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The Determination and Positioning of the Nose, Lens and Ear

I. INTERACTIONS WITHIN THE ECTODERM, AND BETWEEN THE ECTODERM AND UNDERLYING TISSUES [1]

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The nasal organ, the lens of the eye, and the inner ear mechanism are structures whose emergence from the ectoderm during development depends on induction by other tissues. Presently the evidence favors the idea that each of these organs is elicited by a sequence of inductors acting synergistically.

In the amphibian embryo at the open neural plate stage the cell groups that will later form the nasal organs, the lenses, and the ears are parts of the epidermis that lies just lateral to the anterior neural plate and folds (fig. 1). At this stage the mesoderm mantle underlies the neural plate and folds, but in the anterior regions, does not yet underlie the ventrolateral epidermis.

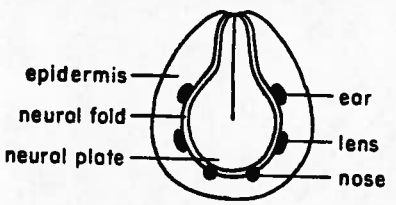

Fig. 1 Drawing of a dorso-anterior view of a salamander embryo at an open neural plate stage showing the positions of the epidermal cell groups that later give rise to noses, lenses, and ears.

Hence, the cells that are to form nose and lens are underlain by endoderm of the pharyngeal wall. The presumptive ear epidermis overlies the most anterior extension of the lateral portions of the mesoderm mantle which is the future heart mesoderm (Wilens, '55).

The endoderm that underlies future nose and lens and the mesoderm than underlies future ear exert an inductive action on the overlying epidermis to initiate the determination of each of these organs. The process of determination is continued, as neurulation is completed, by neural inductors brought by morphogenetic movements beneath the different regions of the epidermis. Forebrain completes the induction of the nose, prospective sensory retina completes induction of the lens, and hindbrain completes the induction of the ear.

During neurulation, the nose, lens, and ear are brought into contact with mesoderm that was previously beneath the neural plate. This mesoderm definitely has a role in ear induction (see Yntema, '55). It probably does in lens induction as well. There has been a belief that prechordal mesoderm participates in nose induction (see Yntema, '55). Among more recent experiments, Orts Llorca and Ferrol ('61) obtained reduced nasal organs in the chick embryo in the complete absence of the forebrain. They attributed their induction to the prechordal plate mesenchyme. Reyer ('62) found that *Amblystoma punctatum* early neurula prospective nose ectoderm differentiated into nasal placodes when transplanted to such sites as larval dorsal fin, eye chamber, or ear region. Reyer's experiments suggest that forebrain is not an essential inductor of the nose. The experiments of Haggis ('56) do not support this idea for the nose of *Amblystoma punctatum*. Reyer ('62) presents a comprehensive discussion and review of the literature on these problems for nose and lens. Mangold ('61) and Lopashov and Stroeva ('61) have also recently reviewed the lens problem.

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At the completion of neurulation, as the prospective nose, lens and ear ectoderm is being brought into contact with the neural inductors, migrating mesenchyme of neural crest, prechordal plate and possibly brain plate origin comes into close proximity to the future placodal structures and may have a role in their determination. Von Woellwarth ('61) found that increased numbers of lenses formed in the absence of the retina if the neural crest was removed as well. He suggests an inhibitory role of the neural crest cells on lens formation.

In the present studies, the epidermal strip that gives rise to the nose, lens and ear has been manipulated in a variety of ways in an effort to discern the relative roles of lateral endoderm and mesoderm, dorsal mesoderm, neural fold and neural plate derivatives in the determination and positioning of the nose, lens, and ear. Some of this work has been reported in abstract form (Jacobson, '61a).

