Mit besten Grüßen.
H. S.

Über Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren

Von

H. Spemann und **Hilde Mangold**

Mit 25 Textabbildungen

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Facsimile reproduction of the cover of an original reprint of the 1924 article by Hans Spemann and Hilde Mangold, with a handwritten dedication by H. Spemann which reads "With best regards, H.S." (Courtesy of K. Sander, Freiburg).

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Induction of Embryonic Primordia by Implantation of Organizers from a Different Species

by

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Freiburg i.B.

With 25 illustrations

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I. Introduction

In a *Triton* embryo, at the beginning of gastrulation, the different areas are not equivalent with respect to their determination. It is possible to exchange by transplantation parts of the ectoderm at some distance above the blastopore that in the course of further development would have become neural plate and parts that would have become epidermis, without disturbing normal development by this operation. This is feasible not only between embryos of the same age and of the same species but also between embryos of somewhat different age and even between embryos of different species (Spemann 1918, 1921). For instance, presumptive epidermis of *Triton cristatus* transplanted into the forebrain region of *Triton taeniatus* can become brain; and presumptive brain of *Triton taeniatus* transplanted into the epidermal region of *Triton cristatus* can become epidermis. Both pieces develop according to their new position; however they have the species characteristics with which they are endowed according to their origin. O. Mangold (1922, 1923) has extended these findings and has shown that prospective epidermis can furnish not only neural plate but even organs of mesodermal origin, such as somites and pronephric tubules. It follows from these experimental facts, on the one hand, that the exchangeable pieces are still relatively indifferent with respect to their future fate; and, on the other hand, that influences of some sort must prevail in the different regions of the embryo that determine the later fate of those pieces that are at first indifferent.

Notes added by the IJDB Editorial Office: 1. The serial number of each experiment, e.g. Um 25, refers to two embryos (a and b), between which transplants were exchanged. Thus "a" usually refers to the donor *cristatus* embryo while "b" typically represents the host *taeniatus* embryo. 2. It is worthwhile noting that all figures in this paper were hand-drawn by Hilde Mangold. The drawings of histological sections are based on photographic paper prints. On these, each nucleus and cell border was traced with Indian ink. Thereafter, the silver halogenide grains were removed chemically, after which the drawing stood out on the white background. This method was described in Spemann (1918, p. 545).

[*Abbreviations used in this paper: Bl*, blastopore; *Oc*, optic vesicles; *pc*, pericardium; *pr. Med*, primary neural tube; *sec. Ch*, secondary notochord; *sec. D*, secondary intestine; *sec. Lab*, secondary otocyst; *sec. Med*, secondary neural tube; *sec. Mes*, secondary mesoderm; *sec. Pron*, secondary pronephric duct; *sec. Uw*, secondary somite; *Um X*, Urmund (meaning "primitive mouth" or blastopore) followed by the serial number "X" of the experiment.]

A piece from the upper lip of the blastopore behaves quite differently. If it is transplanted into the region that would later become epidermis, it develops according to its origin; in this region, a small secondary embryonic primordium develops, with neural tube, notochord and somites (Spemann 1918). Such a piece therefore resists the determining influences that impinge on it from its new environment, influences that, for instance, would readily make epidermis out of a piece of presumptive neural plate. Therefore, it must already carry within itself the direction of its development; it must be determined. Lewis (1907) had already found this for a somewhat later developmental stage, when he implanted a small piece from the upper and lateral blastopore lip under the epidermis of a somewhat older embryo and saw it develop there into neural tissue and somites.

It suggested itself from the beginning that effects might emanate from these already determined parts of the embryo that would determine the fate of the still indifferent parts. This could be proved by cutting the embryo in half and shifting the halves with respect to each other; in this case, the determined part proved to be decisive for the direction that subsequent development would take. For instance, the animal half of the gastrula was rotated 90° or 180° with respect to the vegetal half; determination then spread from the lower vegetal piece, that contained just the upper lip, to the upper animal piece. Or two gastrula halves of the same side, for instance two right ones, were fused together. As a result, the half blastoporal lips completed themselves from adjacent material of the fused other half, and in this way, whole neural plates were formed (Spemann 1918).

Thus, the concept of the *organization center* emerged; that is, of a region of the embryo that has preceded the other parts in determination and thereupon emanates determination effects of a certain quantity in certain directions. The experiments to be presented here are the beginning of the analysis of the organization center.

Such a more deeply penetrating analysis presupposes the possibility of subdividing the organization center into separate parts and of testing their organizing capacities in an indifferent region of the embryo. This experiment has already been performed, and it was precisely this experiment that gave the first indication that the parts of the embryo are not equivalent at the beginning of gastrulation (1918). However, this intraspecific, homoplastic transplantation did not make it possible to ascertain how the secondary embryonic anlage that originated at the site of the transplant was constructed, that is, which part of it was derived from the material of the implant and which part had been induced by the implant from the material of the host embryo. The identification of these two components is made possible by heteroplastic transplantation, as for instance by implantation of organizers from *Triton cristatus* into indifferent material of *Triton taeniatus*.

This experiment, that followed logically from its presuppositions, was performed during the summers of 1921 and 1922 by Hilde Mangold née Pröscholdt. It gave at once the expected result that has already been reported briefly (Spemann 1921, pp. 551 and 568). In the following, we shall present the basic fact in more detail.

II. Experimental Analysis

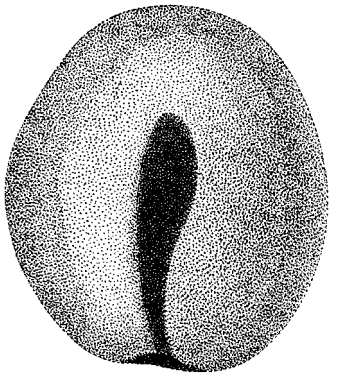
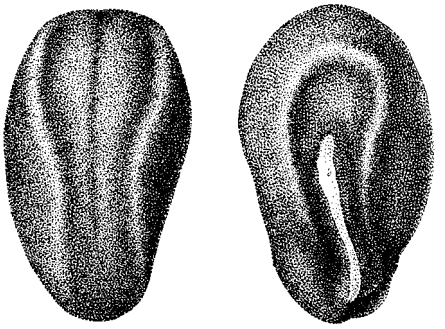


Fig. 1. Um 8 crist. *The* cristatus *embryo at the neurula stage. The* taeniatus *transplant is dark and elongated; it is located in the presumptive neural plate. 20X.*

Nothing new need be said concerning the experimental technique; it was the same as in previous experiments (Spemann, 1920).

Of the species of *Triton* available, *taeniatus* can best tolerate the absence of the egg membrane, from early developmental stages on and it is the easiest to rear. Hence the organizer that was to be tested for its capacities was always taken from a *cristatus* embryo and usually implanted into the presumptive epidermis of a *taeniatus* embryo. The place of excision was marked by implantation of the piece removed from the taeniaius embryo; that is, the pieces were exchanged.

Experiment Triton 1921, Um 8b. The exchange was made between a *cristatus* embryo with distinctly U-shaped blastopore and a *taeniatus* embryo of the same stage. A small circular piece at some distance above the blastopore was removed from the



Figs. 2 (left) and 3 (right). Um 8b. *The* taeniatus *embryo at the neurula stage, with primary and secondary neural plate; the elongated white* cristatus *implant is in the median plane of the latter. 20X.*

cristatus embryo and replaced by a piece of presumptive epidermis of the *taeniatus* embryo. This *taeniatus* implant was found, later on, as a marker in the neural plate of the *cristatus* neurula, between the right neural fold and the midline, and it extended to the blastopore, slightly tapering toward the posterior end (Fig. 1). One could not see in the living embryo whether it continued into the interior, and the sections, which are poor in this region, did not show this either.

The *cristatus* explant (the "organizer") was inserted on the right side of the *taeniatus* embryo, approximately between the blastopore and the animal pole. It was found in the neurula stage to the right and ventrally, and drawn out in the shape of a narrow strip (Fig. 2). ln its vicinity, at first a slight protrusion was observable; a few hours later, neural folds appeared, indicating the contour of a future neural plate. The implant was still distinctly recognizable in the midline of this plate; it extended forward from the blastopore as a long narrow strip, slightly curved, over about two-thirds of the plate (Fig. 3).

This secondary neural plate, that developed in combination with the implanted piece, lagged only a little behind the primary plate in its development. When the folds of the primary plate were partly closed, those of the secondary plate also came together. Approximately a day later, both neural tubes were closed. The secondary tube begins, together with the primary tube, at the normal blastopore and extends to the right of the primary tube, rostrad, to approximately the level where the optic vesicles of the latter would form. It is poorly developed at its posterior part, yet well enough that the *cristatus* implant was invisible from the outside. The embryo was fixed at this stage and sectioned as nearly perpendicularly to the axial organs as possible. The sections disclosed the following:

The neural tube of the primary embryonic anlage is closed through the greater part of its length and detached from the epidermis, except at the anterior end where it is still continuous with it, and where its lumen opens to the exterior through a neuropore. The lateral walls are considerably thickened in front; this is perhaps the first indication of the future primary eye vesicles. The notochord is likewise completely detached, except at its posterior end where it is continuous with the unstructured cell mass of the tail blastema. In the mesoderm, four to five somites are separated from the lateral plates, as far as one can judge from cross sections of such an early stage.

Only the anterior part of the neural tube of the secondary embryonic anlage is closed and detached from the epidermis. Here it is well developed; in fact, it is developed almost as far as the primary tube at its largest cross-section: its walls are thick and its lumen is drawn out sideways (Fig. 4). Perhaps we can see here the first indication of optic vesicles. The central canal approaches the surface at its posterior end and eventually opens to the outside. Then the neural plate rapidly tapers off; its hindmost portion is only a narrow ectodermal thickening (Figs. 5 and 6).

Although the overwhelming mass of this secondary neural tube is formed by cells of the *taeniatus* host that can be recognized by the finely dispersed pigment, a long, narrow strip of completely unpigmented

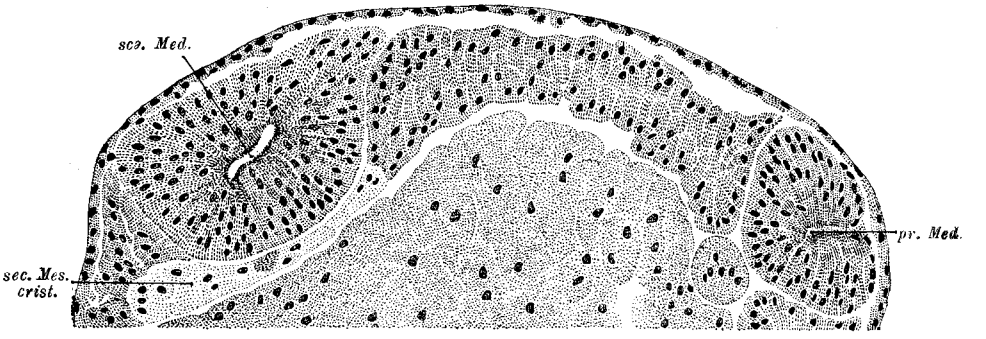


Fig. 4. Um 8b. *Cross section through the anterior third of the embryo (cf. Figs. 2 and 3) pr. Med., primary neural tube; sec. Med., secondary neural tube. The implant (light) is in the mesoderm (sec. Mes. crist.). 100X.*

cells is intercalated in its floor, in sharp contrast to the adjacent regions. This white strip is part of the *cristatus* implant that was clearly recognizable from the outside in the living embryo before the neural folds closed (Fig. 3). The anterior end of this strip is approximately at the point where the thickness of the neural tube decreases rather abruptly; it opens to the outside shortly thereafter. The strip is wedge-shaped, with the pointed edge toward the outside; as a result, only the tapering ends of the cells reach the surface of the embryo (Figs. 5 and 6) or the central canal at the short stretch where they border it.

