Induction by Regions lateral to the Streak in the Chick Embryo

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With two plates

The discovery of the organization centre of Amphibia by Spemann (1918) opened a new field in the outlook of experimental embryology; but there were several technical difficulties to be overcome in the application of the concept of the organizer to avian embryology. In this connexion the tissue culture technique has several advantages over the earlier methods of in situ sectioning or chorio-allantoic techniques, and it has helped a great deal in the understanding of the processes of development in the chick.

It has already been proved that at least the anterior third of the primitive streak has an organizing function (Waddington, 1932); but regions outside the primitive streak have not been systematically tested so far for the inducing capacity. In the present studies an attempt is made to study the extent of the organization centre in the chick embryo at the definitive primitive streak stage.

Materials and methods

Fertilized fresh eggs, obtained from the Poultry Research Centre in Edinburgh, were incubated at 37.5° C., to the desired stage of development.

The embryos were cultivated in vitro by the technique described by New (1955). After about 22–24 hours of culturing, some of the entire specimens were fixed in Bouin and stained with dilute Delafield’s haematoxylin; they were then cleared in cedar wood oil and microphotographed. Sections were serially cut at 10 μ. They were stained in Delafield’s haematoxylin. In all, 303 grafts were made and 270 examined histologically.

The experiments consisted in cutting a single blastoderm of a definitive primitive streak stage of development (the length of the streak varied from 1.7 to 2.1 mm.) into a certain number of median and lateral squares each of about 0.3 mm. side. Tungsten needles were used for cutting the blastoderm, and the measurements were made with an ocular micrometer.

The position of the cuts may be ascertained from Text-fig. 1. The primitive
streak was cut into units of 0.3 mm square; the first cut was made to include the node level of the streak (a) and the subsequent cuts were made with it as an origin. The cuts containing the parts of the streak at different levels are labelled

\[ a, a', a^2, \ldots \] successively hindwards. Those immediately next to them on the right and left at the corresponding levels are referred to as \( b, b', b^2, \ldots \) and \( c, c', c^2, \ldots \), respectively. The squares still farther away from \( b, b', b^2, \ldots \) and \( c, c', c^2, \ldots \) are labelled \( bb, b'b', b^2b^2, \ldots \) and \( cc, c'c', c^2c^2, \ldots \), respectively. The square 0.3 mm anterior to the node is labelled \( HMa \); those on the right and left of it \( HMb \) and \( HMc \) respectively.

Each square was then removed and grafted between the endoderm and the epiblast of a host embryo of the same age (definitive primitive streak) in a manner described by Waddington (1932).

RESULTS

In the description of the specimens, the letters \( HMb, HMc, \ldots \) refer to the level of the donor blastoderm from which the graft was made. The protocol number of the operation is given afterwards. It is not possible to describe here all the important cases. One or two of each level will be described.
**HMa region**

This region is a square of about 0.3 mm. side in front of the node (a) of the primitive streak. It is the region of the presumptive forebrain. Thirty-one grafts from this level were made. Two gave inductions and 11 differentiated into neural tissue.

*HMa 30.* Length of donor streak 2 mm.; of host streak 1.5 mm. Graft placed in the left anterior region. In the section (Plate 1, fig. 1) an induced secondary neural plate was seen. It was continuous with the host neural tube. The graft seems to have fused with the endoderm of the fore-gut of the host to form a thick neural plate. A considerable amount of mesenchyme is seen scattered round about the secondary neural plate and the graft. The fusion of the graft with the endoderm, especially when the graft lies in the head, has been described by Abercrombie (1937).

*HMa 26.* Donor streak 2 mm.; host streak 2 mm. In the section (Plate 1, fig. 2) the graft has differentiated into a complete neural tube and seems to have induced a neural plate above it.

**HMb and HMc regions**

These are the squares of 0.3 mm. side on the right and left sides of the square HMa respectively. Ten grafts were made from HMb level, of which 4 differentiated into neural plates. Fourteen were made from HMc level, 5 of which differentiated into neural plates. In neither level was induction caused. Four grafts developed into hearts.

*HMb 1.* Donor streak 1.8 mm.; host streak 1.5 mm. Graft placed in the left anterior region. A very beautiful heart, derived from the graft, was seen beating in the specimen (Plate 1, fig. 3) cultured for 24 hours. It seems to be of the same shape and size as that of the host. In section (fig. 4), the graft neural plate is fused with the endoderm of the host.