MATERIALS AND METHODS

The experiments were done with the West Coast Newt, *Taricha torosa* (= *Triturus torosus*). Embryos were in ice water for one day while enroute by air express from California to Texas. They were shipped in gastrula or earlier stages. Before the operations, the embryos were kept at either 10°C or 17°C. If kept at 10°, they were transferred to 17° for several hours before the operations to avoid temperature shock during the operations. All experimental embryos and explants were reared at 17° after the operations, which were done at open neural plate stages 15 or 16 (Twitty and Bodenstein stages). The operating medium was Holtfreter's solution. The rearing medium was usually 66% Holtfreter's solution. Embryos and explants were reared in 35 × 10 mm disposable sterile plastic petri dishes. These dishes are excellent for this kind of work. Amphibian tissues seldom stick or spread on the plastic as they will on glass, so there is no need to line the bottoms with cellophane as is usually done when glass dishes are used.

From 11 to 21 days after the operations, experimental material was fixed, serially sectioned, and stained in hemotoxylin and eosin for microscopic examination. Donor embryos were at larval stages 38 to 42 when fixed, and explants were fixed at an equivalent time. In many cases, observations and photographs were made of the embryo or explant between the time of operation and the time it was fixed.

Only ectodermal parts were explanted in some of the experiments to be described. The excised ectodermal tissue was laid on the bottom of the dish on its external surface. In about an hour, the tissue had rounded up into a spherical vesicle with the free edges of the piece of tissue healed together so little or no surface that was not former external surface was exposed to the medium. In cases in which tissues other than ectoderm were included in the explant, these tissues, if subjacent to the ectoderm, were left attached to the ectoderm, and if not from underlying positions, were placed on top of the former inner surface of the excised ectoderm piece. In any case, the non-ectodermal tissues were incorporated inside when the ectoderm rounded up to form a vesicle.

In many instances, donor embryos from the explant experiments were reared to larval stages, then fixed, sectioned, and stained for microscopic examination. The donors, as defect experiments, are sources of additional data.

Some of these experiments were originally done for an analysis of heart determination (Jacobson, '59, '60, '61b).

EXPERIMENTS

Possibly some of the processes that lead to determination and positioning of the nose, lens and ear have already begun by early neurula stages. The following experiment suggests that even if such processes have begun at these stages of development, all factors are still present and capable of inducing normally positioned placodal structures from foreign epidermis.

In two cases, a strip of ventral epidermis was removed from a donor embryo at early neurula stage 15 and transplanted to a

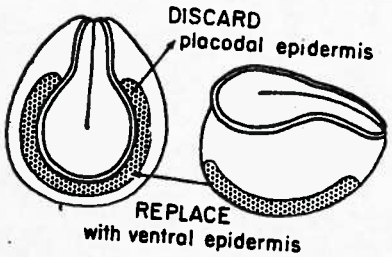

Fig. 2 Operation scheme to replace the prospective placodal epidermis with ventral epidermis from another embryo.

stage 15 host embryo in place of the host's prospective placodal epidermis (fig. 2). The donor embryos had been stained with nile blue sulfate so it could be made certain that donor ventral epidermis occupied the future nose, lens, and ear positions. These embryos formed normally positioned noses, lenses and ears.

Clearly, the causal factors responsible for elicitation and positioning of nose, lens and ear can fully express themselves when acting on epidermis only after the open neural plate stage. These causal factors, in all likelihood, arise from the cells and tissues in the immediate environment of the prospective placodal epidermis.

The following series of operations have been done to test each of these tissues and cell groups for its capacity to induce the nose, lens and ear from the prospective placodal epidermis, and to position the placodal structures within that strip of epidermis. The entire prospective placodal epidermis of the early neurula was excised and explanted either alone, or combined with various tissues of its environment (fig. 3). Donor embryos were examined in many cases to give data on the comparable defect experiments.

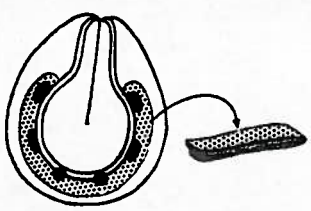

Fig. 3 The stippled area is the prospective placodal epidermis explanted, with or without underlying tissues, for operation series 1a-h.

