

Embryology. — *On the Development of the Tail in the Amphibian Embryo.* By Miss J. H. BIJTEL and Prof. M. W. WOERDEMAN.

(Communicated at the meeting of September 29, 1928).

The development of the hindmost part of the body has been frequently recorded in the literature. This is not surprising, if we bear in mind that this development is closely related to the gastrulation-process, one of the problems of embryology that have given rise to most controversy.

Of late years the views regarding the gastrulation of amphibian embryos have considerably improved by the application of new methods of investigation. It is above all W. VOGT¹⁾ who has added many important contributions to our knowledge of the process of gastrulation. He applied pieces of agar-agar, saturated with a solution of a vital dye to the surface of blastulae and so succeeded in putting small local marks on it. Because the cellgroups, which had attracted the dye, retained it for a long time, VOGT could watch the fate of circumscribed parts of the blastulae during and after the gastrulation. Coincidentally with a better conception of the gastrulation the opinions concerning the formation of the hindpart of the body changed. It is outside the scope of the present paper to dwell at large upon the literature relating to this subject. But for a clear understanding of what follows we consider it well to say something here about the hitherto almost universal conception, and about the opinion of W. VOGT.

According to the present views the different organs (nervous system, notochord, mesoderm-segments) existing in the tail, are supposed to originate from an indifferent cell-mass, the tail-bud, which is supposed to comprise elements of the three germ-layers, and to develop posteriorly from the region of the blastopore, where these three germ-layers are connected with each other. In this indifferent mass differentiation is believed to appear during the growth, so that nervous-system, notochord, myotomes, etc. become visible and thus this mass should add one segment after the other to the existing trunk.

The results of W. VOGT's researches, however, are different. He states this most explicitly, when he says on page 66 of his publication: "Chorda und Medullaranlage werden nach hinten in ihre Schwanz-abschnitte nicht durch Auswachsen verlängert, sondern durch Streckung, wobei das Hinterende dieser Anlagen nach Medullarschluss zunächst vom After gelöst und immer weiter nach hinten entfernt wird." Now BRACHET²⁾ a.o.

1) W. VOGT: Verhandl. Anatom. Gesellschaft. Ergzh. Anatom. Anz. 61, blz. 62, 1926.

2) A. BRACHET: Traité d'Embryologie. Paris, MASSON & Cie. 1921.

had maintained that at the end of the gastrulation strictly speaking only the head of the Amphibian embryo is present (acro- and cephalo-genesis have terminated), and the entire trunk and tail are still to be developed (notogenesis) by apposition from an indifferent mass (zône of growth). VOGT's observations led to the conception that at the end of the gastrulation trunk and tail are already present, but that they obtain their ultimate form through displacement of material and stretching ("Gestaltungsbe-
wegungen").

BRACHET's idea justifies the assumption that directly after the end of the gastrulation the part of the neural plate, lying right in front of the blastopore, corresponds with a place in the fully developed nervous system, which we may imagine to be just a little behind the N. vagus. According to VOGT the anlage of the whole nervous system then already exists on the neural plate, and the caudal end of the nervous system develops through stretching of that part of the plate which lies right in front of the blastopore.

As regards the formation of the mesoderm of the tail, VOGT imparts that just before the closure of the blastopore the invagination-process, characterizing the gastrulation in Amphibians, is still going on in the lateral and the ventral lip of the blastopore. The material which is invaginated then furnishes the somites of the posterior part of the trunk, growing out from the lateral lip, whereas proceeding from the ventral lip, mesoderm of the side-plate in the environment of the anus, and non-segmented mesoderm for the tail is formed. The somites of the tail, however, arise from those parts of the neural folds that encompass the blastopore. They are not used for the formation of the central nervous system. On their coalescence an ectodermisuture appears that will procure the dorsal as well as the ventral part of the caudal fin.

It appears from the above that in the formation of the tail VOGT assumes a stretching for notochord and neural tube, whereas the mesoderm is formed by adding new material to the already existing.

At the same time we may deduce from VOGT's other arguments that during the stretching of notochord and neural tube material is supplied from an indifferent mass, which does not take part in the formation of the caudal ectoderm, but it does in that of the neural tube, notochord, somites, and non-segmented mesoderm. This mass is used up during the formation of the tail and consequently cannot be considered as a "tail-bud", or a "centre of growth".

