slide is now again to be turned over, and the mixture of wax and oil is to be added until a surface is obtained covering the whole of it. This surface, as soon as it is cooled, is separated from the slide, and this succeeds very well if the latter was thoroughly wetted with oil of cloves. The position of the embryo can now be accurately tested under the microscope; and if it does not fully agree with the axis of the wax, it can be corrected by cutting the latter parallel to it. Then I carefully prick the wax surface on both sides of the embryo in order to mark the position of the latter. In the next place a

drop of slightly warmed wax should be carefully laid upon the embryo; and when the latter has become stiff, the whole surface formerly turned to the slide should be covered with wax. To prevent the two wax surfaces, between which the embryo now lies, from again falling apart, as is often the case, a hot needle must be several times run through the wax, though naturally at some distance from the embryo.

The serial sections were made by hand, without the aid of a microtome, though this in the case of such small objects needs a little practice to be done easily. The cutting was made under alcohol. Care should be taken, in cutting, that the place where the section is situated should be accurately marked. The particular piece of wax is to be transferred with a needle from the knife, which is laid flat on the slide. The section is now to be treated with a drop of absolute alcohol; and after removal of the superfluous alcohol, a drop of oil of cloves should be added. The latter is to be preferred to other means, inasmuch as it does not easily evaporate, and the section may remain in it for a longer time. The entire series of sections may now be placed in oil of cloves.

The removal of the wax is now brought about by the warming of the slide over a spirit-lamp. The warmed oil of cloves soon acts as a solvent to the wax. After removal of the superfluous oil of cloves (the section may be easily taken out of the warmed oil of cloves by means of a needle) the section can be preserved in Canada balsam.

The *Amphioxus* begins to spawn in the first warm days of spring, and so far as my observations went, as early as the last days of March. The spawning goes on through the whole summer. But it is, as we will explain later on, dependent on the conditions of the

weather, insomuch that if these are continually unfavourable, one may have to wait fourteen days in April for the spawning, and that without result. Any one, therefore, who intends to study the development of the *Amphioxus* in *Faro*, I should strongly advise not to go there until the beginning of May, when one is sure of having some fine days, in which a very considerable amount of spawning will take place. This will always be found most plentiful when some warm days succeed to a somewhat long period of cold and stormy weather, and it is on the second or third fine day that it mostly begins.

The fertilization can best be studied by taking the animals out of the sand on the shore on some such warm afternoon, whereupon they will be found to spawn plentifully in the glasses. I have not been able to observe the process more accurately.

For the study of the segmentation it is better to fish in the sea after sunset in Pantano itself for eggs liberated under natural conditions, and to allow their further development in the glasses. Too many eggs should not be placed in one glass, since otherwise abnormal stages will result. The segmentation itself may only be studied by night. In order to convince

myself of the normal activity of the later stages, I have even fished in the sea at twelve o'clock at night, at which time the earlier segmentation stages were to be found.

To follow the closing of the gastrula-mouth in fully normal stages, it is a good thing again to fish in the sea on the next morning after the spawning. It is then in the early hours of the morning that embryos are to be found with a wide-open gastrula-mouth, somewhat younger than in Fig. 26. In the case of all these embryos fished on the morning of the first day, there is a certain and complete development to be observed, with the exception of those which have perhaps been injured by the net.

In this way I easily obtained a large quantity of embryos of entirely normal development, and these too underwent in my glasses a further development which was quite normal. This I learnt by a comparison with the further stages fished at Pantano. From the gastrula stage on the eggs are far less susceptible to outside influences than immediately after their liberation, and during the segmentation.

With this material I was able again to pursue the further development in stages following each other in

unbroken succession. Here, so far as the quantity of material and the similarity of age in the stages were concerned, the conditions were favourable to an extent that is reached only in few other cases, as, for instance, when artificial fertilization is used.

In the course of the forenoon, it is possible to follow the closing of the gastrula, and this should be mostly studied in the living object.

The formation of the first mesoblastic somites which then succeeds is of such rapidity that the process must often be studied in the living object and in preserved preparations in order to understand it thoroughly.

The development proceeds with such rapidity, and specially on the first afternoon, that the examination of the object can scarcely keep pace with it.

During the next hours the development becomes more and more slow. After the lapse of about forty-eight hours, we have the formation of the mouth and first gill-slit, and these mark the conclusion of the embryo development. In order to study all the stages previous to this, there must, I think, be for some time night work. Through alterations in the speed of development, this speed depending on differences of temperature, it is quite true that with repeated

examination we may obtain by day a view of almost all the stages, with the exception of those occurring on the first night. And of this the reason is, that when the weather is not cold, the development is almost as far advanced at the conclusion of the first day as in cold weather at the conclusion of the second night. It is, however, always advisable, with a view of obtaining a continuous idea of the development, to follow the embryos obtained from one spawning for thirty-six hours from the gastrula stage.

In order to be thoroughly acquainted with the fully normal activity of all the stages, I have also studied embryos of every degree of development fished out of Pantano.

After the perforation of the mouth and first gill-slit, I was able to keep the larvæ a yet longer time alive in the glasses. The development of the latter is, as might be expected, poor as compared with those which are reared in Pantano.

I preferred, therefore, to examine the organization of the larvæ and their further development in individual specimens fished in the sea.

THE SPAWNING, AND THE DURATION OF DEVELOPMENT IN THE EMBRYO.

A. The Spawning.

In October, 1878, I visited *Faro* for the purpose of investigating the circumstances under which the *Amphioxus* is there seen, and I examined the condition of the animal with reference to sexual maturity. At this time of year the sexual products were not yet fully matured; and though I fished Pantano with Müller's net, there were to be found, on drawing the net up, neither stages of development nor young specimens of the *Amphioxus*. The whole winter through, frequent repetition of the examination yielded the same result. The maturing of the sexual products was but a slow process.

On the 3rd of April, 1879, I fished the surface of Pantano with Müller's net, and I found, on drawing up the net, a large number of embryo stages of development and larvæ, with mouth and first gill-slit. According to my later experiences with regard to the period of development, the first spawning must have taken place about eight days previously.

The spawning continued throughout my whole stay,—namely, till the middle of June,—and I imagine that it lasts a still longer time.

The spawning period thus begins, at any rate in the case of the Amphioxus of *Faro*, somewhat earlier than Kowalevsky stated. He observed in *Naples* on the 18th of May, for the first time, that the animals which he kept in aquariums were liberating their eggs.

The spawning is, in a remarkable degree, dependent upon the conditions of the weather and the time of day. In cool and stormy weather, I had to wait for weeks together for a spawning period, and without result. But on warm sunny days, it was possible on the second or third afternoon to distinguish in females taken from the sand of the shore, masses of eggs situated in the atrial cavity, and glistening through the thin covering of the body. And then the animals which had been placed in glasses began to emit their sexual products through the opening of the mouth. The males sent out whole clouds of sperma, and the females in the same way their eggs in such quantities that a great many remained hanging on their oral cirri.

The sexual products probably made their way,

through bursting the follicle membrane, into the neighbouring atrial cavity, and from there, through the gill-slit, into the inner part of the pharynx, to be pushed thence through the mouth, and so outside. I can fully confirm Kowalevsky's statement, that the sexual products of the Amphioxus are emitted through the mouth—a statement which has, without justice, been called in question.

It was thus possible on such a warm afternoon, by disturbing the animals, to make them emit the sexual products stored up in the atrial cavity. Apart from this interference, however, they kept the sexual products till evening.

It was of no use to fish with the surface-net by day, since then it was not possible to find any of the emitted sexual products. At sunset, however, the spawning began almost simultaneously along the whole stretch of shore where the Amphioxus lives in the sand, so that the eggs could be fished in great quantities with Müller's net. When darkness begins, the spawning advances with great speed, so that all the embryos in Pantano are always found of the same stage of development in the next twenty-four hours, and also on the following day.

We have seen that the spawning depends upon the conditions of the weather, and on the temperature. In the same way other influences appear to have an adverse effect. Thus for weeks together I was unable to obtain any deposit of the sexual products from specimens kept in glasses, since, though containing them in abundance, they were plainly under unfavourable conditions.

B. Duration of Embryo Development.

The development of the Amphioxus may, as I have already said, be divided into two chief parts. The first part may be suitably called development of the embryo, for in this part the development proceeds at the expense of nutriment stored in the egg.

In this first period the development advances very rapidly. Its duration occupies a period of, on an average, something less than forty-eight hours. That is to say, it continues from the liberation of the eggs, immediately followed by the fertilization, up to the perforation of the mouth and first gill-slit, this involving the conclusion of the embryo development.

The speed with which the processes of development advance in this part depends, for the rest, chiefly upon the conditions of the temperature. I will give a

readily intelligible record of several of my observations. From this the alterations in the duration of development will be easily seen.

As I have already said, the eggs are liberated immediately after sunset, that is in the season at which my observation took place, the hour being about 8 o'clock. The segmentation begins about an hour after the liberation of the eggs, and is finished probably a little after 12 o'clock. From an hour and a half to two hours later begins the invagination. After the lapse of another hour the segmentation cavity is fully removed. I now give in a table a statement of the details, as I noticed them follow one after the other.

8.0	o'clock,	Liberation of the eggs.
9.0	„	Stage with two cells.
10.0	„	„ „ four cells.
10.15	„	„ „ eight cells.
10.30	„	„ „ sixteen cells.
11.0	„	„ „ thirty-two cells.
11.30	„	Further steps in the division.
11.45	„	
12.15	„	
12.30	„	The cells begin to assume the character of an epithelium.
1.0	„	Blastula.
1.45	„	Beginning of the invagination.
2.45	„	Segmentation cavity entirely removed.

From now until early morning the lessening of the gastrula-mouth proceeds but slowly.

Several other successive stages which I noticed in the development advanced with similar speed.

On the morning of the first day—after, that is, the lapse of ten hours—the gastrula-mouth was still wide open. The closing of it is a very slow process as compared with the other processes of development, and occupies, as a general rule, the greatest part of the forenoon.

From noontide of the first day till evening the most important processes of development advance with great speed,—I refer to the remarkable folding of the primary hypoblast which introduces the formation of the mesoblastic somites and of the notochord, as well as the formation of the neural canal from the epiblast. So we have a succession of important processes, through which the most important systems of organs are developed from the two primary germ layers.

In the next twenty-four hours nothing further took place beyond a slight increase of the mesoblastic somites, elongation of the form of the body, and histological differentiation. So far as new organs are concerned, there is only to be noticed in this period the

addition of the peculiar gland of the Amphioxus larva. At the end of this period, however, takes place the perforation of the mouth and first gill-slit. Some hours later the anus also appears.

The following table will give more exact information with regard to the times of development and their variability :—

FIRST DAY.

First Series.	Second Series.	Third Series.
o'clock	o'clock	o'clock
	8.15. Stage, Fig. 26.	
8.30. Stage, Fig. 29.		8.30. Stage, Figs. 31-33.
	9.0. Stage, Fig. 29.	9.0. Stage, Fig. 33.
9.30. Stage, Fig. 31.		
	9.45. Stage, Fig. 31 (begin to rotate).	9.45. Stage, Fig. 35.
10.15. Embryos begin to rotate.		
	10.30. Stage, Fig. 33.	10.30. First Mesoblastic Somite.
		11.0. 2 Mesoblastic Somites.
	11.15. Stage, Fig. 35.	
11.30. Stage, Fig. 33.		11.30. 2–3 Mesoblastic Somites.

First Series.	Second Series.	Third Series.
o'clock	o'clock	o'clock
12.0. Stage, Fig. 35.	12.0. First Mesoblastic Somite.	
		12.15. 3–4 Mesoblastic Somites.
12.30. 1 Mesoblastic Somite.		
	12.45. 2 Mesoblastic Somites (leave the egg membrane).	
1.0. 1–2 Mesoblastic Somites.		1.0. 4–5 Mesoblastic Somites.
1.30. 2 Mesoblastic Somites.	1.30. 3 Mesoblastic Somites.	
		1.45. 5–6 Mesoblastic Somites.
2.0. 2–3 Mesoblastic Somites.		
2.30. 3 Mesoblastic Somites.	2.30. 3–4 Mesoblastic Somites.	2.30. 6 Mesoblastic Somites.
3.30. 3–4 Mesoblastic Somites.	3.30. 4 Mesoblastic Somites.	
4.30. 4 Mesoblastic Somites.	4.30. 5 Mesoblastic Somites.	
		4.45. 7 Mesoblastic Somites.
	5.30. 6 Mesoblastic Somites.	
6.0. 4–5 Mesoblastic Somites.	6.0. 6 Mesoblastic Somites.	6.0. 8 Mesoblastic Somites.

SECOND NIGHT.

First Series.	Second Series.	Third Series.
o'clock	o'clock	o'clock
7.0. 5 Mesoblastic Somites.	7.0 6–7 Mesoblastic Somites.	
8.0. 5–6 Mesoblastic Somites.		
	8.30. 8 Mesoblastic Somites.	
9.0. 6 Mesoblastic Somites.		
10.0. 7 Mesoblastic Somites.	10.0. 9 Mesoblastic Somites.	
11.30. 8 Mesoblastic Somites.		
12.0. 8 Mesoblastic Somites.	12.0. 10 Mesoblastic Somites.	
1.0. 8–9 Mesoblastic Somites.		
2.0. 8–9 Mesoblastic Somites.	2.0. 14 Mesoblastic Somites (weak muscular movements.)	
3.0. 8–9 Mesoblastic Somites.		
4.0. 9–10 Mesoblastic Somites.		
	4.30. 12–13 Mesoblastic Somites.	
5.0 9–10 Mesoblastic Somites.		
	6.0. 12–13 Mesoblastic Somites.	

SECOND DAY.

First Series.	Second Series.	Third Series.
o'clock 7-8.0. 10–11 Mesoblastic Somites (weak muscular movement).	o'clock	o'clock
	8.30. 14 Mesoblastic Somites.	
10.0. 11–12 Mesoblastic Somites.		
	11–1. Perforation of mouth and first gill-slit prepared for.	
12.0. 13 Mesoblastic Somites.		
2.0. 14 Mesoblastic Somites.		
	2.30. Mouth and first gill-slit perforated with very fine opening.	
	5.30. Both openings still very small.	
6.0. Mouth and first gill-slit perforated as fine openings.		

From the perforation of the mouth and of the first gill-slit the larva begins to nourish itself independently, since the yolk material contained in the egg is quite used up, and the cells of the larva consist of absolutely transparent protoplasm.

From now onwards the development proceeds very slowly, especially at first, the larva having to make good the material for development, which is quite exhausted.

Thus, while a great part of the most important processes of development takes up only the short space of forty-eight hours, the further development on the other hand, from the time when the larva must begin to nourish itself, occupies months.

C. The Liberated Egg, the Expulsion of the Polar Body, and the Fertilization.

The following is Kowalevsky's account of the eggs just liberated. "The ejected eggs lay at first from ten to twenty together in little lumps. Further and repeated observations always resulted in showing that the ejection of the eggs was preceded on the part of the male by an ejection of semen.

"The eggs just ejected consisted of a dark yolk

and a vitelline membrane but little removed from it. A large amount of water was absorbed, and the vitelline membrane was continually distended, until at last it reached the proportions represented in Fig. 1. The yolk presented itself, by transmitted light, as a quite dark homogeneous round body, which on nearer examination, and when pressed, consisted of an absolutely transparent plasma and very fine fat globules. The diameter of the egg was not more than 0·105 mm. I could not find a nucleus in the fertilized eggs, although in the unfertilized, taken from the ovary, it was always quite plainly to be seen. I do not however at all mean to say that the nucleus vanishes; I know the difficulties in the way of finding it in the fertilized egg."

These statements I can for the most part confirm. In certain points however I went further than Kowalevsky. These mainly are concerned with the observation of a polar body, and in close connection therewith the proof of the polar differentiation in the unsegmented egg.

The first processes of development which I brought within the sphere of my examination, have to do with the eggs just ejected, so far as they were liberated by

the specimens of the *Amphioxus* collected at noon by Pantano.

The eggs were found to be mostly entirely isolated. It was only seldom that they hung a few at a time in a little lump together, though Kowalevsky describes this condition as the regular one. The substance of the egg consists of a clear protoplasm, which is however so darkened by numerous yolk granules that the whole egg appears as a somewhat untransparent body.

The yolk granules were described by Kowalevsky as fat globules. I cannot agree with this account. They are round corpuscles, which do not interrupt the light to so great a degree as fat bodies. Their relation, too, with regard to reagents is quite different from that of fat. They are, indeed, very strongly darkened by osmic acid, and under this process the earlier stages, which still contain a large number of yolk granules, grow far darker than the later stages, in which they are already more dissolved. These corpuscles are not however dissolved through treatment with alcohol and turpentine, or oil of cloves. Carmin will not colour them. I must mention here, that protoplasm itself, also, is made browner by osmic acid in the earlier stages than in the later.

In all the eggs the germinal vesicle had disappeared, and in the living egg, which was but slightly transparent, I could see nothing at all left of it. In one place, namely, the upper pole of the egg, there was to be seen on the surface a tolerably clear mass of protoplasm poorly supplied with yolk, and on the surface of this a clear and already quite sharply defined polar body. As I found the polar body already fully defined a short time (perhaps a quarter of an hour) after the ejection of the eggs, I think I must conclude that already, during the course of the day, isolation of the individual eggs from one another and the ejection of the polar body took place within the atrial cavity.

I could now understand why the artificial fertilization, which had been so often tried, never would succeed. The reason is that the eggs, which were obtained by pulling the ovaries to pieces, could never be completely isolated from one another, and always exhibited a large distinct germinal vesicle with germinal spot and nucleolus. They were not therefore in a condition capable of fertilization, since, as we see, in the *Amphioxus* the fertilization does not take place till after the ejection of the polar body, that is, a considerable time after the expulsion from the ovaries.

The eggs ejected were surrounded by numberless spermatozoa, which turned like radii towards the membrane of the egg, held fast to it by their heads and tried to make their way into it.

At the same time the vitelline membrane began to separate rapidly from the protoplasm of the egg, probably under the influence of the sea water. It was only at one point that the membrane adhered any longer to the protoplasm, it having there in consequence the appearance of being drawn in like a funnel. I think that this is just the place in which a spermatozoon made its way into the egg. This place I regularly found near to the lower pole.

The separation of the vitelline membrane advances with great rapidity, and it expands to many times the diameter of the egg, enclosing a clear liquid, which can certainly be nothing else than diffused sea-water. This expansion of the vitelline membrane advances also further during the first segmentation stages, and reaches such a degree as may be seen in Fig. 1, where a later embryo stage is formed within the egg membrane.

These conditions exhibit to us already the remarkable and extreme elasticity of the vitelline membrane. I will introduce here some further observations, which give

us still further enlightenment with regard to this elasticity, and also make intelligible the manner in which the spermatozoa force their way in through the vitelline membrane. By pressure, and by turning the egg with the coverslip, one may be convinced of the great elasticity of the vitelline membrane, which is widely separated from the egg. A further chance observation gave me a clear idea of the very remarkable consistence of this membrane. In the examination of later stages, in which the already ciliated embryo rotates inside the egg membrane, it happened that through the pressure of the slip, the egg membrane tore in one place, and there a part of the soft embryo-body forced its way outwards, as it were a bag bursting. When the pressure of the cover-slip was removed, the part of the embryo which had been pressed forward was abstricted by the egg membrane. The rest of the ciliated embryo made its way inside the egg membrane again, and the rupture of the latter disappeared so completely that not a trace of it was any longer to be perceived. This remarkable and, one might almost say, plastic activity of the vitelline membrane, explains how it is that without the formation of a micropyle the spermatozoon can force its way into the egg.

I did not here, it is true, follow the processes of fertilization with reference to the alterations of the nucleus with any greater accuracy, as I had already done this before sufficiently with thoroughly favourable objects. Yet I was able to point out observations in accordance with the now generally received views. Among the changes of the germinal vesicle, by which the latter is withdrawn from observation, is to be placed the ejection of the polar body; and next to this follows the fertilization. After the fertilization, and before the beginning of the segmentation a nucleus was again to be noticed in the egg.

FIRST PERIOD OF DEVELOPMENT.

The Segmentation (Figs. 2–20).

The segmentation was previously followed by Kowalevsky in its several features. The first stages apparently he had described very accurately, namely the division of the egg into two, and afterwards into four cells, which then, by an equatorial furrow, are divided into eight. A sixteen-cell stage was afterwards described, as well as the formation of a hollow sphere occasioned by succeeding divisions, this having a large cavity and a thin wall constructed out of a single layer of cells.

