Jacobson, Antone Gardner

1963. The Determination and Positioning of the Nose, Lens and Ear.
III. Effects of Reversing the Antero-posterior Axis of Epidermis, Neural Plate and Neural Fold.

J. Exp. Zool., v. 154, no. 3, December.
Pages 293–304.

PRESS OF
THE WISTAR INSTITUTE
OF ANATOMY AND BIOLOGY
PHILADELPHIA, PA., U.S.A.

Reprinted from THE JOURNAL OF EXPERIMENTAL ZOOLOGY
Vol. 154, No. 3, December 1963

The Determination and Positioning of the Nose, Lens and Ear

III. EFFECTS OF REVERSING THE ANTERO-POSTERIOR AXIS OF EPIDERMIS, NEURAL PLATE AND NEURAL FOLD [1]

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To account for the antero-posterior distribution in the developing embryo of noses, lenses and ears there are several possibilities. An attractive hypothesis is to relate this specificity to the different neural inductors of these organs. Specific inductor substances from forebrain would specify nose formation from the epidermis, substances from the retina would specify lens formation, and substances from the hindbrain would specify ear formation. This hypothesis is not by itself sufficient since noses, lenses and ears can form in reasonably proper order in embryos lacking the entire neural plate (Jacobson, '63a).

Another likely source of qualitatively different inductor substances distributed along the antero-posterior axis is the endoderm which is itself, at early neurula stages, largely a mosaic of future organ parts. However, normally positioned noses, lenses and ears form in endoderm-free embryos and also in embryos in which the antero-posterior axis of the endoderm has been reversed (Jacobson, '63b).

It is not a simple matter to test the mesoderm for its capacity to position nose, lens and ear. However, embryos lacking all mesoderm and all endoderm can form normally positioned noses, lenses and ears; so the mesoderm cannot be all important (Jacobson, '63b).

In explant, the prospective placodal epidermis of the early neurula forms no noses, lenses or ears when isolated alone, and forms normally positioned noses, lenses and ears only when neural plate, neural fold, mesoderm and endoderm are all present (Jacobson, '63a).

It seems there is an involvement of many tissues in nose, lens and ear positioning, with the possibility that one group of tissues alone can accomplish the whole job when the others are removed or misplaced.

The experiments in this paper were designed to examine more critically than heretofore the problem of responsibility for positioning of nose, lens and ear. This has been done by reversing the antero-posterior axis of the prospective placodal epidermis and of associated tissues.

MATERIALS AND METHODS

These experiments were done with the west coast newt, *Taricha torosa* and with *Amblystoma punctatum* from North Carolina. Eggs were shipped to Texas in ice water and were one day enroute. They usually arrived at a temperature of 8°C. Operations were performed with iridectomy scissors and hair loops in Holtfreter's solution. Glass bridges were occasionally required to hold transplanted tissues in place until they healed. Embryos were reared in 66% Holtfreter's solution at 17°C for 10 to 15 days when they reached larval stages then fixed, serially sectioned, stained and examined microscopically.

EXPERIMENTS

Most of the operations involve reciprocal transplantations of tissues between two early neurulae. Figure 1 illustrates the procedure and the results. By exchanging right and left halves of the prospective

[1] This investigation was supported by Public Health Service Research Grants RG-8494 and GM-08494-02, from the Division of General Medical Sciences.

