INDUCTION BY THE PRIMITIVE STREAK AND ITS DERIVATIVES IN THE CHICK

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(With Two Plates.)

It has recently been shown (Waddington, 1932) that in the chick embryo a developing primitive streak can cause any ectoderm lying above it (provided only that the ectoderm is of the right age) to differentiate into a neural plate. It was shown that in all successful cases the grafted primitive streak had yielded both neural tissue and mesoderm, but that in some specimens the grafted neural tissue was very small in amount and was not in contact with the host ectoderm, and from this fact it was concluded that the mesoderm is capable of acting as an organiser in the absence of neural tissue. The first specimen to be described in this paper confirms this conclusion, since in this case the grafted tissue has given rise only to mesoderm, and this uncontaminated mesoderm has induced the formation of a neural plate from the host ectoderm.

The remaining specimens which will be described illustrate two main theses: firstly, that a capacity for induction is retained for some time by the derivatives of the primitive streak, and secondly, that this capacity is still possessed by the differentiated neural tissue at a stage when it appears to have been already lost by the notochord.

The technique used was similar in all respects to that described in the paper mentioned above. The embryos were fixed in Bouin's fluid, sectioned at 15μ and stained with Delafield's haematoxylin.

GRAFT OF MESODERMAL PORTION OF PRIMITIVE STREAK.

32-43. Graft: posterior two-thirds of primitive streak of 18-hour blastoderm which had a medium primitive streak1.

Host: right side of similar blastoderm.

Cultivated: 24 hours.

When the culture was fixed, an L-shaped structure could be seen in the region of the graft. The longer limb of the L was parallel with the embryonic axis of the host, and from its anterior end the shorter limb extended at right angles, with its free end

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1 The stage of development of blastoderms is indicated by a rough classification of the length of the primitive streak, thus: short primitive streak, in round area pellucida; medium primitive streak, in early pear-shaped area pellucida; long primitive streak, in advanced pear-shaped area pellucida, just before the appearance of the head process.
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turned away from the host: the longer limb could be seen to lie in or below the surface of the blastoderm, but the shorter limb consisted of a finger-like process projecting above the surface. The section plane is perpendicular to the longer limb (and to the axis of the host) and cuts the shorter limb longitudinally.

In the entire series of sections, no neural tissue derived from the graft can be found, but there is a clear neural plate induced in the ectoderm of the host. This plate runs along the whole length of the shorter limb of the L, which consists of a bag of ectoderm filled with mesoderm. At the junction of the two limbs of the L, the induced plate curves round and is continued along the longer limb; in fact, the longer limb consists of very little else, since the graft mesoderm is very thin in this region (Fig. 1). The only indication of the formation of somites is just posterior to the junction of the two limbs of the L, and it is not clear how much of the mesoderm making up these structures is derived from the graft and how much is associated with the reaction of the host to the stimulus of the graft. In two separate places, a small piece of tissue is budded off from the host endoderm, and appears to be forming a notochord (Fig. 1). As the induced plate is traced towards the posterior of the host, it becomes very thin and small. At its most posterior end (posterior, that is to say, as regards the host) there are clear indications of the formation of a head (Fig. 2). The host endoderm folds upwards and forms a fore-gut, at each side of which there is a sheet of mesoderm, which appears to be in the early stages of the development of a heart, and which is continuous with the side plate of the host. Further posteriorly, a head fold is present. The actual neural plate, however, remains very small and ill-developed throughout the head region.

GRAFTS OF THE WHOLE EMBRYONIC AXIS.

The endoderm is difficult to remove from the head process or the sinus rhomboidalis, and becomes easier to remove at a slightly later stage of development. It has, however, been found to exert little influence on the inductive capacity of the grafts. In fact, grafts of these two regions, including all three tissue layers, are perhaps the easiest to make of all these which have as yet been performed in the chick, and give the greatest prospect of obtaining a successful induction.

663. *Graft:* part of the head process from a 42-hour chick (head-fold stage) extending two-thirds of the way from Hensen’s node towards the head fold.
*Host:* left anterior region of 17-hour blastoderm.
*Cultivated:* 24 hours.