According to this account of Kowalevsky, the segmentation of the Amphioxus was always treated as the type of an equal segmentation leading to a blastula without any well-defined main axis. My own observations complete our knowledge of the Amphioxus to this extent that we recognise that the segmentation is by no means of exact equality, but

rather unequal. There is a great difference to be noticed between the segmentation spheres of the upper half and those of the lower half. We can continuously observe the main axis drawn from the upper to the lower pole, from the unfertilized stage up to the formation of the blastula.

When we look more closely at the segmentation of the Amphioxus in reference to the characteristic succession of the planes of segmentation, we recognise that it shows in the main the same type as does that of the lower vertebrates, which have a holoblastic development (Petromyzon, Sturgeon, and Amphibia). That is to say, we find an extensive agreement which was not to be recognised from the accounts hitherto published.

The segmentation begins, as Kowalevsky says, about an hour after the liberation of the eggs. The formation of the first furrow and the division of the egg follow then very quickly, that is, in about five minutes. It is just the same with the succeeding divisions. After a somewhat long interval the division of the segmentation spheres begins, its completion being followed by an interval as before.

The First Furrow and Two-celled Stage (Figs. 3, 4.)

The first furrow makes itself primarily noticeable as a depression on the upper pole of the egg in the neighbourhood of the polar body. Then immediately it grows round the whole circumference, and gradually begins to divide the egg in two parts. It is however always deeper on the upper side, where it first appeared (Fig. 3). Before the complete separation of the egg into two halves,[1] we have still a somewhat clear protoplasmic connexion, poor in yolk granules. At last this also is separated into two, and the two parts, which are distinguished from each other by quite a sharp outline, take the spherical form, and touch only at one single point. The formation of the first furrow up to the complete division into two segmentation spheres, occupies scarcely five minutes. The two spherical portions now become flat opposite each other in the plane of the first furrow, so that their contact surface is a far greater one.

The first segmentation plane is accordingly a meridional one, and so far as can be observed divides the egg into two absolutely equal parts. The polar body

[1] Blastomeres.

remains however, as may be seen from Figures 3 and 4, attached to one of these pieces.

The processes which have to do with the cell nucleus next occupied my attention. In the but slightly transparent living object it is to be noticed that the cell nucleus apparently vanishes during the process of division, and after this is completed is again visible as a clear spot in the centre of the segmentation sphere. The same is to be observed in the further divisions.

Four-Celled Stage (Figs. 5–7).

After a pause of about an hour the formation of the second furrow begins. This is likewise a meridional one, and is at right angles to the first. Both segmentation spheres[1] are affected by this division at exactly the same time. We have represented in Fig. 5 the manner in which the furrow makes a deeper cut on the outside, than on the contact surface of the two cells.

As a result of this segmentation we have four spheres of equal size, on one of which the polar body is still attached at the upper pole. The spheres are again flattened against each other, a proceeding which

[1] Blastomeres.

is repeated after each division, so that they lie against one another with the planes of division pushing against each other in a cruciform manner at right angles (Fig. 6). In the middle between the four segmentation spheres there remains however, above and below, at the upper and lower pole, an open hollow space which forms the first indication of the segmentation cavity.[1] The opposite position of the four cells can be best seen in Fig. 6, where this stage is seen from the upper pole, and in Fig. 7, where it was represented from the side, and in such a way that one of the cells is turned towards the spectator. The division into four cells is concluded approximately after the lapse of the second hour from the liberation of the eggs.

Eight-Celled Stage (Fig. 8).

The segmentation process begins, from now on, to advance with much greater speed, for after the lapse of another quarter of an hour we find all the cells again divided.

The division of the four cells follows at the same time, and in a common, in fact, equatorial plane of division. Every one of the four spheres is divided

[1] Blastocœl.

into a smaller part situated at the upper pole, and a greater at the lower.

Simultaneously with this first equatorial furrow there takes place a difference of size in the segmentation sphere, and a clearer distinction between the upper and lower poles results. The segmentation cavity still remains wide open at the upper and lower pole, as is to be seen in Fig. 8.

Sixteen-Celled Stage (Fig. 9).

The next division which again takes place within a quarter of an hour concerns all the eight cells simultaneously; each one of them divides by means of a meridional furrow into two equal parts, so that we see an upper tier of eight smaller cells and a lower of eight larger ones.

This stage arises, as we see, through the simultaneous appearance of four new meridional segmentation planes.

So long as the cells immediately after the division still possess a more or less spherical form, it should be noticed that the stages are considerably increased in breadth and are depressed from the upper to the lower pole.

The opening of the segmentation-cavity on both sides is still wider than in the preceding stage (Fig. 9).

On observing this stage from the upper pole we are struck by the beautiful regularity with which these cells are arranged in two tiers. So far as the activity and order of the cells are concerned only one chief axis can be distinguished, which reaches from the upper to the lower pole. The polar body however is attached to a single upper cell.

Thirty-two Celled Stage (Figs. 10, 11).

At the end of the next half-hour, *i.e.* three hours after the liberation of the eggs, follows the further division of all sixteen cells together, so that we reach the stage with thirty-two cells. This means that the eight upper as also the eight lower cells are all divided simultaneously by means of furrows running equatorially, and each one into two parts.

Of the thirty-two cells which thus result the eight situated at the lower pole are considerably larger than the rest. Towards the upper pole, the size gradually diminishes in the three further tiers

of cells, so that those which are situated at the upper pole are the smallest.

The segmentation cavity becomes considerably larger, owing to separation of the cells, while the cells at the upper and the lower pole begin to close together in such a manner that the segmentation cavity which was formerly open is at once completely closed, as can be seen from Fig. 11.

Cessation of the Process of Division at the Lower Pole (Fig. 12).

The next division, which we have to consider, is the first which does not concern all the cells of the embryo but only a part of the same. What happens is that all the cells of the three upper tiers are simultaneously divided, separating as they do through meridional planes of division, each of them into two equal parts. Thereby the number of the cells in each of the three upper tiers is increased from eight to sixteen, while the fourth and lowest keeps its former number of eight.

Through this cessation in the division on the part of the lowest tier of cells, the difference in respect

of size between the cells of the lower and the upper pole is further considerably emphasized.

In the next place, from these eight large cells of the lower pole eight smaller ones are separated, which lie towards the upper pole, this again being by equatorial division. These eight smaller cells also divide through meridional division into a circle of sixteen. We have now a stage before us in which we may count four upper tiers, each of 16 cells, and a lower one with eight large cells (Fig. 12).

The lowest tier remains then without change throughout a series of stages, composed of eight large cells which considerably surpass all the other cells of the embryo in size.

Further Increase of the Tiers of Cells through a Number of Equatorial Divisions (Figs. 13, 14).

There follows now at constantly shorter intervals a series of equatorial furrows by which the number of the tiers with sixteen cells is increased.

We have, for example, represented in Fig. 13 a stage of this period in which, besides the lower tier with eight cells, five with sixteen are to be counted. In one of the latter we see also all the

cells in equatorial division. In this example can be noticed the remarkable regularity with which all the cells of a tier are simultaneously affected by the division. We see all the cells of this tier taking a biscuit-like form.

During the equatorial division of a tier, the whole embryo takes a form elongated in the chief axis. The purpose of this is that it shall return to the spherical form in which, after completion of the division, the products again lie closer to each other.

In Fig. 14, in which we see represented the same stage in optical section, we may observe the segmentation cavity already considerably increased.

In this stage I was still generally able to see the polar body at the upper pole.

Further Increase of the Cells, and Disappearance of the Regular Tiers (Figs. 15–18).

With the appearance of new meridional divisions there is lost the hitherto regular arrangement of the cells in tiers. I could still, indeed, in individual cases observe stages with more than ten tiers, of which the lowest still counted eight cells, while most of the others, at any rate the lower and middle

ones, were formed of about thirty-two. In most cases, however, the regular arrangement of the tiers had been lost through shifting of the cells, while their number was diminishing.

Despite the loss of the tiers, the chief axis drawn from the upper to the lower pole can still be clearly distinguished in these stages.

Indeed, all about the lower pole, a region of somewhat large dark cells can be distinguished, which for the greatest part at any rate have their origin in the eight cells of the lower pole, and are plainly different from the other and smaller cells.

The smallest cells are to be found at the upper pole.

During this period the transition to the blastula stage is already noticeable, while the dimension of the cells in height gradually surpasses the other dimensions. The cells, too, through a flattening of the ends, first those turned towards the segmentation cavity, and afterwards those outside, acquire an epithelial-like character.

During these processes, which are accompanied by a constant increase of the cells, there takes place a continual growth of the segmentation cavity. This

growth of the segmentation cavity appears to take place at the expense of the cell mass, which becomes somewhat smaller.

The Blastula (Figs. 19, 20).

The transition from the segmentation stages to that stage which we designate by the name blastula is characterized in the following way. The cells, which had formerly approached the spherical form, being therefore flattened against one another only to a limited extent, and which as well outwards as inwards pushed forward towards the segmentation cavity with a decided tendency to being spherical, now lie against one another, and acquire a more epithelial-like character. In the next place epiblast cells, which compose the upper two-thirds of the roof, are changed, while first its inner surface, that, namely, which is turned towards the segmentation cavity, and then, finally, its outer surface are altered in the characteristic way (Figs. 16 and 20).

It is not till somewhat later that the lower third, composed of larger and darker cells representing the hypoblast, is affected by similar processes. The cells had hitherto kept a certain, and for the seg-

mentation sphere, characteristic independence; this they now lose, since as epithelial cells they stand in closer dependence on one another. Thus that stage of the blastula is reached which is characterized by an epithelial layer on every side, surrounding a closed inner cavity. This simple epithelial layer forms the substratum for the later processes of development. We shall see how through foldings and growth of this simple layer the most important organs become separated off. During the whole embryonic development, all changes from the blastula onwards can be traced to these primitive cell-layers. There never occurs a multiplication of the epithelial layers, or division of the same.

SECOND PERIOD OF DEVELOPMENT.

Formation of the Gastrula, and the Closing of the Gastrula-Mouth (Figs. 17–34).

Kowalevsky describes how the round blastula first becomes oval, then through flattening of the one wall and invagination of the same takes the shape of a flat two-layered embryo. My observations of this process do not differ in important points from his, and the segmentation cavity, which Kowalevsky describes even after the invagination as a small slit, according to my observations disappears entirely, so that the two layers touch one another immediately.

After the invagination follows the closing of the gastrula-mouth,[1] whereby the embryo, according to Kowalevsky, gradually takes the shape of "a somewhat elongated hollow globe." The embryo is now covered, according to him, with cilia. Further, it is stretched to a yet greater length, and the gastrula-mouth, considerably narrowed, becomes excentric,

[1] Blastopore.

being pushed to that particular side which by being flattened out becomes the dorsal side of the embryo. According to Kowalevsky, from this stage on can be recognised the bilateral symmetry.

I myself was able to recognise the bilateral symmetry much earlier, that is to say, from the stage of the completed invagination. I also came to the conclusion that the original wide gastrula-mouth belongs entirely to the later dorsal region, and that one part of its edge indicates the hinder part of the body. The closing of the gastrula-mouth proceeds, so far as I could see, from front to back, and at last there only remains the part furthest back.

I will now state the results of my observations in more detail.

After the formation of the blastula is completed, there is a pause in the increase of the cells in order to allow room for another process which now directly begins in the embryo: this is the process of gastrulation.

On our next view of the blastula (Figs. 19, 20), which forms the substratum of the alterations about to commence, we see that at this stage, as from the beginning of the development onwards, there is only a single axis to be distinguished. At the lower pole we see

a surface of somewhat dark cells, which is easily distinguishable, and occupies about a third of the whole extent. These cells are darker, as they contain a greater number of yolk-granules, and therefore allow the cell nuclei to show through less distinctly. This surface now begins at once to flatten itself out (Fig. 21) and then to be indented, the purpose being that the segmentation cavity should be obliterated and that it should gradually become attached to the upper layer formed of the smaller and clearer epiblast cells (Figs. 22, 23). The result of this process is a flat cap-like two-layered stage in which no segmentation cavity is noticeable, but rather only a sharp border line between epiblast and hypoblast (Fig. 24).

If we compare with one another the stages from the blastula up to this two-layered, cap-shaped gastrula, and especially if we regard the number and proportionate sizes of the cells, we shall see that the lower layer of cells, the true hypoblast, corresponds to but little more than a third of the blastula. These cells, however, have increased in size during the invagination process and simultaneously with the disappearance of the segmentation cavity. This is only to be explained by the fact that the hypoblast cells have

partly reabsorbed the moisture found in the segmentation cavity. The mechanical side of the process is also thereby explained to us. The hypoblast cells play a more active part, the epiblast cells remaining passive during the whole process and forming a spherical covering.

By this time, owing to the first enlargement of the hypoblast cells, which take a more columnar form (Fig. 21), the flattening of the lower pole begins. Further, owing to the diminution of the fluid in the segmentation cavity, which we ascribe to an action of the hypoblast cells, this flat surface is bent inwards, since it offers less resistance to such bending than the convex epiblast cells. The continual diminution of the moisture in the segmentation cavity, aided as it is by the change in form of the enlarging hypoblast cells, requires continuous invagination. The enlargement of the hypoblast cells makes it possible that they, which originally occupy a relatively smaller surface, form the whole lower stratum of the flat convex gastrula stage. The extension of this inner surface is, however, still always to be considered as far less than that of the outer one which is formed of the epiblast cells.

The absorbed moisture of the segmentation cavity may indeed have partly contained the albuminous substances separated from the segmentation cells, and which were now again taken up by the hypoblast cells. To a great extent this moisture was diluted indeed with sea water which evidently fills up that space within the vitelline membrane with which the segmentation cavity in the early stages (up to the stage with sixteen cells, Fig. 9) was in open communication.

On a closer examination of the flat gastrula stage (Fig. 24) the bilateral symmetry can already be distinguished, that is to say, the right and left side of the body, while in the blastula only a chief axis was distinguishable.

This may quite well be recognised in profile in the irregularity of the curvature (Fig. 24), as also, on examination of the embryo from the gastrula-mouth, the outline here appearing not circular but somewhat oval (Fig. 25).

Fig. 24 is not, like the former ones, constructed with reference to the axis from the upper to the lower pole, but, like the succeeding figures, according to the later longitudinal axis. It is now time to mention

particularly that so far as my observation went the axis drawn from the upper to the lower pole crosses the longitudinal axis at an acute angle.

In profile we see what is afterwards the anterior end indicated by a place that is more sharply curved, which in relation to the upper pole, occupying as it does the middle of the curve, lies excentrically, so that the greater part of the curvature belongs to the ventral side, the shorter section to the dorsal side.

The understanding of the stages, in which follows the closing of the gastrula-mouth, presents difficulties, especially in reference to their mutual position. I will now describe the changes of form quite objectively, and without attempting any explanation. In the first place during the diminution of the gastrula-mouth the flat cap-formed stage takes a deeper, as it were, hemispherical form (Fig. 26). The bilateral symmetry finds its strongest expression in the flattening of that side which corresponds to the later dorsal side. In the progressive diminution of the gastrula-mouth the embryo gradually takes a form which, on the front view, has nearly a circular outline, and in profile causes the flattening of the dorsal side to be more and more strongly expressed (Figs. 29–32). The

opening of the gastrula-mouth appears situated somewhat in the direction of the dorsal surface.

The embryo takes further an elongated form, so that, on the front view, it appears somewhat oval, and in profile shows a dorsal surface parallel to the longitudinal axis and quite flattened out, on the posterior end of which lies the already considerably narrowed gastrula mouth (Figs. 33, 34).

The manner in which these successive stages follow from one another certainly admits of various explanations. It may be considered, and this is more or less the view of Kowalevsky, that the axis drawn from the upper to the lower pole corresponds to the later longitudinal axis. This view would further say that the gastrula-mouth, from quite the beginning (Fig. 24), corresponds to the posterior end, that during the diminution of the gastrula-mouth the extension of the embryo is continually advancing, and that the gastrula-mouth only in the last stages experiences a movement towards the back. I will not absolutely deny the fairness of this view, although I consider, as far more probable and more consistent with the facts, an explanation which I will now proceed to give.

This explanation finds its expression in the examina-

tion of Figs. 24, 26, 29, 31, and 33. In the examination of the figures, regard is first paid to the more sharply marked part of the curvature, which is called the anterior end. By this means I came to the conclusion that the gastrula-mouth belongs entirely to the later dorsal surface, and that the posterior edge of the same marks the posterior end of the embryo. The longitudinal axis is constructed accordingly, a straight line being drawn from the sharply marked part of the curvature which marks the anterior end through the posterior edge of the gastrula-mouth. This line crosses at an acute angle the axis drawn from the upper to the lower pole.

The closing of the gastrula-mouth starts from its anterior edge, while the posterior edge remains all along unaltered. The growing together of the edges follows in a line which forms the larger and posterior part of the later dorsal line. The most posterior remainder of the gastrula-mouth still persists for a long time as a small opening dorsally situated at the posterior end.

When we compare the stage which is represented in Figs. 24 and 25 with those represented in Figs. 33 and 34, we come at once to the conclusion, that the

mode we have mentioned of the closing of the gastrula is the simplest mechanical process by which the one form can be changed into the other. In this way, without pre-supposing any important cell displacements, is to be explained the change of the broad, wide-open, flat, cap-formed gastrula, into the considerably diminished form of Figs. 33, 34. This process can be easily represented by a flexible model.

We arrive also at the same conclusion on a careful comparison of the stages. Comparing Fig. 26 with the earlier stage of Fig. 24, we see that the originally short dorsal part has become considerably lengthened. At the same time the flattening of the dorsal surface appears more prominent. In the further stages also, Figs. 29 and 31, we see that the dorsal side becomes continually longer and longer, while the ventral side of the curvature shows only an alteration of its curve occasioned by diminution of the gastrula-mouth. To this diminution of the gastrula-mouth it is also due that the angle at which the ventral and dorsal walls meet one another towards the anterior end becomes continually smaller and smaller, until at last the dorsal surface gains a direction parallel to the longitudinal axis (Fig. 33).

Other circumstances may also be observed, and the

conclusion drawn therefrom that the posterior edge of the gastrula-mouth remains unaltered, and, what is most important, the anterior edge suffers alterations during the closing of the gastrula-mouth. The transition from the epiblast to the hypoblast is, that is to say, not similar in all parts of the gastrula-mouth. It is at the posterior edge that the removal of the epiblast from the hypoblast takes place most prominently, since there the hypoblast cells are most strikingly distinguished by their size from the epiblast cells. There we can quite early distinguish two specially large hypoblast cells[1] situated towards both sides of the central line. These two mark for us the posterior pole of the body, and they serve for us as a starting point, in order to recognise that during the closing of the gastrula-mouth the posterior edge of the latter remains unaltered and corresponds to the later posterior pole of the body. In the remaining periphery of the gastrula-mouth the removal of the epiblast from the hypoblast is less clearly defined, and this gradual transition of the epiblast to the hypoblast is most striking at the anterior edge. This retains the character of a rounded edge, as distinguished from the condition of the hinder edge.

[1] Polar mesoblast cells.

Thus the closing of the gastrula along the central line cannot be here quite so exactly observed as is possible in other cases (*e.g.* Mollusca and Annelida). A conclusion may however be drawn as to it by means of exact consideration of the alterations in form.

During the diminution of the gastrula-mouth in the stage of Fig. 31 it may be noticed that the epiblast becomes ciliated. This is at first exceedingly slight, and in the beginning only to be observed with difficulty. By this the embryo gradually becomes subject to a slow rotation.

Kowalevsky stated that the embryo was first covered with thick cilia, which he in some cases observed to appear somewhat earlier than has here been stated. In much later stages every epiblast cell bears only a single flagellum. So far as my observation goes every cell bears from the very beginning only one single flagellum, very delicate, and later on continually becoming longer. Of just the same condition are the cells of the archenteron of the larva, which only assume a ciliated appearance in a much later stage. In this way no ciliated cells are to be seen in the Amphioxus nor in the grown animal even during the development, but only flagellate cells.

THIRD PERIOD OF DEVELOPMENT.

In this period of development we would comprehend those stages in which takes place the formation of the most important organ-systems, of the mesoblastic somites, of the nervous system, and of the notochord (Figs. 35–53).

From the standpoint of philogenetic consideration this period may be divided into two sections. Of these the one comprises the formation of the mesoblastic somites and of the neural canal. It reaches, that is to say, up to the stage of Fig. 47. The second period, on the other hand, in which these organ-systems attain to still sharper separation and further formation, is characterized principally by the formation of the notochord.