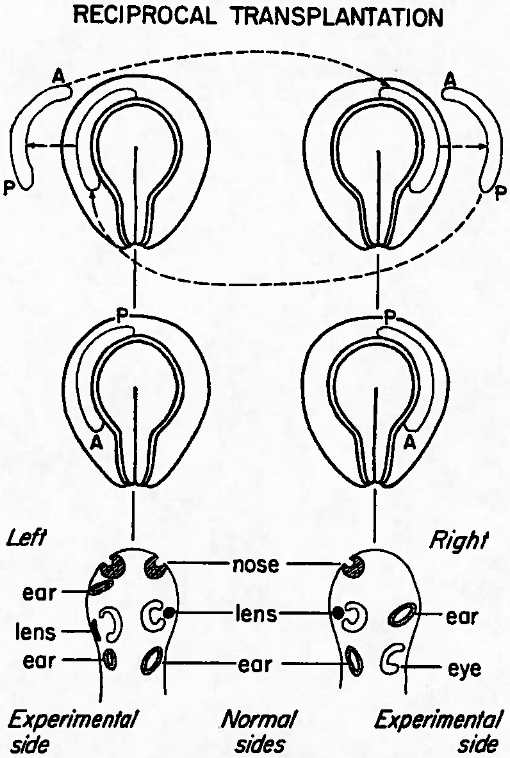


Fig. 1 Operation scheme of reciprocal transplantations reversing the antero-posterior axis of the prospective placodal epidermis. When the operated embryos reached larval stages serial sections were made and examined. The positions of the placodal structures that formed are indicated in the lower drawings.

placodal epidermis between two embryos as shown, the antero-posterior axis of each half is reversed without reversing any other axes. The drawings illustrating the results are derived from examination of either serial frontal sections or serial transverse sections of the operated embryos made after they reached the larval stages.

Figures 2 through 13 describe and illustrate the rest of the operations and their results. In operations two and three a slit was made in the epidermis just lateral to the neural fold on the right side of one embryo and the left side of another. A third embryo was the donor of the tissue. The prospective placodal epidermis from the donor's left side was put in the slightly gaping wound prepared in the right side of the host embryo. The antero-posterior axis of the implant was reversed before putting it in position. The donor's right placodal epidermis was similarly placed in the host with the slit on the left. These embryos therefore had their own placodal epidermis ventral to the implanted piece with the reversed antero-posterior axis. Operation four was done in a similar fashion to operations two and three, with the exception that the donor's neural fold was included along with the prospective placodal epidermis and the slit in the host was made by removal of the neural fold where the implant was to be made. Operations 5, 6, 7, 8, 9 and 10 were done in the same way as the operation illustrated by figure 1. Operations 11, 12 and 13 involved removing the tissues indicated, rotating them 180° and reimplanting them in the same embryo from which they were cut out.

The operations illustrated by figures 1 through 4 were done on *Taricha torosa*. The rest of the operations were done on *Amblystoma punctatum*. Operations illustrated by figures 5, 6, 7, 11, 12 and 13 were done on *T. torosa* as well as *A. punctatum*, but results were so similar with *T. torosa* to those shown for *A. punctatum* that I have not illustrated them.

Reversal of the prospective placodal epidermis (operations 1, 2 and 3) put prospective ear epidermis next to forebrain and other nose inductors, and prospective nose epidermis next to hindbrain and other ear inductors. The lens region was not shifted; it was pivoted on. Ears formed both anteriorly out of the prospective ear epidermis (figs. 14 and 15) and posteriorly out of the prospective nose epidermis. The ear region mesoderm and hindbrain would account for induction of the posterior ears, but the anterior ears that form next to nose, eyes and forebrain must have been determined before the epidermis was reversed. This early determination of the ear is not detected in explant experiments (Jacobson, '63a). Noses that formed from the reversed epidermis in their normal anterior positions were presumably induced there by endoderm and forebrain. Since the noses and ears formed adjacent to one another, it is clear that there are no interactions between nose and ear fields that prohibit formation of one or another of these organs.

No important differences were noted when neural fold with prospective placodal epidermis was reversed (operation 4). An ear formed anteriorly and seemed to compete with the eyecup for space (fig. 16).

When the placodal epidermis and neural fold were reversed at a late neurula stage (stage 19, operation 8), ears and noses formed both anteriorly and posteriorly. It is apparent that at late neurula stages the ear and nose epidermis is sufficiently determined for ears to form in the reversed epidermis next to the forebrain and other nose inductors (fig. 17) and noses to form next to hindbrain and other ear inductors (fig. 18). At the same time the epidermis is still sufficiently labile to respond in its new position to the nose and ear inductors, so noses are induced next to the anterior ears and ears are induced next to the posterior noses.