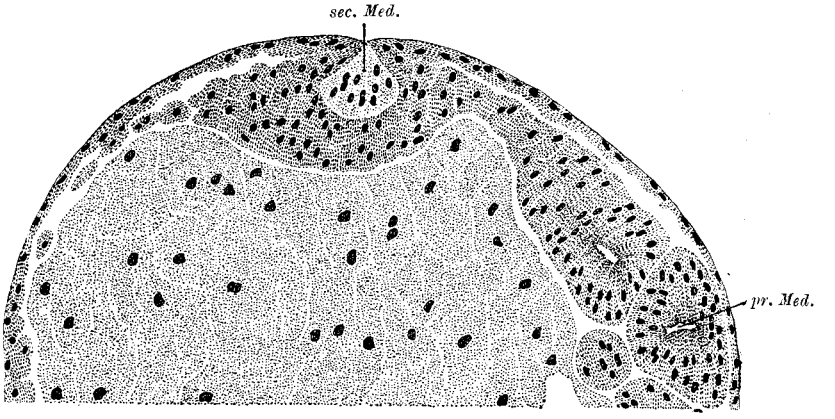


Fig. 5. Um 8b. *Cross section through middle third of the embryo (cf. Figs. 2 and 3). pr. Med., primary neural tube; sec. Med., secondary neural tube. The implant (light) is in the secondary neural tube.*

At its posterior end, the *cristatus* strip reaches the blastopore, and it is continuous with a mass of *cristatus* cells that is located between the secondary neural tube and the mesoderm on one side, and the endoderm on the other (Fig. 6). Because of their position one would be inclined to consider these cells as endoderm; but in size they resemble more the mesoderm of the *taeniatus* embryo, with which they are associated. At any rate, this cell mass, which extends a bit farther rostrad, has reached its position by invagination around the blastoporal lip. There is yet another mass of *cristatus* cells still farther rostrad. It has the form of a thin plate underlying the anterior part of the induced neural tube, as far as it is closed; at its anterior end and at its sides, it coincides approximately with the edge of the tube, and at its posterior end, it extends to the ectodermal strip of the implant. This plate is incorporated in the normal *taeniatus* mesoderm (Fig. 4). It is not differentiated further into notochord or somites.

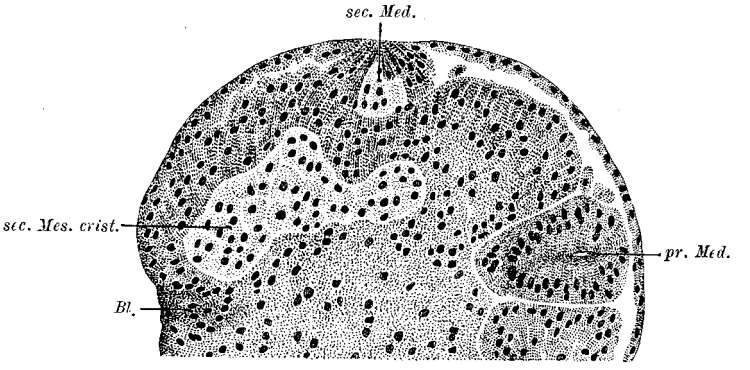


Fig. 6. Um 8b. *Cross section in the region of the blastopore (Bl.) (cf. Figs. 2 and 3). pr. Med., primary neural tube; sec. Med., secondary neural tube. The implant (light) has several cells in the secondary neural tube, with its main mass in the mesoderm (sec. Mes. crist.). 100X.*

Altogether, a rather substantial part of the implant remained in the ectoderm. This portion was greatly stretched in length; as a result, the circular white disk that was implanted has become a long narrow strip that turns inwards around the blastoporal lip. Shifting of cells in the surrounding epidermis may have played a role in these form changes; the extent to which this occurs would have to be tested by implantation of a marker of indifferent material. A piece from a region near the upper lip of the blastopore could handily be considered as suitable for this purpose. We know from earlier experiments (Spemann 1918, 1921) that convergence and stretching of the cell material occurs at the posterior part of the neural plate. It is improbable that the cells of the neural plate are entirely passive in this process; rather, they may have an inherent tendency to shift that perhaps has been, together with other characteristics, induced by the underlying endo-mesoderm. This tendency would be retained by

the piece in the foreign environment. In this way we might also explain the fact that the piece gains contact with the invaginating region of the normal blastoporal lip, although it was originally far distant from it. Once it has arrived there by active stretching, it could be carried along, at least in part, by local cell shiftings.

Whereas this posterior cell mass is continuous with the cell strip that has remained on the surface, it is separated from the more anterior *cristatus* cell plate by *taeniatus* mesoderm. Therefore, this anterior plate that underlies the neural tube cannot have arrived at its position by invagination around the upper blastoporal lip; it must have been located in the deeper position from the beginning. Undoubtedly it derives from the inner layer of the implant; hence it was originally just under the *cristatus* cells, some of which are now formed partly in the neural plate as a narrow strip, and others of which had migrated inside around the blastoporal lip. These displacements carried it along and brought it forward to such an extent that now its posterior margin is approximately level with the anterior end of the *cristatus* cell strip in the neural tube.

Although a piece of presumptive neural plate taken from a region a little anterior to the actual transplant would have become epidermis after transplantation to presumptive epidermis, this implant has resisted the determinative influences of the surroundings and has developed essentially according to its place of origin. Its ectodermal part has become part of the neural plate and the endo-mesodermal part has placed itself beneath it.

Furthermore, not only did the implant assert itself, but it made the indifferent surroundings subservient to it and it has supplemented itself from these surroundings. The host embryo has developed a second neural plate out of its own material, that is continuous with the small strip of *cristatus* cells and underlain by two cell plates of *cristatus* origin. This secondary plate would not have arisen at all without the implant, hence it must have been caused, or induced, by it.

There seems to be no possible doubt about this. However, the question remains open as to the way in which the induction has taken place. In the present case it seems to be particularly plausible to assume a direct influence on the part of the transplant. But even under this assumption, there are still two possibilities open. The ectodermal component of the transplant could have self-differentiated into the strip of neural plate, and could have caused the differentiation of ectoderm anterior and lateral to it progressively to form neural tissue. Or the determination could have emanated from the subjacent parts of the endo-mesoderm and have influenced both the *cristatus* and *taeniatus* components of the overlying ectoderm in the same way. And finally, it is conceivable that the subjacent layer is necessary only for the first determination, which thereafter can spread in the ectoderm alone. A decision between these possibilities could be made if it were possible to transplant successfully pure ectoderm, and pure endo-mesoderm from the region of the upper lip of the blastopore, and, finally, such ectoderm which had been underlain by the endo-mesoderm. In such experiments, heteroplastic transplantation offers again the inestimable advantage that one can establish afterwards with absolute certainty whether the intended isolation was successful.

In our case, such a separation of the factors under consideration has not been accomplished. Nevertheless it seems noteworthy that the induced neural plate is poorly developed in its posterior part where it is in closest and most extensive contact with the ectodermal part of the transplant; and, in contrast, that it is well developed at its anterior end where it is remote from the *cristatus* cell strip, but underlain by the broad *cristatus* cell plate.

We shall discuss later a second possibility of a fundamentally different nature that is particularly applicable to more completely formed secondary embryonic primordia.

A second experiment, similar to the first, confirms it in all essential points. They both have in common that the implant remains estodermal to a considerable extent, and therefore later forms part of the neural tube. The situation is different in the following experiment.

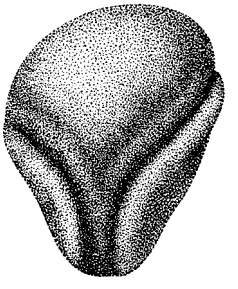


Fig. 7. Um 25b. *The* taeniatus *embryo at the neurula stage. On the right is the primary and on the left the secondary neural tube.* 20X.

Experiment Triton 1922, Um 25b. A median piece of the upper blastoporal lip was taken from a *cristatus* embryo at the beginning of gastrulation (sickle-shaped blastopore). It came from directly above the margin of invagination and was implanted into a *taeniatus* gastrula of the same stage in the ventral midline at some distance from the future blastopore. Twenty-two hours later, when the *taeniatus* embryo had completed

its gastrulation, the implant had disappeared from the surface, which looked completely smooth and normal. Another 24 hours later, the embryo had two neural plates whose folds were about to close. The secondary neural plate starts from the same blastopore as the primary one; at first it runs parallel to the primary plate, adjacent to its left side, and then it bends sharply to the left (Fig. 7). Shortly thereafter, the embryo was fixed; the sections were cut perpendicular to the posterior part of the axial organs.

The primary neural tube is completely closed and separated from the epidermis; its optic vesicles are protruding. The notochord is separate down to its posterior end which becomes lost in the indifferent zone. Seven or eight somites are formed.

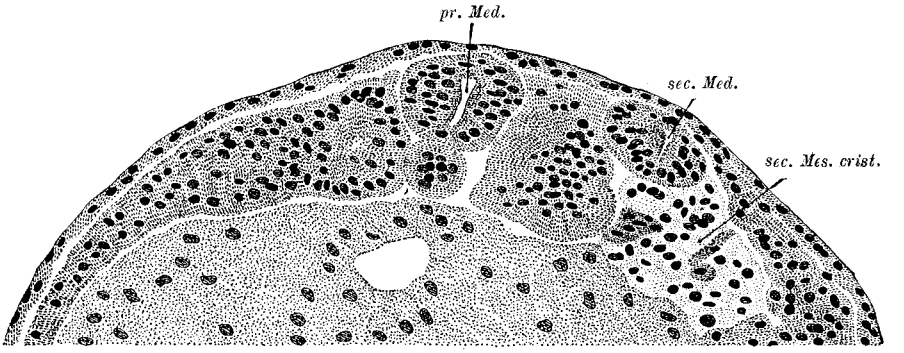


Fig. 8. Um 25b. *Cross section in the middle third of the embryo (cf. Fig. 7). In the figure the secondary neural tube is seen to the right of the primary tube. The implant (light) is in the right primary mesoderm (sec. Mes. crist.). 100X.*

The secondary neural tube is also closed and separated from the epidermis; anteriorly its walls are broad and its lumen is transverse (probably an indication of optic vesicles). It decreases in thickness posteriorly. In its anterior one-third, it is bent sharply to the left and is therefore at some distance from the primary neural tube: but more posteriorly, at its posterior two-thirds, it approaches the latter and eventually fuses with it. However, the lumina, as far as they are present, remain separate. This secondary neural tube is formed completely by *taeniatus* cells, that is, by material supplied by the host embryo. *Cristatus* cells, that is, material of the organizer, do not participate in its formation.

The implant has moved completely below the surface. Its most voluminous, anterior part is a rather atypical mass located directly under the secondary neural tube (Fig. 8), between it and the large yolk cells of the intestine. Separate somites cannot be seen, but the contour of a notochord can be delineated; in the anterior sections, where the axial organs curve outward, it is cut longitudinally, but transversely in the more posterior ones (Fig. 8). Toward its posterior end, the implant tapers off; it forms only the notochord and a few cells that merge with the endoderm (Fig. 9). Thereafter, the notochord disappears also, and the implant lies entirely in the endoderm and forms the upper covering of a secondary intestinal lumen that extends over a few sections. In its entire posterior part, the implant is separated from the secondary neural tube by interposed mesoderm of the *taeniatus* embryo (Fig. 9). The neural tube extends considerably farther caudal than the implant.