*HMb 6.* Donor streak 2 mm.; host streak 1.9 mm. Graft placed in the right anterior region. The graft differentiated into a large neural tube (Plate 1, fig. 5). Even though the neural tube derived from the graft is in contact with the host ectoderm, no induction was caused.

**Node region (a)**

This is the square of 0.3 mm. side containing the node of the primitive streak. In all 20 grafts were made from this region, of which 17 caused inductions. The capacity for induction seems to be highest at this level of the primitive streak.

*a 11.* The graft appears to have been incorporated into the host (Plate 1, fig. 6) and has induced a complete secondary axis with neural tube, notochord, and fore-gut. The specimen was fixed after 46 hours of culturing.

**b and c regions**

These regions are the 0.3 mm. squares immediately to right and left of the node respectively. Thirty grafts of b were made, of which 2 caused inductions
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and 8 differentiated into neural tissue. Four differentiated only into mesenchyme. Thirty-nine grafts were made from c, of which 3 gave inductions; 14 differentiated into neural tissue and 4 into mesenchyme alone.

b 11. Donor streak 2 mm.; host streak 1·4 mm. Graft placed in the left anterior region. In the section (Plate 1, fig. 7) the induced neural tube is seen confluent with that of the host. The graft is differentiated into a neural tube, which is not complete, and there is much mesenchyme. No notochord seems to be associated either with the induced neural tube or the graft.

b 28. Donor streak 1·8 mm.; host streak 1·9 mm. In the section (Plate 1, fig. 8) the graft seems to have fused with the endoderm of the host to form a neural tube, though not a complete one. Associated with the graft is mesenchyme as well as a well-developed heart. On the inner side of the heart there is a tubule lined by endodermal cells. The graft is separated from the host ectoderm by a thick layer of mesoderm, and there is no induction.

c 6. Graft placed in the left anterior region. In section (Plate 1, fig. 9) the graft has differentiated into a neural plate fused with the endoderm, and has induced a neural plate above.

c 28. Donor streak 1·9 mm.; host streak 1·5 mm. Graft placed in the right anterior region. The graft has differentiated (Plate 1, fig. 10) into a neural plate, a notochord, and a group of mesenchyme cells on either side of it, probably forming a somite. The section is an interesting instance showing the inter-relationship between the formation of somites and the notochord, which will be considered in the discussion. In this case, although the graft has differentiated into neural tube and notochord, and lies close to the host ectoderm, it has not produced an induction.

c 27. Donor streak 2 mm.; host streak 1·5 mm. Graft placed in the left anterior region. In the section (Plate 2, fig. 11) the graft is seen to have differentiated into a well-developed heart, some mesenchyme and a round mass of cells, probably a somite. At the time of fixation two hearts were seen beating. The heart of the host is larger in lateral extent than that of the graft, and lies opposite to it.

Second level (a 1) of the primitive streak

This is a square of 0·3 mm. side behind the node. In all, 16 grafts were made from this region, 9 of which caused inductions.

b 1 and c 1 regions

These are the 0·3 mm. squares immediately to the right and left of the second anterior level of the primitive streak (a 1). Out of 25 grafts made from the b 1 region, only 1 caused a weak induction, and 5 differentiated into neural tissue. Thirty-three grafts were made from c 1, of which 5 caused inductions and 8 differentiated into neural tissue.

b 1 25. Donor streak 2 mm.; host streak 1·7 mm. Graft placed in the anterior region. The graft seems to have differentiated (Plate 2, fig. 12) into a complete
neural tube; due to the presence of the graft the diverticulum of the fore-gut on that side is withdrawn and is divided into two short diverticula.

\(c^1 15\). Donor streak 1-8 mm.; host streak 1-2 mm. Graft placed in the left anterior region. A good induced neural plate is seen in the entire specimen (Plate 2, fig. 13a). In section (fig. 13b) the induced neural plate is seen widely separated from that of the host. There is no trace of the graft other than a mass of mesoderm.

**Third level of the primitive streak (a\(^2\))**

This region is a square of about 0·3 mm. sides posterior to the second level of the primitive streak (a\(^1\)). Eight grafts were made, of which 3 gave inductions.

\(h^2\) and \(c^2\) regions

These are the 0·3 mm. squares immediately to the right and left of the third level (a\(^2\)) of the primitive streak. Out of 16 grafts made from \(b^2\) none caused induction and only 2 differentiated into neural tissue. Thirteen grafts were made from \(c^2\), of which 1 caused induction and 3 differentiated into neural tissue.