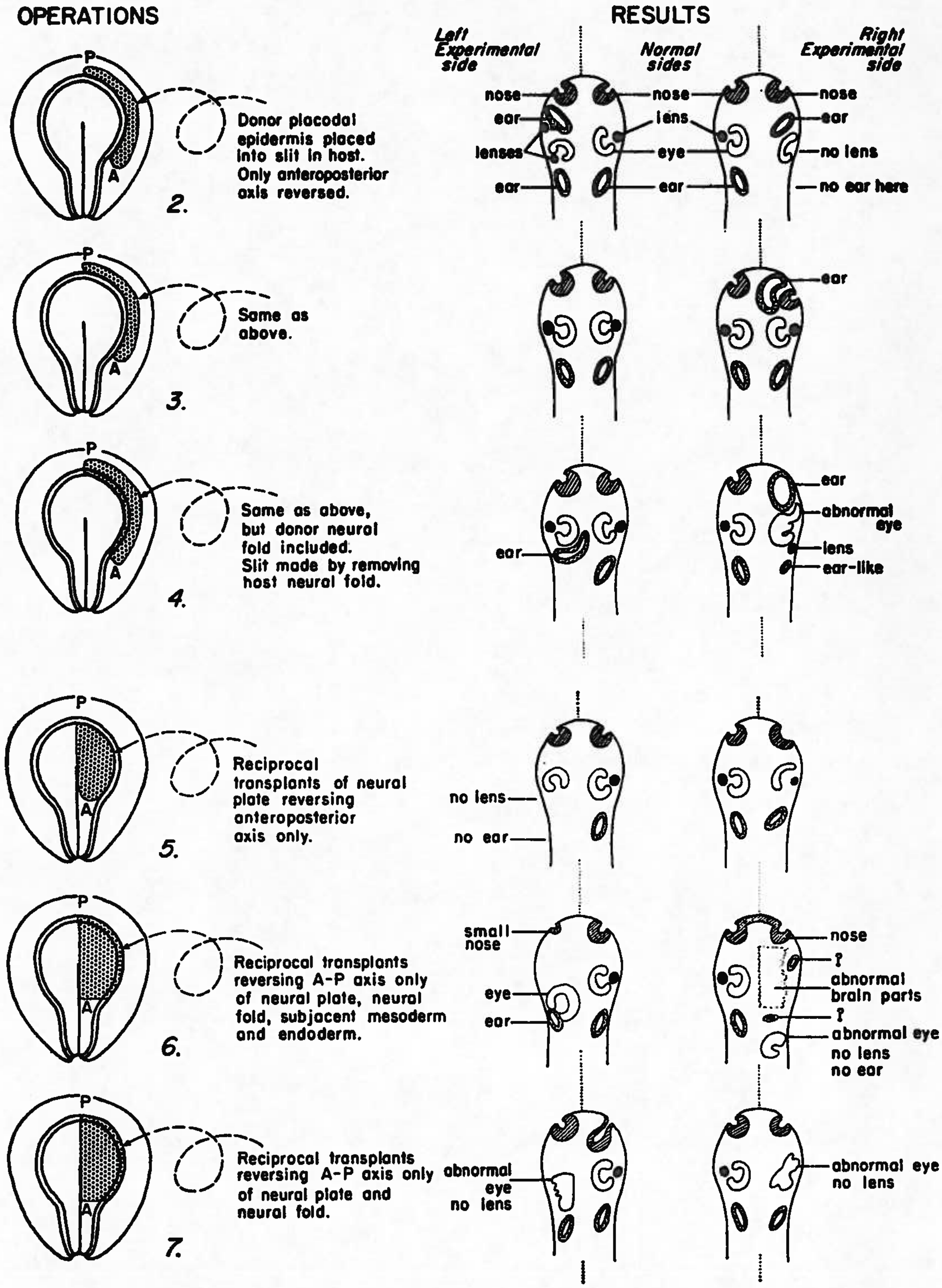
When one-half the neural plate (operation 5), or neural plate, fold and subjacent mesoderm (operation 6), or neural plate and fold (operation 7), were reversed, noses, lenses and ears still formed in their normal sites for the most part. They did this despite the inappropriateness of the adjacent neural inductors. When the brain plate is reversed unilaterally (operations 5, 6, 7, 9, 10), there are some accommodations made along the midline where the reversed part of the brain fuses with the half not reversed, giving the appearance of some regulation in the reversed part (figs. 19, 20, 21). This may be more appearance than fact. In some examples, even though the reversed half has fused to the normally positioned half, each of the two sides gives the appearance of having reconstituted some contralateral parts, so the brain is broader and the canals are separate and laterally displaced.