This indifferent material is provided by the lips of the blastopore through further invagination of the cell-groups that originally lay on the surface of the lips. The final result of his research is summarized by VOGT in the following words: "Damit scheint mir erwiesen, dass das hintere Körperende und speziell die Schwanzknospe nicht einem örtlichen Wachstumszentrum, sondern einer Gestaltungsbewegung ihre Entstehung verdankt; die Materialverschiebung, die sich dabei abspielt, ist die Fortsetzung der während der Gastrulation begonnenen Mesoderminvagination; longitu-

dinale Streckung und Konvergenz des Materiales nach der dorsalen Mitte sind die weiteren Hauptmomente, die der Gestaltung des Hinterendes zugrunde liegen und dieselbe als Fortsetzung der Gastrulationsbewegungen verstehen lassen."

By marking Amphibian embryos with vital dyes, one of us, Miss BIJTEL, has carried out an investigation into the prospective significance of the various parts of the neural plate, and has recorded it already in a short memoir ¹⁾. The caudal part of the neural plate has also received her attention, and she was thereby induced to consider the tail-problem. Her observations give a somewhat different picture of the tail-formation from that of VOGT. Before long they will, as is to be hoped, be published in detail. It will do to report here only a few general results of her research.

Whe, therefore, take a single example out of the many experiments on embryos of *Amblystoma mexicanum*, *Triton taeniatus*, as well as *Rana esculenta*. Now there are no essential differences between Urodela and Anura, as the marking experiments bore out, but there is only a difference in degree. Since there is no unanimity on this subject and BRACHET ²⁾ has tried, after the appearance of VOGT's study, to partly account for the difference in their opinions by the different circumstances viz. that VOGT worked with Urodela and he himself with Anura, it will be well to state here at once that, although we speak about a case relating to Urodela, in principle the same can be found with experiments on Anura.

In an embryo of *Amblystoma mexicanum* with a neural plate, that begins to close already, and with a slitlike blastopore three stained marks are put the one behind the other in the manner shown in Fig. 1a. The hindmost lies before and by the side of the blastopore, and overlaps the extremities of the neural folds; the other two marks lie more in front and also overlap the folds. The middle mark being of a different colour from the other two, the development of the areas of each of the marks can very well be watched. In Fig. 1b we see that the neural folds are moving towards each other, and will soon coalesce. In Fig. 1c we see the embryo after the coalescence of the folds is accomplished. (Side view of the embryo.) As the marks also stretched over part of the folds, the ectodermal suture, originated with their fusion, is also coloured. Now, in the living animal (Fig. 1d and 1e) it could be observed that the area of the anterior mark is considerably elongated. The middle mark has stained the ectoderm of the end of the tail and a small portion of the dorsal and the ventral margin of the tail-fin, while the hindmost mark can be seen again quite on the ventral margin of the tail. Fig. 1e shows that in the living animal also coloured somites are looming through the ectoderm and we can see that the hindmost somites have the colour of the central mark and the segments lying between the last-named and the unstained trunk-segments, have the colour

¹⁾ J. H. BIJTEL: Nederl. Tijdschr. v. Geneesk. 72. I. 26. 1928.

²⁾ A. BRACHET: Arch. f. Entw. mechan. 111. 1927.

of the other two marks. After the embryo had got a little older, it was prepared under an anaesthetic, i.e. after the ectoderm had been prepared away, also the myotomes were removed, in order to observe the staining of the neural tube and notochord. The results of this inquiry have been