The processes with which this chapter deals were in their important points well described by Kowalevsky in his "Further Studies." My own observations constitute therefore for the most part only a further prosecution of his ideas. The most important differ-

ences to be found in my observations concern the employment of the first mesoblastic somite, from which Kowalevsky erroneously thought that the peculiar club-shaped gland took its origin. The development too of the notochord I have found to be somewhat otherwise. It originates, as Kowalevsky has stated, from well marked folds of the hypoblast, but not till somewhat later than would appear to be the case according to his account. I have also in my own investigation devoted more strict attention to some processes at the anterior end of the embryo, to which he gave but slight consideration, as well as to the further increase of the mesoblastic somites at the posterior end.

First Section of the Third Period of Development.

(Formation of the Mesoblastic Somites and of the Neural Canal.)

Kowalevsky describes very well for us the processes of formation of the mesoblastic somites and of the neural canal, which fall under this section of the development. First we have a sinking of the flattened dorsal side of the gastrula. The base of the furrow which originates in this way is overgrown by its sides. The neural plate is

separated at the edges of the furrow, and therefore is cut off long before the edges unite with each other. The formation of the neural canal is distinguished from that of the other vertebrate animals, in which its separation only follows after union of the edges of the neural furrow. In the Amphioxus "the dorsal channel, although on the outside fully covered, is on the inside, beneath this covering, still open." The growth of the edges of the neural furrow begins at the posterior end, where the gastrula-mouth is at once enclosed, and advances towards the anterior end, where an opening [1] is found to persist.

The remains of the gastrula-mouth continue accordingly as an opening between archenteron and neural canal, and this opening is still to be observed even in much later stages. This is the neuro-enteric canal which is generally typical in the development of the vertebrate animals.

Together with the formation of the neural canal there appears the formation of the mesoblast in the form of mesoblastic somites. In the posterior part of the hypoblast there arise two lateral longitudinal folds which represent the beginnings of the mesoblast.

[1] Neuropore.

These, starting from the front, separate into single mesoblastic somites, whose cavities are parted from one another.

The cavities of the mesoblastic somites are nothing else than diverticula of the archenteron.

These discoveries of Kowalevsky which I here describe are confirmed in their most important respects by my own observations, and in some points prosecuted with greater exactitude.

Let us now consider again more exactly the last stage of the former period of development, in which the closing of the gastrula-mouth has advanced till only a small remnant is left (Figs. 33, 34). The form of the body is that of an egg with flattened dorsal part (Fig. 33). The archenteron corresponds with the outer form. The body wall consists of two layers, the first being the epiblast layer, which is considerably thinner, and composed of smaller cubical clearer ciliated cells. The other is the primary hypoblast layer, which is considerably thicker, and is composed of darker columnar cells. At the posterior end of the embryo, just at the posterior edge of the gastrula-mouth, lie two large hypoblast cells, which are distinguished by their round form from all other cells, and

from all other hypoblast cells by a somewhat more granulated appearance, and a larger nucleus. We shall further see that these cells, which always mark the posterior pole, constitute in the formation of the mesoblast its posterior extremity. We will therefore describe them as the polar mesoblast cells.

In the transverse sections, also made from the embryos of this stage, it can be seen that the body wall is everywhere composed only of two cell layers (Fig. 71). The characteristic flattening of the dorsal surface, which introduces the important changes of the next stages of the development, is well seen in the transverse section (Fig. 71).

From this stage on begin changes which run their course with great rapidity. These are scarcely to be thoroughly investigated in the living object. The examination must further be made with suitable re-agents of special preparation. Further, a methodical examination of transverse sections is absolutely necessary.

The Living Object.

I will in my description proceed from those observations which can be made on the living object.

The first change which we see is a deep depres-

sion of the dorsal surface, which even in the living object may be plainly enough seen, especially in optical transverse sections. This depression extends from the anterior fourth of the body as far as the gastrula mouth situated at the posterior end (Figs. 35, 36, 39). The larva shows upon the optical transverse sections a now almost triangular outline. The two edges which constitute the boundary of the dorsal surface require the formation of two longitudinal folds of the hypoblast.

These folds, which are in no way more sharply separated from the rest of the hypoblast, form the material which becomes the mesoblast. We will from now on characterize these longitudinal folds of the hypoblast as mesoblast folds.

In these mesoblast folds the anterior part begins to separate itself from the posterior and greater part by a sharper contour. On this anterior section the folding is at once more sharply marked, and it appears therefore as a distinct and prominent fold which represents the anterior mesoblastic somite (Figs. 36, 38).

The most anterior mesoblastic somite appears accordingly marked off by a sharp boundary from

the posterior and undifferentiated part of the mesoblast fold, while towards the anterior, and in the direction of the ventral side, it passes into the wall of the archenteron without any distinctly visible boundary in the living object. The lumen too of the first mesoblastic somite is still connected with the lumen of the archenteron by means of a wide opening.

The second mesoblastic somite is separated by means of a second boundary which arises in a similar way (Figs. 37, 38, 39).

During the formation of the two first mesoblastic somites the neural plate, forming the floor of the neural canal, is arched over by the edges of the latter.

This is however a process which can only be followed with difficulty in the living object. It can nevertheless be seen in these stages that the gastrula-mouth is not open any more, and optical tranverse sections teach us that the neural plate has separated underneath the superficial layer of cells. The dorsal surface of the embryo is furthermore still continually deepened in the form of a channel, and it is only gradually that its epithelium is raised above

the neural plate, the dorsal surface at the same time again assuming a rounded form.

With regard to the details in the formation of the neural canal, which are not to be recognised in the living object, subsequent information will be given by means of the other methods.

It is at about this stage (Fig. 39) that the embryos leave the egg membrane. This is however often burst somewhat earlier, while in other individual cases embryos are again to be found whose third mesoblastic somite is already in course of formation inside the egg membrane. The bursting of the latter seems to me in part at least to be brought about by the ever increasing rotation of the embryo. Perhaps too we have to deal with a gradually altered consistency of the egg membrane. When further distended, it is generally seen to be split, and to have fallen asunder into two parts.

The movement of the embryos inside the egg membrane, and also after leaving it, is a quite peculiar one. They swim continually with the anterior end of the body bent forwards, and revolve at the same time on their axis, this revolution always following the same direction from right to left. Thus all the points of the

body which are not situated in the longitudinal axis describe a spiral [1] line in their forward movement.

The embryos maintain this peculiar movement for still quite a long time and do not give it up till the body has assumed a very extended fish-like form.

The eggs which have attained to their development in the glasses lie on the bottom of the latter. After leaving the egg membrane the embryos make their way to the surface of the water.

In the next stages, in which the formation of the third mesoblastic somite takes place, there can as yet be observed in the living object only a sharper optical appearance of the mesoblastic somites (Fig. 41).

So far as concerns the alteration of the outer bodily form, during the formation of the three first mesoblastic somites a continual elongation of the embryo may be observed. The dorsal surface too, which also after the enclosure of the neural plate was further deepened, becomes gradually flat, and at the same time the cavity of the neural canal underneath the superficial epithelial layer becomes visible.

[1] A similar spiral movement may also be observed in other bilaterally formed larvæ (Echinoderm larvæ, Worm larvæ, etc.), as was lately pointed out by Metschnikoff.

In the next stages, with four and five mesoblastic somites, the elongation of the embryo advances, and at the same time there takes place an alteration of its transverse section, the dorsiventral diameter being in the meantime enlarged at the expense of the transverse diameter. Thus there takes place a lateral compression; at the same time the dorsal surface, which was originally sunken and afterwards flat, becomes at last convex.

The most important advances which we are able to observe in the stages with four and five mesoblastic somites are concerned however with the further development of the latter. Their cavities situated one behind the other, which hitherto stood in open communication with one another, are separated so that there is formed a double-layered wall of cells between two contiguous cavities. At the same time however the individual cavities of the mesoblastic somites still stand in open communication with the archenteron. Especially large is the opening of the first mesoblastic somite (Fig. 47).

A further and important step which is to be remarked in the mesoblastic somites in the living object is the appearance of a sharp lateral boundary between

the mesoblastic somites and the cells which form the archenteron.

We will now gain a closer acquaintance with the processes which fall under this section, while we take into consideration the results yielded by the improved methods of investigation.

Formation of the Mesoblast Folds and Mesoblastic Somites.

We will first explain more accurately the formation of the mesoblastic somites.

If we examine stages in which the dorsal surface begins to sink in, that is to say, stages which are somewhat older than that represented in Fig. 33, upon artificially prepared transverse sections, we can then obtain a closer knowledge respecting the state of the mesoblast folds.

We see such a transverse section represented in Fig. 72. The material of the still very flat mesoblast folds passes indeed directly over into the hypoblast plate. The boundary line however of the mesoblast folds is in the direction of the median plane already sharply defined. On the outer surface of the hypoblast two distinct furrows are to be found, which form the

median boundary of the mesoblast folds; we shall see the same in the later stages coming into continually sharper prominence.

We will now take into consideration the separation into mesoblastic somites which begins at once in the mesoblast folds. In the most anterior region of the embryo, into which the mesoblast folds do not extend, the hypoblast becomes smaller (Figs. 35, 36). Just behind this flat hypoblast lies dorsal-wards the first mesoblastic somite which originates from a slight cross-fold of the mesoblast. In the optical longitudinal section this fold makes itself noticeable through a stronger concavity turned towards the inside in the direction of the archenteron (Fig. 36). The anterior and posterior edge of the first mesoblastic somite is marked by smaller transverse indentations, which especially concern its outer surface. Just behind the lower hypoblast of the anterior extremity lies dorsal-wards the transverse shallow indentation which marks the anterior end of the first mesoblastic somite. A sharper separation inside the cells is however not to be observed here. On the other hand, at the posterior edge of the mesoblastic somite a well-defined separation of the cells is to be observed. This

is accompanied by a sharp furrow running transversely over the mesoblast fold on its outer as well as on its inner surface. It is this transverse and posterior boundary of the first mesoblastic somite which specially attracted our attention in the living object. A third indentation forms the side boundary of the mesoblastic somite. This is only to be observed on transverse sections (Fig. 74).

The next mesoblastic somites are formed by means of a similar transverse fold, and the posterior boundary of the mesoblastic somite, as well as the indentation which forms the lateral boundary, are repeated in quite a similar way (Figs. 42–45). The process however of the formation is, as we shall subsequently see, a very much shorter one when the later mesoblastic somites begin to appear.

During the formation of the second and third mesoblastic somites the neural plate continually sinks in deeper between the two mesoblast folds. Thereby the longitudinal folding as well in the region of the mesoblastic somites as in that of the posterior undifferentiated mesoblast foundation, is much more sharply marked than can be seen from the series of transverse sections of Table VII.

In the transverse sections it can also be seen how the series of cells of the mesoblastic somites pass at once medianwards and no longer continuously into the series of cells of the middle hypoblast plate (Fig. 80). Now too in the arrangement of the cells the median separation of the mesoblastic somites is far more sharply expressed. Finally, in the transverse sections too, the lateral separation of the mesoblastic somites is correlated also to the arrangement of the cells.

In the stage with three mesoblastic somites it can be seen on transverse sections that in the region of the anterior mesoblastic somites a universal sharper definition of their cell material has made its appearance towards the hypoblast plate (Fig. 84). Most sharp is the differentiation of the mesoblastic somite towards the median hypoblast plate, which we saw was earliest introduced. There, what was at first a shallow indentation becomes a sharp-edged furrow, along which is to be seen a distinct differentiation of the series of cells of the mesoblast from those of the archenteron.

We will now acquaint ourselves with the alterations of the mesoblastic somites of the next stages, in

which four or five mesoblastic somites have been formed. This we will do through a consideration of the whole embryo, and afterwards by looking at them in sections.

In Fig. 46 we see from the side an embryo formed with five mesoblastic somites. These appear on a side view already completely defined. From front to back they diminish in size and formation, the cavity of the mesoblastic somite especially being much smaller in the posterior mesoblastic somite. Behind the region of the mesoblastic somites lies the undivided mesoblast fold. This, as compared with the mesoblastic somites, does not appear sharply defined laterally. It passes there straight over into the ventral hypoblast. It is only with regard to the further course of the development that it can be stated how far the mesoblast folds stretch backwards. They reach right away over the gastrula-mouth, and end with two large cells which form the hinder boundary of the latter, and which we described as the posterior polar mesoblast cells.

In viewing the embryo from the back (Fig. 47), one can, by different positions of the microscope, get an insight into the condition of the mesoblastic

somites in such a way as has already been described by Kowalevsky. By raising the object glass they may be seen already defined on every side and shut off; by lowering it, the connexion of their lumen with the archenteron may be distinguished (cp. also Fig. 45). Especially wide is the opening through which the first mesoblastic somite opens into the archenteron.

Behind the region of the mesoblastic somites there can be recognised moreover on the back view the anterior and more sharply defined section of the undivided mesoblast fold. At the same time the latter, when being thus considered, appears towards the posterior, where it is flatter, to pass direct over into the wall of the archenteron. It is just the lateral dorsal edges of the archenteron, ending at the posterior edge of the gastrula-mouth with the two large cells, which represent the cells forming the still undivided mesoblast.

We will examine the conditions of a stage with four or five mesoblastic somites by a series of transverse sections. On Table VIII. Figs. 86–92, is represented such a series of sections from a stage which was somewhat later than that of Fig. 46.

On the section from the most anterior region of the body (Fig. 86) nothing is to be seen of the formation of mesoblastic somites.

Let us now consider a section through the first mesoblastic somite (Fig. 87). We see that here the folding is still much sharper defined than before. The lumen of the mesoblastic somite is in continuous connexion with the cavity of the archenteron. The series of cells of the mesoblastic somite is seen in its arrangement to be already quite out of continuity with the hypoblast plate. The cells of the latter are at the slit where the mesoblastic somite lumen opens inwards already about to unite with one another, whereby the mesoblastic somite becomes completely separated from the hypoblast. The form too of the cells of the mesoblastic somite has altered, while these cells, as opposed to the columnar ones of the archenteron, have assumed a low and more cubical form. A histological differentiation too makes itself in so far noticeable that in the cells of the mesoblastic somite the yolk granules are dissolved more quickly than in the hypoblast. This becomes noticeable in comparison with the hypoblast cells through their not being made so brown by osmic

acid, as well as by a stronger carmin staining of the mesoblastic somite cells.

The histological differentiation, as too the separation of the mesoblastic somites, is continually less and less defined the further back the mesoblastic somites lie, which we observe in the series of transverse sections. If we consider the section represented in Fig. 89 through the third mesoblastic somite, and that represented in Fig. 90 through the fourth, we can see how gradually the movement of the cells take place which lead to complete separation of the mesoblastic somite.

As we have already recognised from the back view of the whole embryo (Fig. 47), the lumens of the individual mesoblastic somites are fully separated from one another. Those sections therefore also, which are constructed in the region between two mesoblastic somites, must show an altered appearance. We see here the mesoblastic somites cut straight through, and we see too that here the slit which leads into the inside of the mesoblast fold is closed by union of the median dorsal hypoblast plate with the ventral hypoblast. Nevertheless the place where the closing happens is still distinctly to be seen.

Further back in the section (Fig. 91, left side), where the fifth mesoblastic somite is just in process of formation, we see conditions similar to those which we saw when the first mesoblastic somite came to view. The process is only in so far a shorter one that the folding at once appears much sharper. This is owing to the fact that the dorsal channel is already constructed during the formation of the mesoblastic somites now arising, and thereby we have altered conditions of form.

Still further back (Fig. 91, right side) the hypoblast fold is lower, and on sections which are taken near to the posterior end of the embryo (Fig. 92) the more sharply defined dorsal lateral edges of the hypoblast form merely an intimation of the mesoblast formation.

Formation of the Neural Canal.

We will now turn our attention to the development of the neural canal, which has been taking place during the formation of the mesoblastic somites.

While the mesoblast fold and the first mesoblastic somite are beginning to form, the neural plate is becoming separated. The latter does not stretch to the whole extent of the back, but reaches only

a little further forward than the mesoblast folds. Along the dorsal ridges, which form the sides of the dorsal furrow, the median plate,[1] which constitutes the base of the dorsal furrow, is now divided off from the lateral epiblast by a sharp separation inside it. This separation is chiefly to be seen in sections, and is especially remarkable for the fact that a discontinuity occurs in the arrangement of the cells (Fig. 72). The cells begin to move from the side over the neural plate.

We can too in Fig. 36, in the posterior region of the body, view the edges growing forward towards the median line as wavy border lines running upon the surface. In the living object, where this superficial arching layer lies right on the neural plate, nothing is to be noticed of this. In the objects treated with reagents the wavy boundary of the arching layer comes most prominently to view, when it has separated itself somewhat from the neural plate through the action of the reagents.

This arching begins on the sides of the gastrula-mouth, which lies at the posterior end of the medullary plate. The gastrula-mouth is now bridged over by

[1] Neural plate.

this arching, so that on the optical longitudinal section, if one follows the epiblast at the posterior end from the ventral side towards the dorsal side, it is seen not to end at the gastrula-mouth, but can be followed over the same dorsal-wards some considerable distance, covering the neural plate (Fig. 37).

We can observe this advance of the arching from back to front in the series of transverse sections also. We already see in section, Fig. 72, which is drawn somewhat obliquely, the arching further advanced upon its left than upon its right side, which answers to a region situated further forward.

If we further observe a series of sections from a somewhat older embryo, in which the first mesoblastic somite is already formed, we shall in just the same way see the arching further advanced in the section (Figs. 75, 76) taken through the posterior third of the embryo than in the neighbourhood of the first mesoblastic somite (Fig. 74). In the region immediately in front of the mesoblastic somite we find too the neural plate separated laterally by sharp boundaries. Nevertheless the arching has as yet not begun at all here (Fig. 73). This is the place in

which the nerve channel later on too remains still for a long time open.

In a still older embryo we see in one section of the posterior region of the body the arching cells pressed forward from both sides as far as the middle line, and there coming into contact with one another (Fig. 78). In the anterior region, on the other hand, the middle line is still uncovered (Fig. 77).

The arching over the medullary plate advances, according to my observations, far more rapidly than has been stated by Kowalevsky. I found it indeed in embryos with two well formed mesoblastic somites, that is, in that stage in which the embryos leave the egg membranes, advanced along the whole back as far as the anterior end of the first mesoblastic somite (Figs. 42, 43). Through the action of reagents we can easily get a separation in the line of growth, so that then the wavy edges along which the arching cell plates are united come plainly to view. I have made a representation of this in drawing in Figs. 43 and 45.

The arching plates, so far as they have touched one another in the middle line and are then grown into each other, are raised from the neural plate

lying underneath them, so that a flat neural canal is constructed underneath the outer covering, but still wide open (Figs. 79–81). This opens outwards more widely, in the region in front of the first mesoblastic somite.

This opening diminishes but slowly in the later stages, and that too while advancing from back to front. The result of this is that the close of this opening may be viewed as a very lengthened continuation of the process of closing the neural canal which we have just observed.

The cavity between neural plate and outer covering is thus a narrow slit, which in front, in the part before the first mesoblastic somite, opens outwards. Behind, owing to the remnant of the gastrula-mouth continuing in existence, it stands in communication with the lumen of the archenteron as a neurentic canal.

The hollow space, at first narrow, which we will now call the neural canal, becomes deeper for two reasons. The first is that the neural plate in the course of the development shrinks closer. The second is that the dorsal covering, which, even after the arching of the neural canal is completed, is still

sharply concave in the stage with three mesoblastic somites, first of all flattens, and at last in the stage with four and five becomes convex, and thereby rises far more from the base of the neural canal.

We will observe more closely the shrinking of the neural plate. This is closely connected with its diminution, the latter being due to the fact that its cells alter their form while changing to high and sharply pointed wedge-shaped cells. This process begins in the region of the first mesoblastic somite, and continues in a backward direction. We see it in the stages of this section of development advanced just about as far backwards as the formation of mesoblastic somites.

We see in the series of sections, represented in Figs. 86–92, first of all in Fig. 86, the neural plate in that place where the neural canal opens outwards, flat and not as yet arched over. Further back we meet the deepened neural canal in all sections which are drawn through the mesoblastic somite region (Figs. 87–91). In the region behind the mesoblastic somites (Fig. 92) we find again the neural plate quite flat.

We see that the processes of formation in the

neural canal, answering to the metameric type, advance from in front, where are the older mesoblastic somites, backwards to the region of the younger ones, while the arching of the neural plate followed in a reverse direction. However, in the later stages, this differentiation of the neural canal, which advances from in front backwards, is obliterated by the mechanical part of the development, and cannot be so clearly seen.

Remarks upon the Mechanical Part of the Development Processes.

We will now make some remarks about the mechanical part of the development processes just observed, in so far as they force themselves upon our notice in the close observation of the embryos and their series of sections. This perhaps may not be without use for the theoretical considerations to follow later; for it may perhaps be concluded from the great simplicity of the mechanical processes that the whole development is very simple and short.