On *A. punctatum*, the same species used in my experiments, Roach ('45) examined brains following unilateral or bilateral reversal, with or without underlying mesoderm, at preneurula stage 13 and early neurula stage 14 and reported that brain parts always developed in the order appropriate to their original position in the embryo. In my material, the brain develops without regulation in the reversed position following reversal of the entire brain plate (operations 11, 12, 13; figs. 23, 24, 25), but following unilateral reversal the brains are so abnormal in appearance that I would hesitate to say whether the reversed part has regulated or not. The brain is much more abnormal when the substratum is reversed along with half of the brain plate (operation 6, fig. 20) than when half the brain plate without substratum is reversed (opeeration 5, fig. 19, operation 7, fig. 21). So far as the induction of placodal structures is concerned, the results following reversal of the entire brain plate are essentially the same as the results after reversing half the brain plate so the abnormal differentiation of the reversed brain halves is most likely not of particular importance for this study of placode induction.

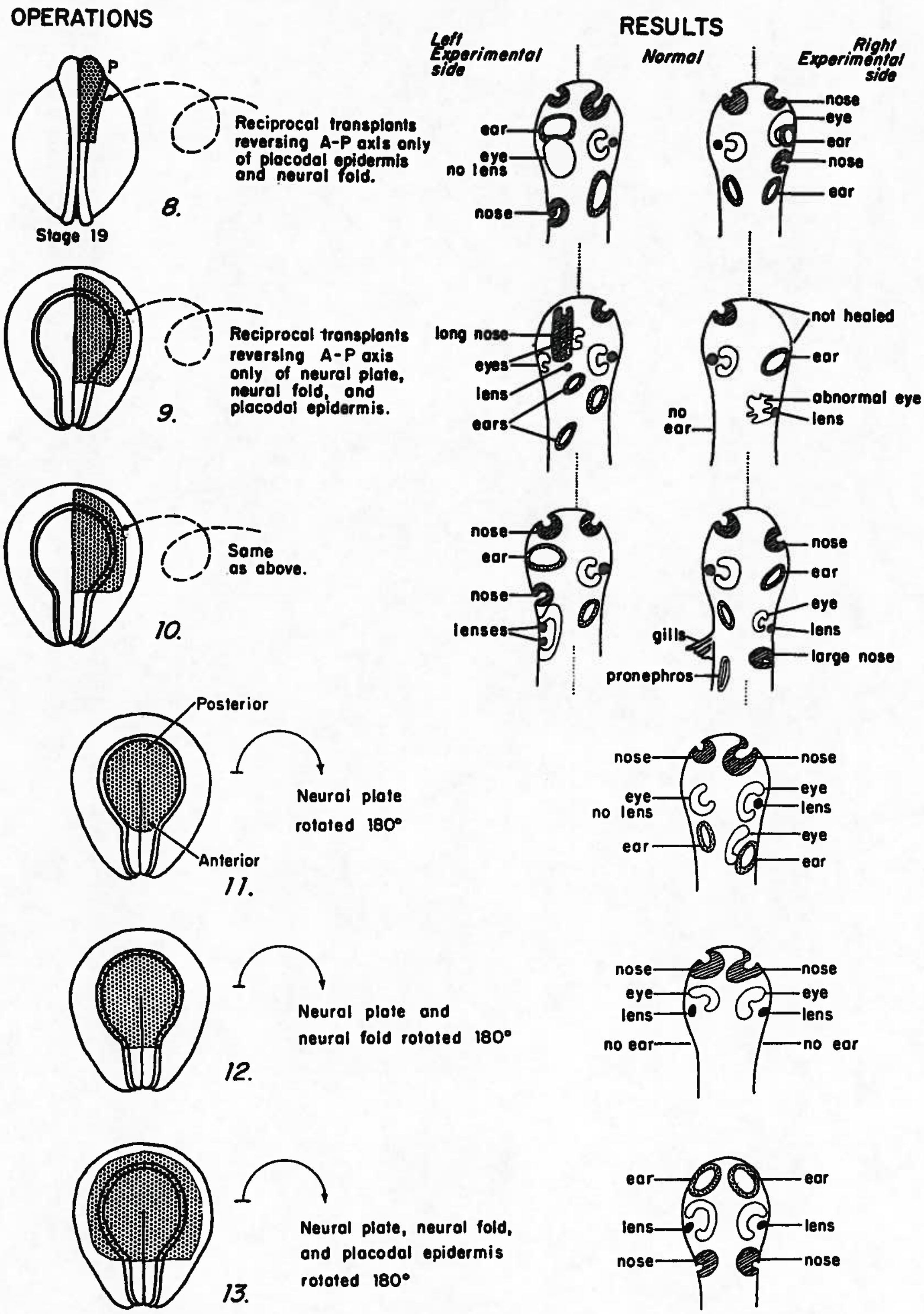
Reversal of half the neural plate, fold and placodal epidermis (operations 9, 10) resulted in ears forming anteriorly in the reversed epidermis along side noses; and noses appearing as far back as the pronephros region (fig. 22). In this operation the epidermis is reversed at a time when to some extent it is already determined for ear and nose. The neural components, reversed along with the epidermis, can continue the process of determination of nose and ear in their reversed positions. The non-neural inductors of nose and ear, the endoderm and mesoderm, are left in their original positions where they induce from the reversed epidermis noses anteriorly and ears posteriorly. These results emphasize again the primary importance of the non-neural inductors in the elicitation of nose and ear.