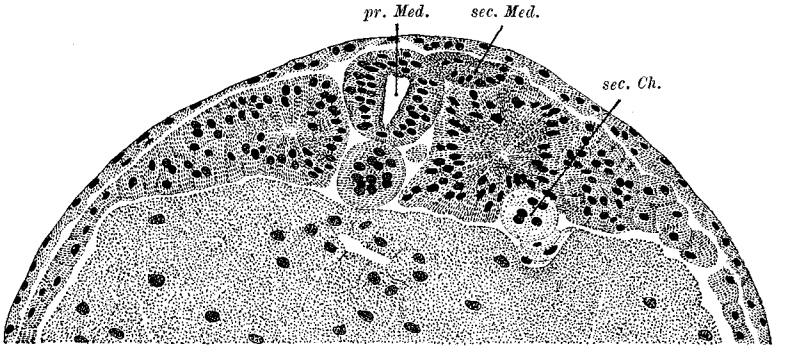


Fig. 9. Um 25b. *Cross section in the posterior third of the embryo (cf. Fig. 7). The secondary neural tube is attached to the left side (right in the figure) of the primary tube. The implant (light) forms secondary notochord (sec. Ch.). 100X.*

In contrast to the first experiment, the implant in the present case forms a uniform mass; it is not separated into two sections by intervening mesoderm. This must have something to do with the way in which it was shifted to below the surface. However nothing definite can be ascertained concerning this point. The fact that the two embryonic anlagen share the remainder of the blastopore proves that the implant has been invaginated in the normal way around the blastopore. However, it is doubtful whether the implant was entirely passive in this process. It comes from a region whose cells normally participate actively in invagination; and in other instances they have retained this capacity after transplantation. For this reason, the situation becomes complicated.

The implant has formed the entire notochord, the greater part of the mesoderm, which however is not typically segmented, and a small part of the intestinal primordium. It is not clear in the present case whether it has also exerted an inductive effect on the adjacent mesoderm. However, it has certainly evoked the formation of the entire secondary neural tube; but in which way this has occurred remains undecided. A direct influence would be possible in the anterior region where the implant lies directly under the neural tube (Fig. 8). However this explanation is improbable farther back where the implant is displaced by host mesoderm (Fig. 9) or is entirely missing. One would have to assume that this mesoderm has been altered by the organizer and has, in turn, initiated the formation of the neural plate in the overlying ectoderm. However, it could be that the organizer had exerted its entire effect on the ectoderm before it had moved to the interior.

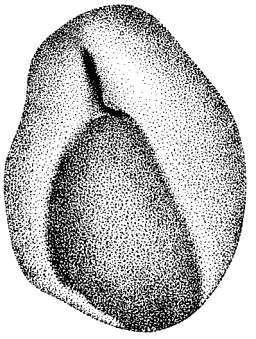


Fig. 10. Um 131a. *The* cristatus *embryo at the neurula stage. The* taeniatus *implant (dark), in the shape of a triangle with unequal sides, lies in the posterior dorsal half. 20X.*

In summary, it is characteristic of this case that implant cells are completely absent in the secondary neural tube, and that the notochord is formed completely by cells of the implant. The same thing is shown, perhaps even more beautifully, in another case (*Triton* 1922, Um 214), in which the notochord formed by the implant, and also the induced neural tube, extend almost over the entire length of the host embryo, and are both near the normal axial organs. But this case again fails to indicate whether the implant can form somites or induce them in host mesoderm. The next case gives information on that point.

Experiment Triton 1922, Um 131b. The exchange of material was done in advanced gastrulae, after formation of the yolk plug. A large piece of *cristatus*, derived from the median line directly above the blastopore, was interchanged with a piece of *taeniatus* whose origin could not be definitely determined.

The *taeniatus* implant has not participated in invagination in the *cristatus* embryo; it has caused a peculiar fission (Fig. 10). The neural tube is closed anteriorly; at the point where it meets the *taeniatus* piece, it divides into two halves, one to the left and one to the right. At this point, a bit of endoderm comes to the surface, perhaps as the result of incomplete healing or of a later injury. The cross-sections show a neural tube and notochord in the anterior part back to the point of bifurcation. The two divisions of the neural tube are still distinct for a few sections, but then they become indistinguishable from the surrounding tissue. The same is true, to a greater degree, of the notochord.

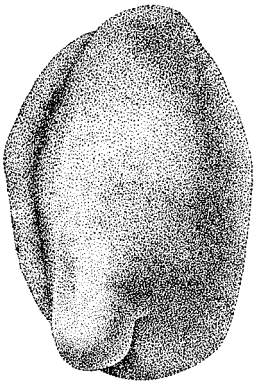


Fig. 11. Um 131b. *The* taeniatus *embryo at the neurula stage. The neural folds are closing. The implant (light), in the middle and posterior third, to the right of the dorsal median plane, is visible through the surface layer, and continues into the protuberance.*

The *taeniatus* embryo has reached the neurula stage 20 hours later. The implant is located on the right side, somewhat behind the middle, and next to the right neural fold. Its original anterior half is still on the surface and strongly elevated over the surroundings; its original posterior half is invaginated and appears as a light area underneath the darker cells of the *taeniatus* embryo. The piece is stretched lengthwise and directed from posteriorly, and somewhat above, to anteriorly and somewhat downward. Invagination still continues; a half-hour later, a strip of *cristatus* cells is visible only at the outer margin of invagination. Twenty-five hours later, the neural folds are almost closed; the implant is

visible to their right as a long, stretched out pale strip shining through the epidermis. At its posterior end, it continues into an elevation above the surface of the embryo that has the shape of a small blunt horn (Fig. 11). After another 22 hours, the neural tube is noteworthy for its breadth. The implant is still visible at its right side. It apparently participates in the formation of somites; it continues posteriorly into the outgrowth. The embryo was fixed 11.5 hours later when a small area of disintegration appeared on the head. The sections were *[cut]* perpendicular to the longitudinal axis.

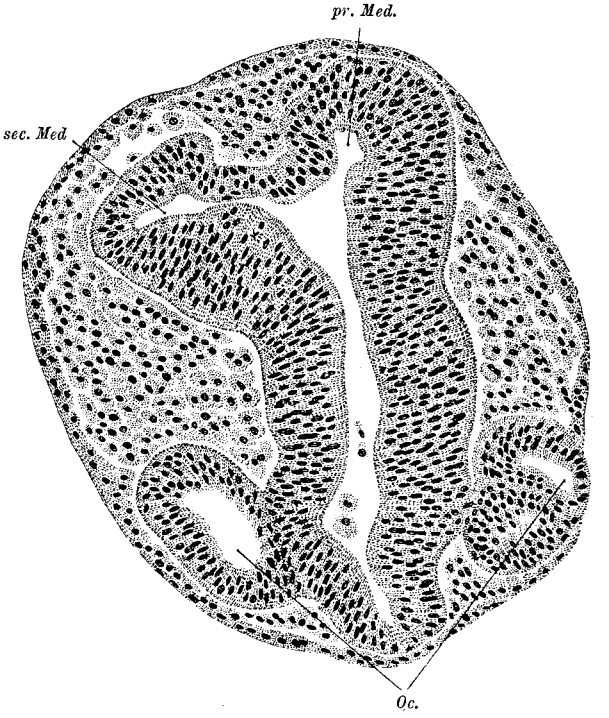


Fig. 12. Um 131b. *Section through the head (cf. Fig. 11). Primary and secondary neural tubes are fused and their lumina are continuous. Oc., optic vesicles of the primary neural tube. 100X.*

We shall consider the axial organs, at first disregarding their different origin, and we begin in the middle region, where they show the typical appearance of a duplication (Fig. 14). The neural tube is incompletely duplicated; the upper outer walls and the lower inner walls of the two individual parts merge in such a fashion that their median planes converge dorsally and meet at a right angle ventrally. There is one notochord underneath each of the two halves. There is an outer row of somites lateral to each notochord, and between them a third row, not quite double in size, that is common to both embryonic anlagen. Also, the intestine shows a double lumen in this region.

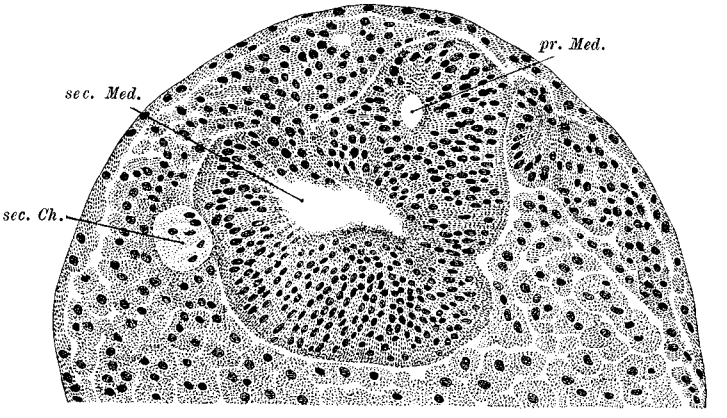


Fig. 13. Um 131b. *Cross section in the anterior third of the embryo (cf. Fig. 11). Primary and secondary neural tubes are fused but their lumina are separate. The implant (light) has differentiated into notochord (sec. Ch.). 100X.*

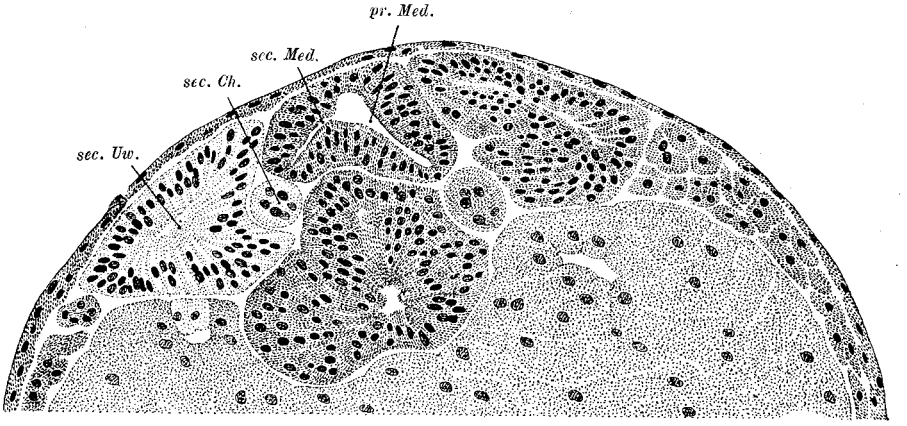


Fig. 14. Um 131b. *Cross section in the middle third of the embryo (cf. Fig. 11). Primary and secondary neural tubes are fused and their lumina continuous. The implant (light) forms the secondary somite (sec. Uw.) and the secondary notochord, and in addition the roof of the secondary gut. 100X.*

We now follow the different organs forwards and backwards from such a middle section.