In that stage of the development which we have just described there took place a series of processes which may be referred to *foldings*, *stretchings*, and

over-archings. These foldings and stretchings can be explained through the mutual relation of the neighbouring parts, through the pushing and pulling which the latter exercise upon one another. What are the powers however which call into play this pushing or pulling? The mechanical causes are of various sorts. In the end they may all be referred to the activity of protoplasm. We can however classify certain appearances according to common significations.

First of all we can distinguish between processes which are occasioned by contractions of protoplasm, which can, in a stricter sense, be described as *active changes of form*, and such processes as are to be referred to growth. In this respect, the difference between the energies of growth in neighbouring portions plays an important part. Thereby foldings as well as stretchings are effected. Many alterations, as for instance growths, may be *immediately* referred to the *finer processes in protoplasm* which are less accessible to our understanding.

The active changes of form are indeed confined to short periods of development, where they often effect rapid and important appearances of development. In the section of development which lies before us we

appear to have an active change of form, especially in the formation of the dorsal furrow, which stands in close mechanical connexion with the beginning of the mesoblast folds.

Differences in the energies of growth come before us in far more manifold fashion, and stand in close relation with all the more important processes of development. We shall see that in the separation of the neural plate, and in the arching of the same, the differences between its energy of growth and that of the neighbouring epiblast play an important part. We shall further recognise that, although the first foundation of the mesoblast folds was introduced through an active change of form, yet later the energy of growth of the mesoblast folds, which is greater in comparison with the neighbouring parts, causes their further formation and their division into mesoblastic somites.

The energy of growth is in general at first greater in the anterior region of the body, and keeps equal pace with the differentiation which advances towards the posterior end.

We will now go somewhat more closely into the details in the mechanical part of the development processes.

During the formation of the first two mesoblastic somites, when the arching over the neural plate at once takes place, the mechanical processes are quickest. They lead to an important dorsiventral depression of the embryo, which is very distinctly to be recognised in Fig. 37, and, as the series of sections tell us, is marked most clearly at the posterior end of the body (Fig. 78, 81). From the stages, with two and three mesoblastic somites, whose dorsiventral diameter is in consequence of the depression less than the transverse diameter, a lateral compression becomes noticeable.

I would be inclined in the mechanical part of these processes, to ascribe to the hypoblast the preponderatingly active part. On a superficial consideration of the representations, we shall at once attribute an active part in the changes of form far sooner to the thick hypoblast layer than to the thin epiblast-covering layer. The chief part in the invagination of the dorsal side, and the considerable flattening of the embryo may proceed from the hypoblast. Still the arching over the neural plate, as we shall see later on, must be referred to energies of growth inside the epiblast itself. In the lateral compression too of the

embryo, and in the foldings which are continually forming more sharply in the internal parts, processes of growth and movement in the hypoblast are the preponderating cause.

If we consider more closely the details of the mechanical processes, it is apparent that quite various formations are brought into connexion through one and the same mechanical process. We shall thus recognise that the appearance of the dorsal furrow not only introduces the formation of the neural canal, but stands in just as intimate a relation to the formation of the mesoblast folds. Thus we can also see, on a consideration of the series of sections, that those two longitudinal furrows which form the median bounding of the mesoblast folds and play an important part in the sinking of the dorsal surface stand in just as intimate relation to the shrinking of the neural canal as to the formation of the mesoblast folds.

The formation of the mesoblast folds is now to be referred to a more important surface extension of the hypoblast in the posterior region.

The formation too of the mesoblastic somites may be referred to mechanical processes in the hypoblast. We see that the formation of the first mesoblastic

somite is introduced by a flattening of the hypoblast cells in front of the mesoblastic somite region. Therewith an extension of this layer is necessarily occasioned, by which the cells are pushed backwards, and through this pressure the transverse folding is caused, which leads to separation of the mesoblastic somite.

The formation of the succeeding mesoblastic somites, I would be inclined to refer to a pressure of the cells of the mesoblast folds in the longitudinal axis similar to that in the formation of the first mesoblastic somite. The embryos elongate especially during the formation of the next mesoblastic somites. This elongation appears now in the neighbourhood of the mesoblastic somite region of the mesoblast folds a more productive one than in the neighbouring parts of the embryo.

The preponderating elongation in the neighbourhood of the mesoblastic somites, owing to which the foldings are caused, as well as the elongation of the embryos in general, appears to be brought about partly through change of form in the cells, partly through growth occasioned by using up the yolk corpuscles.

The change of form in the cells may be recognised in Figs. 43–47, as also in the series of sections. We see indeed that the cells of the mesoblast folds become

lower, especially in the neighbourhood of the mesoblastic somites, and therefore require a more important surface extension of the cell plate which is composed of them.

On the other side, we see from the sections (Figs. 87, 89) that the yolk corpuscles in the mesoblastic somite cells are more quickly used up than in the remaining part formed of the primary hypoblast. We can therefore conclude a preponderating energy of growth in the neighbourhood of the mesoblastic somites.

The energies of growth and their differences in the epiblast can in the same way be concluded from the condition of the yolk granules in the neural plate and in the rest of the epiblast. If we for instance consider the series of sections (Figs. 86–92, on Table VIII.) of an embryo of 4–5 mesoblastic somites, it must occur to us that the neural plate contains yolk granules far more richly than the rest of the epiblast, so that the cells of the neural plate show more similarity with the hypoblast cells than with the more nearly related epiblast cells, and this gives us a deeper insight into the mechanical part of the processes which have taken place. We shall indeed explain the separation of the neural plate from the

neighbouring epiblast cells by the fact that the growth energy in the neighbouring epiblast, when the yolk granules are dissolved more quickly, is a stronger one than in the neural plate. This preponderating growth energy of the neighbouring epiblast parts leads first to a break between these and the neural plate, and then to arching over the latter.

There are thus some processes explainable by ordinary mechanical conditions. We must however keep in view that by far the larger number of appearances can only be referred to such causes as we without closer knowledge must comprehend under the quite general name protoplasmic activity.

Second Section of the Third Period of Development.

(Formation of the Notochord.)

In the second section of the epoch described as a third period of development the body-form elongates in a higher degree simultaneously with lateral compression.

Of internal processes are to be noticed: the further increase of the mesoblastic somites and the further differentiation of the same—especially the processes

in the first mesoblastic somite, from which a continuation as a mesoblast formation of the head (*sensu strictiore*) grows into the anterior end of the body. There we have, as specially characteristic of this epoch, the formation of the notochord, of the neural canal, and finally of the anterior outgrowths of the alimentary canal which are then gradually abstricted from one another.

We already know the alteration of the body-form in general through Kowalevsky's investigations. The alterations which the mesoblastic somites experience in this epoch have been indicated by him only cursorily and in part. The formation of the notochord we only know through his work in so far as the same was proved to take its origin from the hypoblast as a dorsal canal derived from it. We will return to Kowalevsky's statements and deal with them more fully when explaining the developmental conditions of the individual organs.

Formation of the Notochord.

We will now describe more closely that which characterises this section, namely the formation of the notochord.

In his first treatise Kowalevsky states that the notochord, even during the closing of the dorsal ridge, arises like a row of cells composed of an inconsiderable number of large ones. He does not in his second work explain these inaccurate statements, but passes over them in silence.

It may well be gathered from this second work that the notochord is commenced somewhat later. It was also recognised that it arises from the hypoblast, and indication was given of the importance of a median dorsal hypoblast fold for the development of it.

Kowalevsky states that the hypoblast in its upper half falls into three folds. He describes further that in one section through an embryo with about four mesoblastic somites "in the middle line is found a small fold, or more exactly a channel, since the latter is scarcely marked off above." In an older embryo with perhaps eight mesoblastic somites (its transverse sections are not correctly represented) is seen "the chorda dorsalis, sharply separated from the surrounding tissues, and under it a very fine lamina of the glandular layer of the alimentary canal." Respecting the number or size of the cells which

compose this notochord foundation, he gives no information here, and mentions too nothing with respect to the relation of these matters to his earlier statements.

In material points I can confirm his statements; I will however mention that the middle fold of the hypoblast is not entirely expended on the formation of the notochord, but that its side cells form the dorsal portions of the alimentary canal. The notochord, too, at the time when Kowalevsky represents it as completely split from the membrane of the archenteron, is still in continuous connexion with it. And even, in later stages, when it has already attained to its separation, it still for some time takes part in forming the wall of the alimentary canal. Further differences, which I do not intend to describe more closely here, may be gathered from a comparison of the figures.

I will now proceed to give an exhaustive description of my own observations.

In the living object the first processes of the notochord formation are not to be followed very exactly. It may be seen, indeed, that a strand is marked off dorsally from the archenteron in the neighbourhood

of the mesoblastic somites, which at first does not reach as far as the anterior end of the body, but only as far as the anterior end of the first mesoblastic somite, and gradually extends anteriorally. Towards the posterior, too, the formation of the notochord advances during the further separation of the mesoblastic somites. The study of the living object yields thus in these stages but few results. In later stages, during the histological differentiation of the notochord, the study of the living object is again, however, of special importance in order to follow the appearance of the vacuoles in the notochord.

We make a far greater advance through the study of prepared objects (preparations made with osmium-carmin-glycerine). It can there be recognised that the notochord arises from the upper wall of the alimentary canal. For in the first stages, where the notochord foundation can be noticed, it still takes a direct share in forming the wall of the archenteron (Fig. 48). It may also be seen that the foundation which appears with several layers in the profile passes over into the simple hypoblast epithelium behind the mesoblastic somite region.

In the later stages, with nine mesoblastic somites the

notochord appears in the region of the mesoblastic somites shut off from the bounding surface of the archenteron, and lengthened towards the front into the anterior end of the body (Fig. 50).

On a consideration of the whole embryo one would be inclined to hold the view, that the notochord in the region of the mesoblastic somites is formed from the hypoblast, and then, through a secondary outgrowth of this foundation, extends into the anterior end of the body (Figs. 48, 50, 54). On consideration of the transverse sections we shall recognise that this is not the case, but that the anterior end of the notochord is formed in this position out of the hypoblast in front of the mesoblastic somites.

The most important method for the examination of the notochord formation is the study of the transverse sections.

We must here revert to the consideration of the transverse sections of the early part of development, in order to take notice of some conditions which are preparatory for the formation of the notochord. It can indeed be already recognised in the stage with three mesoblastic somites (Figs. 83, 84) that the dorsal median portion of the alimentary canal which lies

between the mesoblast folds shrinks to a flat channel, whose concavity is turned towards the lumen of the alimentary canal. This channel deepens considerably in the stages with four and five mesoblastic somites (Figs. 88, 94), and the result is finally in the stage with six mesoblastic somites (Fig. 97) the formation of a sharply marked fold, first in the region of the most anterior mesoblastic somites, and gradually, too, in that of the posterior ones. Further back, in the region of the unseparated mesoblast fold, the notochord fold gradually flattens (Figs. 91, 92). The lumen of the channel becomes in the process of folding a narrow slit, so that the internal ends of the cells of the right and left half of the fold finally touch one another (Figs. 98, 101).

At the time when this folding is sharply marked, the slits which connect the cavity of the mesoblastic somite with the lumen of the archenteron are already closed, and are only indicated through the sharper separation of the cells in this place. The formation of the notochord is thus first anticipated at a time in which the mesoblastic somites are already completely separated from the hypoblast.

The notochord is now in the following stages formed

at the expense of this dorsal fold of the archenteron; that is to say, out of the material of that median dorsal hypoblast plate, which in the formation of the mesoblast folds and mesoblastic somites obtained its separation between them. This hypoblast plate, however, as already mentioned, is not entirely used up in the formation of the notochord, but the lateral cells of the latter become connected with the ventral portion of the hypoblast. Now, since this connexion takes place before the notochord becomes split off, it is difficult to give this proof with precision for the region of the anterior mesoblastic somites. For this purpose the exact comparison of the drawings of numerous series of sections is necessary. This is easier in the later shortened development of the succeeding mesoblastic somites. This we can quite well recognise in Fig. 101 and Fig. 102 (cp. also Figs. 118 and 141), that the middle part of the hypoblast plate belongs to the foundation of the notochord.

We will now proceed to view the changes which the foundation of the notochord in general undergoes in the region of one of the anterior mesoblastic somites.

The notochord fold consists, according to its development, of two rows of cells, which are inclined in a somewhat oblique direction (Figs. 98, 101) towards the central line which has resulted from the former lumen of the channel. A cell situated in the dorsal middle line forms the transition from the right to the left row of cells upon the transverse section. These dorsal cells of the notochord foundation lie behind one another in a series, which on a consideration of such an embryo from the dorsal surface strikes our notice, owing to the regular arrangement of its cells (Fig. 49).

The straight slit of the notochord foundation, which may be referred to the process of folding, vanishes directly, and that, owing to the fact that the cells from both sides begin to grow in between one another (stage with six mesoblastic somites, Figs. 99, 100). The cells, as well of the right as of the left side, grow out over the middle line. The character of a fold is thereby lost, and the notochord foundation has now the appearance, with reference to the arrangement of its cell position, of a many layered dorsal thickening of the archenteron. This thickened dorsal part now becomes by degrees sharply marked off from the

neighbouring cells of the latter (stage with eight primitive segments), and that in such a way that this marked-off strand, which may now be described as notochord, still takes direct part in the bounding of it (Figs. 109, 110, 111).

The notochord foundation becomes, for the first time in the succeeding stages, with nine and ten mesoblastic somites, gradually shut off from the bounding of the archenteron. It remains, however, at first regularly wedged into its upper wall (Fig. 116).

The alteration in the arrangement of the cells of the notochord makes progress simultaneously. Indeed, the union of the originally right and left row of cells progresses so far that all the cells at last pass over the whole transverse diameter of the notochord (Figs. 113–117). And with that a point is reached which is of importance for the later structure.

On a consideration of the whole embryo we notice on a dorsal view more than before the arrangement of the series of cells which extend along the whole transverse diameter of the notochord (Fig. 52), this arrangement having previously concerned only the dorsal cells of the notochord. On the side view

the transverse section of the cells are seen, of which about four comprise the thickness of the notochord.

There may, indeed, be observed a lower and an upper series of cells, and about two less regularly arranged middle series (Fig. 50).

In this stage, also, the histological differentiation of the notochord begins to be expressed by the appearance of numerous small vacuoles. We will return to this point on a closer consideration of the histological differentiations, which are included in the next period of development.

We have now still to explain in what manner the advance of the notochord proceeds backwards and also towards the anterior end. The changes we have described of the dorsal hypoblast fold take place in the stages from the formation of the sixth to that of the tenth mesoblastic somite, and in general it may be said that the differentiations proceed more quickly in the neighbourhood of the anterior mesoblastic somites. For instance, while in the stage with six mesoblastic somites, in the anterior mesoblastic somites the median slit of the notochord foundation has already vanished (Figs. 99, 100), the latter is still preserved in its entirety in the last but one,

namely, in the fifth mesoblastic somite (Fig. 101); and still further back, in the sixth, the cavity of the fold may be seen (Fig. 102). In the neighbourhood of the posterior mesoblast folds the middle hypoblast plate is still completely flattened (Fig. 103); thus the formation of the notochord has not yet begun.

The advance of the differentiation from front to back, as characteristic for the metameric type, finds its expression here.

In the formation of the later mesoblastic somites the process of the formation of the notochord appears abbreviated, as we shall later on explain still more fully.

Of special interest is, too, the formation of the notochord in the anterior part of the body, namely, that situated in front of the mesoblastic somite region, especially having regard to the fact that the Amphioxus is distinguished from all vertebrates and also from the Ascidians, thus from the whole race of the Chordata, by means of the notochord, which reaches right into the anterior end.

We will here mention that as a matter of fact in the region of the first mesoblastic somite the develop-

ment of the notochord is to a slight extent later than that of the second mesoblastic somite. This may indeed be seen on a comparison of Figs. 87 and 88, where the folding of the median hypoblast plate in the neighbourhood of the first mesoblastic somite is much less expressed than in the region of the succeeding section.

Further, we see in the stage with six mesoblastic somites in the region of the first of these the notochord slit still well marked (Fig. 98), while it has already vanished in the region of the second and following mesoblastic somites, through the ingrowth of the cells (Fig. 100).

The delay in the notochord formation in the region of the first mesoblastic somite is, however, quite unimportant, and only to be seen in a few stages on good transverse sections.

Far more remarkable is the delay of the development of the notochord in the most anterior end of the body. We see, reverting to earlier stages, in Fig. 86, where a section is represented which has to do with the anterior opening of the neural canal, no trace here, as yet, of that channel which introduces the formation of the notochord.

Later on we see in the sections which correspond to the same region, the folding already introduced, while the formation of the notochord in the region of the mesoblastic somite of the same embryo has advanced considerably further. Figs. 95 and 96 answer more or less to the same place of the embryo as Fig. 86, taken from an earlier stage. In the sections belonging to the later stage, Figs. 95 and 96, the representation is remarkably different, owing to the fact that the closing of the neural canal has now advanced further, and also to the fact that the anterior prolongations of the first mesoblastic somite are cut by the section, while on Fig. 86 nothing was to be seen of the formation of the mesoblast. Quite in the front, the condition of the hypoblast in this stage is similar to that in Fig. 104 (which belongs to a later stage). There is still no trace of the channel of the notochord to be found.

We should now observe the series of sections from the anterior end of a still older embryo. There we see that in the region which corresponds to Figs. 95–98, the slit of the notochord is now entirely vanished (Fig. 107). Further forward, however, we still continue to find the open channel (Fig. 106), which

towards the front becomes quite flattened (Fig. 105).

We can further also see in the older stages (see 112–115), that the closing of the fold, the growing over of the cells, and the separation of the notochord foundation from the bounding of the lumen of the archenteron follows in the anterior end the general process, except that here the advance of the process is observed from back to front.

The complete separation of the notochord in the anterior end of the body is only completed in the first stage of the next period of development (Figs. 120–123).

The details of this process I do not wish to describe more fully. I will only point to the comparison of the figures: Figs. 87, 94, 97–99, 109 correspond to each other, also Figs. 93, 96, 108, 115, 123, also Figs. 86, 95, 107, 122, also Figs. 105–106, 113–114, 121, also Figs. 104, 112, 120, each in their own region of the body.

Further Formation of the Mesoblastic Somites.

We will now proceed to the consideration of those processes of development which concern the mesoblastic somites.

We can, through the study of whole embryos, at once recognise that the cavity of the anterior mesoblastic somites is completely shut off from that of the archenteron (Fig. 49). Also on a consideration of the embryos from the dorsal surface, it may be recognised that the cavities of the mesoblastic somites are enlarged through flattening of the cells (Fig. 49, 52).

At once the mesoblastic somites penetrating between epiblast and hypoblast broaden out ventral-wards. This may be followed step by step in the representations of an embryo with seven mesoblastic somites, Fig. 48, and also one with nine, Fig. 50.

Further, in the arrangement of the cells a condition has made its appearance which arouses our special attention. The cells, which are situated towards the epiblast, and those which form the division walls between the individual mesoblastic somites (Fig. 52) appear mostly flattened. Those seem higher which are in contact with the notochord. These last are arranged now, that is, while the complete abstriction of the mesoblastic somites is taking place, in such a manner that they are continuous through the whole length of the mesoblastic somite. This is to be ob-

served as well in the optical anterior sections (Fig. 52), which we get on a dorsal view of the embryo, as on the lateral view (Fig. 51).

These elongated cells in contact with the notochord, which later on provide the muscular system, are found in definite numbers and in regular arrangement. This is the case in so far as they are arranged lengthwise in regular rows, so that the individual cells of the primitive segments, which follow one another, are in contact with each other.

We can, too, on a lateral view of the stage with nine primitive segments, recognise a further advance in the posterior undifferentiated region of the body, while, too, the undifferentiated mesoblast fold experiences a distinct lateral separation.

In the whole embryos another and important appearance may be followed, which concerns the first mesoblastic somite. This is the formation of a hollow continuation from it, which grows gradually forward, right into the end of the body, and breaks through that region of the body in which no mesoblast was formed.

Let us now consider, on transverse sections, the changes which the mesoblastic somites experience in this period of development.

In the stages, with five or six mesoblastic somites, there follows in the anterior region of the body the complete closing of the mesoblastic somite cavities.

After the closing, too, of the slit communicating beween the mesoblastic somite and the archenteron canal, still, for some time its place may be traced in the arrangement of the cells at that point, in consequence of a feebly expressed discontinuity in the cells of the hypoblast. It can then be seen (Fig. 101) that the hypoblast slit is moved somewhat dorsally, and this is in connexion with the folding of the notochord.