Reyer ('62), in *A. punctatum*, obtained noses from prospective nose and lens epidermis transplanted to the ear region at open neural plate stages, suggesting that the nose epidermis is already determined at these stages. He came to the same conclusion from transplantation of the early neurula prospective nose epidermis to the larval eye or dorsal fin. Many of my experiments support the idea of an early determination of nose, lens and ear.

After reversal of the entire brain plate (operation 11), noses, lenses and ears formed at reasonably normal levels along the antero-posterior axis. One ear sat in an extra eye-cup (fig. 23) suggesting, as did other examples in operations 6, 8, and



Figs. 2–7 Operation schemes and results for operations 2–7. See text for further explanation.



Figs. 8–13 Operation schemes and results for operations 8–13. See text for further explanation.

11, that there is no rigid relationship between retina and lenses.

When the entire brain plate and adjacent neural fold was reversed (operation 12), noses formed anteriorly next to the hindbrain (fig. 24). When the entire brain plate, neural fold and placodal epidermis were reversed (operation 13), noses, lenses and ears formed next to their appropriate neural inductors and were thus in reverse order in the animal (fig. 25). These larvae had head parts raised dorsally giving them the appearance of having their heads on backwards and slightly raised to look down their backs towards their tails. When the parts were turned, anterior epidermis and neural fold were interposed between spinal cord and forebrain so these parts of the nervous system are not fused to each other. Noses opened posteriorly and ears formed at the anterior end of the larva.

The results of operations 11, 12, and 13 are instructive in showing that the most important forces in the elicitation and positioning of nose, lens and ear are not the neural inductors, but rather the endoderm and mesoderm that through inductive interactions have already, at open neural plate stages, to some extent fixed the positions in the epidermis of the nose, lens and ear.

DISCUSSION

The ear is induced by a sequence of inductors, principally the underlying mesoderm during gastrula and neurula stages, and later by the medulla of the brain. The ectoderm acquires competence to respond first to the mesodermal inductor and later to the neural inductor. These different ear inductors can elicit ear formation in competent foreign epidermis when acting alone, but complete differentiation of the ear is achieved only when the two inductors as well as the rest of the normal ear environment are all present and functioning together (Harrison, '45; Yntema, '50, '55).

The lens is similarly induced by a sequence of inductors; by endoderm and mesoderm during gastrula and neurula stages and by prospective sensory retina following outgrowth of the optic vesicle. Either inductor can be itself elicit lens formation, but normally the non-neural inductors must act before the neural inductor is effective. Completely differentiated and persistent lenses form only when all inductors act in their normal sequence in the normal environment of the lens (Liedke, '51; Jacobson, '55, '58, '63a, '63b).