\(c^2 5\). Donor streak 1·5 mm.; host streak 1·8 mm. Graft placed in the left anterior region. In the entire specimen (Plate 2, fig. 14a) as well as in section (fig. 14b) an induced neural plate can be seen attached to that of the host in the hind-brain region. No neural tissue has developed in the graft, which is seen in section as a strand of mesoderm beneath the induced neural plate. It seems that the presence of the graft in the heart region has upset the formation and extension of that organ in the host.

\(c^2 11\). Donor streak 2 mm.; host, early head process stage. Graft placed in the right anterior region. In the section (Plate 2, fig. 15) the graft is seen to have differentiated into an almost complete neural tube. Round about are a few scattered mesenchymal cells.

**Peripheral grafts**

The peripheral grafts are difficult to distinguish from the tissues of the host. It seems from the results that none of the peripheral grafts have a capacity for induction and that they differentiate mostly into myoepicardium or mesenchyme.

\(bb\) and \(cc\) regions

These grafts were taken 0·4-0·6 mm. laterally from the node of the primitive streak on right and left respectively. Out of 8 grafts of \(bb\), 1 can be recognized as mesenchyme. Fourteen grafts of \(cc\) were made, of which 3 were recognized as forming either mesenchyme or myoepicardium. The remaining grafts of \(bb\) and \(cc\) could not be identified.

\(bb 3\). Donor streak 2 mm.; host, early head process stage. Graft placed in the right anterior region. In the section (Plate 2, fig. 16) the graft differentiated into a mass of mesenchyme arranged round roughly circular cavities.
**b₁b₁ and c₁c₁ regions**

These regions are 0·4–0·6 mm. to the right and left sides of the second level of the primitive streak. Seven grafts of b₁b₁ were made, of which 2 differentiated into myoepicardium. Of 9 grafts from c₁c₁, 2 differentiated into myoepicardium and mesenchyme.

b₁b₁ 1. Donor streak 2 mm.; host streak 1·7 mm. Graft placed in the left anterior region. In section (Plate 2, fig. 17) the graft has differentiated into a round thin-walled structure surrounded by a thin layer of mesenchyme. It was beating at the time of fixation; thus it may perhaps be myoepicardium: The surrounding layer looks like somatic mesoderm, but its nature cannot be definitely ascertained.

c₁c₁ 4. Donor streak 2 mm.; host streak 1·9 mm. Graft placed in the left anterior region. The graft (Plate 2, fig. 18) seemed to have differentiated into a well-developed heart (endocardium and myoepicardium) and a group of mesenchyme cells. Beneath the mesenchyme is seen a small, yet complete neural tube. It has probably been formed by regulation. The heart derived from the graft is almost as big as the heart of the host.

**b₂b₂ and c₂c₂ regions**

These levels are 0·4–0·6 mm. to the right and left respectively of the third level of the streak (a²). Six grafts of b₂b₂ were made, of which 2 differentiated into recognizable mesenchyme or myoepicardium. Of 11 grafts of c₂c₂, 3 developed into myoepicardium or mesenchyme.

**DISCUSSION**

From the results of the present work it appears that besides the anterior third of the primitive streak, the regions at least 0·3 mm. in front and to the side of it have an inducing capacity, though to a much lesser extent. This does not seem unlikely from consideration of the results obtained by Abercrombie (1950), who found that after reversal of a variety of different parts of the streak many cases of regulative development were obtained, showing that the primitive streak is subject to control by the surrounding blastoderm.

Bautzmann (1926) also found that in amphibia the organizing capacity is not restricted to the blastopore alone, but is spread over a quadrant of the egg.

On the basis of the results obtained in the present work, a map showing the extent of the organization centre in the chick blastoderm at the definitive streak stage may be presented. Text-figs. 2 and 3 show that the frequency of induction is highest at the node of the primitive streak, and falls off laterally as well as along the axis. It seems that there are antero-posterior and medio-lateral gradients of inducing activity. From the present work nothing can be said about the nature of the gradients, but it is clear that the regions (b, c, c₁) near the notochord have a greater inducing capacity than the parts (bb, b₁b₁, cc, c₁c₁, &c.) away from it.
### Regions of Chick Blastoderms

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**Text-fig. 2.** A map showing extent of the organization centre in a blastoderm of a definitive streak stage. The two figures at the top of each square give, first, the number of inductions obtained when that region was used as a graft, and, second, the total number of such grafts made. In the lower part of the square, the number of inductions is expressed as a percentage of the number of grafts.