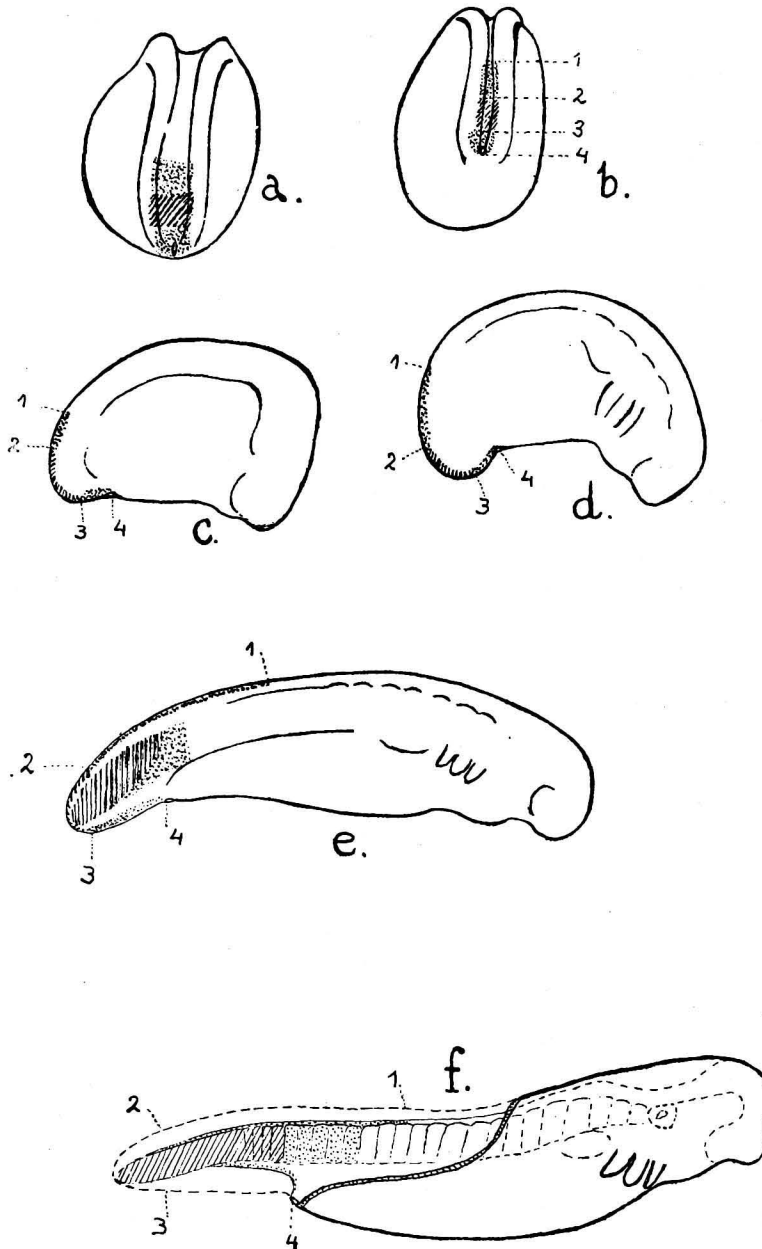


Fig. 1. Six serial developmental stages of an embryo of *Amblystoma mexicanum*; stage a provided with three stain-marks. In f part of the skin has been removed. (Zeiss. Object. $F=55$. Ocul. 2).

The figures 1, 2, 3 and 4 indicate the limits of the stain-marks.

brought together in Fig. 1f. First of all let us point to the very large extension of the ectoderm-area, which has arisen from the coalescence of the neural folds in the region of the anterior mark. Only at the tip of the tail has the ectoderm been formed from the central mark. This area also extends a little way over the dorsal margin of the tail, while it overlaps the ventral margin only for a very small distance. Almost the whole ventral margin has originated from the ectoderm of the posterior mark. From this it may be concluded that in the stage illustrated by Fig. 1a part of the ectoderm of the tail already exists on the neural folds, and is elongated during its further development. There is no question about a complement of this part of the ectoderm from an indifferent mass. Secondly it appears that the neural tube in the tail has entirely the colour of the anterior mark up to the very end. The caudal part of the spinal cord must, therefore, be formed from a part of the neural plate that is situated inside the area of the anterior mark. This observation, substantiated by numerous other observations, leads to the conception that after the end of the gastrulation the whole of the central nervous system is already projected on the neural plate. This, indeed, has also been communicated by Miss BIJTEL¹⁾.

The conception of BRACHET, who assumes that after the gastrulation notogenesis is still to take place, is no longer tenable now. The caudal part of the neural tube originates through intense stretching of the part of the neural plate (Fig. 1a), lying in the anterior mark. Later on nothing is added to this material, as the whole tailpart of the neural tube has in Fig. 1e the colour of the anterior mark. But it also appears from it that the caudal extremity of the neural tube must be situated inside the anterior mark, and that, therefore, the part of the neural plate, occupied by the central and the posterior mark does not partake in the formation of the central nervous system. What, then, has become of the last-named regions?

It is certain that in stages such as are illustrated in Fig. 1a the invagination of material in the neighbourhood of the blastopore has not yet terminated. So, in the case described, the area of the posterior stained spot is still rolled inwards totally, or perhaps for the greater part, with the exception, of course, of that part of the mark that lay on the continuation of the neural folds, and that after the closure of the neural tube was to be found in the ectoderm-suture, from which part of the skin-ectoderm of the tail arose.