The left half of the neural tube (at the right of the sections), which already in this middle region is somewhat larger than the right one, becomes relatively larger more and more anteriorly and continues eventually into a normal brain primordium with primary optic vesicles (Fig. 12). Thus the right half becomes reduced to an increasingly more insignificant appendage and terminates finally without

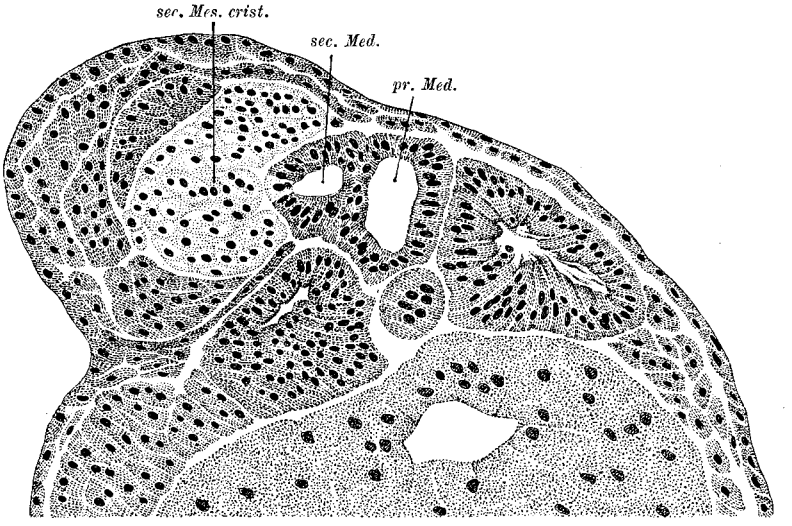


Fig. 15. Um 131b. *Cross section at the base of the secondary tail (cf. Fig. 11). The primary and secondary neural tubes are fused; their lumina are separate. The implant (light) is in the floor of the secondary neural tube and forms mesoderm (sec. Mes. crist.) in the secondary tail. 100X.*

forming optic vesicles. The two tubes continue to have a common lumen; where it seems to be divided into two (as in Fig. 13), we are dealing with a curvature of the tubes resulting in tangential sections through their walls. Toward the posterior region, the two tubes separate from each other; at first their lumina separate (Fig. 15), and then also their walls. As far as one can make out, mesoderm intervened between them. The larger left tube (at the right in the sections) continues into the normal tail bud and the smaller right tube into the secondary tail-bud outgrowth. The greater width of the neural tube had already been observed in the living embryo; but in the stage of the open neural plate neither the larger size nor the duplication of the folds, that must have been present, had been noticed.

The left notochord runs medially, in typical fashion, under the left part of the neural tube (Figs. 14 and 15, right). The right notochord extends even farther forward than the left one (Fig. 13). It is clearly delineated (Fig. 14) up to the point where the secondary tail bud begins (Fig. 15). Here, its contour becomes indistinct and eventually it disappears entirely.

Of the somites, only the outer left row (Fig. 14, right) is typically developed in its entire length. The outer right row, which is its symmetrical counterpart in the middle region (Fig. 14, left), anteriorly decreases in size considerably. Toward the posterior end it becomes symmetrical within itself, so that the notochord primordium lies approximately in its median plane (Fig. 15). It fades out eventually in the secondary tail bud. The middle row of somites seems, in its middle portion, to belong equally to both sides (Fig. 14). Toward the posterior end, where the right row achieves its own symmetry, the middle row becomes more and more the mirror image of the left row (Fig. 15). The primary plane of symmetry of the duplication therefore no longer bisects the middle row, as is the case in the middle region, but it passes between it and the right row.

Parts of these primordia derive from the *cristatus* cells of the transplant. In the neural tube, there are only a few *cristatus* cells in the median floor of the right half (Fig. 15). Furthermore, the entire right notochord and the entire outer row of somites are formed by *cristatus* cells (Figs. 13-15). In the gut, again, there are only a few such cells, located dorsally, forming the border of a small secondary lumen for a short distance (Fig. 14).

Besides these parts whose material derives from the implant, others have received the stimulus for their formation from the implanted organizer. This is certainly the case with respect to the entire right neural tube. But also the middle row of somites, in its symmetrical portion, has apparently been influenced from both sides, that is, from the normal and the implanted center; and it, in turn, seems to have affected the outer row of *cristatus* somites that are symmetrical to it.

The peculiarity of this case lies in the formation of somites from implanted material and, furthermore, in the interference of the implanted organizer with the normal organization center over a long distance. In the next case, this interference is limited to the anterior most parts of the two embryonic primordia. Furthermore, the *cristatus* organizer was implanted into the very dark *alpestris* embryo, and the difference in pigmentation is, in part, very sharp.

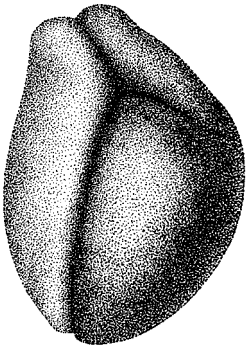


Fig. 16. Um 83. *The* alpestris *embryo at the neurula stage. Dorsal view. The secondary neural tube branches off laterally from the primary tube and deviates to the right. 20X.*

Experiment Triton 1922, Um 83. The organizer was taken from an early gastrula of *cristatus*, medially, close to the blastopore, and implanted at the animal pole of an *alpestris* embryo in the blastula stage. The *cristatus* embryo disintegrated.

Gastrulation in the *alpestris* embryo begins after 23 hours. The implant is located in the animal half; it is large and curved inwards. Gastrulation is not yet complete after another 23 hours; the implant has disappeared completely into the interior. In its place, a little horn composed of *alpestris* cells protrudes on the dorsal side of the embryo. After another 21 hours, the folds have just begun to form. The little outgrowth is on the right neural fold, at the posterior border of the broad plate. After another 24 hours, the neural folds are in the process of closure; the little horn has disappeared. In the position where it had been visible, a small secondary tube branches off the neural tube; it extends obliquely toward the caudal end (Fig. 16). After further development for 24 hours, the embryo was preserved and the sections were cut as nearly transverse to the two branches of the neural tube as possible.

The primary neural tube is closed and separated from the epidermis for almost its entire length (Fig. 18); it is still continuous with the epidermis in the midbrain region where it opens to the outside. The optic vesicles are indicated by compact protrusions of the brain wall.

The primary notochord is delineated in normal fashion for the greatest part of its length (Fig. 18); at the posterior end, it merges with the indifferent tissue of the tail bud.

Of the somites, the left or outer row is normal (Fig. 18, to the right); 7 to 8 somites are separate from the lateral plate. The right or inner row (Fig. 18, to the left) seems to be somewhat deranged at the anterior end, in front of the bifurcation, as if dammed up.

The secondary neural tube is closed in its middle portion and separated from the epidermis (Fig. 18). It meets the primary tube anteriorly at an acute angle and fuses with it at approximately the level of the future midbrain (Fig. 17); at this point, its lumen opens to the outside. Posteriorly, it becomes lost

indistinguishably in the surrounding mesoderm of the secondary embryonic anlage, as it would in a normal tail bud.

The notochord is likewise distinctly delineated in the middle portion (Fig. 18); it lies directly above the wall of the intestine. Anteriorly, it passes without clear demarcation into the mesoderm formed by the implant (Fig. 17), and caudally it merges in the same way with the *alpestris* mesoderm that it has induced.

In the middle region, the secondary somites are symmetrically arranged with respect to the secondary notochord and neural tube (Fig. 18). Anteriorly, near the bifurcation point, a mesoderm strip of

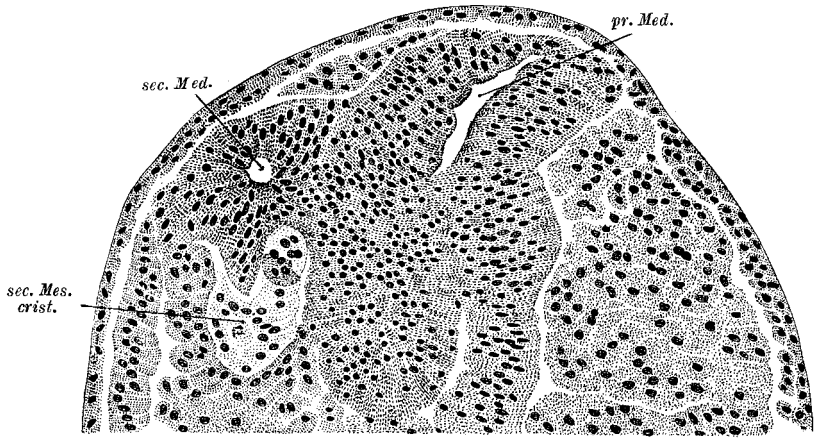


Fig. 17. Um 83. *Cross section in the anterior third of the embryo (cf. Fig. 16). In the upper right of the figure may be seen the primary neural tube, from which the secondary tube branches off. The implant (light) is in the mesoderm (sec. Mes. crist). 100X.*

cristatus cells appears between the somites; it connects the lower edges of the somites and separates the notochord from the intestine. In the same region the somites become smaller and indistinct, the left (inner) row earlier than the right (outer) row. Farther back, the somites merge with the unidentifiable tissue in which the notochord and neural tube also loose their identity.

The lumen of the intestine in its middle portion is shifted toward the side of the secondary embryonic anlage, so that it comes to lie in the overall median plane of the duplication (Fig. 18). In this case, the cell material of the implant participates only in mesodermal structures. The neural tube is composed purely of *alpestris* cells, at least as far as it is delimited from other parts. The notochord, on the other hand, is formed principally of unpigmented cells derived from the *cristatus* implant. But, here and there, distinctly pigmented cells are interspersed along its entire length; they are of the same color as the cells of the neighboring somites (Fig. 18). Since they were never observed in a *cristatus* notochord, they undoubtedly derive from the *alpestris* embryo. Lateral to the notochord, the implant is in an

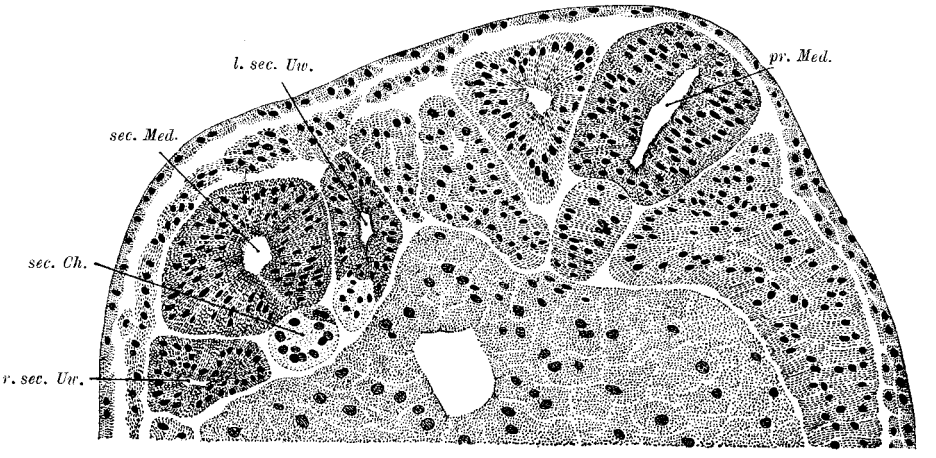
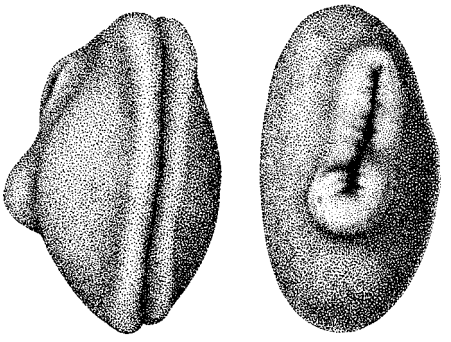


Fig. 18. Um 83. *Cross section in the middle part of the embryo (cf. Fig. 16). The primary axial organs are at the upper right of the figure and the secondary axial organs are at the upper left. The implant (light) is in the left secondary somite (l. sec. Uw.) and in the secondary notochord (sec. Ch.). 100X.*

asymmetrical position; in its middle portion it appears in the edges of the left somites (Fig. 18, to the right of the notochord), but in its anterior portion in the right somites *[to the left of the notochord, (see Fig. 17)]*. In addition, the transplant furnishes the mesoderm strip mentioned above that connects the two sides.