Operation series 1

Operation number	Tissues explanted
1a	Prospective placodal epidermis explanted alone.
1b	Placodal epidermis explanted together with the prospective heart mesoderm that is subjacent to the ear portion of the epidermis.
1b′	Placodal epidermis of ear and lens regions only, together with heart mesoderm.
1c	Placodal epidermis explanted together with anterior endoderm that is subjacent to the lens and nose portions of the epidermis.
1d	Placodal epidermis explanted together with anterior endoderm and heart mesoderm.
1d′	Placodal epidermis explanted together with heart mesoderm and ventral endoderm.
1e	Placodal epidermis explanted together with heart mesoderm, posterior neural plate and adherent notochord.
1f	Placodal epidermis explanted together with anterior endoderm, posterior neural plate, and adherent notochord.
1g	Placodal epidermis explanted together with heart mesoderm, anterior endoderm, prechordal plate and notochord.
1h	Placodal epidermis explanted together with posterior neural plate and fold.

Results of these experiments are summarized in table 1.

Operation series 2

This series differs from series 1 by including the adjacent neural fold in the explant of prospective placodal epidermis (fig. 4). The placodal epidermis and adjacent neural fold was explanted either alone (2a) or together with subjacent endoderm (2b). Donors were examined for data on the comparable defect experiments. Table 2 summarizes the results of these experiments.

TABLE 1

Summary of results of operation series 1

Explants				Operation	Donors			
Cases	Numbers of: Noses	Lenses	Ears		Cases	Numbers of: Noses	Lenses	Ears
21	0	0	0	1a	16	1	2	10
68	0	7	0	1b	2	1	0	1
16	0	0	0	1b′	—	—	—	—
7	0	0	0	1c	9	0	5	9
18	2	3	1	1d	7	1	1	1
7	1	0	1	1d′	—	—	—	—
6	0	3	4	1e	—	—	—	—
2	0	0	5	1f	—	—	—	—
8	0	0	1	1g	2	1	2	1
4	0	0	4	1h	—	—	—	—

TABLE 2

Explants				Operation	Donors			
Cases	Numbers of: Noses	Lenses	Ears		Cases	Numbers of: Noses	Lenses	Ears
7	0	0	0	2a	8	6	5	8
7	9	1	0	2b	7	1	2	7

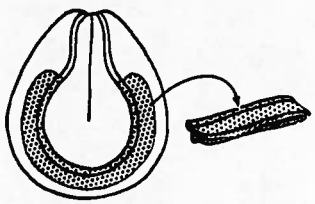

Fig. 4 The stippled area indicates the prospective placodal epidermis and adjacent neural fold explanted, with or without subjacent tissues, for operation series 2a, b.

Operation series 3

In this series, the outer portion of the neural plate was excised along with the neural fold and prospective placodal epidermis (fig. 5). In *operation 3a*, this strip of epidermis, neural fold, and neural plate was explanted alone. In *operation 3b*, the prospective heart mesoderm that is subjacent to the prospective ear epidermis was explanted together with the ectodermal strip. In *operation 3c*, all of the tissues immediately subjacent to the ectodermal components were included in the explant. These explants therefore included the prospective placodal epidermis, the neural fold and strip of neural plate adjacent to this epidermis, the heart mesoderm underlying the future ear epidermis, the pharyngeal endoderm underlying the prospective lens and nose epidermis, and the mandibular and prechordal plate mesoderm underlying the neural fold and strip of neural plate. *Operation 3d* consisted of the prospective placodal epidermis, neural fold, strip of neural plate, future heart mesoderm, and endoderm taken from the ultimate site of heart formation on the anterior ventral face of the embryo.

Donors of the explants were examined; there was never complete healing after such extensive tissue removal. When underlying tissues were excised, as well as the ectoderm, healing was more often nearly complete. Removal of the underlying tissues undoubtedly allowed the free edges of the ectoderm to come nearer to one another. The numbers of noses, lenses and ears that formed in the explants and donors are recorded in table 3.