From the invaginated material have been formed the caudal part of the intestine, and some somites, viz. first the 16th somite of the trunk. It goes without saying, that this border is a matter of chance and depends on the extension of the central-mark. But the most caudal mesoderm segments show the stain of the central mark. They must have originated from the area of the neural plate which is indicated by stripes in Fig. 1a (central mark). It has already been stated that the caudal end of the neural tube

¹⁾ J. H. BIJTEL: *Nederl. Tijdschr. v. Geneesk.* 72. I. 26. 1928.

is not situated near the blastopore, but a little more cranial to it. The part of the neural plate, occupied by the two posterior marks is, therefore, only in appearance a part of the neural plate. If, then, by the term neural plate is meant exclusively the anlage of the central nervous system (which would be quite reasonable) it should be borne in mind that the neural plate ends some way cranial to the blastopore, and that the neural folds also end there, although it would seem as if the folds are continued on either side of the blastopore.

In what way the posterior tail-myotomes have been developed could be fully ascertained in several cases. Change of situation and extension of stain marks, and comparison of conditions afterwards revealed in the experimental objects, enabled us to watch the fate of different cell-groups in the formation of the tail. It then appeared, that, a small part excepted, (in Fig. 1a the area of the posterior mark) the cell-material belonging apparently to the neural plate, which lies before the blastopore, (in Fig. 1a the area of the central mark), is not rolled inward round the rim of the blastopore, but, after the coalescence of the folds that encompass it, is found again in the interior of the tail-anlage, and adds new somites to those that have originated from the invaginated mesoderm-material.

In the investigation of the Axolotl-embryo, just discussed, the notochord appeared to be unstained, and to lie among the stained somites, as an unstained cell-strand. This can be understood only when assuming that the notochord did not originate from the same material as the somites of the tail; in other words that neither the material of the posterior mark, which is rolled inward, nor the area of the second mark has contained cellular material for the notochord in the tail. Then, probably, the notochord in the stage of Fig. 1a, as well as the tail part of the neural tube is already present in its entirety and proceeds (when the tail is formed) in the tail-anlage through stretching. From the fact that notochord, neural tube, and the greater part of the somites were of a different stain in the tail, it follows avowedly that they cannot have originated from one and the same indifferent cell-mass (the tail-bud).

It remains for us to say that by means of vital-dye marking in *Rana esculenta* the same development of the tail could be demonstrated as in *Urodela*, of which we discussed, for the sake of brevity only the *Amblystoma*, but of which *Triton* also yielded results in harmony with what has been said above. It only became evident, that in *Rana esculenta* more somites arose from the material round the blastopore than in *Amblystoma*, while the more cranial part of the neural plate, which no longer partakes of the invagination-movements, contributes less to the formation of the tail-mesoderm.

As Miss BIJTEL will soon discuss more at large all sorts of questions bearing on those treated briefly in this paper (conception of gastrulation, *canalis neurentericus* etc.) we shall no longer expatiate on the stainmarking experiments.

In 1927 WOERDEMAN had performed some experiments on *Amblystoma*-embryos, which also throw some light on the development of the tail, although they were carried out with a different object. But not before the results of the stain-marking experiments became known, could these experiments be fully explained. They corroborated those results. Hence we decided to report our results jointly.

Of *Amblystoma* embryos with clearly visible neural plate, as illustrated in Fig. 2, a large part of the neural plate was excised (outlined in Fig. 2).

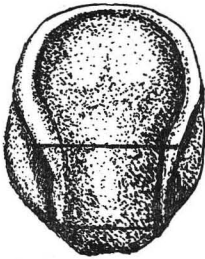


Fig. 2. Embryo of *Amblystoma mexicanum*. The outlined area was excised, rotated through 180° and then implanted again.

The incision was so deep that the gut-cavity was opened, and the material lying under the neural plate was also cut out. The whole rectangular, excised piece was then rotated through 180° (so that the caudal line of incision became cranial, and the cranial line caudal). After that it was grafted in the open wound, and made to coalesce again as well as possible in the rotated position. The caudal line of incision remained at some distance before the blastopore, and, as projected in Fig. 1a, will as a rule have lain in the area of the central mark, now slightly anteriorly, now again slightly posteriorly. The position of the anterior line is of less importance.

A number of animals operated upon now developed in a very peculiar manner. In Fig. 3 four of such animals have been represented.