At the time of fixation a structure derived from the graft could be clearly seen, lying beside the head of the host. In the sections, the most anterior part of the grafted tissue is found to consist only of neural plate and notochord. Farther posteriorly, somites appear which are derived at least in part from the graft (Fig. 3); these somites, together with the notochord, disappear again in the most posterior part of the graft. The graft neural plate is folded into a groove at its anterior end, while posteriorly it is larger and becomes a closed tube (Fig. 3), which increases in diameter in the most posterior part where the mesodermal tissue is lacking: it disappears at about the level
of the heart of the host embryo. These facts suggest that the antero-posterior axis of the graft is reversed as regards the axis of the host; that is to say, that the most "posterior" part of the graft, lying opposite the heart of the host, is really the head region, while the part of the graft which lies opposite the head of the host is the posterior part. There is also some indication that the orientation of the induced embryo is reversed like that of the graft. Thus opposite the head of the host, the induced neural plate consists of a simple groove, which, farther posteriorly (as regards the host), becomes larger and more deeply folded (Fig. 3), and finally, opposite the heart region of the host, is very large and flat, losing its folding and spreading out into a broad shallow groove. No definite head fold is present, so one must suppose that the most anterior part of a neural plate is nowhere represented in the induced plate: but the appearances strongly suggest that the part of the induced plate opposite the heart of the host represents a more anterior region than that opposite the head, and thus that the graft and not the host has determined the orientation of the induced plate.

713. **Graft:** head process (all three tissue layers) from a blastoderm shortly before the appearance of the head fold.

**Host:** left anterior region of 13½-hour blastoderm (short primitive streak).

**Cultivated:** 23 hours.

In the cleared specimen, a structure derived from the graft could be seen; it ended anteriorly in a projection raised above the surface of the blastoderm in the manner of a head.

In the sections, the grafted and induced structures appear to the left of the host, stretching from the level of the first somite to the level of the head. In the posterior part, the grafted tissue appears as an irregularly shaped neural tube, above which the host ectoderm has formed a shallow neural groove, which is bounded on the right side by a deep fold of normal ectoderm (Fig. 4). The host neural tube is misshapen. Farther anteriorly, the induced neural groove is raised on the top of a slight swelling on the surface of the blastoderm. A mass of apparently induced neural tissue appears for a few sections below the main induced neural groove, with which it fuses anteriorly. In the most anterior region, the grafted neural tissue is absent, and the two masses of induced tissue, now fused together, form a typical head (Fig. 5). Throughout the series of sections, very little mesoderm derived from the graft can be seen, but in one place a patch of notochordal tissue is present.

32-15. **Graft:** posterior part of 50-hour embryo.

**Host:** left anterior region of 22-hour blastoderm, with medium primitive streak.

**Cultivated:** 23 hours.

An attempt was made to use only the notochord as the graft, and this was removed from the donor embryo from the most posterior region in which a definite notochord was present. The removal was, however, not a success, and a large amount of the overlying neural plate has remained attached to the notochord.

In the sections, the graft lies to the left of the host neural plate, which is flat in this region, not having folded in to make a groove. Similarly the graft and induced
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neural plates are flat, except that in one place the induced plate forms a small groove extending for four or five sections (Fig. 6). The induced neural plate lies immediately above the grafted neural plate, and is co-extensive with it, both plates stretching through twenty-five sections. The grafted notochord is confined to eight sections, and is swollen up into a large mass which is displaced to the left. The induced neural plate does not spread out to the left in the region of the graft notochord, and in fact does not appear to be affected in any way by its presence. The induced neural plate lies immediately against that of the host, but the two are never actually fused.

Owing to its flatness, it is impossible to be certain whether the induced neural plate represents a head or not, but if it does, one would have expected some indication of the presence of a head fold.

32-61. Graft: embryonic axis (all three tissue layers) from just posterior to the last somite of a 14-somite embryo.
Host: right anterior region of a 20-hour blastoderm.
Cultivated: 27 hours.

In the sections the grafted material is found lying to the right of the head of the host. It consists of a flat piece of neural tissue which has united with the endoderm of the host, of several strands of notochordal tissue and of some irregularly arranged somitic mesoderm. The neural tissue extends considerably farther anteriorly than the notochord and mesoderm (Fig. 7). The host ectoderm above the complex has formed a neural groove of moderate size, the posterior part of which lies just beside the host neural plate. In this region both the host and the induced plates are flat, but farther anteriorly the host plate becomes separated off from the surface of the blastoderm by the head fold and here both plates are more deeply folded (Fig. 7). The induced plate merges anteriorly in a fold of non-neural ectoderm which persists through several sections after the disappearance of the graft tissue. There is no indication of the formation of a head by the induced neural plate.