![Graphic representation of the extent of the organization centre](image)

**Text-fig. 3.** Graphic representation of the extent of the organization centre.
It is interesting to note that the frequency of induction as well as of differentiation, in the grafts from the right \((b^1, b^2, \&c.)\), is less than that at the corresponding levels from the left \((c^1, c^2, \&c.)\). The grafts from the right at these levels have not caused any induction. It is probable that the asymmetry of structures shown in the adult bird is seen even at such an early stage of development. Reference to the asymmetry of the node at the primitive streak stage has been made by Jacobson (1938). The superior developmental capacity of the grafts from the left over those from the right, at the stage of the head process blastoderm, has been shown by Rawles (1936, 1943) and Rudnick (1932), with the chorio-allantoic technique.

A correlation between induction and the self-differentiation of the graft into neural tissues was suggested by Waddington (1952). The present results agree in some respects with this suggestion. The more peripheral grafts, from \(bb, b^1b^1, b^2b^2, \text{ and } cc, c^1c^1, c^2c^2, \&c\) (Plate 2, figs. 16, 17, \&c.) which have never differentiated into neural tissue have not caused any induction, while some grafts from \(b, c, c^1, \&c\), close to the anterior levels of the primitive streak, have differentiated into neural tissue, and have induced (Plate 1, figs. 7, 9, \&c.). At the same time it must be remembered that some grafts (Plate 1, figs. 5, 10, \&c.) which have differentiated well have not produced inductions.

**Text-fig. 4.** Maps of the presumptive areas in a chick blastoderm of a definitive streak stage. A, after Pasteels (1937); B, after Spratt (1947 a, b, 1952, 1955); C, after Waddington (1952). In Figs. A and C the presumptive neural tissue is indicated by vertical lines, the presumptive chorda by dots, the presumptive axial mesoderm by close horizontal lines, and non-axial mesoderm by spaced horizontal lines; already invaginated mesoderm is shown by crosses. In Fig. B the presumptive neural plate is shown by the lined area in the left figure, while on the right there are indications of the presumptive chorda (Pch.), somite (PS), and lateral plate (PLP).

It was suggested by Hoadley (1927), Gräper (1929), Wetzel (1929), Umanski (1931), and Dalton (1935) that induction should be primarily due to the axial mesoderm; and this view was adopted by Waddington (1932) when he showed that the streak is able to induce. It is thus worthwhile discussing the position and extent of the various presumptive tissues with the help of the results obtained in the present work. But in any isolation experiments (chorio-allantoic, \textit{in vitro}, intrablastodermal grafts) there may be regulation within the isolate, and organs may appear from regions which are not their presumptive origin. With this
caution in mind, an attempt will be made to discuss the position and extent of the various presumptive tissues.

The localization of the presumptive tissues in the early and definite streak stages has been mapped out by several investigators, such as Gräper (1929), Wetzel (1929), Pasteels (1937), Waddington (1932, 1952), Rudnick (1938a, b, c), Spratt (1942; 1947a, b; 1952; 1955), and several others. It is beyond the scope of the present work to discuss all the maps. The maps given by Pasteels (1937), Spratt (1947a, b; 1952; 1955), and by Waddington (1952) are shown in Text-fig. 4.

Presumptive neural tissue

In the definitive streak stage, according to Pasteels, the presumptive neural tissue is crescentic in shape, the two arms tapering off and extending towards the middle of the streak. In Spratt's map the neural plate is ‘oval’ in outline (the node lying in the centre), extends about 0.25 mm. anterior, 0.4 mm. posterior, and 0.45 mm. lateral to the primitive pit (Spratt, 1947a, 1952). In Waddington's maps the presumptive neural area is in the shape of a horseshoe, the two arms extending along about one-third of the streak. No measurements are given either by Pasteels or by Waddington.