They are all characterized by a rudimentary development of the tail, while the rotated part has also developed a tail, but this one grows cranially. Other particulars are observable, but they may be left out of consideration. On closer inspection it appears that the true tail of the animals consists chiefly of skin-ectoderm, some mesenchyme, and in a few cases also a little mass of axial mesoderm. In all these cases microscopical examination of the slice-series revealed that behind the posterior border of the grafted piece the neural tube and the notochord are lacking, but that myotomes occur. Their number differs. In the tail, however, that grows cranially from the rotated piece the normal structure can be observed in all cases up to the very end. There a neural tube, a notochord and axial mesoderm has developed. This is illustrated in Fig. 4. Fig. 4a is a section of the animal represented in Fig. 3a at the place marked by arrow 1, while Fig. 4b represents a section at the place marked by arrow 2. In Fig. 4a the anteriorly directed tail has been cut; (also the nervous system has just been cut, which has developed in the head from the anterior part of the neural plate). In this anlage of the tail we clearly see neural tube, notochord, and myotomes. But in the tail proper (Fig. 4b) only small myotomes and some mesenchyme are to be seen, but no neural tube and no notochord.

The animal represented in Fig. 3b has in the tail proper numerous myotomes, but no notochord, and no neural tube, as is shown in Fig. 5

which represents a section at the place, indicated by the arrow in Fig. 3b. As the parting organs between left and right myotomes (neural tube and

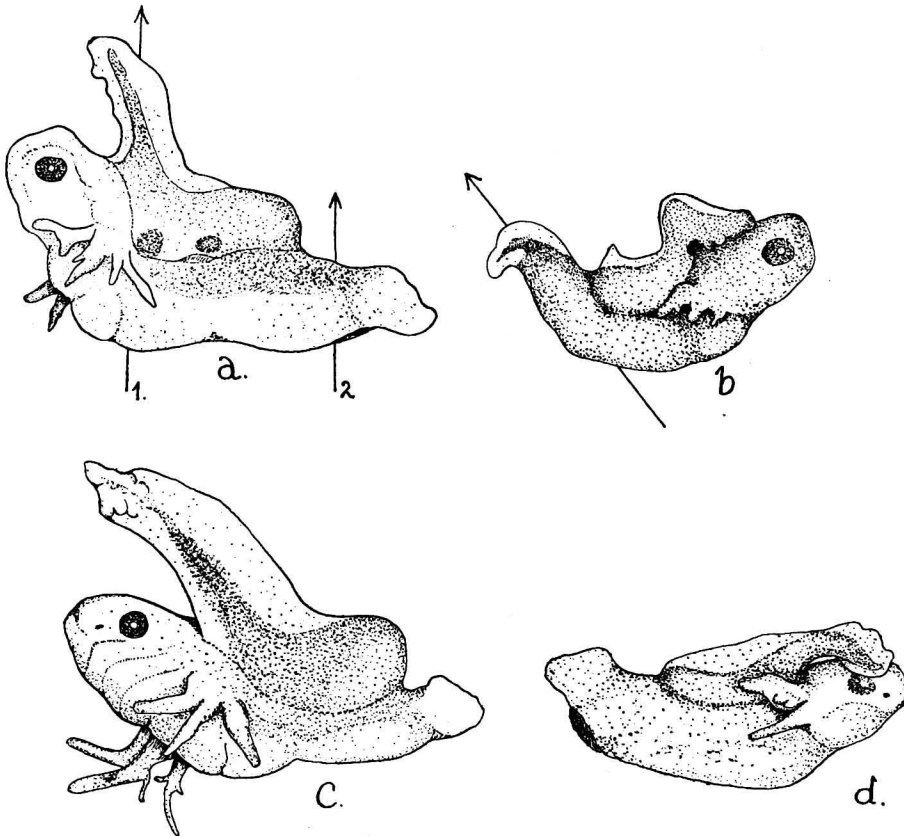


Fig. 3. Four embryos of *Amblystoma mexicanum*, Eleven days after operation according to Fig. 2. (Zeiss. Object $F=55$, Oc. 2. $\times 3/4$).

notochord) were wanting, in a number of places the muscle-fibres, which have been formed from the myotomes, have grown across the median line.

In explaining the result of the experiments it should be remembered that the caudal line of incision lay during the operation some way before the blastopore. Behind this line no neural tube nor a notochord has developed. The anlage of the two organs must, therefore, terminate caudally at some distance before this caudal line of incision.