The neural tube and somites of the secondary embryo are definitely induced by the transplant, as far as they are composed of *alpestris* cells. The pigmentation of the primary and secondary neural tubes is equally deep in both. However, it is surprising how dark the secondary somites are, in comparison to the primary somites (in Fig. 18, however, the difference is exaggerated). It might be assumed that they are formed of different material, that is, of the deeply pigmented cells of the animal half. The experiments of O. Mangold (1922, 1923) have proved that the latter are capable of forming somites. The implant would have carried these cells along with it when it invaginated; this would have been facilitated by the early age of the host embryo (blastula). We shall return to this possibility later. We shall then also discuss the remarkable fact that the implant does not lie in the longitudinal axis of the organs induced by it, but at an acute angle to it.

Experiment Triton 1922, Um 132. The organizer was taken from a *cristatus* embryo in advanced gastrulation (medium-sized yolk plug). The median region, directly above the blastopore, was transplanted into a *taeniatus* embryo of the same stage. The implant moved inward in the shape of a shallow cup. The *cristatus* embryo, with the exchange implant from *taeniatus*, developed to a larva with primary optic vesicles; it was lost by accident before sectioning. In the neurula stage, the implant had been located medially in the posterior part of the neural plate and extended to the blastopore. Closure of the neural folds was delayed and not quite complete at the caudal end; it was similar to, but not quite as abnormal as that in *Triton* 1922, 131b.



Figs. 19 (left) and 20. Um 132. *The* taeniatus *embryo at the neurula stage; the secondary neural folds are viewed from the right side (Fig. 19), and from above (Fig. 20). 20X.*

In the neurula stage *taeniatus* embryo, 19.5 hours after the operation, the implant is no longer visible. In its place are two short neural folds surrounding a groove. They extend obliquely across the ventral side of the embryo, from left posterior to right anterior in top view. Twenty-five hours later the neural folds have approached each other (Fig. 20). The two folds mentioned above and the groove between them are on the left ventral side of the embryo; they are lengthened, and they approach the anterior ends of the host neural folds at an acute angle (Figs. 19 and 20). After another 22 hours, this secondary embryonic primordium has flattened out anteriorly, but posteriorly it projects considerably above the surface. In this region, somites seem to form. Approximately 28 hours later, the embryo has primary optic vesicles, otic pits and a tail bud. In the secondary embryo, at least on the right side, somites can be quite clearly recognized. After another 20 hours, paired otocysts are seen at its anterior end; they are at the same level as those of the primary embryo. The free posterior end has grown somewhat and is bent toward the primary embryo. Four hours later, a pronephric duct is visible in the induced anlage. The embryo was fixed 6 hours later, when a blister appeared on the dorsal surface; the sections were cut transversely.

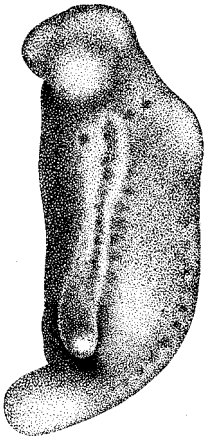


Fig. 21. Um 132b. *The* taeniatus *embryo shown in Figs. 19 and 20, developed further; viewed from the left side. Surface view of the secondary embryo, with tailbud, neural tube, somites, and otocysts. 20X.*

Immediately before fixation, the living object showed the following features:

The embryo is stretched lengthwise, but its tail is still bent ventrad (Fig. 21). The optic vesicles are strongly expanded, the otic pits distinct, and a large number of somites is formed. The head is continuously bent to the left, probably due to the secondary embryonic anlage which is on the left side. The latter is rather far ventral,

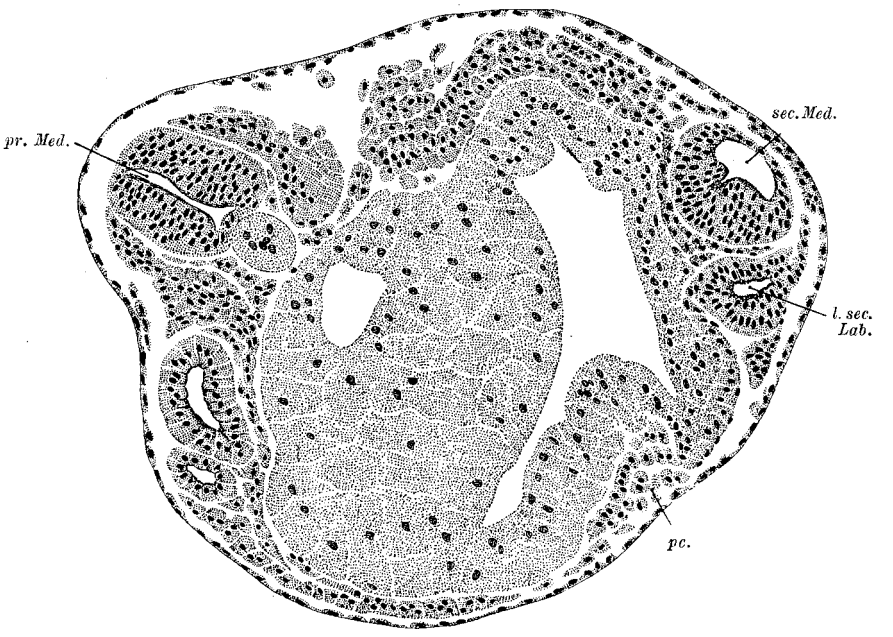


Fig. 22. Um 132b. *Cross section at the level of the primary pronephros (cf. Fig. 21). The primary axial organs are at the upper left of the figure and the secondary axial organs at the right. l. sec. Lab., left secondary otocyst; pc., pericardium. 100X.*

and approximately parallel to the primary axial organs, which it approaches anteriorly at an acute angle. It extends over a considerable part of the length of the primary embryo, from the posterior border of the left optic vesicle to the level of the anus. Its posterior end is lifted up like a tail bud. The central canal of its neural tube is visible through the epidermis, and likewise the lumen of the otocysts and of the right somites. The left somites are not recognizable.

The evaluation of the finer structures is facilitated by the almost complete independence of the normal and the induced embryonic primordia, in contrast to the two previously described cases.

Of the axial organs of the primary embryonic anlage, the neural tube, notochord and somites are entirely normally developed; so is the right pronephros. The left pronephros, however, which faces the

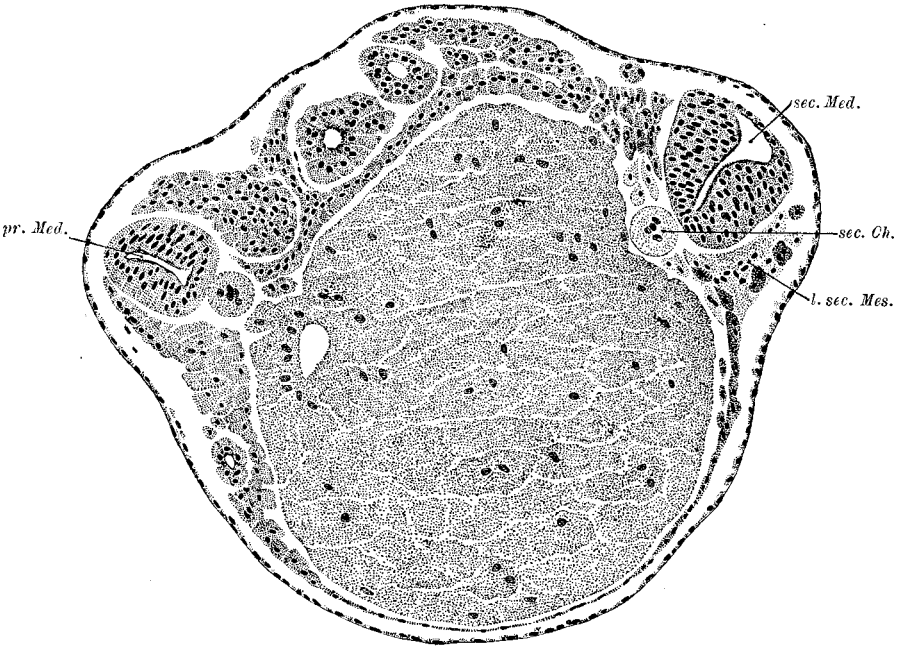


Fig. 23. Um 132b. *Cross section in the anterior third of the embryo (cf. Fig. 21). The primary axial organs are at the left of the figure and the secondary axial organs at the right. The implant (light) has differentiated into notochord and left secondary somite. 100X.*

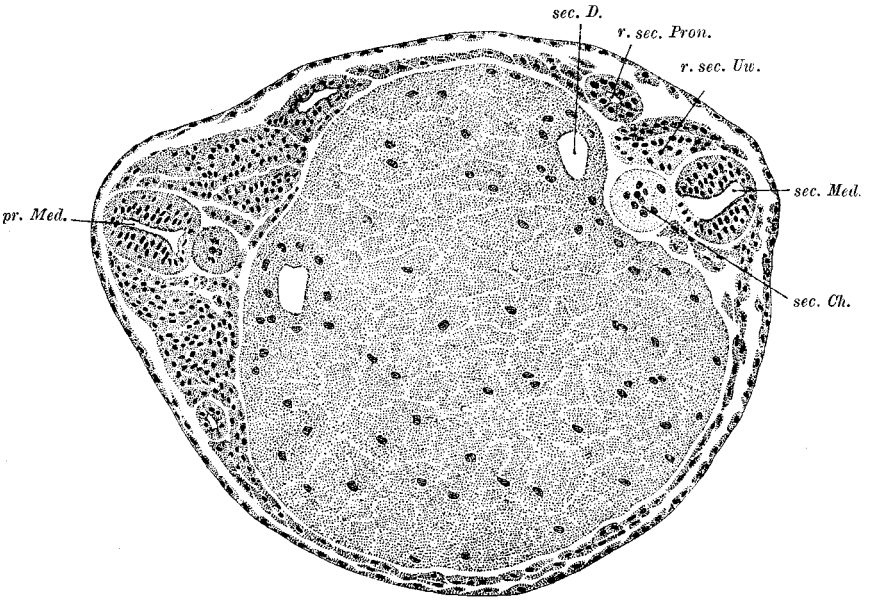


Fig. 24. Um 132b. *Cross section through the middle of the embryo (cf. Fig. 21). The primary axial organs are to the left of the figure and the secondary axial organs to the right. r. sec. Pron., right secondary pronephric duct. The implant (light) has formed notochord and part of the right secondary somite. 100X.*

secondary primordium, shows a minor irregularity. In the brain primordium, the primary optic vesicles are already transformed into cups, and the lens primordia are recognizable as slight thickenings of the epidermis. The otic pits have closed to form vesicles, but they are not further differentiated, except for the indication of a *ductus endolymphaticus*. The notochord is separated from the adjacent parts throughout almost its entire length. Between 11 and 13 clearly segregated somites can be counted. Neural tube, notochord and somites pass into undifferentiated tissue at the tip of the tail. The primordium of the pronephros consists on each side of two nephrostomes with associated tubules (Figs. 22 and 23). These open into pronephric ducts, in a normal fashion (Figs. 23 and 24). The left duct has a larger diameter anteriorly than has the right one. The pronephric ducts can be traced far posteriorly, but not to their opening to the outside.