If we observe the transverse section of a fully separated mesoblastic somite, we find that it possesses a more or less triangular form (Figs. 97–100). Three sides can be distinguished in it, that is, a base[1] which is formed of those cells which constitute the edges of the communicating slit, and which are in contact with the archenteron; an inner side,[2] which is in contact with the notochord and the nervous system; and an outer side,[3] which is in contact with the outer boundary.

The base[1] of the triangle furnishes the fibrous layer of the alimentary canal.

[1] Visceral. [2] Notochordal. [3] Parietal.

The cells in contact with the notochord, which, as we saw earlier during the consideration of the whole embryos, stretch through the whole length of the segment, are intended to provide the lateral muscles of the body.

The remaining part of the interior side of the mesoblastic somites, which is in contact with the medullary plate, takes no part in the formation of the muscles, but its cells become in the later stages a plate-like epithelium.

In just the same way, too, the cells of the outer mesoblastic somites which are in contact with the outer surface become flattened.

We can see these differentiations quite plainly expressed in the stage with six mesoblastic somites (Fig. 100).

In the following stages, with nine mesoblastic somites their extension towards the ventral side may be followed (Fig. 116). We see that this extension is dependent on the outer surface, which is constantly flattening more and more, and on the fibrous layer of the alimentary canal. These layers, while intruding like a wedge between hypoblast and epiblast, grow towards the ventral middle line. While

these flat-celled layers extend more and more the opposition between these and the columnar muscle cells becomes more and more noticeable. The latter preserve their limited extension on the lateral surfaces of the notochord.

The form of the muscle cells becomes club-like on the transverse section, while their interior ends, which are in contact with the notochord, become considerably smaller in comparison with the exterior ends turned towards the lumen of the mesoblastic somites. Thereby the transverse section of the series of muscle cells assumes a fan-shaped form. On the transverse section are found the cell nuclei in the club-like extremities of these cells. On one section, however, these are not visible in all cells, since, indeed, as we have seen before (Fig. 51), each cell extends through the whole length of the mesoblastic somite; and in all this extension possesses but one single cell nucleus.

We will now, in transverse section, examine the anterior outgrowth of the end of the first mesoblastic somite. It can be seen that during the closing of the communicating slit the first mesoblastic somite extends forward into a blunt prolongation (Figs. 93,

94). In these sections it may be recognised that the first mesoblastic somite has at once fully attained its separation and given up its continuity with the wall of the archenteron. It may be gathered from this that the continuation which grows out does not arise through a new folding of the hypoblast as was the case with the notochord, but is developed from the already existing mesoblastic somite. Further, if the series of sections of the different stages be followed, it may be seen how the hollow continuation penetrates continually further forward in regions where there was formerly no mesoblast formation to be seen.

The mesoblast continuation shows, then, like the mesoblastic somite, in general a triangular form (Figs. 95, 96), and its parts differentiate later on in a similar way. The extension of the individual parts, especially of that part which is in contact with the notochord, is, however, here much slighter. Later on its cells change here, just as in the body, into the muscular band lying by the side of the notochord. These muscle cells, in the region of the first somite, and especially in its anterior prolongation, are already much smaller than in the other somites.

We can thus indicate in the anterior prolongation of the mesoblast, a cavity which is connected with the cavity of the first mesoblastic somite. Further, as in the region of the body, so here there are lamellæ, corresponding to the muscular layer in contact with the notochord, and to the muscular layers of the body-wall and alimentary canal.

Some important conditions are still to be observed in the side view of the embryo on a closer consideration of the boundaries of the mesoblastic somite. The latter run at first in a straight line from the dorsal surface to the ventral side (Fig. 48). Gradually they begin in their ventral section gently to bend backwards (Fig. 50). Thereby is introduced the later characteristic curvature of the segment boundaries.

Another important process, too, may be indicated on a closer observation of the mesoblastic somite boundaries. In the early stages, indeed, with eight mesoblastic somites the beginning of a process may already be seen which profoundly influences the construction of the Amphioxus, and explains to us many a hitherto unintelligible peculiarity of it.

This is an unsymmetrical movement of the mesoblastic somites. This gains a continually sharper

expression in the further development. We will get a more exact view of this in the stage with nine mesoblastic somites, where it is already quite distinct.

If the embryo be considered from the side, and the microscope be first directed to the mesoblastic somite boundaries of the left side, and next, to those of the right side of the body, it may then be noticed that they do not cover one another, but that those of the right side come to lie somewhat further back than those of the left. This condition may be most clearly represented through drawing by means of the Camera lucida. In the same way this movement may be indicated by a consideration of the embryo from the back (Fig. 52). This movement advances gradually, in the first stages of the next period of development, so far forward that it extends as far as half a mesoblastic somite. We see, then, the first mesoblastic somite boundary of the right side fall nearly between the first and second of the left side, the movement of the first mesoblastic somite being not quite so important as that of the rest. Then we see the second between the second and third, the third between the third and fourth, and so on (Fig. 54). At the posterior end, where the latest mesoblastic somites lie, the move-

ment is not yet so important, but only begins in the development, and is only completed simultaneously with the differentiation of these mesoblastic somites The originally symmetrical foundations always experience simultaneously with the differentiation such a movement that the alternation of the mesoblastic somite boundaries is restored.

Neural Canal.

In the living object the closing of the neural canal may be recognised by the appearance of a contiguous layer of the outer surface, at first very thin, which closes the canal, thus converting it into a tube. Further, in the living object, if the growth be very marked, it may be seen that the cells of the neural canal, just as the outer epiblast cells, are ciliated. The long feeble cilia, whose direction is posterior, may be followed from those stages in which the lumen of the neural canal becomes distinct. Probably these cells have kept their character as ciliated cells, even during the invagination. The transverse sections provide us with more exact information with respect to the closing of the neural canal.

In the foregoing section of development the med-

ullary plate, which is already deepened like a channel, shrinks together, so that we have the closing of the channel which was originally open underneath the epiblast.

We will now follow this process in a single mesoblastic somite.

On comparing with one another sections from the same region of the embryo, but of various stages, we see that the neural plate grows considerably smaller during the deeper incurvation. This diminution is partly attributable to the fact that the single cells alter their form, while becoming columnar and smaller; otherwise this is effected through the elongation of the embryo. During this latter process a movement of the material of the cells takes place; for we see that a smaller number of cells composes the transverse section of the neural in the earlier, than in the later stages.

When the incurvation has reached to such a degree by the edge cells of the neural plates advancing more strongly towards the middle line, the closing is at last completed by these cells elongating towards the middle line, and there growing together (Figs. 116, 119).

The lumen of the now closed neural canal is at first dorsiventral and elliptically elongated, and does not, till later, become circular in transverse section. The transverse section of the whole neural canal in its form continually reminds one of the earlier stages (Figs. 116, 118), while its dorsal edges still have a wide lateral extension. It is only gradually, as we shall see in later stages, and through shortening of these cells (Fig. 117 is very instructive), that the transverse section of the neural canal becomes spherical (Figs. 125, 128). Its transverse section is at first more or less trapezoidal (Figs. 116, 118). The ventral side is a little deepened into the shape of a channel, and this channel is in contact with the dorsal surface of the notochord. This quadrilateral form is at once altered, owing to the diminution of the dorsal surface. The transverse section of the neural canal is now spherical, with a ventral segment for the upper surface of the notochord (Figs. 126–128).

The closing of the neural canal does not follow from front to back, as would be conjectured according to the differentiation which in general moves in this way, but the shrinking together and closing, succeed at the hinder end somewhat earlier, and advance towards the

front. This is perhaps to be explained through a connexion with the mechanical process of the formation of the mesoblast folds, and the diminution of the body form.

It still remains to explain the condition of the neural plate in the anterior region of the body, where there are conditions before us which differ from those of the typical mesoblastic somite. The neural plate reaches, as we have seen in the sections of the previous stages, forward beyond the region of the mesoblastic somites, and its last prolongation forms there the ground of the channel, which in this place is open outwards (Fig. 86). The closing of this opening still makes slow progress during this period of development, and, as already mentioned, in a direction from back to front, as may be seen from a comparison of the serial sections, and from carefully comparing the side views of the embryos.

The front end of the neural foundation is also distinguished by other peculiarities from the parts which follow further back. It can already be seen in the earlier stages that the neural plate in the region of the first mesoblastic somite is formed somewhat more thickly than further back. During the

closing of the neural canal, this condition comes continually into sharper prominence; through the considerable elongation of the embryo, the neural canal also becomes considerably thinner. The anterior end of the neural canal is now less affected by this elongation, so that proportionately it is continually appearing of greater thickness. While the neural canal, from the second mesoblastic somite on, shows a smaller transverse, section the neural plate is found far thicker and considerably widened in the region of the anterior half of the first mesoblastic somite, and still further forward in the region of the prolongation of the anterior end of the mesoblast, where the neural canal is still open outwards (cp. Figs. 113–115 with 116; and Figs. 122–124 with 125). The central canal, too, possesses in the neighbourhood of the first mesoblastic somite a considerable diameter. The neural foundation thus shows an unmistakable swelling on the anterior end of the body, especially on from the anterior half of the first mesoblastic somite.

This is also to be recognised in the side view of the embryo, and is remarkable on a back view, owing to its being wider than the notochord (Fig. 56).

In an anterior direction the neural plate has not

even yet attained to its complete separation. There may, indeed, be seen on the side view of the embryo, the series of cells of the neural plate, passing over in unbroken continuity into the lower epithelium of the front end of the body.

Formation of two Anterior Endoderm Diverticula.

In the stage with seven mesoblastic somites a formation makes its appearance in the anterior region of the hypoblast of the body, that is, in front of the mesoblastic somite region, whose remarkable destinies will attract our special attention in the later stages. This formation consists of two dorsal folds of the hypoblast. These appear more or less simultaneously with the formation of the notochord fold in this region, and relations of their positions to the notochord fold remind one of those of the mesoblast folds in the region of the body (Figs. 105, 106).

In the foregoing section of development, the completion of these folds becomes continually more pronounced, and they form two lateral, and at first symmetrical, diverticula, at the anterior end of the alimentary canal. The conditions may be recognised as well on the side view (Figs. 48, 50), and the

ventral view (Fig. 53) of the embryos, as in the transverse sections (Figs. 105, 106, and Figs. 113, 114).

In the transverse sections of the older embryos at this period of development, we are struck by the relation of these folds to the prolongation of the anterior end of the mesoblast of the first mesoblastic somite. We see that this prolongation inserts itself dorsally between the hypoblast fold and the nervous system.

These dorsal folds separate later from the alimentary canal, and show in the further development a very remarkable unsymmetrical condition. We will gain some acquaintance with these processes in the next period of development. I will only mention here, that the unsymmetrical formation begins as early as in the stages with nine, ten, and eleven mesoblastic somites.

The Epithelium.

We will further add some remarks with regard to the changes in the outer epithelial layer. In view of the extension which the embryo experiences during this last period of development, the epithelium becomes continually lower, so that the individual cells change continually more and more from the form

of a cylindrical, to that of a cubical epithelium, only on the anterior point of the body, and on the posterior end the cells remain columnar.

The cilia, which, as already mentioned, every cell bears, grow in the course of development to a considerable length, as has been represented in Fig. 50.

Absorption of the Yolk Granules.

The absorption of the yolk granules, and therewith the transparency of the embryos, make continual advance during this period of development.

In general, the cells of the posterior end of the body contain in all layers more abundant yolk granules than those in the remaining parts.

With regard to the layers of the body, it may be said that the yolk granules attain their absorptions most quickly in the outer epithelium, where they at the end of this period of development are now not to be found by any means plentifully. The mesoblast follows next, then the medullary plate, and the hypoblast last, so far as the speed is concerned with which the yolk granules attain their absorption (compare the sections on Table VIII.).

FOURTH PERIOD OF DEVELOPMENT.

Period of the histological differentiation.

Just as the third period of development may be described as that in which the differentiation of the organs follows from the two primary germinal layers, so the histological differentiations which concern these organs may be described as the most important processes of the fourth period of development. Especial account is to be taken of the formation of the muscles, the histological differentiation of the notochord, and of the fibres in the neural canal.

The formation of new organs is in this period of development only of secondary importance. The two evaginations of the alimentary canal, which we previously mentioned, are completely separated from the alimentary canal, and are altered in a remarkable way. There is further originated a gland which was noticed in older larvæ by Leuckart and Pagenstecher, and minutely described by Kowalevsky. The formation of the ventral blood-vessel now begins.

Finally, at the end of this period of development, preparation is made for the perforation of the mouth and first gill-slit, and of the anus.

Although the important part of this period of development depends upon the histological differentiation, yet the most striking appearance is the considerable change in the outer form, *i.e.* of the larva. The ovoid shape was in the former period of development somewhat altered through elongation and lateral compression, without, however, leading to a characteristically expressed form of body. In the period of development, however, which is now before us, the body, through considerable elongation, receives continued lateral compression. Through the growing out of the epiblast cells of the posterior end to a caudal fin, and through nose-like elongation of the anterior end of the body, it acquires a fish-like form, which vividly reminds us of the vertebrate animal type (Table V., Figs. 54–61).

The Formations of the Mesoblast.

We will now review the alterations which concern the mesoblast formations.

Kowalevsky's observations have yielded but little

information with regard to the differentiations of the mesoblastic somites. He has only told us that they serve for the formation of the muscular system. His conjecture, moreover, is to be mentioned that the cavity of the mesoblastic somites becomes the body cavity. I will proceed to give my own experiences.

The multiplication of the mesoblastic somites proceeds more slowly in this period of development. The elongation of the body is also to be referred not to a growing out of the posterior end, but especially to extension of the body segments which we already have, and of them, chiefly those which are anterior. The number of the mesoblastic somites up to the perforation of the mouth opening, which for us marks the end of this period, only increases to fourteen (Figs. 59, 61).

The formation of these mesoblastic somites follows in the same way as before. The mesoblastic somite cavities remain for some time in open communication with that of the archenteron (Figs. 128, 141).

Posteriorwards the fourteenth mesoblastic somite is followed by a quite short and undivided mesoblast fold, which ends off at the posterior end of the

gastrula-mouth, with the two large round pole cells of the mesoblast. The lumen of the mesoblast folds still remains in open communication with the lumen of the archenteron (Fig. 143); and it is only at the conclusion of this period of development that we have a complete separation of the undifferentiated mesoblast folds from the archenteron, and it is then that they present themselves as formations fully separated from the hypoblast.

The alterations which the mesoblastic somites experience in this period concern (1) alterations of form, and (2) histological differentiations.

So far as concerns the alterations of form, we must mention in the first place that the mesoblastic somites gradually grow forward until they reach the ventral middle line. At the same time not only do the outer surface and archenteron grow, but also the dissepiments, so that the mesoblastic somite cavities in their ventral part also become separated by the dissepiments into segmental divisions (Fig. 57). At the end of this period of development, but not till then, the dissepiments are curved backwards in the ventral portion, and remain confined to the dorsal part of the body.

The bending of the mesoblastic somite boundaries, which at first showed itself only in a slight backwardly turned curvature of its ventral part, becomes continually more pronounced, till at last it becomes an angular bending of these lines. The apex of the angle is in the region of the notochord (Figs. 54, 61), and gradually moves a little further dorsally. The two sides, both the shorter and dorsal, as well as the longer and ventral, point in the posterior direction. The angle becomes, in the course of development, continually more acute, and more especially the ventral side, which, after retroformation of the ventral part of the partitions no longer reaches so far, and is turned more sharply towards the back (Fig. 61 A).

We will now review the histological differentiations of the mesoblast. As we have previously seen, all the parts of the mesoblastic somite as well as the outer surface, and also the archenteron and the part in contact with the neural canal, are composed of cells which experience a considerable flattening. Only the cells in contact with the notochord, which are to form the lateral body muscles and which on each side compose a small band running the whole length of the body,

consist of columnar cells, whose behaviour we have previously described in detail.

The differentiation of the muscles has now its origin in these cells, more or less in the stages with ten mesoblastic somites. In the larvæ stages with eleven mesoblastic somites I could already observe slight lateral shrinkage which is to be referred to the action of the muscles. The fibrilla, which at first were very faint, became continually more distinct in the course of this period of development.

It may be seen that each cell forms at first only a single fibrillum, moreover the muscle cells close together in longitudinal series and a segmental interruption is not to be seen in the separate fibrilla. It may therefore really be said that a cell now forms a common fibrillum which may be followed straight on through the length of the body, and that each separate cell becomes a segment in the formation of such a long fibrillum.

There may be seen transverse stripes of the fibres both in the living object and in preparations, this being especially the case when they become more distinctly prominent.

In order to become acquainted with the manner

in which the fibres become separated from the cells we will take into consideration single preparations as well as sections.

In Fig. 59, we see isolated a part of the notochord with the muscle cells attached. It may there be seen that the fibrilla are formed in contact with the median side of the cells, *i.e.* they are immediately in contact with the notochord. On a surface view of the muscle band (Fig. 58) we may with one position of the microscope see the elongated protoplasmic bodies of the cells which even yet contain single yolk granules. In the places where the cell granules lie the cells are swollen in a spindle-like shape. It may further be here seen how the cells of contiguous mesoblastic somites are directly continuous the one into the other. On lowering the microscope the small elongated muscle fibrilla are seen whose number agrees with that of the cells.

With reference to this agreement we are readily liable to mistakes in single preparations, since a number of the cell granules may be easily torn away from the muscle band, while the fibrilla belonging to it lie before us. Owing also to disarrangement of the contours a larger number may be indistinctly

seen. So far as this goes, the transverse section make things thoroughly clear. In these we are able also to gain further information regarding the form and growth of the muscle fibrilla.

The muscle fibrilla come under our notice in the transverse sections first as quite small bright granules on the surface of the cells in contact with the notochord (Figs. 124–127). The fibrilla grow in the further course of the development to such an extent that from being thread-like in form they become strap-shaped. These strap-shaped fibrilla are parallel to one another and in a somewhat acute angle on the lateral surface of the notochord. They occupy the interior half of the cells, at whose expense they grow. Between the muscle bands remains at the most only a very small remnant of protoplasm. This is stored up in the outer club-shaped portion of the cells (Figs. 130–136).

These histological differentiations proceed from the anterior to the posterior end.

The prolongations of the anterior end of the first mesoblastic somite differentiate also in the main in the same way, with this difference, that the number of the muscle fibrilla is there smaller, answering

to the smaller number of muscle cells. In these parts also the mesoblast does not grow forward till it reaches the ventral line, but remains limited to the dorsal half (Figs. 129, 131).

When the extension of the mesoblastic somites has advanced as far as the ventral middle line, a simple mesoblast lamella is seen between epiblast and alimentary canal. This lamella has arisen through growth of the prolongations of the mesoblastic somites on both sides. It extends, however, further back into those regions in which the mesoblastic somites have not yet grown forward as far as the ventral line. This may be observed on the side view (Fig. 60) as well as in the transverse sections of the embryos (Fig. 139). It is at the end of this period of development that the first indications of the system of blood vessels show themselves, in this mesoblast lamella in the ventral line. There can be here seen in the latest stages a clear canal, which may be followed from the posterior end forwards. This is bounded by extremely flat endothelium-like cells which form its wall. In the region of the second somite, where in the ventral middle line a disc-like thickening of the hypoblast takes place, which forms the foundation of the first

gill, the course of the blood-vessel foundation experiences a deviation. The clear canal is pushed to the right through the foundation of the gill, it runs the length of the latter's outer edge on the right side of the body, and ends off in the region of the club-shaped gland (Fig. 61 A). Contractions of this blood vessel are not to be observed till somewhat later, after the mouth opening and first gill slit have already perforated. Kowalevsky described this blood vessel in somewhat older stages. When these are reached, we will examine his account more closely. With regard to the origin of the vessels, he conjectured that they "originate from cells which lie freely in the cavity of the body, which at first collect in a firm strand, the lumen being only a secondary formation."

Further Formation and Histological Differentiation of the Notochord.

Kowalevsky's account of the histological alterations of the notochord must be regarded as incorrect.

He speaks of a special notochord sheath; this is however not to be seen; perhaps it was the dorsal and ventral cell series of the notochord which led him to make this mistake.

He further describes the origin of the notochord plates in a manner not answering to the reality. In common with Max Schultze he regarded them as products separated from the cells. In the notochord foundation which "consists of a distinct notochord sheath and a central part of homogeneous substance" bodies should make their appearance, these being at first very small and interrupting the light, afterwards coalescing with the notochord plates.

We shall see that the histological differentiation of the notochord is introduced in a way similar to that usual with vertebrate animals, *i.e.* by the formation of vacuoles in the cells. The notochord plates constitute the walls of separation which lie between the elongated vacuoles.