This corresponds with the experience in the staining experiments: that the anlage of the central nervous system does not reach as far as the blastopore (in Fig. 1a the presumptive posterior end lies in the anterior mark). The material lying between the presumptive end of the anlage of the neural tube and the blastopore does not take part in the formation of the notochord of the tail, and the caudal part of the neural tube, otherwise the experimental animals, in which this material was not displaced, should

The capacity of stretching intensely appears distinctly in these *Amblystoma*-embryos, for the notochord and the neural plate extremities, rotated through 180° , are growing far beyond the anterior border of the rotated graft towards the head, and thus develop a tail in opposite direction.

Also the relations shown by the myotomes in the true tail, and in the one that grows anteriorly, are conceivable. If we assume that in a stage, such as is represented in Fig. 1a, a part of the neural plate was rotated, and the posterior incision went through the middle of the central mark, then after the rotation one part of this mark will be directed anteriorly, and another part will remain behind the incision. This latter part contributes together with the material lying round the blastopore to the formation of mesoderm for the true anlage of the tail. But notochord and neural tube cannot be formed from it. The part of the central mark placed anteriorly after the operation also supplies mesoderm for a tail, which, owing to the capacity of stretching of notochord and neural tube anlage, grows anteriorly.

Now, if we assume that in the embryo, pictured in Fig. 1a, the posterior border of the piece to be rotated, is lying close to the posterior mark, so that nearly the whole of the central mark is rotated along with it, then a condition will arise as in Fig. 3c, in which behind the posterior border of the rotated graft a very small piece of the trunk is to be seen, and a very rudimentary tail. But the tail directed anteriorly is well developed.

If, on the contrary, the posterior border of the graft should lie closer to the anterior mark, the central mark would for the greater part keep its own place, and the larva would receive a smaller quantum of mesoderm in its anteriorly directed tail, and a larger one in its true tail (Fig. 3a and 3b).

In all experiments in which parts of the dorsal half of the embryo were rotated, the blastopore and a rather large area before it, kept its own place. Part of the material, which lies between the posterior extremity of the neural anlage and the blastopore, and which according to the stainmarking experiments supplies tail-mesoderm, takes up an anterior position after rotation of the graft through 180° , and now forms mesoderm for the tail that grows in a cranial direction. This proves at the same time that it is not necessary for this presumptive mesoderm to be carried in with the invagination in the process of gastrulation. It should be remembered that the staining-experiments also led to the opinion that part of this presumptive mesoderm in case of normal development was not invaginated.

Finally let it be pointed out that the true tail, as well as the rotated one, exhibits a more or less distinct tail-fin. The skin for this fin has originated with the coalescence of the neural folds. Also the tail proper has this fin-formation, for at the operation the caudal continuations of the neural folds have retained their place.

Thus it is evident, that the rotation-experiments are in perfect harmony with the results of the staining experiments and in large measure lend support to the conceptions based on the staining experiments.

Summary.

1. The conception that the tail arises from an indifferent cell-mass (tailbud) which, while growing caudally, gives origin to the different organs of the tail through differentiation, is not in keeping with our experience.

2. The notochord of the tail and the caudal portion of the neural tube are totally present in stages with slitlike blastopore, and without tail-bud, but take their final shape through intense stretching of their anlage. In this process, however, no new material is assimilated.

3. The posterior part of the anlage of the notochord and the anlage of the central nervous system lies at some distance in front of the blastopore, so that between the blastopore and the posterior extremity of the neural anlage (which is not visible outwardly), an area is left, of which the material appears to provide mesoderm for the tail.

4. To this end the material lying in the environment of the slitlike blastopore is first carried inward round the rims of the blastopore (continuation of the cell-displacements in gastrulation). It contributes to the formation of the hindmost trunk-segments and somites of the tail-root, together with non-segmented mesoderm of the same region.

5. The presumptive mesoderm that lies more cranial from the blastopore is not concerned in this invagination. It provides the posterior somites of the tail.

6. Cell-groups that lie ventrally and laterally to the blastopore (in stages with slitlike blastopore) and which are carried inward, contain the anlage of the tail gut.

7. The ectoderm for the skin of the tail lies in stages with slitlike blastopore partly quite ready on the neural folds and on their caudal continuation on each side of the blastopore. Part of the skin of the tail is supplied through stretching of this ectoderm of the folds.

8. The folds just alluded to also supply mesenchyme for the tail.

*From the Anatomical and Embryological Institute
of the State-University of Groningen.*