GRAFTS OF THE NOTOCHORD.

It is difficult to separate the notochord from the neural plate until the latter has folded in to make a closed tube. In earlier stages the two tissues do not separate from one another by mere pulling, and the boundary between them is difficult to see, so that the operation of cutting away the neural tissue is none too simple. In all about twelve successful operations have been performed. Of the two grafts described here, which are the two which have given the best suggestions of induction, the first was derived from a region in which the neural tube was already closed, while the second represents one of the few successful isolations from an earlier stage.

667. Graft: notochord from the middle of the somite region of a 25-somite embryo.
Host: left anterior region of a 21-hour blastoderm (long primitive streak).
Cultivated: 26 hours.

The grafted notochord, which lies anterior to, and to the right of, the head of the host embryo, is accompanied by a certain amount of mesoderm the origin of
which is doubtful. The host ectoderm above the graft is swollen into a small blister, and the whole surface of this is thickened. The cells of the thickened ectoderm are similar to the normal non-neural ectoderm cells, and the whole thickening bears no resemblance in gross morphology to a neural plate (Fig. 8).

695. _Graft:_ notochord from the posterior region of a 20-somite embryo.
_Host:_ left anterior region of 20-hour blastoderm (long primitive streak).
_Cultivated:_ 20 hours.

The grafted notochord lies on the left side of the host embryo, and extends parallel with it from the level of the heart to just anterior to the head. The host ectoderm lying above and slightly to the right of the graft is thickened, and the thickening extends throughout the whole length of the graft (Fig. 9). It may indicate a feeble attempt at induction, since the cells of the thickening cannot be distinguished from those of a neural plate; but the gross morphology bears no resemblance to that of a neural plate, and the development has not proceeded far enough for a certain identification to be made on the basis of the histology.

GRAFTS OF THE NEURAL PLATE.

As has been said above, it is difficult to separate the tissue layers in the early stages of development of the embryonic axis, but by the stage when the neural plate has become folded to form a closed tube, it is fairly simple to isolate it for grafting. These tubular grafts are, however, very liable to become clothed in a layer of host mesoderm and thus insulated from the ectoderm on which they are expected to act. This encystment is probably due to the comparative inactivity, as regards outwandering, of their edges: an exactly similar phenomenon is seen when coagulated tissue is used as a graft, and has up to the present made it impossible to obtain any definite information as to the inductive capacity of such material. The difficulty with neural tissue can be minimised by placing the grafts in comparatively mesoderm-free parts of the host, such as the head region, and by using flat, not tubular, pieces of neural tissue for the grafts: such pieces of tissue are most easily obtained from the head region of embryos with four or five pairs of somites.

733. _Graft:_ left wall of neural tube from head of 4-somite embryo, not including fore-brain.
_Host:_ right anterior region of 16½-hour blastoderm.
_Cultivated:_ 20 hours.

Two head-like structures could be seen in the cleared specimen. One of these appeared superficially as if it were a prolongation of the host embryonic axis; actually this is the graft and the accompanying induction, and the real head of the host is bent away to the left. It was impossible to make out the details of the construction of the composite induced and inducing structures in the entire specimen.

As the host neural plate is traced through the sections forwards from the posterior end, it is found to make a sudden bend to the left at the region of the posterior end of the fore-gut. Immediately anterior to this, the grafted neural plate appears, ac-
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companyed by an induced neural plate formed from the host ectoderm (Fig. 10). For a short space the host neural plate and the induced neural plate are confluent, but they soon separate from one another; in this region the induced plate is broad and irregularly folded, but anteriorly it narrows down to a small shallow groove (Fig. 11). At its most anterior end it juts forwards from the surface of the blastoderm in a manner which recalls a head.


The grafted neural tissue can be seen in the sections lying to the right of the head of the host. The induced and the host plates are confluent in the posterior part, forming a very large area of neural tissue, at least twice as wide as the normal host plate in other parts of the embryo. Anteriorly this structure splits into two, and the host plate becomes separated off from the rest of the blastoderm by the head fold, soon after which it disappears (Fig. 12). The induced plate continues through another fifteen sections accompanied by a small tubular mass of grafted neural tissue: at its most anterior end a head fold appears. Both the host plate and the induced plate are quite flat and unfolded throughout this region: this abnormality is quite common in the heads of chicks grown in vitro, and is usually found when two neural plates are parallel and lie near together.

DISCUSSION.