Kowalevsky held the vacuoles, which on their first appearance are very small and round, to be secretions, and later on too confused vacuoles and notochord plates with one another.

His mistakes are to be referred to his insufficient methods, as well as to the then insufficient views respecting the histological formation of the developed notochord.

When dealing with the further formation of the

notochord in this period of development, we shall take especial note of two historical points: first, the morphology; and, secondly, the histological differentiation.

Taking the first as our starting-point, we will first of all observe the condition of the notochord in a convenient myotome of the body, then the growth of the notochord at the posterior end, and finally the condition in the anterior end of the body.

In the transverse sections of the last stages of the former period of development in the region where the myotomes are completed we saw the oval notochord transverse section still wedged in between the cells of the mesenteron. In the next stages the notochord is being pressed out of the wall of the latter, though it still to a considerable extent continues to lie inside it (Figs. 132–139). In the region of the later segments we still find the former condition of the wedging (Figs. 138, 139). Still further back we find the notochord even yet not sharply distinguished from the mesenteron (Fig. 140). In the neighbourhood of the latest mesoblastic somite it passes over into the dorsal fold of the mesenteron (Fig. 141), and this, too, becomes at last flattened

in the neighbourhood of the neurenteric canal (Fig. 142). The growth of the notochord at the posterior end follows, owing to an abstriction of the fold formation of the dorsal wall which continually progresses backwards. Just as however the undifferentiated mesoblast folds from which the foundation of numerous mesoblastic somites is still to proceed, are completely abstracted from the hypoblast as separate formations at the end of this period of development, so in the same way, at the conclusion of the embryonal development, follows the abstriction of the undifferentiated notochord fold from the archenteron. This now completely isolated foundation forms the material at whose expense the notochord later on also continues to grow at the posterior end during the formation of new myotomes. Mesoblast and notochord are thus further increased by new formations, which, as before, originate at the expense of the undifferentiated foundations of the posterior end of the body, except that these undifferentiated parts are no longer connected with the hypoblast, but are completely separated.

In the anterior end of the body the notochord had already become almost completely separated

at the conclusion of the former period of development. This separation and removal from the wall of the mesenteron is completed at the end of the period of development which we have now been considering. With the trunk-like outgrowth of the anterior end of the body is united an elongation of this part of the notochord. The notochord appears here to grow more extensively than the neighbouring tissues. Its anterior point seems quite wedged in between the epiblast cells of the end of the body; through excessive growth it often experiences here swelling or curving.

The histological differentiation of the notochord is introduced by the appearance of numerous vacuoles at first small in the interior of the notochord cells. The vacuoles are especially numerous in the middle cells; in the dorsal and ventral cell series of the notochord their number is very small. These little vacuoles make their appearance as early as the end of the former period of development in embryos with nine to ten mesoblastic somites (Fig. 50).

The vacuoles become continually larger, and their number in consequence smaller. This is to be explained by the fact that several small ones coalesce together.

The different destiny of the vacuoles is furthermore of considerable importance. While in the dorsal and ventral cell series they keep a round or somewhat irregular form, increase but slightly, and thereby become fainter and decrease in number, in places even entirely vanishing, those of the two middle series of cells come into continually sharper prominence, increase considerably, and experience a characteristic change of form (Figs. 54, 60, 61A).

In consequence of the increase, these vacuoles do not retain their round form, but they appear longitudinal, both on the side and back view of the embryo; it is only on transverse sections that their outline is round. They are thus flattened in the direction of the longitudinal axis of the embryo (Figs. 54, 60). While (on a side view of the embryo) they become continually higher, they are moved in such a manner forwards on to one another that they form one single series. The middle cells of the notochord, which contain the vacuoles, have also naturally to do with this movement. Whereas these cells were originally to be found in two series, they form later on by moving into one another merely one (Figs. 60, 61). The notochord consists now of three cell series, a

dorsal, a ventral and a middle, the latter containing the large flattened vacuoles (cp. Figs. 131–134).

The vacuoles extend so much that there remain between them only thin perpendicular walls of separation. These are the notochord plates, which through the thickening of their outer layer appear with a sharp contour.

On the dorsal and ventral cell series the cell boundaries and granules are to be plainly distinguished.

In the middle cell series, penetrated by large vacuoles, which represents the peculiar and characteristic notochord tissue, the cell boundaries are no more to be seen. The cell granules too become there less distinguishable, still they may be seen by means of proper reagents.

The histological differentiation of the notochord may quite well be followed on complete stained preparations of the stages following one upon the other. For the thorough comprehension of the formations before us, transverse sections also are necessary. A further examination too is of great importance for the control of results so gained, namely, the examination of the posterior end of the notochord of older embryos

or larvæ. At the posterior end where the notochord continues to grow and its tissues become again more and more differentiated, all the stages of this differentiation may be observed one after the other. We may follow step by step the appearance of the vacuoles, their elongation, their movement into one single series, so that perpendicular walls of separation are formed between them as notochord plates, to which we add the alterations of the cells. Here, where we see the stages of development near one another in direct transition, we find the correctness of the above statements established.

The continuation of the histological differentiation from anterior to posterior is thus very sharply stamped upon the notochord.

The general picture of the histological differentiation of the notochord already shows plainly enough the same type which we find in the developed animal. The transverse fibring too of the notochord plates appears, as we shall soon see, very early.

The Neural Canal.

The neural canal had become closed at the end of the former period of development. It assumes a rounded form on the transverse section with ventral channel for the notochord.

During the further elongation of the embryo the transverse section of the neural canal becomes continually smaller, the canal diminishing chiefly in its dorsal part, so that the ventral surface which is in contact with the notochord is broadest. The edges of the neural canal appear then as though drawn out laterally (Figs. 134–140).

The small lumen of the neural canal (primary central canal) is spherical in the transverse section. The cells which compose the canal surround this cavity as a single layer. In the living object the ciliation which has a posterior direction may always be followed in the central canal.

Since the elongation of the embryo takes place principally in the region of the myotomes, in the same way this is the region too in which the neural canal becomes thinner. In the region of the unsegmented posterior end it forms therefore a considerable swelling (Figs. 60,

61). This posterior thickened end of the medullary tube forms the undifferentiated material which, on the completion of the growth and the further multiplication of the myotomes, is employed on the formation of new sections of the neural canal.

This terminal section of the neural canal, which belongs to the undifferentiated region curves during this period of development ventralwards round the posterior end of the notochord, as has been well shown by Kowalevsky, the communication with the mesenteron still remaining.

The thickening of the anterior end of the neural canal is more prominent too in this period of development. The latter is also towards the anterior sharply separated from the thinner epithelium of the surface of the body. The brain-like thickening of the neural canal is readily to be observed on a side view of the embryo. It is seen how owing to it the notochord experiences a very considerable indentation (Figs. 60, 61). It may be recognised also how the central canal, which is here somewhat wider, opens outwards towards the front into a funnel-shaped depression of the outer surface. The brain-swelling can be shown still better in transverse sections,

especially since the widening of the wall as well as of the central canal is very important (Figs. 122, 123 and Figs. 129–132).

At a quite definite place, in the fifth myotome in the ventral wall of the medullary tube, a black pigment spot makes its appearance at a fixed time (Fig. 54, etc). Much later, at the end of the embryonal development, there appears also a pigment spot, which unmistakably has the appearance of an eye spot. At the end of the embryonal development the first nerve fibre strands make their appearance in the neural canal more exactly in the two ventral laterally drawn edges. We will gain a closer acquaintance with these later on, when considering the larva.

The Transformation of the Anterior Hypoblast Diverticula.

For the formation of the anterior end of the body, the destinies of those anterior evaginations of the alimentary canal, which we saw originating at the conclusion of the former period of development, are of great importance.

These evaginations, which appeared in pairs and symmetrically, are further formed in a curiously

unsymmetrical manner. They both abstrict completely from the alimentary canal, which then is completely withdrawn from the anterior end of the body. The diverticulum on the right side extends considerably, and its cells become flattened like an endothelium. This thin-walled diverticulum encloses then a large triangular open space occupying the anterior body end of the notochord ventralwards. The diverticulum on the left side remains round and thick-walled; it is composed of columnar cells. While that on the right side moves more towards the anterior, the other remains at the posterior end of the head-prolongation somewhat further back than the brain swelling of the neural canal. At the beginning of the larval stage this diverticulum, which is covered on the inner surface with cilia, breaks through on the left side of the body with a small opening outwards—the *preoral pit.*

The diverticulum on the left side was described by Kowalevsky as "a peculiar sense organ" of the larva. He did not recognise its development.

We will now observe these processes more closely.

The alterations of both hypoblast diverticula can be studied in the living object by observation of a con-

tinuous series of developments. The appearances are there very clear and convincing, and we can easily get a view of all the transitions.

After the formation of the ninth mesoblastic somite, the two diverticula, which are still in open communication with the mesenteron, begin to assume an unsymmetrical form. That which is on the right side enlarges, its cells flatten in the manner of an endothelium, and it moves more in an anterior direction. Through the extension of this diverticulum the alimentary canal is pushed back from the anterior end of the body. The diverticulum on the left side is at the same time moved somewhat backwards.

In the next stages, during the trunk-like growth of the anterior end, the diverticulum on the left side is completely abstricted from the mesenteron. That on the right side, which has extended still more, still continues by means of a small opening in open communication with the anterior end of the mesenteron (Figs. 54, 55). This opening may thus be observed at a time when the endothelium-like change of the cells has already made a very considerable advance, and when the varied formation of the evaginations of the mesenteron, which were originally

in pairs and similar, is already to be quite clearly recognised. At the end of this period of development the difference of formation is so considerable that without knowledge of the development the original similarity of both formations could hardly be imagined (Figs. 60, 61).

The diverticulum on the left side lies obliquely under the notochord, so that its blind end reaches over to the right side. This end, even before the perforation of the outer opening, begins to differentiate into two parts. These are a larger, wider, and more strongly ciliated section, which lies towards the left, and receives the outer opening later on, and a smaller and narrower one having a direction towards the right, which also forms the blind end of the organ.

The perforation of the outer opening of the internally ciliated organ falls under the post-embryonal period of development, following, as it does, shortly after perforation of the mouth opening.

The results arrived at through examination of the living object are confirmed and completed by the study of osmic-carmin-glycerine preparations. The study of transverse sections, especially, supplies us with information as to their first alterations, their

relations of position to notochord and alimentary canal, and their relations to the first mesoblastic somite. I will not describe this here in detail, but merely refer to the plates (Figs. 113, 114, 121, 122, 129, 130).

Development of the Club-shaped Gland.

We will now observe more closely the development of another organ, which is formed from out of the alimentary canal. This is the peculiar gland which all previous observers had found in the Amphioxus larva, with regard to whose formation, however, we have entirely erroneous statements, and whose structure was hitherto not thoroughly recognised.

This gland originates through a folding from out of the alimentary canal, in the region of the first myotome.

In the embryos, even with 9–10 mesoblastic somites, a very shallow and transverse folding of the alimentary canal may be recognised in this region (Fig. 50). This advances ventralwards, from the right side wall of the alimentary canal, where it is most sharply marked, and reaches over to the left side wall. In

the next stages this fold is deepened, and in its appearance it soon reminds us of the completed gland, although still, in its whole extent, it is open towards the alimentary canal (Figs. 55, 57). It is strongest on the right side of the body, where along the whole surface of the wall of the alimentary canal it descends somewhat obliquely in an anterior direction, and continues, on the left side, considerably smaller, and only as far as the middle of the left wall of the alimentary canal (Fig. 60).

Towards the end of the embryonal period follows the closing of the canal, and the abstriction of this formation from the alimentary canal (Fig. 61). This formation now exhibits a gland situated on the right side. This continues into a thin duct, which ventralwards curves round the alimentary canal, and on the left side rises till near the middle of the latter (Fig. 63). There, later on, the thin duct opens outwards. Just as, later on, the mouth perforates in this part of the left wall of the body, so the gland then opens at the exterior edge of the mouth.

This canal, which runs obliquely over the alimentary canal, and which divides into a glandular portion

and a duct, has thus not been formed through outgrowth from the place of opening, but has reached its abstriction along its whole length.

The cells of the club-shaped, thickened glandular portion enlarge, and assume a granular appearance and yellowish colour. The thin duct is composed of a small number of somewhat flat cells, and on that side of these, which is turned towards the lumen of the canal, faint cilia are at once formed.

Kowalevsky speaks, in his treatise, of two glands, and gives representations of them. He was misled by a thickening of the alimentary canal in front of the gland, a ciliated organ, of which we shall speak again later on.

With regard to the development of this gland, Kowalevsky fell into the extraordinary mistake of supposing that it originated through change of the primitive vertebræ. We have already shown above that the first mesoblastic somite differentiates much earlier in just the same way as those which succeed it, having, at the same time, nothing to do with the formation of this gland.

The Alimentary Canal.

The alimentary canal begins to ciliate in this period of development on its inner surface, as on every cell a cilium is formed. The formation of the mouth and of the first gill-slit foundation is thus prepared.

Kowalevsky thus writes: "On the right side more or less, on the place where the diverticulum of the alimentary canal ends in front, the walls of the interior canal (alimentary canal) and of the body coalesce on a little space. There arises first, from the thickening of the tissue, a somewhat dark spot. In the middle of this an opening is soon formed originating from the separation of the cells which have coalesced. The opening so formed is surrounded by edges raised like walls. This aperture is the opening of the mouth."

He says, further: "Soon after the formation of the mouth, it may be noticed that at the lower edge the wall of the alimentary canal coalesces with that of the body, and soon there is here seen an opening; this is the first gill-slit. This newly arisen opening does not remain long in this place, but moves to one side of the body, that, namely, opposite to the opening of the mouth."

Kowalevsky has given us no information respecting the relation of position of mouth and gill-slit, with reference to the segments.

I will now myself describe the processes belonging to the end of this period of development, which are preparatory of the formation of the mouth and first gill-slit.

In the region of the first two segments, the anterior blind end of the alimentary canal is thickened in a club-like manner, whereby the body appears raised in this region. The raising of this section of the alimentary canal is connected with the formation of mouth and first gill-slit.

The formation of the mouth is introduced by a disc-like thickening of the epiblast. The latter consists here of somewhat high cells, in contrast to the other and larger flat cells.

This thickening of the epiblast lies on the left side of the body, corresponding more or less to the middle of the side wall of the alimentary canal. It is also in immediate contact with the hypoblast, since here the mesoblast does not grow forward so far ventralwards (Figs. 132, 133).

While the formation of the mouth opening princi-

pally owes its origin to a thickening of the epiblast, the formation of the first gill-slit begins with remarkable alterations in the hypoblast.

We were already able, in the stage of Fig. 54, to observe a small indentation of the hypoblast, on the ventral side, in the region of the second segment. The cells multiply here very rapidly. While the wall of the anterior end of the alimentary canal, with the exception of a small-celled streak in front of the club-shaped gland, is composed of large granulated cells, the foundation of the gill comes at once into view, owing to the fact that it consists of somewhat high thin columnar cells of clear appearance (Fig. 60). These form a disc-like thickening of the wall of the alimentary canal.

This thickening lies at first, more or less, in the ventral middle line, but before the perforation of the gill-opening moves a little to the right; and when we think that the boundaries of the segments are elongated, we see that this opening is situated in the second segment. This disc is distinguished also by its stronger ciliation.

We have already described above the relation of this foundation to the blood vessel.

The Exterior Epithelium.

We have still to mention the function of the exterior epithelium, and the formation of the caudal fin which originates from it.

The epiblast epithelium becomes continually thinner. Its cells are at the conclusion of the embryonal period very thin, extended, and flattened. It is only in a few places that an exception can be seen. We must here particularly mention the anterior end of the body where the thick epithelial layer seems to form a sort of tactile organ.

Finally, at the posterior end the cells grow out to an extraordinary height, while they here introduce the formation of an epithelial caudal fin. This primary caudal fin, which is only a provisional formation, does not originate as a fold, but is a pectinate elevation of the epithelium. These epithelial cells contain, as a general rule, numerous fine black pigment granules.

All the cells of the exterior epithelium, even those which compose the caudal fin, carry each one of them a long cilium, by means of which the embryo moves itself slowly forward, although it is now capable of really strong muscular activity, which, however, does not appear except on special provocation.

FIFTH PERIOD OF DEVELOPMENT.

Transition to the Larvae Stage.

We will include together in this fifth period of development those stages which form the transition from the development of the embryo, which takes place at the expense of the yolk granules stored up in the cells, to the larvae stages which are self-nourishing.

The processes which characterize this fifth period of development consist in the perforation of a number of apertures, which first render possible the functional activity of the organs. These openings are: The mouth, and the first gill-slit, the opening of the ciliated organ, which has developed from the anterior diverticulum of the alimentary canal, on the left side the opening of the club-shaped gland, and finally, that of the anus. These openings originate chronologically in the order in which they have been here enumerated.

This period of development, which thus comprises the stages from the first perforation of the mouth opening up to that of the anus, occupies one and a half to two days, which should be added to the previously stated duration of the embryonal development.

Formation of the Body Apertures.

We will now take into consideration the origin of the above-named apertures, which is described as characteristic of this period. Kowalevsky states that the formation of the mouth follows somewhat earlier than that of the first gill-slit. I myself saw these two openings make their appearance simultaneously as very fine slits.

The formations introducing the perforation of the mouth and first gill-slit have already been described.

The mouth appears as a very fine opening, which lies in the middle of the disc-shaped thickening of the hypoblast, and places the alimentary canal in communication with the exterior. This fine opening lies, as has already been mentioned, in the region of the first somite. During the following days this opening enlarges very slowly. This enlargement

follows in such a way that the place of the original opening corresponds to the anterior edge of the later and subsequent one. The opening of the mouth remains continually surrounded by a thickened epiblast edge (Fig. 62).

The origin of the first gill-slit is due to the fact that a funnel-shaped depression is now formed on the interior surface of the disc-shaped gill foundation, which is turned towards the lumen of the alimentary canal (Fig. 61A). This depression presses forward as far as the outer surface, which is here in immediate contact with the alimentary canal, and there is now formed in this place a fine opening, which gradually enlarges, similarly to the opening of the mouth. The epiblast remains thin on the edge of the gill-slit; the hypoblast, however, which previously manifested here a disc shape now forms a broad ring-shaped wall of thin columnar ciliated cells, representing the interior edge of the gill-slit. The latter is already on its first perforation moved slightly to the right, and during its enlargement makes its way further and further up on the right side of the body.

The perforation of the outer opening of the ciliated organ follows soon after that of the mouth and first

gill-slit. The opening, which at first is small (Fig. 62), lies immediately underneath the region of the notochord, on the left side of the body. The opening soon enlarges very considerably.

The ciliated duct of the club-shaped gland perforates on the upper surface of the body, somewhat ventralwards from the anterior edge of the mouth.

The perforation of the anus follows later than that of the other organs mentioned here. Its place is the posterior end of the alimentary canal.

In this place there was, a short while previously, to be observed a connexion of the cavity of the archenteron, not only with the neural canal, but also with the cavity of the mesoblast folds, and with the slit of the notochord. After the mesoblast folds and the notochord foundation have completely separated from the archenteron, there only now exists the connexion with the ventrally curved end of the neural canal. The two canals, which are histologically plainly distinguishable through the size and clearness of the cells, have as their point of connexion the morphological posterior end. On both sides of this point are found the posterior pole cells of the mesoblast folds.

The interruption of the communication between the archenteron and the neural canal takes place, more or less simultaneously with, or perhaps somewhat later than, the perforation of the anus.

The anus perforates outward ventrally from this opening of communication, which represents the last that is left of the gastrula-mouth, it is at the same time moved unsymmetrically to the left side of the body. The perforation is immediately in front of the caudal fin, which bounds the hinder end of the body.

Kowalevsky has most admirably described these relative positions of the anus in his "Further Studies."

Further Alterations.

The form of the body becomes more marked. Its elongation continues taking place, through extension of the already formed somites, for the multiplication of the latter is now, as is the case with all internal processes of development, extraordinarily delayed. Indeed, during this proportionately lengthy period, only one additional mesoblastic somite is formed, this being the fifteenth. The lateral compression also

becomes more marked. The anterior end of the body, which grows out triangularly, and the caudal fin continue their formation.

The embryos, which are covered with long flagella, move only exceptionally by a lateral twisting of the body. Whereas the flagellated embryos, however, continued hitherto on the upper surface of the water, they now begin to sink some distance down. In the glasses they sink to the bottom.