The nose too is induced by a sequence of inductors, first by underlying endoderm in late gastrula and neurula stages, then by the forebrain. Noses can be elicited by either of these inductors alone, but normal differentiation requires that both act in sequence (Reyer, '62; Jacobson, '63a, '63b).

From the evidence presented in this paper I conclude that induction of the nose, lens and ear begins before neurula stages. From the open neural plate stage to late neurula stages the prospective placodal epidermis is both labile enough to respond to new inductive situations and to form appropriate organs, and determined enough to follow its original prospective fate despite being placed in new situations. Whether a nose, lens or ear will form in some particular site depends on the state of determination of the epidermis resulting from past inductive relationships and on the balance of inductive directions continuing to act from all sources. The position that one of these organs will occupy is fixed, therefore, by its entire history of inductive interrelationships with its entire environment.

SUMMARY

1. The determination and positioning of the nose, lens and ear was studied in the salamanders *Taricha torosa* and *Amblystoma punctatum*.

2. Reversing the antero-posterior axis of the prospective placodal epidermis or placodal epidermis and neural fold at early neurula stages results in ears forming both anteriorly and posteriorly. Placodal structures are already determined to some extent at these early stages.

3. Reversing the antero-posterior axis of the brain plate at early neurula stages does not affect the site of formation of nose, lens or ear.

4. If placodal epidermis is reversed along with half the brain plate and neural fold, noses and ears will form both anteriorly and posteriorly. When the entire brain plate and adjacent neural fold and

placodal epidermis are reversed, the noses, lenses and ears form in accord with the reversed tissues.

5. There is no evidence for competitive interactions in adjacent nose, lens and ear fields. These organs often formed next to one another. Specific neural inductors of one organ do not prevent another from forming at that site. Ears formed in eye cups and next to forebrain, noses formed next to hindbrain.

6. Where a nose, lens or ear forms in the embryo depends on instructions from all parts of the environment. When the instructions are conflicting, both organs may form side by side. The major conclusion from these experiments is that the course of differentiation of a group of cells is determined by its history of interactions with all the tissues in its environment, and not by one or another specific inductor tissue alone.

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PLATE 1

EXPLANATION OF FIGURES

14 In figures 14–22 the animal's left is to the reader's right and the animal's right is to the reader's left. This cross section is through a larva resulting from operation 1 in which the prospective placodal epidermis is reversed on the left side (fig. 1, left). An ear lies next to the forebrain.

15 Reversed placodal epidermis implanted into a slit on the right side (operation 3, right) has formed an ear between forebrain and eye.

16 Reversed placodal epidermis and neural fold implanted into a slit on the right side (operation 4, right) has formed an ear between forebrain and eye.

17–18 The left placodal epidermis and neural fold was reversed at late neurula stage 19 (operation 8, left). An ear has formed anteriorly (fig. 17) next to nose, eye, and forebrain. A nose has formed posteriorly (fig. 18) next to hindbrain.

19 The right half of the brain plate was reversed (operation 5, right) and a nose has formed next to what was prospective hindbrain.