In the present work neural differentiation was obtained from the regions HMa (Plate 1, figs. 1, 2) (up to 0.3 mm. in front of the node level); HMb, HMc (figs. 5, 6) (lateral to HMa), and up to 0.6–0.9 mm. (Plate 2, fig. 15) behind the node level of the primitive streak. The posterior extension of the neural tissue in the definitive streak stage has been studied by several workers. Wetzel (1924) at first failed to get neural tissue from the posterior regions behind the node, though later (1926) he obtained it from some of the posterior pieces. Hunt (1932), using the chorio-allantoic technique, could not obtain neural differentiation behind the node level, but Dalton (1935) using the same technique succeeded in getting neural differentiation from about 0.5 mm. behind the node. Waddington (1935) obtained neural tissue in vitro from parts of a blastoderm as far back as 0.7 mm. posterior to the pit. On the other hand, Rudnick (1938b) and Spratt (1952), using the in vitro technique, claimed that neural plate never developed in posterior pieces cut from about 0.4 mm. behind the pit. As mentioned above, the region 0.6–0.9 mm. behind the node has formed perfect neural tube (Plate 2, fig. 15), and, moreover, one of the grafts from the same level has caused induction as well (fig. 14a, b). Waddington (1952) has suggested that the boundary between the inducing and non-inducing region is shown by the limit at which the neural tissue can develop in isolates in vitro; however, Abercrombie & Bellairs (1954) and Islam (1953) have reported inductions through the posterior third of the primitive streak, which is the site of presumptive lateral plate mesoderm, and which itself has not so far been described as forming neural tissue.

The capacity for self-differentiation into neural tissue independently of the mesoderm has been shown only for the fore-brain, by Waddington (1932),
Rudnick (1938b), and Spratt (1942, 1947c). In the present work the graft HMa 28 (not shown), which was labelled with radioactive methionine, clearly shows the self-differentiation of the graft (presumptive fore-brain) independently of the mesoderm. However, no other part of the central nervous system besides the fore-brain is described so far as possessing this capacity, and thus the differentiation of neural tissue from the posterior levels may be due to its induction by the underlying mesoderm as suggested by Waddington. In those cases in which neural tissue is produced by fragments from the posterior end of the streak, the mesoderm has probably regulated to a more 'anterior' condition before inducing the neural tissue.

**Presumptive axial mesoderm (somites and notochord)**

In the definitive streak stage the presumptive axial mesoderm in Pasteels's map is seen as a narrow band occupying more than half the length of the streak. This corresponds more or less with the position and extent of that tissue shown by Gräper, Wetzel, and Waddington. Spratt (1955) makes a further distinction between the somite centre, restricted to about 0.1-0.2 mm. posterior to the primitive pit, and the prospective somite cells found as far as 0.6-0.8 mm. behind the node.

In the present study it is observed that the differentiation of somites and notochord, the typical structures of the axial mesoderm, is rarely seen except in a few grafts from region c (Plate 1, fig. 10; Plate 2, fig. 11). The mesenchyme in these grafts tends to arrange itself into round masses appearing like somites.

The tendency of the mesoderm to form somites in some grafts from c may be due to the fact that these grafts lie adjacent to the node level of the primitive streak, which is perhaps the region of the notochord. Interrelation between the chorda and somites has been suggested by Spratt (1942) and Islam (1953) in the chick, and by Yamada (1940) and others in Amphibia, although Abercrombie & Bellairs (1954) have doubts about the situation in the chick.

The lateral extent of the axial mesoderm seems to correspond to a narrow band, as shown in Pasteels's mapping scheme. The more peripheral grafts, bb, b1b1, b2b2, cc, c1c1, &c., in the present study have shown poor differentiation, except for myoepicardium.

In the present study the notochord is not seen to differentiate from any region other than the node, except in one single instance (Plate 1, fig. 10), in which it is formed from the region adjacent to the node level on the left (c). It is difficult to say whether in this case the notochord is formed by regulation, or if its presumptive area is spread out laterally as far as this.

It is also seen that the inductions, when caused by lateral grafts, were mostly neural inductions with little or no accompanying mesenchyme. The induced neural plate was not associated with notochord in any case (Plate 1, fig. 9; Plate 2, figs. 13b, 14b). It may be that the grafts from these various lateral levels did not contain the region of the presumptive notochord and that the absence of
notochord in the induction is to be accounted for by this fact. On the other hand, one might ask, are the notochordal inductions specific? Or, in other words, are there different substances for neural and mesodermal inductions?

Heart-forming areas

In the definitive streak stage Hunt (1932) found the highest frequency of heart formation in the pieces lying about 0·3 mm. behind the primitive pit. Rudnick (1938b), with in vitro culturing technique, reported heart differentiation as far back as 1 mm. behind the pit. In the head process stage Rawles (1943) obtained the formation of heart anteriorly up to 0·5 mm. and posteriorly 0·4 mm. from the primitive pit. The lateral extent in that stage was found to be from 0·2 mm. to each side of the pit, to a region at least 0·4 mm. distant.