The secondary embryonic anlage also possesses all the axial organs; they are in part very well formed. The neural tube is closed in its entire length and detached from the epidermis. It is sharply delimited

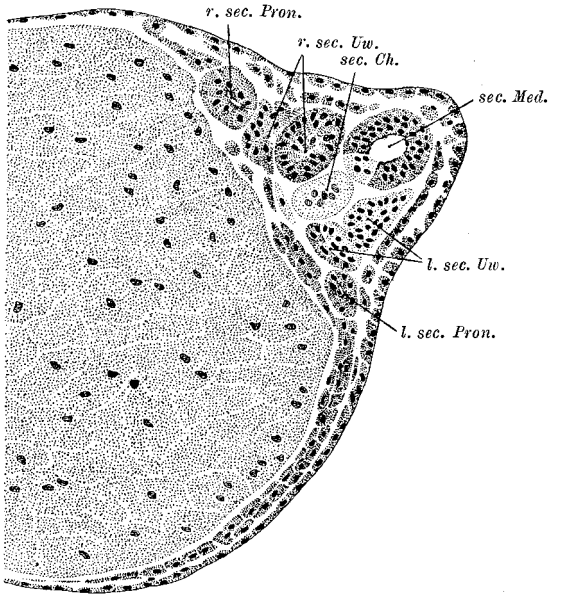


Fig. 25. Um 132b. *Cross section through the secondary axial organs, slightly anterior to the secondary tailbud (cf. Fig. 21). The implant (light) is both in the floor of the secondary neural tube and in the left secondary somite, and has formed the notochord. 100X.*

except for its caudal end where it becomes continuous with the undifferentiated mass of the secondary tail bud. In its middle part, the right side is somewhat more strongly developed than the left side (Fig. 24). Toward its anterior end, the diameter increases, and the roof becomes broader and thinner, as in a normal medulla (Fig. 22). At this level, two otocysts are adjacent to it. The right otocyst is shifted forward; it lies at the level of the anterior end (compare the surface view, Fig. 21), and the left one is slightly more posterior (Fig. 22). They are still attached to the epidermis, and the formation of the endolymphatic duct seems indicated. The notochord extends less far craniad than normally. It is not yet found at the level of the posterior otocyst (Fig. 22); it does not begin until 90 μ behind this section. Otherwise it is well formed, and sharply delimited all the way to its posteriormost part in the tail bud. Somites are formed on both sides; there are more (4 to 6) on the right side facing the primary embryo than on the left side (2 to 3). On the right side, they extend farther forwards (Fig. 24). A pronephric duct is formed on both sides; again, the left one is longer (about 300 μ) than the right one (about 500 μ) *[figures probably erroneously reversed]*. Caudally they are not yet separated from the mesoderm, and anteriorly, tubules and funnels are not formed, or not yet. The two adjacent ducts, namely the left one of the primary embryo and the right one of the secondary embryo, are in communication with each other directly behind the second pronephric tubule.

Both embryos share the intestine which is mainly directed toward the primary embryo. It cannot be ascertained with certainty to what extent the secondary embryo has a share in it in all regions. In the pharynx, primordia of visceral pouches may belong to the secondary embryo (Fig. 22); however, they could also belong to the primary embryo and merely be shifted slightly by the secondary embryo. This holds, at any rate, for the heart primordium (Fig. 22 pc, in section through the posterior end of the pericardium). In contrast, a secondary intestinal lumen is distinctly induced beneath the axial organs of the induced anlage, although it can be traced for only a very short distance (about 60 μ; Fig. 24). The anus is somewhat expanded, so that the endoderm is exposed; it is also shifted toward the left side.

The secondary embryonic anlage is again a chimera formed by cells of the host and of the implanted organizer. The two posterior thirds of the neural tube have a ventral strip of *cristatus* cells (Figs. 24 and 25). The notochord is formed entirely of *cristatus* cells. In the somites, the *cristatus* contribution is in the anterior and posterior sections of the left row (Figs. 23 and 25, right) and in the middle part of the right row (Fig. 24, left); there are no somites at all in the middle of the left row (Fig. 24, right). The implant has remained continuous throughout its length (Figs. 23-25).

All the other structures of the secondary embryo that are not formed by *cristatus* cells have been undoubtedly induced in *taeniatus* material by the organizer.

Hence, in this case the two embryonic anlagen have interfered with each other only to the extent that some of the organ primordia are somewhat more strongly developed on the inner side than on the outer side, and that the pronephric ducts are connected with each other. In other respects, the induced embryonic primordium is entirely independent. This is perhaps one of the main conditions for its complete development.

III. Discussion of the Results

1. *Origin and prospective significance* [normal fate] *of the organizer and site of its implantation*

In all these experiments, the organizer was taken from a *cristatus* embryo and inserted into a *taeniatus* embryo, except for one case, where *alpestris* was the host. This combination has proved to be advantageous. The unpigmented *cristatus* cells can be clearly distinguished, over a long period of time, from the pigmented *taeniatus* or *alpestris* cells, and the part supplied by the organizer can thus be sharply delimited from the regions induced by it. Of course, the same would have been true for the implantation of an organizer from the pigmented *taeniatus* or *alpestris* embryo into the unpigmented *cristatus* embryo. This reciprocal experiment would have offered the additional advantage that an organizer could have been implanted into the considerably larger *cristatus* embryo, thus more easily avoiding its interference with the primary organization center; and, on the other hand, there would have been room for the implantation of several organizers, side by side, and their mutual interference could have been tested. However, several considerable disadvantages cancel out these advantages. For one, the *cristatus* embryos proved to be in general more delicate, as was mentioned above; they seemed, therefore, to be less suitable as host embryos. The larger embryo has probably more difficulty in gastrulation after removal of the vitelline membrane. Furthermore, the neural plate of *cristatus* does

not become distinct in early stages, by pigmentation, as is the case in *taeniatus* and *alpestris*. Even after elevation of the neural folds, it is much less conspicuous; and for this reason, the small and even less distinct induced neural plates are very difficult to see in the living embryo.

The *region* from which the organizer is taken can easily be ascertained in early gastrulae because the crescent-shaped blastopore gives safe points of orientation. Once the blastopore has become circular, a definite orientation is often no longer possible in the undisturbed embryo. Hence, the piece of host embryo for which the organizer was substituted was implanted in the donor as a marker. This would be an ideal method to determine the normal fate, that is, the prospective significance, of the organizer, if one could be certain that development continues undisturbed despite the operation. As a matter of fact, development is probably somewhat altered (once in a while this can be directly observed), in that gastrulation is impeded. It could be that parts that normally invaginate remain on the surface. The opposite, that is, that more material invaginates than normally, can be excluded almost with certainty. However, this marker is not useless. Even in the most unfavorable case, it will show the position of the organizer with respect to the median plane, whether it was in this plane, or lateral to it; and it will show, furthermore, the *minimal* posterior extent of the organizer. We shall disregard those cases in which a more far-reaching disturbance of development, that is, *spina bifida*, was caused by the implant.

To judge from these markers, or from direct observation, the organizers were all derived from the median plane, closely above the invaginating border of the upper blastoporal lip, or at a short distance from it. They always belonged to the zone of invagination, at least in their posterior part. Accordingly, probably in some cases they would have formed the posteriormost part of the neural plate, but they would always certainly have formed notochord and somites. It cannot be said with the same certainty whether they would have also formed the roof of the intestine. This depends on the lateral extent of the piece, that is, on its width when it was a median piece.

The *age* of the host embryos was variable; it ranged from blastula to advanced gastrula with medium-sized yolk plug. Implantation was always into the animal half of the embryo, but at different places, partly within, and partly outside of the zone of invagination.

Although all this could be determined exactly, the same has not been possible, so far, with respect to the *orientation* of the implanted pieces, since they are exactly circular as is the opening of the micropipette with which they were punched out. This is a disadvantage that will have to be overcome in future experiments. Several different methods suggest themselves, for example, marking the organizer by implanting into it some cells with different pigmentation before it is lifted out; or perhaps an implant with a more characteristic contour can be obtained. Only when the organizer has been implanted in an exactly determined orientation is it possible to establish with certainty the relations between its structure and the direction in which it exerts its effects on its surroundings.

2. *Behavior of the organizer after implantation*

All cases observed have in common the fact that the organizer, which is at first on the surface and level with its surroundings, moves later into the interior, either entirely or in its greater part. The manner in which this occurs differs according to the site of implantation.

If the implant is within the normal zone of invagination, then it passes inwards around the blastoporal lip together with its surroundings. This could be established frequently by direct observation; the piece was seen moving toward the margin of invagination, or immediately in front of it. In other instances, it could be deduced from the result of gastrulation.

Such an invagination of implanted pieces has been observed recently by W. Vogt (1922) and O. Mangold (1922, 1923). In the latter experiments, the implant was indifferent material from the animal hemisphere; it demonstrated its capacity for transformation by becoming mesoderm when carried inside, even though it was presumptive ectoderm. It was also remarkable that an implant taken from a young gastrula seemed to participate more readily in invagination than one from an advanced gastrula (O. Mangold, 1923, p. 286 ff).

Our experiments cannot be compared directly with these experiments because our implants, derived from the upper blastoporal lip, have brought with them their own invagination tendencies which, depending on the orientation of the piece, might affect the invagination *[of the host mesoderm]* by either impeding or promoting it. Definite conclusions cannot be expected until it is possible to control the orientation of the implant.

The implant also moves into the interior if it lies outside the zone of invagination. There can be no doubt but that this is caused by forces which the piece brings with it from its region of origin, namely the upper blastoporal lip. Perhaps the first stage in this process is the formation of a depression by the implant; this occasionally appears immediately after implantation and it is frequently still visible on the following day (see *[p. 26]*). We have also often observed the gradual disappearance of the implant. The details of this process of independent invagination require more precise investigation. During and after invagination, the implant undergoes a stretching which corresponds approximately in amount to that demonstrated recently by W. Vogt in reimplanted parts of the upper blastoporal lip (cf. v. Ubisch, 1923, Fig. 9). The remarkable protrusion of the piece, which was observed repeatedly (for instance in 1922, Um 131, see *[p. 21]*), can probably be ascribed to an obstruction of this invagination which is combined with stretching.

Once arrived in the interior, the implant almost always forms a coherent complex. Only in one case (1921, Um 8, see *[p. 18]*) did the mesoderm consist of two portions separated by intercalated host tissue. It was shown that the anterior part probably derived from the deeper layer of the implant.

Even though the process of invagination has to be studied in more detail, the end result is completely clear; it can be read off, directly, from the sections. Depending on its origin and perhaps also on its place of insertion, the implant is brought into the interior more or less completely. That is, part of it remains in the ectoderm and can then be recognized in the neural plate by direct inspection or in sections, where it is found in the wall of the neural tube; or it is completely sunk into the interior where it forms only mesoderm and perhaps endoderm.

3. *Structure of the secondary embryonic primordium*

The structure of the secondary embryonic primordium is quite complete and can be interpreted most easily when it does not interfere with the primary one. In such cases as that described above (1922, Um 132), all organ primordia, such as neural tube with otocysts, notochord, somites, pronephros, and perhaps also intestine, can be present and relatively well developed. The only deficiencies are in the neural tube, the anterior parts of the brain with the optic vesicles; in the pronephros, the tubules and nephrostomes; in the gut, the anus. It does not seem impossible to expect more nearly complete embryos in the course of continued experimentation.

Part of this secondary embryonic primordium always derives from the implant, which can always be sharply distinguished from its surroundings by virtue of its different histological characteristics. The size and position of this component are very variable, depending, undoubtedly, on the size and point of origin of the implant. Host tissue prevails in the neural tube; *cristatus* cells are either absent (e.g. 1922, Um 25; Figs. 8,9; 1922, Um 83, Fig. 18), or they form only a narrow strip (e.g. 1921, Um 8, Fig. 3; 1922, Um 131, Fig. 15; 1922, Um 132, Figs. 24,25). This strip is of very different length in the individual cases, but as observed so far, it is always in the median plane; this is of theoretical significance. In contrast, implant tissue predominates in the notochord; in fact, the notochord consisted completely of *cristatus* cells in all cases except one (1922, Um 83), where small cell groups of the host are interspersed (Fig. 18). The somites assume an intermediate position: they can be composed completely of *cristatus* cells (Fig. 14), or completely of host cells (Figs. 18 and 25, left); or they can be chimeric, i.e., composed of both (Figs. 18 and 25 right).