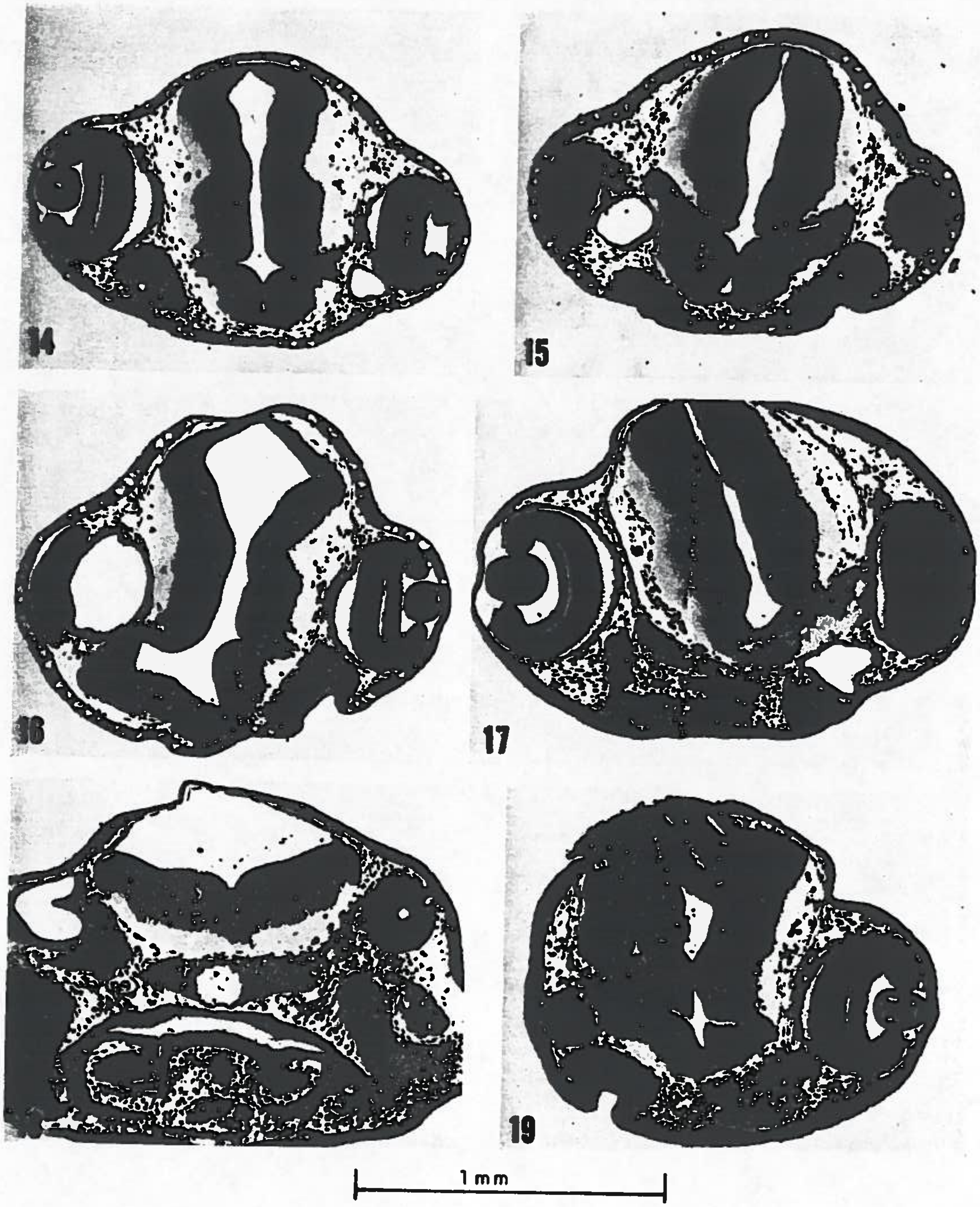
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PLATE 2

EXPLANATION OF FIGURES

20 Section through the eye of the normal side on an animal in which the right half of the brain plate and fold and subjacent mesoderm and endoderm was reversed (operation 6, right). The reversed brain part is badly malformed and fills the area all the way to the epidermis.