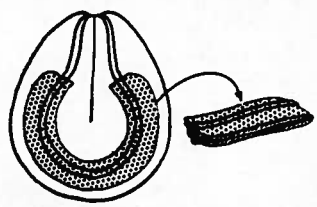

Fig. 5 The stippled area indicates the prospective placodal epidermis, adjacent neural fold and lateral portion of the neural plate explanted, with or without subjacent tissues, for operation series 3a-d.

TABLE 3

Explants				Operation	Donors			
Cases	Numbers of: Noses	Lenses	Ears		Cases	Numbers of: Noses	Lenses	Ears
5	5	6	7	3a	6	0	0	0
7	2	10	10	3b	5	0	1	2
7	13	12	14	3c	5	0	0	4
5	3	8	16	3d	–	–	–	–

These explants included epidermis and often inductors from both sides of the head; in scoring results, if normal conditions were attained, two noses, two lenses, and two ears would appear in each explant.

In operation series 1, group 1d, consisting of explants of prospective placodal epidermis together with anterior endoderm and heart mesoderm, was the only combination of tissues that produced in one explant or another all three organs under study. In one case, a nose, a lens, and an ear were all formed in the same explant (figs. 7, 8, 9).

Operation series 2a produced no recognizable noses, lenses, or ears, but there were some epidermal thickenings that may have been the beginnings of noses. Series 2b with endoderm included formed good noses and in one case a lens vesicle (figs. 10, 11).

The numbers of, and distribution of each type of placodal derivative produced in explants of series 3 is given in table 4. There were too few noses produced in all explant series except 3c. Most explants produced too few lenses, but in four cases there were too many. Most explants had two or more ears. There was an inordinate abundance of ears in series 3d.

In all series of operations, except 3c, the relationship of noses, lenses, and ears to each other were inappropriate when compared to the normal relations in the intact embryo. The vesicular explants were serially sectioned, and in studying the sections placodal derivatives were encountered in a variety of forms, none of them normal. Since the explants were mostly spherical vesicles, there was no way to orient them for sectioning. Even with random orientation, it appeared that the noses, lenses and ears had formed from the epidermal walls of the vesicle in a spiral, reminding one somewhat of similar cases in phyllotaxy. Perhaps some sort of available space rule is operating. There was no uniformity in the order in which noses, lenses, and ears appeared in the spiral. A few cases illustrating this are: from series 3a; reading through the explant in a spiral there appeared ear, lens, lens, lens, nose. From series 3b; an example was ear, nose, lens, ear. From series 3d; Example 1: ear, nose, ear, ear, ear. Example 2: nose, lens, ear, lens, ear, ear. Example 3: ear, lens, ear, ear.

In two cases, one from series 3a and the other from series 3d, noses, lenses, and ears were found in positions that approximated their normal bilateral arrangement; but they were not in correct relationship to each other. In the case from series 3a, there was a single lens (fig. 14), then two ears side by side (fig. 15), then two noses. In the explant from series 3d, there was a nose, then two ears, then two lenses.

Only in explants of series 3c was the positioning of the noses, lenses, and ears similar to that of the intact embryo. All

TABLE 4

Numbers in each explant of:	Operation 3a 5 cases					Operation 3b 7 cases							Operation 3c 7 cases							Operation 3d 5 cases				
	A	B	C	D	E	A	B	C	D	E	F	G	A	B	C	D	E	F	G	A	B	C	D	E
Noses	1	1	2	0	1	1	1	0	0	0	0	0	2	2	1	2	2	2	2	1	1	1	0	0
Lenses	0	1	1	1	3	0	1	1	0	1	4	3	2	2	1	1	2	2	2	0	2	2	3	1
Ears	0	1	2	2	2	0	2	1	3	2	1	1	3	2	1	2	2	1	3	4	2	3	4	3

seven of the explants in this series had placodal derivatives in fairly normal numbers and relationship. In two cases, the noses, lenses, and ears were correctly arranged in relationship to each other and also properly situated bilaterally. This is the only explant series that produced noses, lenses, and ears that were as well differentiated as in an intact embryo (figs. 19, 20, 21).