With regard to the flagellation of the body, we must mention a phenomenon already noticed by Kowalevsky. He describes it as two tactile threads, which are formed on two little warts on the lower side not far from the mouth. "Treatment with acetic acid," he says, "will show that these tactile hairs consist of two long cilia which have coalesced."

I myself found the same, consisting of a number of separated, somewhat strong and motile cilia, which are situated upon a small wart-like projecting thickening of the epiblast, a collection, that is to say, of small columnar epiblast cells. This thickening of the epiblast is on the left side of the body, immediately in front of the anterior edge of the mouth, and its cilia incline towards the mouth.

The very faintly marked boundaries of the mesoblastic somites are only to be followed as far as the region of the ventral edge of the notochord. Further in a ventral direction, the partitions are formed backwards, as was already explained, and the body cavity is not there divided into segmental divisions. It is however no longer formed of open hollows, since the layers are here in immediate contact. It can however be seen that they do not grow together, since treatment of various kinds, pressure, and application of reagents, causes the outer surface, with its thin mesoblast layers, to separate from the inner layers. The ventral blood vessel, and its continuation on the right side, reaching up to the club-shaped gland, begins slow contractions, whose direction is from back to front. The notochord shows a more strongly marked form of the vacuoles and the notochord plates.

The absorption of the yolk granules is concluded with this period. All the tissues are formed of protoplasm, which is clear as glass and transparent.

Explanation of the Figures on Plates I.–IX.

All the Figures on Plates I.–IX. are given by means of the Camera lucida.

The enlargement of the Figures is as follows:—

On Plates I.–V.

The enlargement of Figs. 1, 38, 39, 40, 41, 63, is $\frac{66}{1}$.

„ „ „ „ 58, 59, is $\frac{272}{1}$.

All the other Figures on Plates I.–V. are enlarged 140 times. Wherever the figures are specially small, as for instance 7, 9, 48, 49, and wherever specially large, as for instance 50–53, reference must be had to individual distinctions of the embryos.

On Plate VI.

The enlargement of Figs. 64, 65 $= \frac{66}{1}$.

Fig. 66 $= \frac{206}{1}$.

Fig. 67 $= \frac{140}{1}$.

Figs. 68, 69, 70 $= \frac{272}{1}$.

On Plates VII.–IX.

The enlargement of Figs. 111 and 151 is $\frac{272}{1}$. All the other figures are enlarged $\frac{206}{1}$ times.

I have not in the text given any proportions of individual parts, since they may be computed from the drawings by means of compass and rule.

Plate I.

Fig. 1.

Fig. 2.

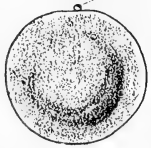


Fig. 3.

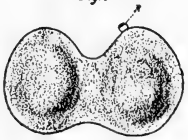


Fig. 4.

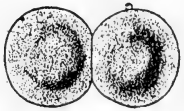


Fig 5.

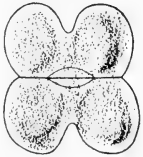


Fig. 6.

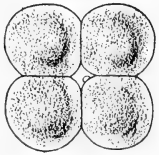


Fig. 7.

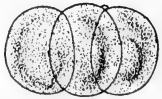


Fig. 8.

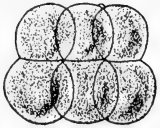


Fig. 9.

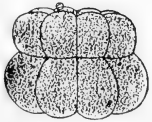


Fig. 10.

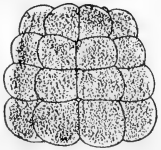


Fig. 11.

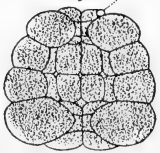


Fig 12.

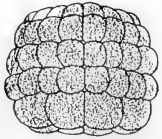


PLATE I.

All the Figures are drawn from the living object.

Fig. 1. An embryo (from about the stage of Figs. 29, 30) inside the yolk membrane, which is separated from it in order to show the relative size of the latter. Em., Embryo; dm., vitelline membrane.

Fig. 2. Egg with polar body (R.) before the fertilisation, vitelline from the surface.

Fig. 3. Egg shortly before the division into two segmentation spheres. There is still a bridge between the two parts, though it is deficient in yolk granules. The polar body (R.) adheres to the one half. Lateral view.

Fig. 4. Two-celled stage. The two cells have, since the division, come again into somewhat closer contact. A lateral view.

Fig. 5. Transition to the four-celled stage, as seen from the pole.

Fig. 6. Four-celled stage, after the cells have come again into closer contact, as seen from the pole.

Fig. 7. The same stage on a lateral view (from one corner). A somewhat smaller individual.

Fig. 8. Eight-celled stage examined in the same way as the former figure.

Fig. 9. Sixteen-celled stage (a small individual), from the side, but somewhat inclined, so that the eight upper cells can be seen.

Fig. 10. Thirty-two-celled stage. Lateral view (a large individual).

Fig. 11. The same in optical section.

Fig. 12. Further stage, with four upper sixteen-celled tiers and one lower eight-celled. Lateral view.

PLATE II.

All the Figures are drawn from the living object.

Fig. 13. Further stage with eight large lower cells and five sixteen-celled spheres. On one of the latter all the cells are biscuit-shaped, in the act of division.

Fig. 14. The same embryo in optical section.

Fig. 15. Further stage, lateral view.

Fig. 16. The same in optical section.

Fig. 17. Further stage, lateral view.

Fig. 18. The same in optical section. The epiblast cells by this time assume the character of an epithelium.

Fig. 19. Further stage (Blastula), lateral view.

Fig. 20. The same in optical section.

Fig. 21. The lower pole begins to flatten out; optical section.

Fig. 22. The invagination is in process; optical section.

Fig. 23. The invagination has advanced further; optical section.

Fig. 24. Stage of the completed invagination in optical longitudinal section.

PLATE III.

All the figures, with the exception of 35, 36, and 37, are drawn from the living object.

Fig. 25. Stage of the completed invagination (comp. Fig. 24), as seen from the gastrula-mouth.

Fig. 26. Further stage in optical longitudinal section.

Fig. 27. The same stage turned 90° round the primary axis (drawn from the upper to the lower pole); optical section.

Fig. 28. The same stage, as seen from the gastrula-mouth.

Fig. 29. Further stage in optical longitudinal section.

Fig. 30. The same in optical front section, as seen from the dorsal surface.

Fig. 31. Further stage in optical longitudinal section. On the upper surface minute cilia make their appearance.

Fig. 32. The same stage in optical front section, as seen from the dorsal surface.

Fig. 33. Further stage of elongated body form: shortly before the formation of the neural plate and the mesoblast folds; optical longitudinal section. In this, as in most of the succeeding figures, the cilia are not drawn upon the upper surface.

Fig. 34. The same stage in optical front section, as seen from the dorsal surface.

Fig. 35. Stage with dorsal furrow and first primitive segment in optical longitudinal section. In all the optical sections hitherto drawn, the cell mosaic has been represented in the background of the cavities. In the succeeding figures this is omitted.

Fig. 36. The same stage, as seen from the dorsal surface. Before everything else is drawn the optical front section. The first mesoblastic somite (I. US.), as well as the mesoblast folds, are indicated by shading. Further, the distinct posterior border of the first mesoblastic somite is drawn. We have, moreover, the borders of the layer (V.) which grows forward to the middle line over the neural plate and the gastrula-mouth (GM.).

Fig. 37. The second mesoblastic somite is by this time in formation; optical longitudinal section, mesoblastic somites, and mesoblast fold are indicated.

Fig. 38. Stage with the first mesoblastic somite, as seen from the dorsal surface, from the living object.

Fig. 39. Stage with two mesoblastic somites, as seen from the dorsal surface, from the living object.

Fig. 40. The same stage on a lateral view.

Fig. 41. Stage with three mesoblastic somites, as seen from the dorsal surface, from the living object.

PLATE IV.

All the Figures are drawn from osmic-carmin-glycerine preparations.

Fig. 42. Stage with two mesoblastic somites in optical longitudinal section. Mesoblastic somites and mesoblast fold are indicated.

Fig. 43. The same stage, as seen from the dorsal surface. Together with the optical front section, the mesoblastic somites and mesoblast folds and the neural canal are indicated in the drawing. The covering of the neural canal is ruptured in the line of union owing to the influence of the reagents, just as in Fig. 45.

Fig. 44. Stage with three mesoblastic somites in optical longitudinal section. The mesoblastic somites which can be seen by raising the microscope are here drawn in.

Fig. 45. The same stage, as seen from the dorsal surface. Representation as in Fig. 43. The region of the mesoblastic somites and mesoblast fold is in the optical front section, drawn on the right if the microscope be raised, on the left if it be lowered.

Fig. 46. Stage with five mesoblastic somites in optical longitudinal section. The mesoblastic somites are drawn in.

Fig. 47. The same stage, as seen from the dorsal surface. The openings of the mesoblastic somite cavities into the cavity of the archenteron are indicated, which are to be seen by lowering the microscope.

Fig. 48. Stage in which the eighth mesoblastic somite is in formation, mesoblastic somites and anterior diverticula of the archenteron are indicated (small individual).

Plate II.

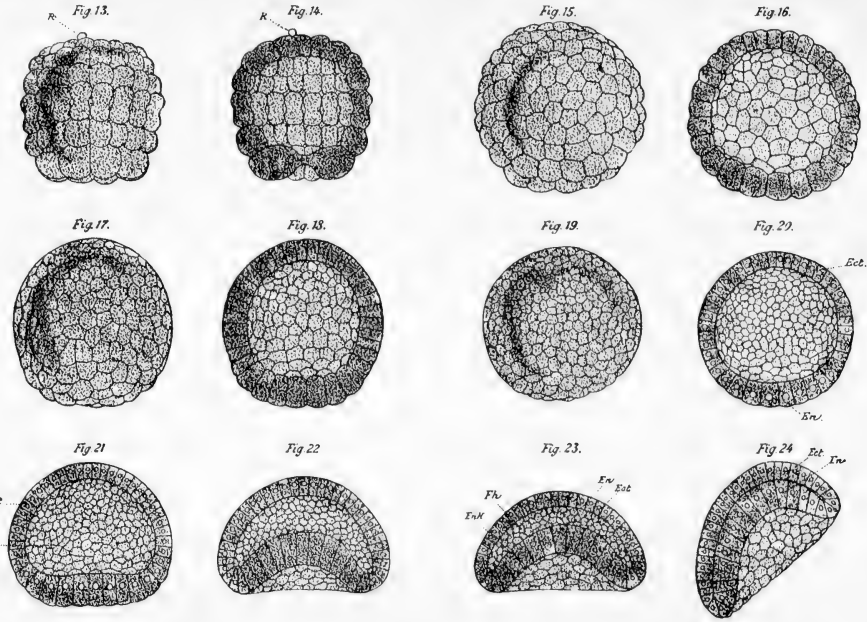


Fig. 13.
R.
Fig. 14.
R.
Fig. 15.
Fig. 16.
Fig. 17.
Fig. 18.
Fig. 19.
Fig. 20.
Ect.
En.
Fig. 21
Ect.
FK.
Fig 22
Fig. 23.
En
Ect
Fh
EnK
Fig. 24
Ect.
En
Waterlow's Engr.

Plate III

Fig 25

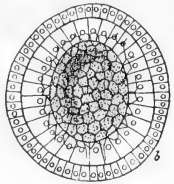


Fig 26.

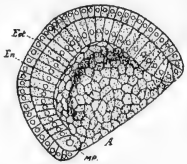


Fig 27

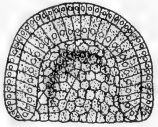


Fig 28.

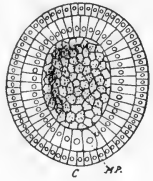


Fig 37.

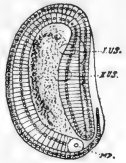


Fig 29.

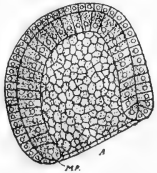


Fig 30.

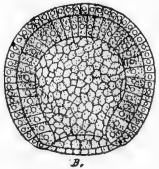


Fig 31

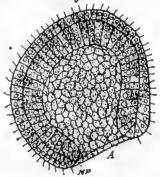


Fig 32

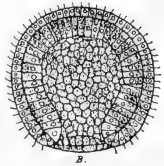


Fig 38.

Fig 39.

Fig 33

Fig 34

Fig 35.

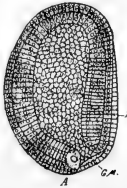


Fig 36

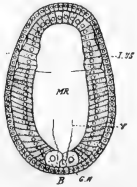


Fig 40.

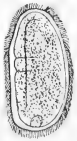
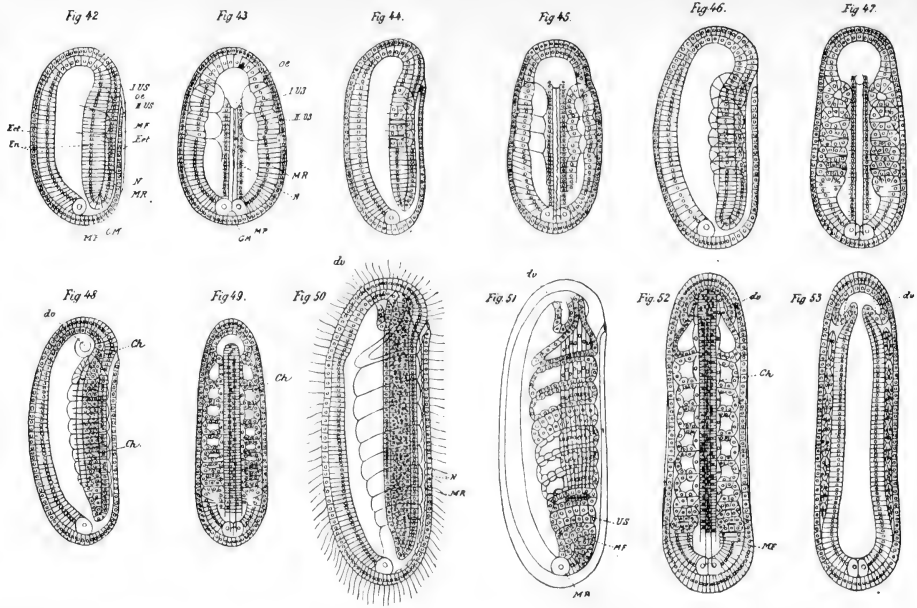


Fig 41.



Vinassa's lag.

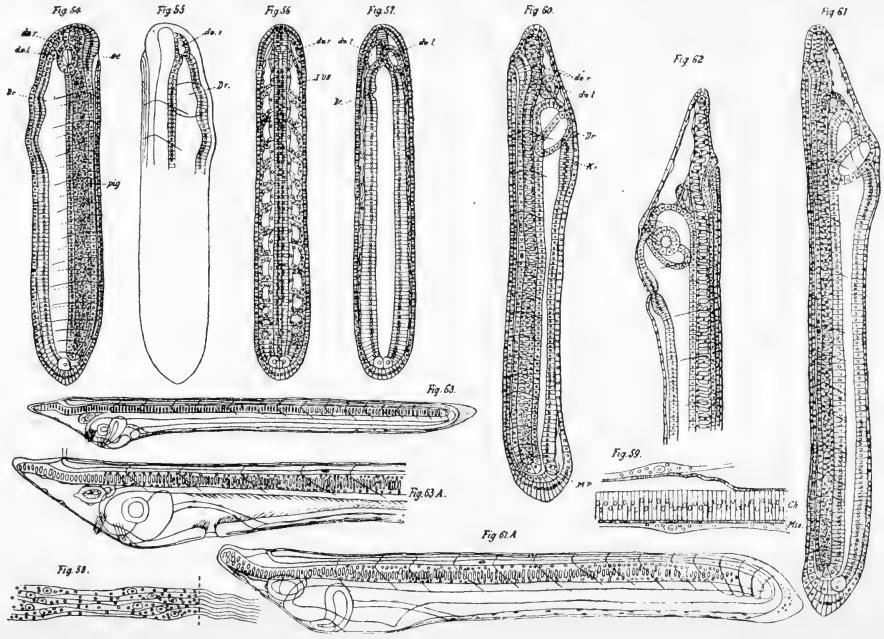


Fig 54
Fig 55
Fig 56
Fig 57
Fig 60
Fig 61
Fig 62
Fig 63.
Fig 63.A.
Fig 59.
Fig 61.A
Fig 58.

Fig. 49. The same stage, as seen from the dorsal surface. The dorsal cell series of the notochord foundation is drawn in, the neural canal is only indicated.

Fig. 50. Stage with nine mesoblastic somites in optical longitudinal section. Mesoblast formations and anterior diverticulum of the archenteron are drawn in.

Fig. 51. The same stage on a lateral view, with especial regard to the mesoblastic somites and the mesoderm fold. The first, second, third, and eighth mesoblastic somites are represented in the optical longitudinal section. The cells forming the muscular system, which are in contact with the notochord, are only indicated here. In the fifth, sixth, and seventh somites, these cells are focussed. The fourth and ninth somite, as well as the undifferentiated mesoblast fold, are represented as seen from the upper surface.

Fig. 52. The same stage, as seen from the dorsal surface. The notochord is drawn in section; the neural canal which runs over it is only indicated by shading.

Fig. 53. The same stage as seen from the ventral side. In the region of the anterior diverticula of the archenteron the course of the archenteron is indicated by punctuated lines, as may be seen by raising the microscope higher, and so getting nearer to the ventral surface.

PLATE V.

Figures 61A, 62, and 63A, are drawn from the living object; all the others from preparations.

Fig. 54. Stage with thirteen mesoblast somites; optical section. Both diverticula of the archenteron are drawn in. The mesoblastic somite boundaries of the left side are given in continuous lines; those of the right side are punctuated.

Fig. 55. The same stage, as seen from the right, to show the

foundation of the club-shaped gland and the diverticulum on the right side, which is still in connection with the archenteron.

Fig. 56. The same stage, as seen from the dorsal surface. The notochord is drawn in, and the swelling of the brain and the central canal are also indicated.

Fig. 57. The same stage, as seen from the ventral side.

Fig. 58. Some isolated muscle cells of the same stage, on a lateral view. On the left hand of the ruptured line the protoplasmic body is focussed; on the right the fibrilla, which are situated deep down.

Fig. 59. Notochord with adhering muscle cells, as seen from the dorsal surface. Isolated preparation. The vacuoles of the notochord are not drawn in.

Fig. 60. Stage with fourteen mesoblastic somites, as seen from the right. The dorsal and ventral series of small vacuoles is not represented in the figures drawn from preparations.

Fig. 61. Further stage, as seen from the right.

Fig. 61A. The same stage, from the living object.

Fig. 62. Anterior end of an embryo, with a still very small opening of mouth, and first gill-slit.

Fig. 63. Just such an embryo from the living object.

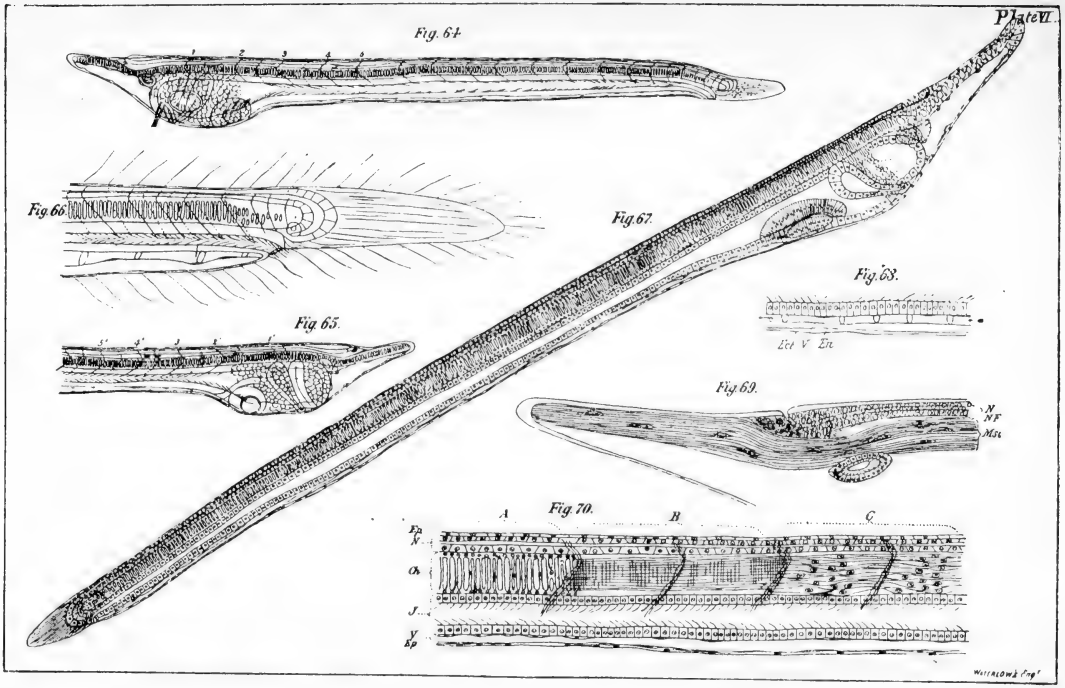
Fig. 63A. Anterior end of the same stage, from the living object.