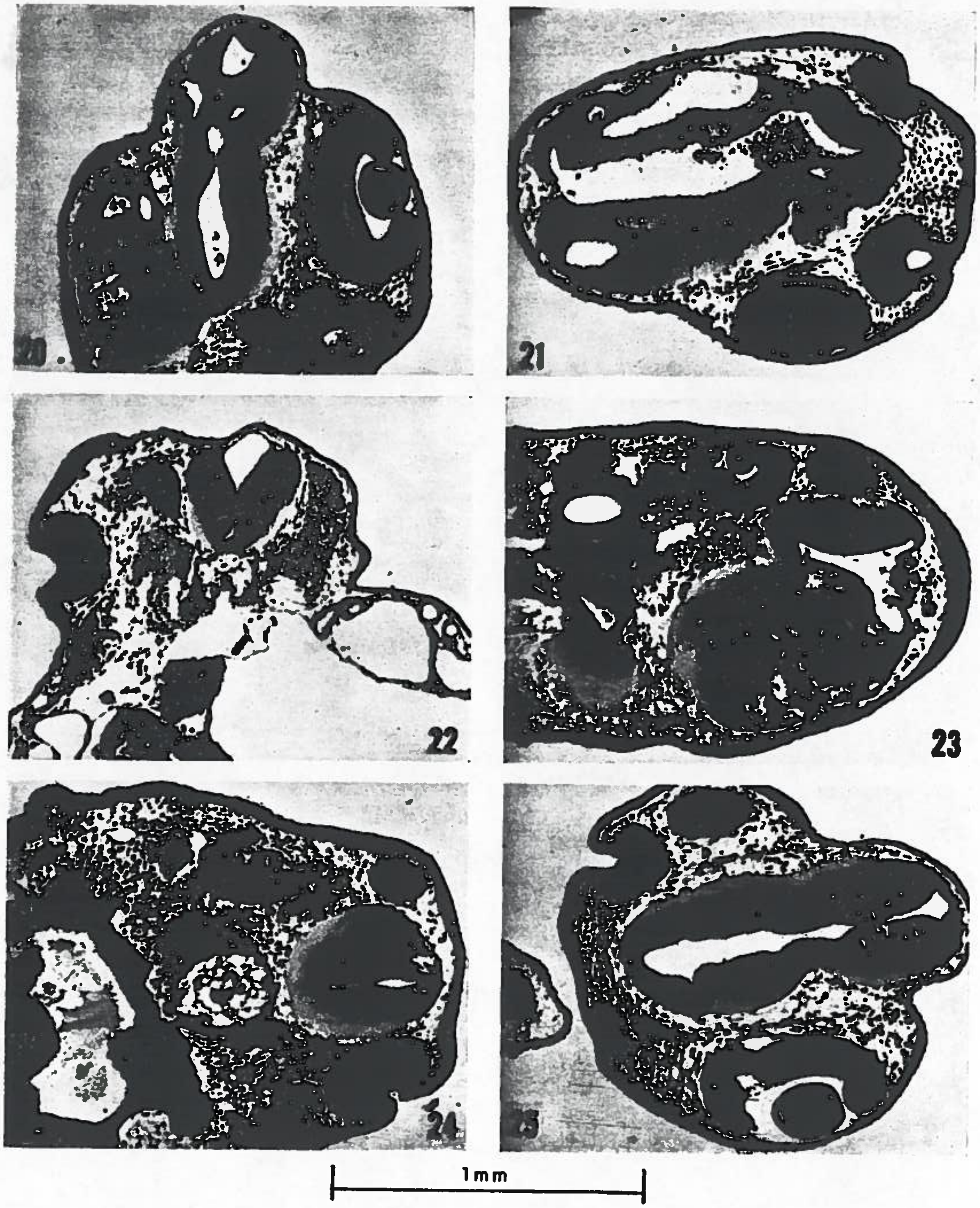
21 The left half of the brain plate and fold was reversed (operation 7, left) and a nose has formed in its usual anterior position next to what used to be the posterior part of the brain (at the top in the photograph).

22 The right half of the brain plate, neural fold and placodal epidermis was reversed (operation 10, right). A nose has formed posteriorly at the level of the pronephros. The neural tissue between the nose and the hindbrain is the most posterior extension of the reversed forebrain. Several sections anterior in the embryo this brain tissue fuses with and occupies the position at the right of the normally positioned hindbrain.

23 Frontal section of a larva resulting from operation 11. The entire brain plate was reversed. Along the top of the picture a nose, lens and ear can be seen in their normal antero-posterior distribution. The nose is next to hindbrain and the ear lies in an extra eyecup.

24 Frontal section of a larva resulting from operation 12. The entire plate and neural fold was reversed. The two noses shown are in their usual anterior positions, but lie next to the reversed hindbrain. Some adherent notochord has been reversed with the brain.

25 Frontal section through a larva resulting from operation 13. The entire brain plate, neural fold and placodal epidermis was reversed. The reversed head projected dorsally so although this section is frontal through the embryo, it is a cross section through the head. The nasal sac opens toward the larva's posterior. Ears appear in other sections at the most anterior extent of the larva.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