The specimen 32-43 raises several points of interest. Firstly, it is the only specimen which has been obtained in which an extensive neural plate has been induced by a graft which did not itself produce any neural tissue. Previously, where there has been not even a trace of neural tissue in the grafts, the induced plates have been very unsatisfactory. It has been pointed out that the reason for this is probably that the boundaries of the presumptive axial mesoderm and neural tissue are not exactly known, so that in making a graft it is largely a matter of chance whether one obtained both tissues together, or pure axial mesoderm, as in this case, or axial mesoderm and side plate mesoderm, as in the grafts which have given unsatisfactory inductions. It is remarkable how little mesoderm is necessary to carry out the induction: in some parts the induced plate is underlain by very little mesoderm indeed. The same phenomenon can be seen in specimen 572 (described Waddington, 1932, p. 211, see Pl. 26, figs. 53, 54, 55). It is possible that further investigation will show that it is always the anterior end of the induction which is lacking in mesoderm, and that this is due to the backward movement of all the available supplies of this tissue in the late development of the primitive streak and head process.

It is also interesting to note that the head to tail axis of the induced embryo runs in the opposite direction to that of the host. It must therefore have been controlled by the orientation of the graft, which shows that this axis can be fixed in the mesodermal portions of the primitive streak and does not depend on the spatial relations of the mesodermal and neural portions of the streak.
The formation of a head by the induced plate is also of interest, since it is certain that the graft did not include the part of the streak which would normally form the head. It is very unlikely that the graft contained even the heart region. It seems clear that the formation of the head, and probably of the heart, depends on a tendency of the induced embryo to become a whole. A similar tendency is shown by organiser grafts in Amphibia, where, as Spemann (1927, see also 1931) puts it, the induced embryo strives after wholeness. It cannot, in this specimen, be decided whether the formation of the whole goes on in the graft or in the induced embryo. In connection with the latter possibility, it may be pointed out that it has already been shown (Waddington, 1932, p. 202) that the anterior part of the primitive streak, containing both cephalic neural tissue and mesoderm, can induce the formation of a fore-gut from foreign endoderm. If an explanation of this type is to be applied here, the determination of the heart must be added to the accomplishments of the neural plate.

The other specimens described above make it clear that a capacity for induction is still possessed by such derivatives of the primitive streak as the head process and the sinus rhomboidalis. In both these areas the notochord is in the process of separating off from the endoderm below and the ectoderm above. If one may accept the tentative conclusions of Wetzel (1929) and Gräper (1929) that the mesoderm is actually invaginated through the primitive streak, one can say that the areas we are considering differ from the primitive streak chiefly in the fact that this invagination has been completed and that the determination of the presumptive medullary plate by the mesoderm is more advanced. The inductive capacity of these regions is thus to be compared with that of the gut roof with its overlying neural ectoderm in a late gastrula of Triton.

In the Urodeles, Mangold and Spemann (1927) have shown that, during and just after the process by which the roof of the archenteron comes to lie under the presumptive medullary plate, the latter acquires the capacity to induce the formation of a neural plate in foreign ectoderm: it becomes capable of performing what they call a homoiogenetic, or assimilating, induction. The specimens 733 and 755 show that a similar state of affairs is found in the chick. Both of these are examples of induction of neural tissue by the agency of already determined neural tissue.

In the Amphibia this capacity for homoiogenetic induction is retained for a very considerable time, but the data for a comparison with the chick in this respect are not yet available.

The amphibian notochord also retains for some time the inductive capacity proper to the mesoderm (Bautzmann, 1929), but loses it earlier than the neural plate loses the homoiogenetic inductive capacity. In the chick it has not yet been possible to prove that the notochord ever possesses an inductive power, but it should be pointed out that owing to the difficulty of isolating this tissue from early stages, the material which has been used in these grafts comes from embryos which are, developmentally, only slightly younger than the stage at which the capacity is lost in the Amphibia (20 somites in the chick, tail-bud stage in the Amphibia). However, even in grafts of the head process and sinus rhomboidalis, it is very noticeable
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that the induced neural plates are usually co-extensive with the graft neural plate and appear to be quite unaffected by the presence of the graft notochord. This contrasts markedly with the conformatons found in grafts of the primitive streak, where it is the mesoderm with which the induced plate is co-extensive, and the graft neural tissue which appears to have little effect. This suggests that even as early as the head process stage, the notochord has either no inductive capacity or at least much less than the neural plate.