In the present study differentiation of heart has occurred in the grafts from various levels. To give a few instances, the graft HMb (0·15–0·45 mm. lateral to the axis) has formed a beautifully developed heart beating at the time of fixation (Plate 1, fig. 3). Hearts were seen to differentiate from the grafts from regions b and c (Plate 1, fig. 8; Plate 2, fig. 11, &c.) and from a number of peripheral grafts from $b^1b^1$, $c^1c^1$, $c^2c^2$ (Plate 2, figs. 17, 18, &c.). The peripheral grafts $c^1c^1$, $c^2c^2$, in which the heart-muscle seems to develop, are 0·45–0·75 mm. lateral to the various levels of the primitive streak, and some $c^2c^2$ (not shown), were a similar distance behind the node. It appears from this that in the primitive streak stage the heart-forming area is larger than in the head process stage as described by Rawles (1943); this reminds one of the gradual contraction of the area found by Ebert (1953) to contain cardiac myosin.

SUMMARY

1. Blastoderms of the definitive primitive streak stage were cut into as many as eighteen squares of about 0·3 mm. side (cf. Text-fig. 1). The inducing capacity of each small piece was tested by transplanting it into the area pellucida of a host blastoderm (in the definitive streak stage), cultured by an in vitro technique. In all, 303 grafts were made and 270 histologically examined.

2. From the results obtained it seemed probable that the grafts from the level immediately (0·15–0·45 mm.) in front of the node, and a similar distance lateral to the node and the anterior third of the primitive streak, have an inducing capacity, though to a much lesser extent than the node itself.

3. The results of the present work make it probable that the inducing capacity falls off not only along the axis of the blastoderm but also medio-laterally. It appeared as if there were gradients in inducing capacity along the longitudinal and transverse axes of the blastoderm. A map showing the extent of the organizer in the blastoderm of a definitive streak stage is given (Text-figs. 2 and 3).

4. The frequency of induction and differentiation was higher in the grafts
from the left side of the blastoderm than in those from the right. This is perhaps the earliest manifestation of the asymmetry of structures found in the adult bird.

5. Peripheral grafts appeared to differentiate only into myoepicardium or mesenchyme.

6. The map of presumptive areas in the definitive streak stage chick blastoderm is discussed with reference to the results obtained.

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REFERENCES


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Plate 1
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Plate 2
REGIONS OF CHICK BLASTODERMS


EXPLANATION OF PLATES

Abbreviations: c, cavity; fgt., fore-gut; hd., head; ht., heart; mes., mesenchyme; my.ep., myocardium; n., notochord; n.p., neural plate; s., somite; T., tubule lined by endoderm. The prefixed letters H, G, and I denote the structures of the host, graft and induced axis respectively.

PLATE 1

Fig. 1. HMa 30. Section through anterior region (x 80).
Fig. 2. HMa 26. Section (x 70).
Fig. 3. HMb 1. Whole mount fixed after 24 hours of culturing (x 10).
Fig. 4. HMb 1. Section of the same passing through two hearts (x 70, sides reversed).
Fig. 5. HMb 6. Section (x 70, sides reversed).
Fig. 6. a 11. Section showing the induction of a complete secondary axis (x 70).
Fig. 7. b 11. Section showing host, induced and graft neural plates (x 70 sides reversed).
Fig. 8. b 28. Section showing the structures of the host and those derived from the graft (x 80).
Fig. 9. c 6. Section (x 75).
Fig. 10. c 28. Section (x 70, sides reversed).

PLATE 2

Fig. 11. c 27. Section through hearts (x 80, sides reversed).
Fig. 12. b 1 25. Section (x 80).
Fig. 13a. c 1 15. Whole mount showing the two embryonic axes (x 10, sides reversed).
Fig. 13b. c 1 15. Section of the same through neural plates (x 70, sides reversed).
FIG. 14a. c^2 5. Whole mount (x 10).
FIG. 14b. c^2 5. Section of the same through the heart region (x 70, sides reversed).
FIG. 15. c^2 II. Section (x 70).
FIG. 16. bb 3. Section through the graft (x 320).
FIG. 17. b^4 b^4. Section (x 80).
FIG. 18. c^4 c^4. Section (x 80).

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