Operation series 4, 5, and 6

Transverse incisions were made across the neural plate and fold and into the epidermis (fig. 6). In *operation series 4a*,

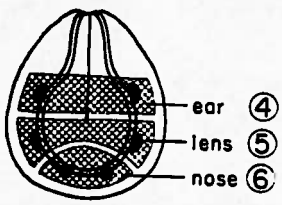

Fig. 6 The hatched areas indicate the ear, lens, or nose epidermis with adjacent neural fold and plate, that was explanted, with or without subjacent tissues, for operation series 4, 5, and 6.

the prospective ear epidermis from both sides was explanted attached to the intervening neural plate and fold. *Operation 4b* included the underlying mesoderm in the explant. In *operation series 5a*, the prospective lens epidermis was explanted with the intervening neural plate and fold. *Operation 5b* consisted of the same ectodermal component together with the endoderm that underlies the lens epidermis and the mesoderm from beneath the neural plate and fold. *Operation series 6a* consisted of the prospective nose epidermis and adjacent neural plate and fold. *Operation 6b* included in addition the endoderm underlying the nose epidermis and the prechordal plate from beneath the anterior brain plate and fold. Donor embryos were reared and later examined for each of these series.

These operation schemes do not actually put together the ear, lens, and nose epidermis with their respective neural inductors as encountered at the end of neurulation. At the stage of isolation, most of the eye field is actually anterior to the area medial to the prospective lens epidermis and was therefore included in operation series 6 with the nose epidermis, rather than in operation series 5 with the lens epidermis. Furthermore, there are forward cell movements in the medial portions of the neural plate isolated in series 4 and 5 that are yet to occur at the time of isolation, so some cells in series 4 would have ended up in neural plate of series 5 in the intact embryo. Likewise some cells posterior to the neural plate in series 4 which would normally move into that area in the intact embryo are excluded in these explants.

Interpretation of the results, presented in table 5 must include the above considerations.

Where there is some doubt about the identification of a placodal derivative in the sectioned explants, the figure in the table is followed by a question mark.

In operation series 4, 5, and 6, the explants of prospective ear epidermis and associated tissues formed ears, and in one case, a lens; prospective lens epidermis formed lenses and an occasional nose or ear; prospective nose epidermis formed noses and quite often lenses as well. Each epidermal area formed the organ it normally would and also, on occasion, the adjacent types.

The high frequency of lenses in operations 6a and 6b (explants of nose epi-

TABLE 5

Explants				Operation	Donors			
Cases	Numbers of: Noses	Lenses	Ears		Cases	Numbers of: Noses	Lenses	Ears
10	0	1	5	4a	10	10	10	2
1	0	0	1	4b	4	3	3	1
12	1	2	1?	5a	6	6	4	6
3	0	1	0	5b	6	3	3	5
8	2?	6	0	6a	10	2?	0	10
8	7	7	0	6b	9	0	1?	9

dermis and associated tissues) was expected, since the retinal rudiments are located in the associated neural plate. Each lens that formed was in an optic cup, except for one case. In series 4 and 5, all lenses that formed were associated with optic cups.

DISCUSSION

Experiments have for the most part been performed on the early open neural plate stages of *Taricha torosa* giving information about the state of determination of the prospective placodal epidermis at that stage of development and helping to define what tissues must be associated with the early neurula prospective placodal epidermis to bring about the formation of the nose, lens, and ear in their normal sites.

Prospective placodal epidermis explanted alone does not form nose, lens, or ear (operation 1a). Explants of just the presumptive lens epidermis from the early neurula of the same species (Jacobson, '55, '58) yielded no lenses or other placodal structures in 49 cases. In this species, it appears that the epidermis is not yet sufficiently determined at the open neural plate stage to form nose, lens, or ear in explant. Probably most induction occurs after the open neural plate stage. The transplantation of prospective belly epidermis to the site of, and in place of, the prospective nose, lens, and ear epidermis yielded normally situated noses, lenses, and ears in the foreign epidermis. Obviously there are sufficient inductors present after the beginning of neurulation to bring about the normal situation.