PLATE VI.

Fig. 64. Larva with mouth and first gill-slit, as seen from the left side, drawn from the living object. The first, second, and third mesoblastic somite boundary of the opposite (right) side of the body is indicated by punctuated lines. The gill-slit situated on the right side is indicated, as seen through the body. Enlargement $\frac{65}{1}$.

Fig. 65. Just such a larva, anterior end, as seen from a right

Plate VI.



lateral view from the right side. Mouth and the first mesoblastic somite boundaries of the left side indicated with punctuated lines. Enlargement $\frac{65}{1}$.

Fig. 66. Posterior end of a larva, in which the second gill-slit has just perforated, from the living object. Enlargement $\frac{206}{1}$. The mesoblastic somite boundaries of the right side of the body indicated with punctuated lines. The pole-cell of the mesoblast still distinguishable. The ventral wall is, by reason of the pressure of the cover-slip, slightly removed from the ventral contractile blood vessel.

Fig. 67. Larva with mouth and first gill-slit, from an osmic-picro-carmin preparation. Enlargement $\frac{140}{1}$.

Fig. 68. Ventral region of such a larva from the living object. The epiblast (Ect.) has, owing to the pressure of the cover-slip, become removed from the ventral blood vessel adhering to the alimentary canal. Enlargement $\frac{272}{1}$.

Fig. 69. Anterior end of such a larva from a preparation. The lateral masses of fibrilla (N. F.) of the neural canal do not reach as far as its anterior end. Enlargement $\frac{206}{1}$.

Fig. 70. A section of such a larva from a preparation. In region A the notochord is represented in optical longitudinal section. In region B the microscope is directed to the muscle fibrilla, in region C to the protoplasmic bodies of the muscle cells. Enlargement $\frac{206}{1}$.

Plates VII., VIII., and IX. contain representations of sections. The enlargement is, with the exception of Figs. 111 and 151, universally the same ($\frac{206}{1}$). The drawings are as far as possible true to nature, though the carmin staining is represented by a darker colour, and the cell granules in the lithography are from reasons of economy kept engraved. Further, the drawing differs from the microscopic representation in that the cell boundaries and

the separation of the individual parts from one another appear black in the former, while in the latter they show clear and bright.

All the sections of a series are recognisable as belonging together, by abbreviated designation of the stage (*i.e.* the number of the mesoblastic somites of the latter), as well as by a series of letters. Where the representations of a stage are taken from two different series of sections, that is from different individuals (for instance, Plates IX., XI., US.), this may be seen from the index given with the letters.

PLATE VII.

Fig. 71. Transverse section from the middle of the body, from an embryo of the stage of Figs. 33, 34.

Fig. 72. Transverse section from the middle of the body from an embryo between the stage of Fig. 33 and that of 34.

Figs. 73–76. Transverse sections from an embryo with the first mesoblastic somite. Stage of Figs. 35, 36.

Fig. 73. Section immediately in front of the region of the mesoblastic somite.

Fig. 74. Section through the region of the mesoblastic somite.

Fig. 75. Section from the middle of the body.

Fig. 76. Section from the posterior third of the body.

Figs. 77, 78. Transverse sections of an embryo in which the second mesoblastic somite is in formation. Stage of Fig. 37.

Fig. 77. Section through the region of the first mesoblastic somite.

Fig. 78. Section from the posterior quarter of the body.

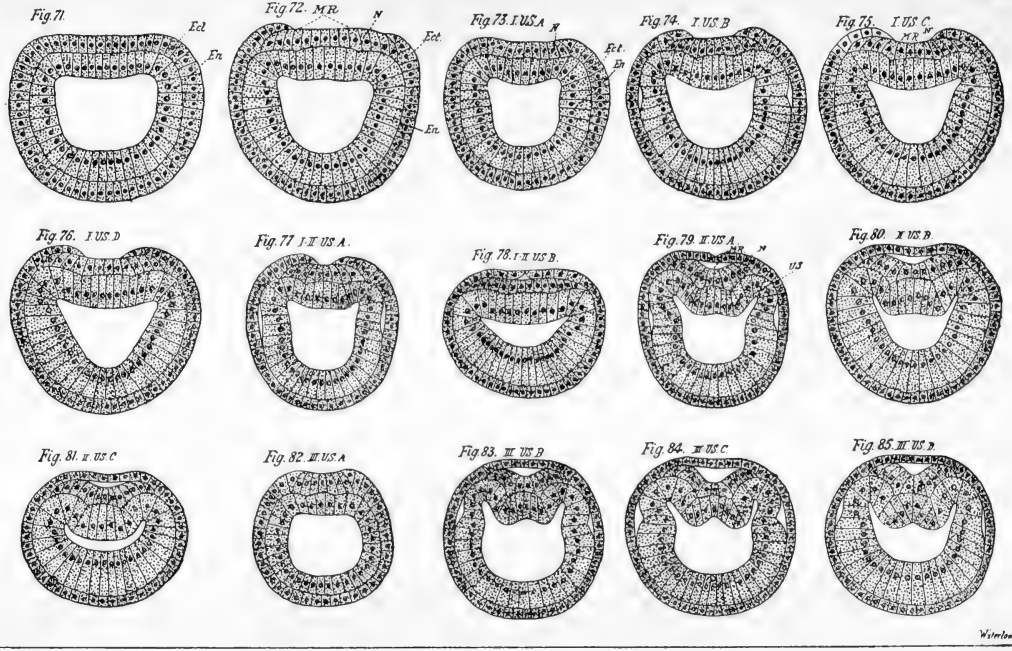
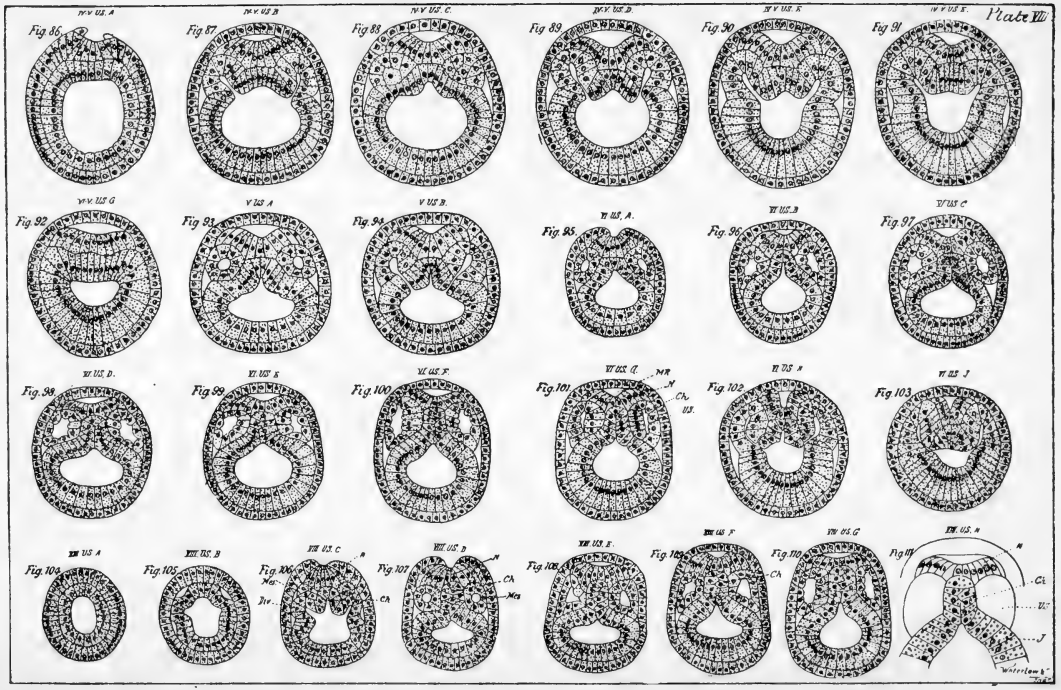


Fig. 71.
Ect.
En.
Fig. 72. MR.
N
Ect.
En.
Fig. 73. I. US. A
N
Ect.
En.
Fig. 74. I. US. B
Fig. 75. I. US. C
MR
N
Fig. 76. I. US. D
Fig. 77 I. II. US. A.
Fig. 78. I. II. US. B.
Fig. 79. II. US. A
N
US
Fig. 80. II. US. B.
Fig. 81. II. US. C
Fig. 82. III. US. A
Fig. 83. III. US. B
Fig. 84. III. US. C
Fig. 85. III. US. D.

Plate VII


IV V US. A
Fig 86.
IV V US. B
Fig 87.
IV V US. C.
Fig 88.
IV V US. D
Fig 89.
IV V US. E.
Fig 90.
IV V US. F
Fig 91.
IV V US. G
Fig 92.
V US. A
Fig 93.
V US. B.
Fig 94.
VI US. A.
Fig 95.
VI US. B
Fig 96.
VI US. C
Fig 97.
VI US. D.
Fig 98.
VI US. E
Fig 99.
VI US. F
Fig 100.
VI US. G.
Fig 101.
VI US. H
Fig 102.
VI US. J
Fig 103.
VII US. A
Fig 104.
VII US. B
Fig 105.
VII US. C
Fig 106.
VII US. D
Fig 107.
VII US. E.
Fig 108.
VII US. F
Fig 109.
VII US. G
Fig 110.
VII US. H
Fig 111.
Ch
Mx
Waterlow

Figs. 79–81. Transverse sections of an embryo with two well-formed mesoblastic somites. Stage of Figs. 42, 43.

Fig. 79. Section through the region of the first mesoblastic somite.

Fig. 80. Section through the region of the undifferentiated mesoblast fold, immediately behind the second mesoblastic somite.

Fig. 81. Section from the posterior third of the body.

Figs. 82–85. Transverse sections from an embryo with three mesoblastic somites. Stage of Fig. 44, 45.

Fig. 82. Section from the region immediately in front of the first mesoblastic somite.

Fig. 83. Section from the region of the first mesoblastic somite.

Fig. 84. Section from the region of the second mesoblastic somite.

Fig. 85. Section from the region behind the third mesoblastic somite.

PLATE VIII.

Figs. 86–92. Transverse sections from an embryo in which the fifth mesoblastic somite is seen in process of formation. Stage somewhat later than that of Figs. 46, 47.

Fig. 86. Transverse section immediately in front of the region of the first mesoblastic somite.

Fig. 87. Transverse section through the region of the first mesoblastic somite.

Fig. 88. Section through the boundary between first and second mesoblastic somite.

Fig. 89. Section through the region of the third mesoblastic somite.

Fig. 90. Section through the region of the fourth mesoblastic somite.

Fig. 91. Section taken somewhat obliquely, on the left passing through the fifth mesoblastic somite, which is in process of formation, on the right the undifferentiated mesoblast fold.

Fig. 92. Section from the posterior end of the body. (There follow two that are similar in the series of sections.)

Figs. 93, 94. Transverse sections from an embryo with five well-formed mesoblastic somites.

Fig. 93. Transverse section passing through the anterior part of the first mesoblastic somite.

Fig. 94. Transverse section passing through the posterior half of the first mesoblastic somite.

Figs. 95–103. Transverse sections from an embryo with six mesoblastic somites.

Fig. 95. Transverse section through the anterior opening of the neural canal. The continuations of the first mesoblastic somites have advanced to this point.

Fig. 96. Succeeding section through the region of the first mesoblastic somite. Notochord fold open.

Fig. 97. Succeeding section through the region of the first mesoblastic somite. Notochord slit closed.

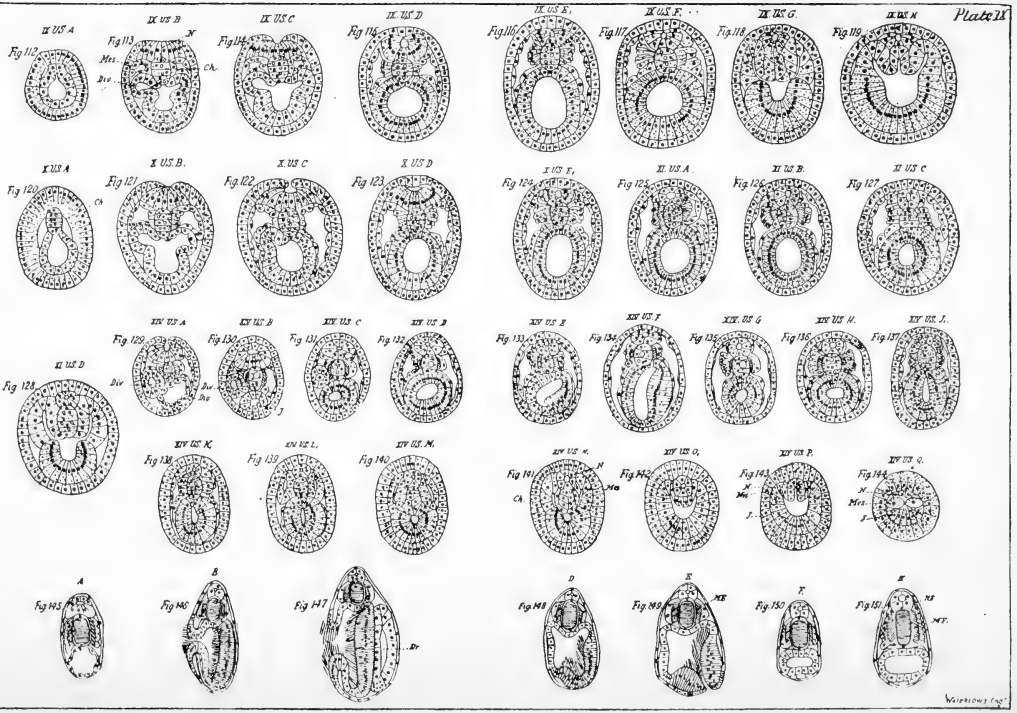
Fig. 98. Succeeding section from the region of the first mesoblastic somite.

Fig. 99. Section through the posterior end of the first mesoblastic somite; notochord slit having disappeared.

Fig. 100. Section from the region of the fourth mesoblastic somite.

Fig. 101. Section from the region of the fifth mesoblastic somite.

Plate IX



Waterlows Lop

Fig. 102. Section from the region of the sixth mesoblastic somite.

Fig. 103. Section from the region of the undifferentiated mesoblast fold.

Figs. 104–111. Transverse sections from an embryo with eight mesoblastic somites. Stage of Figures 48, 49.

Fig. 104. Transverse section from the most anterior end of the body.

Fig. 105. Succeeding transverse section.

Fig. 106. Succeeding transverse section through the anterior part of the neuropore.

Fig. 107. Succeeding section through the posterior part of the neuropore.

Fig. 108. Succeeding transverse section immediately behind the region of the neuropore.

Fig. 109. Transverse section through the posterior part of the first mesoblastic somite.

Fig. 110. Transverse section through the second mesoblastic somite.

Fig. 111. Part of a transverse section through the fourth mesoblastic somite, considerably enlarged. The notochord is indeed cut off, but still shows a flat ventral canal, which takes its part in the bordering of the cavity of the mesenteron.

PLATE IX.

Figs. 112–119. Transverse sections from embryos with 9 mesoblastic somites. Figs. 112–115 belong to one series of sections. Figs. 116–119 to another.

Fig. 112. Transverse section from the most anterior end of the body, notochord slit still open.

Fig. 113. Transverse section through the anterior end of the neural plate, notochord completely differentiated, but still continuing to border the cavity of the mesenteron.

Fig. 114. Transverse section somewhat further back through the anterior opening of the neural canal.

Fig. 115. Transverse section through the posterior part of the first mesoblastic somite.

Fig. 116. Transverse section from the middle of the body.

Fig. 117. Transverse section from the posterior third of the body It passes through on the left hand the cavity of a somite, on the right the boundary of a somite, and this is to be explained by the want of symmetry of these boundaries.

Fig. 118. Transverse section from the region of the ninth mesoblastic somite, whose cavity still communicates with that of the archenteron. The notochord is in this region not yet separated, the notochord slit being distinct.

Fig. 119. Transverse section through the region of the undifferentiated mesoblast folds. In the middle is the open notochord fold. If this section be compared with that from the corresponding differentiated region of an earlier stage, Fig. 92 and 103, the shortening of the development can be clearly seen. Compare also Fig. 141.

Figs. 120–124. Transverse sections from embryos with 10 mesoblastic somites.

Fig. 120. Transverse section through the most anterior end of the body. Notochord differentiated, but still bounding the cavity of the mesenteron.

Figs. 121 and 122. Succeeding sections through the neuropore.

Fig. 123. Transverse section from the posterior part of the first mesoblastic somite.

Fig. 124. Transverse section from a second series of sections, from the middle of the body.

Figs. 125–128. Transverse sections of an embryo with 11 mesoblastic somites.

Fig. 125. Transverse section from the middle of the body; on the right side the section passes through the mesoblastic somite boundary running obliquely, so that here the cavities of two successive stages are passed through.

Fig. 126. A succeeding transverse section.

Fig. 127. Transverse section through the last mesoblastic somite but one.

Fig. 128. Transverse section through the most posterior, *i.e.* eleventh, mesoblastic somite. Mesoblastic somite cavities in open communication with the cavity of the mesenteron.

Figs. 129-144. Transverse sections of two embryos of similar age, with 14 mesoblastic somites. Figs. 129–136 belong to one embryo, Figs. 137–144 to the other.

Fig. 129. Transverse section through the anterior opening of the neural canal. The two diverticula of the mesenteron are passed through by the section.

Fig. 130. Succeeding transverse section. The right diverticulum is lacking here, but the anterior end of the mesenteron (J) is passed through.

Fig. 131. Succeeding transverse section. The left diverticulum also is not more than touched.

Fig. 132. Transverse section from the region in which the first small opening of the mouth perforates later on.

Figs. 133 and 134. Transverse sections through the region in which the club-shaped gland is formed.

Fig. 135. Transverse section from the middle of the body.

Fig. 136. Transverse section, where on the left the oblique mesoblastic somite boundary is touched.

Fig. 137. Transverse section from the posterior quarter of the embryo, twelfth mesoblastic somite.

Fig. 138. Succeeding somite, on the right side the somite boundary is passed through.

Fig. 139. Transverse section through the thirteenth mesoblastic somite.

Fig. 140. Transverse section through the most posterior, *i.e.* fourteenth, mesoblastic somite. Notochord not fully separated.

Fig. 141. Transverse section through the undifferentiated mesoblast folds which are still in open communication with the lumen of the mesenteron.

Fig. 142. Transverse section immediately in front of the neurenteric canal.

Fig. 143. Transverse section passing through the opening of the neurenteric canal into the alimentary canal.

Fig. 144. Succeeding transverse section through the most posterior point of the body.

Figs. 145–151. Transverse sections through a larva with mouth and first gill-slit.

Fig. 145. Transverse section immediately behind the anterior opening of the neural canal.

Fig. 146. Transverse section through the anterior edge of the opening of the mouth.

Fig. 147. Transverse section through the posterior part of the opening of the mouth.

Fig. 148. Transverse section immediately in front of the first gill-slit.

Fig. 149. Transverse section through the gill-slit.

Fig. 150. Transverse section through the middle of the body.

Fig. 151. Transverse section through the same region more enlarged ($\frac{272}{1}$). N.F. nerve fibrilla, M.F. muscle fibrilla.

Signification of Letters in Plates I.–IX.

Ch Notochord.

dm Vitelline membrane.

Div, div Anterior diverticula of the alimentary canal.

dv.r right diverticulum of the alimentary canal.

dv.l left diverticulum of the alimentary canal.

Dr Club-shaped gland, or its foundation.

Em Embryo.

Ect Epiblast.

En Hypoblast.

En H Hypoblast cavity = Primitive alimentary canal cavity.

F H Segmentation cavity.

G M Gastrula-mouth.

J Alimentary canal.

K First gill-slit.

Mes Mesoblast.

M F Undifferentiated mesoblast fold.

M P Polar mesoblast cells.

M R Neural canal.

Msc Muscle fibres.

N Neural canal, neural plate.

N F Fibre strand of the alimentary canal.

O Mouth.

Oe Anterior opening of the neural canal.

pig Pigment spot.

R Polar body.

U S Mesoblastic somite.

I. US, II. US First mesoblastic somite, second mesoblastic somite.

V Vessel.

v Edge of the layer growing over the neural plate.

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