The implant as a whole is not rigidly limited to the median plane; this is again of theoretical importance. For instance, in one case (1922, Um 83) its posterior part extends farther to the left (Fig. 18, right) and its anterior part farther to the right *[with respect to the notochord, cf. Fig. 17]*; hence it forms an acute angle with the median plane (see *[pp. 25, 33]*).

The orientation of these secondary embryonic primordia with respect to the primary axial organs of the host embryo varies considerably. They may be almost parallel to them and nowhere contiguous (1922, Um 132, (see *[pp. 26 ff]*); or they may meet at a more or less acute angle and fuse with them either at the tip, or laterally over a long stretch. To the extent that they are not formed by the *cristatus* cells of the implant, they must have originated from the parts of the host that either were already on the spot, or that came there under the influence of the organizer. This is quite evident for the neural tube; it is formed of cells which otherwise would have formed epidermis of the lateral body wall. The situation is less simple for the more deeply located parts, that is, notochord, somites and pronephros. Sometimes it seems as if they were carved out, as it were, of the lateral plates of the host (e.g. 1922, Um 132. Figs.

24 and 25). In one case however (1922, Um 83, see *[p. 25]*), the secondary somites were so much more darkly pigmented than the primary ones that the idea suggested itself that they might have been formed by presumptive ectoderm, like the secondary neural tube which they resemble with respect to pigmentation. It would then have to be assumed that the organizer had evoked intensive invagination in the blastula cells of the animal pole where it had been implanted, and had subsequently determined them to form somites. The basis for this possibility is undoubtedly provided by the previously mentioned experiments of O. Mangold. The details of these processes would have to be elucidated by investigations directly aimed at this point.

4. *The causes for the origin of the secondary embryonic anlage*

The causal relationships in the origin of the secondary embryonic anlage are still completely in the dark. The only point that is certain is that somehow an induction by the implant occurs. But even the question of the stage of development at which this takes place, hence, whether it is a direct, or a more indirect influence, cannot yet be decided.

It is very probable that the inducing action of the implant already begins very early and that it consists at first of inducing its new environment to participate actively in the invagination. That something like this is possible is proved by an earlier experiment (Spernann 1918, pp. 497 ff) in which the bisected blastopore of a medially split gastrula had been fused with material of a different prospective fate and had drawn this latter material into invagination.

The inducing action of the implant could have run its course with this instigation of invagination; everything else could be merely the consequence of this secondary gastrulation. It would then have to be assumed that the general condition imposed on the cells participating in the gastrulation, and by virtue of this process, would in turn provide the stimulus by which further developments are initiated. The different components of the composite chimeric gastrula would then be subjected to this determination process irrespective of their origin. This is actually the case in those chimeras produced by the implantation of indifferent material.

But there is another possibility, namely that after the termination of gastrulation the implant continues to exert determinative influences on its surroundings. For instance, the long, narrow strip of *cristatus* cells in the neural plate could have caused the adjacent cells, which otherwise would have become epidermis, to differentiate likewise into neural plate. And if it should turn out that this is not the correct causal relationship because the development of the neural plate is perhaps evoked by the underlying endo-mesoderm, it is still conceivable that the mesodermal parts of host origin were formed under the influence of the implanted parts.

Both explanations are based on the assumption that the implanted parts have become, by and large, what they would have formed in normal development. According to the first notion, their differentiation would be merely the result of their inherent tendency toward a certain degree of invagination; according to the second notion, the transplants were, in addition, determined with respect to their future differentiation tendency, though perhaps only within the range of a certain degree of variation. These already determined parts would then have the capacity to supplement themselves from the surrounding indifferent parts. It is on this point that the experiments would have to focus that could decide between the two possibilities.

The question of whether decisive facts are already available may be left in abeyance; instead, keeping both possibilities in mind, we shall discuss the factors on which the orientation, the size and the completeness of the secondary embryonic primordia depend.

The first question of interest concerns the *orientation of the secondary primordium* in the host embryo. These are three possibilities: the orientation could be caused entirely by the host embryo, or entirely by the implant, or by a combination of both.

Assuming the first notion to be correct, then the implant would have to be without structure and to behave passively during its submersion. Its form and position would be imposed on it entirely by the relations of the host embryo; it would be simply towed along by the cell movements of the latter. Furthermore, the determinative effect would proceed exclusively from this underlying endo-mesoderm; and this effect would be somehow symmetrical with respect to the shape that had been imposed on it from the outside. In this instance, it would probably have to be expected that the secondary primordium would always be similarly oriented with respect to the primary one, and, more specifically, probably

parallel to it; but this obviously is not the case. Furthermore, the capacity of the organizer to invaginate autonomously when implanted outside of the normal invagination zone of the host cannot be reconciled with lack of structure within the organizer.

According to the second and third assumptions, the implanted organizer would have a definite structure of its own. On this would depend the direction of invagination and longitudinal stretching and finally, sooner or later, its determinative effect. In this event, the host embryo, in turn, could be either purely passive, or it could participate in the final form and position of the implant by virtue of its own structure or cell movements.

The assumption of an inner structure in the organizer is supported by the fact that the random orientation of the secondary embryonic primordium with respect to the primary one corresponds to the random orientation of the implant. A definite decision will not be possible until the orientation of the organizer can be manipulated at will.

A cooperation of the host embryo seems to be indicated by a peculiarity in the position of the implant to which attention has already been called: namely, the longitudinal extent of the implant does not necessarily coincide exactly with the median plane of the secondary embryonic primordium, nor is it necessarily parallel to it; it may form an acute angle with it. This fact would be surprising if the longitudinal stretching of the implant were attributed exclusively to forces residing in it, and if it were assumed at the same time that the implant alone fixes the direction of the determination emanating from it. Under these premises the implant would be expected to stretch exactly in its own sagittal plane and then to supplement itself anteriorly and laterally from adjacent material. It would then be expected to lie exactly in the median plane or at least sagittally in the induced axial organs. The deviation from such a position should probably be attributed to an influence of the host embryo. Either the elongation of the implant is influenced by the cell shifts of the environment, in which event it would then be the resultant of inherent tendencies and extrinsic forces, or the determination itself could be diverted by an inner structure of the host embryo.

These considerations suggest the experiment of destroying the suspected structure of the organizer to test whether the latter can then still have a determinative effect. For instance, a piece of the upper blastoporal lip would have to be crushed, and the attempt would have to be made to place it between the two germ layers of the gastrula by introducing it into the blastocoel of the blastula.

Obviously, the parts of the upper blastoporal lip possess a definite structure by virtue of which they invaginate in a definite direction and perhaps also release stimuli that cause the more indifferent parts to differentiate further in a specific manner. It is irrelevant whether these parts are normally adjacent to the blastoporal lip or brought in contact with it by the experiment. These indifferent parts may also have a directional structure of their own; however this is by no means sufficiently fixed to abolish the influence of the organizer or even to modify it decisively. Depending on the orientation of the implant in the deeper layer of the host embryo, the direction in which its determinative influence pervades the host tissue will differ. For instance, it will pass through the ectoderm in a direction oblique to that of the primary neural plate in cases where the secondary neural plate later forms a more or less acute angle with the primary plate. Whether determination within the induced neural plate, and in the primary as well as the secondary one, is initiated at the posterior or anterior end; that is, whether it progresses cephalad or caudad, as von Ubisch (1923) believes, or whether the entire ectoderm area underlain by organizer is simultaneously affected cannot yet be decided by definite arguments. It may suffice for now to refer to the noteworthy discussions by von Ubisch.

The *size* of the secondary embryonic anlage may depend on several circumstances. The thought immediately comes to mind that it increases with the size of the implant. In addition, its origin, that is, its prospective significance may be of influence and, in this connection, its shape too. It could make a difference whether the implant is short and wide, or long and narrow. Furthermore, the site of implantation could be of importance; and also the age of the implant, either in itself or in relation to the host embryo. These considerations suggest numerous experiments that are feasible; they promise much further insight, quite apart from the surprises on which one can always count from such experiments. One very important factor will be pointed out shortly.

The *completeness* of the secondary embryonic primordium may depend on factors similar to those that influence its size. Again, either the conditions in the host embryo or those in the organizer could

be of primary importance. With respect to the first alternative, there come to mind not only the instances of a very obvious interference of the primordia, where the development of the secondary primordium is impeded by the precocious encounter of its anterior end with that of the primary primordium and by its subsequent fusion with it. It could also be that, despite an apparent independence of the secondary primordium, the completeness of its formation depends on the primary primordium; or, more precisely, the primary organization center could co-determine the mode of action of the implanted secondary center. In this respect, it is noteworthy, for instance, that in experiment 1922, 132 (Fig. 21), the two otocysts of the secondary primordium are at almost exactly the same level as the primary otocysts, and that the secondary neural tube ends there, blindly. The reason for this could be that the primary organization center caused the ectoderm at this level to form the respective sections of the neural tube and the otocysts. And the reason for the absence of the anterior portion of the secondary neural tube and the optic vesicles could be that the secondary primordium did not extend to the level of the optic vesicles of the primary one. Although, according to this version, the primary organization center would, in the final analysis, also be responsible for the degree of completeness of the secondary primordium, the other assumption could also be correct, namely that the defect is to be traced back to deficiency in the implanted organizer. The latter could have been deficient in certain parts of the organization center which would be necessary for the induction of anterior neural plate with eye primordia.

Quite similar considerations had been made previously in the discussion of peculiar defects in duplications that originate after a somewhat oblique constriction in early developmental stages (cf. Spemann 1918, pp. 534-536). The neural tube of the deficient anterior end can be so seriously defective that it ends blindly at the level of the otocysts, without widening, exactly like the neural tube of the secondary primordium of the experiment just discussed. It is remarkable that here again the four otocysts of the two heads are at the same level. The same possibilities, in principle, were considered as an explanation: the new method *[i.e. of heteroplastic transplantation]* will perhaps permit an exact decision between these possibilities.

Interferences between the two organization centers, the primary one and the implanted secondary one, are complications that should be avoided for the time being, as far as possible. Once the analysis has progressed, valuable information concerning the finer details of the mode of action of the centers can be expected of them.

Of particular theoretical importance is the question of whether, apart from visible interference, the two embryonic primordia *mutually influence*, or more precisely, limit each other's size. Simple experimental facts show that this is entirely within the realm of possibility. One could have assumed from the beginning that the presumptive neural plate is already determined, in sharp outline, in the ectoderm of the beginning gastrula. This, however, is ruled out by its interchangeability with presumptive epidermis. Then, it could be the size of the organization center which determines the size of the neural plate by the magnitude of its effect. But this is also refuted by the fact that we can remove the ventral half of the embryo without disturbing the organization center and then the size of the neural plate is also reduced to such a degree that it maintains approximately its normal proportion to the reduced whole (Ruud-Spemann, 1923, p. 102 ff). Therefore there must be some retroaction of the whole on the part. We could imagine, for instance, that different primordia require a certain specific degree of saturation which is naturally reached earlier in an embryo of reduced size than in a normal embryo. If something of this sort actually occurs, then we should expect a secondary primordium to exert an inhibitory effect on the first. To test these relationships, more precise measurements would be necessary; these will be tedious but rewarding.

The possibilities that have been discussed presuppose partly one and partly the other of the two basic concepts concerning the mode of induction. It is therefore necessary to find out whether facts are already available to permit a decision in one direction or the other, and to discuss the type of experiments that would have to be designed to bring to light such facts.

It will not be easy to decide by unequivocal experiment whether the process of invagination itself, as the first assumption holds, can create an overall situation which guides further development in a certain direction. We could try to find out whether passive submersion *[of presumptive endo-mesoderm]* under the surface has the same effect as active invagination. This could be investigated by implanting endo-mesoderm of a very early gastrula under the ectoderm of another embryo and

then observing whether it can produce there the same effect as the endomesoderm of a completed gastrula that has already gone through the process of invagination. However, even if the results were clearly positive, the main problem, i.e. the harmonious patterning subsequent to gastrulation, would not be brought much closer to its solution.