The reversed orientation of the induced neural plate in specimen 32-43 has already been adduced as evidence of the fixation of an antero-posterior axis in the mesoderm of the primitive streak. There is other evidence to be gleaned from the specimens which have been described which indicates the existence of a similar regional differentiation in the structures derived from the primitive streak. Thus it has been suggested, on somewhat meagre evidence, it is true, that the induced neural plate in specimen 663 (graft of a head process) was also reversed in orientation. Moreover, specimens 713, 733 and 755, all of which are grafts of the anterior part of the embryonic axis, give clear indications by the formation of a head fold that the induced neural tissue represents the anterior part of a neural plate. Such indications are lacking in specimens 32-15 and 32-61, which are grafts of the posterior parts of the embryonic axis. All the grafts lie roughly in the same part of the host, namely in the anterior region near the head. We may conclude that in this region the anterior part of the embryonic axis can induce the formation of the anterior part of the neural plate, and that the posterior part probably cannot, although for the latter conclusion more experiments are desirable. Work is at present in progress to confirm this and to test the influence, if any, of the different regions of the host on the nature of the induction performed by any region of the embryonic axis. It will be noticed that the results at present indicate that, in the chick, a "posterior" organiser cannot induce a head even in the immediate neighbourhood of the head of the host, whereas Spemann (1931) has shown that in Triton it can.

The two pieces of neural tissue which were used as grafts in specimens 733 and 755 were both lateral halves of the neural tube. Nevertheless, the induced neural plates are bilaterally symmetrical, or nearly so. This is parallel with the finding of Mangold and Spemann (1927) in the Amphibia. In the chick, at any rate, it is probable that this bilateral symmetry is dependent on mechanical causes: it is very likely that any area of ectoderm which is differentiating as neural plate tissue will fold into a symmetrical tube, unless the tensions applied to it are asymmetrical.

SUMMARY.

1. Specimens are described which demonstrate the induction of neural plate by (a) the mesodermal part of the primitive streak, (b) the head process and sinus rhomboidalis, and (c) neural plate.

2. The neural plate which was induced by the mesodermal part of the primitive streak was in reversed orientation as regards the host embryo. Thus the orientation
of the embryo must be already fixed in the mesodermal part of the streak, and must
in this case have overcome any influence which the host may be able to exert.

3. The same embryo was more complete than indicated by the presumptive
date of the tissue which induced it, whence it is concluded that the chick
organiser, like the amphibian, shows a tendency to complete itself, and to this extent behaves
like part of a harmonious equipotential system.

4. Grafts of the anterior part of the embryonic axis (head process and neural
plate) into the anterior part of the host blastoderm, have induced structures which
in nearly all cases give indications of being heads. Inductions by posterior parts of
the axis (sinus rhomboidalis) have never given such indications.

5. Grafts of the notochord have not, as yet, given satisfactory inductions.

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EXPLANATION OF PLATES.

PLATE I.

Fig. 1 (32-43). Graft of mesodermal part of primitive streak. At n a piece of tissue which is
probably notochord is being budded off from the host endoderm (left and right side reversed).
(×105.)

Fig. 2. Same specimen. (×105.)

Fig. 3 (663). Graft of head process (sides reversed). (×90.)

Fig. 4 (713). Graft of head process (sides reversed). (×75.)

Fig. 5. Same specimen. The induced neural plate is forming a head. (×105.)

Fig. 6 (32-15). Graft of sinus rhomboidalis (sides reversed). (×90.)

PLATE II.

Fig. 7 (32-61). Graft of sinus rhomboidalis. (×90.)

Fig. 8 (667). Graft of notochord. (×105.)

Fig. 9 (693). Graft of notochord. (×90.)

Fig. 10 (733). Graft of neural plate (sides reversed). The host neural plate is cut longitudinally.
(×105.)

Fig. 11. Same specimen. (×105.)

Fig. 12 (755). Graft of neural plate. (×90.)

(Explanation of lettering. Host, graft and induced tissues are distinguished from one another by
the capital letters H, G and I placed before the other symbols.)
e.t. ectodermal thickening; f.g. fore-gut; h.f. head fold; h.r. heart rudiment; mes. mesoderm;
n. notochord; n.p. neural plate; n. somite.
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WADDINGTON—INDUCTION BY THE PRIMITIVE STREAK AND ITS DERIVATIVES IN THE CHICK (pp. 38–46).