As to the other assumption mentioned above, which implies that the implant not only invaginates but also differentiates further by virtue of its inherent developmental tendencies, a qualifying remark should be made at this time. The possibility was present from the beginning that the implanted piece may undergo pure self-differentiation and develop into exactly the same parts which it would have formed at the place where it came from, and that in order to form a complete whole, it would appropriate from the indifferent surroundings the parts that were missing. However, such complete self-differentiation of the organizer almost certainly does not occur, because the implant would then have been too large for the smaller secondary primordium. Insofar as it adapts itself harmoniously to the secondary primordium, its material has been disposed differently than in normal development.

The results of W. Vogt (1922) also argue perhaps against its complete self-differentiation. He found that a piece from the neighborhood of the blastopore becomes ectoderm or endo-mesoderm, depending on whether it remains outside or invaginates inside during the process of gastrulation. The most recent experiments of O. Mangold (1922 and 1923) have shown the same very clearly for the indifferent embryonic areas (i.e. presumptive ectoderm); the experiments of W. Vogt extend this result *[namely, lack of rigid self-differentiation]* to the parts near the blastopore.

But complete self-differentiation does not seem to be necessary for the implant to enable it to exert an inducing influence beyond the stimulus for gastrulation. Definitely directed inherent developmental tendency and capacity for regulation are not mutually exclusive. Clarification of this point could be achieved by experiments that would test the effects of different regions of the organization center. If, for instance, a piece taken from its lateral margin should be found later to occupy a lateral position in the embryonic primordium induced by it, then it could be concluded that it was already determined as lateral at the moment of implantation, and that it retained this characteristic after implantation and influenced its surroundings correspondingly. Or, if the degree of completeness of the secondary embryonic primordium should differ according to the exact place of origin of the grafted organizer, this would also indicate differences within the organization center that could hardly have been transmitted to the induced embryonic primordium by stimulation of gastrulation alone.

This much at least is probable: that the possibility exists of a determining effect progressing from cell to cell, not only as suggested by the first assumption, during the period shortly after implantation, when the assumption of an effect on the environment can hardly be escaped, but also during later developmental stages. Among the most recent experiments of O. Mangold (1922, 1923) already mentioned several times, there are some whose continuation could contribute to a decision between the points in question. If presumptive epidermis, after implantation into the zone of invagination of a beginning gastrula, comes to lie within the somite region, it participates in somite formation. It is not possible to decide when and how the determination of these indifferent cells took place. They could have acquired the characteristics of somites soon after implantation into the upper blastoporal lip, and, on the basis of this first determination, could have participated in all the later destinies of their surroundings. However, this explanation meets with difficulties in the cases where the implant later does not seem to fit smoothly into its environment but forms supernumerary structures. This gives the direct impression that the determining influence emanated from the somite and determined the adjacent indifferent tissue in the same direction. This suggests a new experiment, the implantation of indifferent tissue such as presumptive epidermis of the beginning gastrula, into an older embryo that has completed gastrulation, so that it reaches its destination without having been part of the blastopore lip. Moreover, the same situation would have prevailed in those cases in which the presumptive epidermis was implanted in the yolk plug, and then moved first into the floor of the archenteron, apparently shifting secondarily into the somite region where it was subjected to determination to form somites (O. Mangold, 1923, p. 258).

If wishful thinking were permissible in questions of research, then we might hope in this case that the second of the previously discussed assumptions would prove to be the correct one. For, if

induction should be limited to a stimulus for gastrulation, then the problem of the harmonious equipotential system, which had just seemed to become accessible to experimental analysis, would right from the start confront us again in all its inaccessibility.

Concerning the *means* of the determinative influence, no factual clues are yet available. The experiment proposed above (implantation between the germ layers of crushed organizer that is thus deprived of its structure) could lead us further into this subject.

We would have assumed that the species whose embryos can interact with each other should not be too widely separated in their taxonomic relationship. *Triton cristatus*, *taeniatus*, and *alpestris*, between which mutual induction is feasible, belong at least to the same genus. However, surprises of great importance seem to be in prospect; Dr. Geinitz in our laboratory has just very recently succeeded (May, 1923) in inducing embryonic primordia in *Triton* by organizers of *Bombinator* and *Rana*. Thus he brought anurans and urodeles into determinative interaction. With this discovery, experimental ideas which seemed to be more dreams than plans (Spemann, 1921, p. 567) have passed into the realm of feasibility.

5. *The organizer and the organizing center*

The concept of the organization center is based on the idea that determination proceeds from cell to cell in the embryo. Such an assumption suggests itself whenever differentiation, that is, the visible consequence of determination, does not start in all parts simultaneously but, beginning at one place, progresses thence in a definite direction. However, pure observation is by no means sufficient evidence of progressing determination. We might be dealing merely with a chronological sequence in the absence of causal relationship. One way of testing this consists in the interruption of spatial continuity. If such separation does not result in a disturbance, that is, if development that had started on one side of a separating transection continued on the other side, then differentiation in the latter would have been independent, at least from the moment of severance.

A clear example of such a situation in the field of amphibian development is the progressive formation of the blastopore in gastrulation. This begins medially with the formation of the upper blastopore lip; it progresses from there to both sides, and finally reaches the median plane again when the circle is closed at the lower blastopore lip. The observer, quite naturally, gets the impression that the part that is in the process of invagination always draws the adjacent cells of the marginal zone with it. However, if the dorsal half of the embryo including the upper blastopore lip is removed, this does not prevent the formation of the lateral and ventral blastoporal lips, which is not even perceptibly retarded. This holds not only for frontal bisection at the beginning of gastrulation, when determination possibly emanating from the upper lip might already have transgressed the line of transection, but it holds also after frontal ligation in the two-cell stage. Failure of the ventral half to gastrulate would still not have been stringent proof for progressive determination. The fact that gastrulation does occur excludes at least the necessity of assuming such a causal relation.

Braus (1906) followed the same method, in principle, when he analyzed the skeletal development of the pectoral fin of elasmobranch embryos. It is known that the first primordium of fins is a skin fold into which grow muscle buds from the myotomes of the trunk. However, the skeletal rods of the fin differentiate from the mesoderm which fills the skin fold; the rods in the middle form first, then differentiation progresses craniad and caudad. If the tissue that is still indifferent is separated by a cut from the skeletal rods that are already in the process of differentiation, then histological differentiation of pre-cartilage and cartilage proceeds in the former, but organization into separate skeletal rods does not take place. The spatial and temporal progression of this patterning apparently depends on determination that progresses into the indifferent tissue.

We can call this difference in the degree of differentiation at a given moment a differentiation gradient, as does von Ubisch (1923). A gradient is an obvious presupposition for progressive differentiation, although the latter is not a necessary consequence of the former.

This conception of progressive determination leads of necessity back to the conception that there are points in the developing embryo from which determination emanates. It is therefore not surprising to find that this idea has been advocated before. For instance, several sentences in the

paper of Boveri on the polarity of the sea urchin egg (1901) hint at an idea akin to ours. Boveri considers the possibility (*op. cit.*, p. 167) that in the sea urchin embryo

> *"every region of the blastula is prepared to form mesoderm or to invaginate and the restriction to one point is effected by the fact that at this point these processes are more readily initiated than at all other points. Once differentiation has started here, then from this point all other regions are determined for their fate by a process of regulation. The existence of such a preferential region is explained by the demonstrable differences in the properties of the cytoplasm in the different regions of the egg".*

These sentences are qualified later (*op. cit.*, p. 170) in the sense

> *"that beyond a certain zone in the animal region of the egg, the cytoplasmic quality which is necessary for gastrulation is not represented at all, or at least not in sufficient quantity".*

Shortly thereafter a similar possibility was considered for the *Triton* embryo (Spemann, 1903, p. 606).

The facts that were known earlier sufficed only to establish the concept of a starting point for differentiation, but not to demonstrate the real existence of such centers. To obtain this evidence, it is not enough to separate the region to be tested, which is believed to be such a center, from its potential field of activity. It must be brought into contact with other parts, normally foreign to it, on which it can demonstrate its capacities. This has apparently been done for the first time in the embryonic transplantations at the gastrula stage. In these experiments the organization center was left in its normal position, and indifferent material was presented to it, so to speak, for further elaboration. A much more penetrating analysis is made feasible by the transplantation of the organization center itself, and of its parts, the organizers. The present investigation makes a first beginning of this analysis. The new possibilities now opened up, particularly in combinations with heteroplastic transplantation, are not yet foreseeable. Several possible approaches to further advances have been indicated in the preceding pages.

For the moment, it is of subordinate significance whether the concepts of organizer and organization center will still prove to be useful when the analysis has advanced further, or whether they are to be replaced by other terms which would be more exact. We can already state that the concept of the organizer is the fundamental one, and that the term organization "center" shall be used only to designate the embryonic area in which the organizers are assembled at a given stage, but *not* to designate a center from which development is being directed. The designation "organizer" (rather than, perhaps, "determiner") is supposed to express the idea that the effect emanating from these preferential regions is not only determinative in a definite restricted direction, but that it possesses all those enigmatic peculiarities which are known to us only from living organisms.

IV. Summary of Results

A piece taken from the upper blastopore lip of a gastrulating amphibian embryo exerts an organizing effect on its environment in such a way that, following its transplantation to an indifferent region of another embryo, it there causes the formation of a secondary embryo. Such a piece can therefore be designated as an organizer.

If the organizer is implanted within the normal zone of invagination, then it participates in the gastrulation of the host embryo and, afterwards, shares the blastopore with it; if transplanted outside the zone of invagination, it invaginates autonomously. In this case, part of it may remain on the surface and there participate in the formation of the ectoderm and, specifically, of the neural plate; or it may move altogether into the interior and become endo-mesoderm entirely. In this event it is likely that cells of the host embryo can also be invaginated along with the transplant. Indeed, this might be considered already as a determinative effect of the implant on its environment.

In the host embryo, a secondary embryo originates in connection with the implant; it can show different degrees of differentiation. This depends, in part, on whether it interferes with the primary

axial organs, or whether it remains completely independent. In one case in the latter category, a neural tube without brain and eyes, but with otic vesicles, and also notochord, somites, and pronephric ducts developed.

These secondary embryonic primordia are always of mixed origin; they are formed partly by cells of the implant and partly by cells of the host embryo. If, in the experiments under discussion, an organizer of another species is used for induction, then the chimeric composition can be established with certainty and great accuracy. This was demonstrated for most organs, for the neural tube, somites, and even for the notochord.

There can be no doubt but that these secondary embryonic primordia have somehow been induced by the organizer; but it cannot yet be decided in what manner this occurs and, above all, when and in what way. The inductive effect could be limited to a stimulation to gastrulation, whereupon all else would follow, as in normal development. In this event, the different parts of the secondary zone of gastrulation would be subjected to determination without regard to their origin. But the induction by the implant could also continue beyond the stage of gastrulation. In this case, the organizer, by virtue of its intrinsic developmental tendencies, would essentially continue its development along the course which it had already started and it would supplement itself from the adjacent indifferent material. This might also hold for the determination of the neural plate; but it is more likely that the latter is determined by the underlying endo-mesoderm. But the development of the implant could not be pure self-differentiation; otherwise it could not have been harmoniously integrated with the secondary embryonic primordium which is smaller than the primary primordium. Apparently the inducing part, while in action, was subjected to a counter-action by the induced part. Such reciprocal interactions may play a large role, in general, in the development of harmonious equipotential systems.

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