Most of the rest of the experiments were done in order to investigate the relative roles of the different environmental tissues in the induction and positioning of the nose, lens, and ear. Results indicate that the embryo does not rely on any single inductor for the elicitation of these organs. In general, two or three tissues seemed to have the principle inductive role for each of the organs. A nose was formed only in those explant combinations that included either anterior endoderm or anterior neural plate and fold. A lens formed in those explants that included either neural plate and fold or heart mesoderm, with the exception of a single lens that formed in the presence of neural fold and anterior endoderm. Ears formed in explants that included adjacent neural plate and fold, or heart mesoderm. Ear formation was also favored by the presence of neural plate and fold from regions posterior to the ear site (operations 1e, 1f, and 1h) (fig. 12).

Differentiation of induced noses, lenses, and ears was best when both neural and non-neural inductors were present in the explant. With just neural plate and fold present in the explant along with the prospective placodal epidermis, differentiation of the placodal derivatives was somewhat poorer. Differentiation was still poorer if only mesoderm and endoderm were the inductors. Figures 7 through 21 are arranged for easy comparison of the size and differentiation of noses, lenses, and ears in the various explant combinations.

The positioning of the noses, lenses, and ears in the epidermal strip is subject to great variation in explant, but it is apparent that the more normal inductors there are present in their normal relationships, the more normally are the placodal derivatives positioned. Only when the explant was so inclusive that it contained nearly the entire environment of the strip of prospective placodal epidermis were the noses, lenses and ears present in normal numbers and correctly positioned bilaterally and in relationship to each other.

Donors of the tissues for the explant experiments served as defect experiments. In operation series 1, 2, and 3, noses, lenses, and ears formed in a number of cases in the donors in epidermis that healed in from more ventral regions. Cases in which the placodal epidermis and also the underlying tissues were removed (operations 1b, 1c, 1d, 1g, 2b, 3b, and 3c) in some cases formed noses, lenses and ears. In these cases some of the normal inductors were removed so appearance of the placodal structures implies that there must have been regulation in the inductor system as well as recruitment of nearby epidermis as reacting tissue.

In past studies of lens induction (Jacobson, '55, '58), a number of experiments were done and analyzed for results relating to the lens problem. Some of the experiments were equally pertinent to the problems of nose and ear determination, but

TABLE 6

Operation	Stage	Number of cases	Number and per cent that formed noses	Number and per cent that formed lenses	Number and per cent that formed ears
Defect experiment: Anterior neural plate and fold removed from early neurula	15–16	46	8 17%	12 26%	46 100%
Defect experiment: Entire neural plate removed from early neurula	15–16	7	1 14%	3 43%	6 86%
Explant of anterior neural plate, neural fold, and epidermis from early neurula	15–16	9	1 11%	3 33%	2 22%
Explant of anterior neural plate, neural fold, and epidermis from late neurula	18–19	13	10 77%	10 77%	12 92%

these aspects were not pursued at that time. Table 6 contains data derived from a re-examination of the serial sections of relevant material from those studies. The experiments were on the same species used in this study. In two defect experiments, the anterior neural plate and fold was removed, or the entire neural plate was removed from the early neurula. In both experiments, well formed noses, lenses, and ears were obtained. These formed despite the lack of their normal neural inductors, which were removed long before they grew out to underlie the placodal epidermis.

In an experiment in which the anterior end of the early neurula was cut off and the mesoderm and endoderm removed leaving the neural plate, fold, and epidermis as an explant; noses, lenses, and ears were obtained. These structures formed despite the absence of their normal non-neural inductors indicating that the neural inductors alone are effective to some extent. In a similar experiment done at late neurula stages, many more noses, lenses and ears formed showing that the presence of the non-neural inductors during neurulation greatly augments the production of these structures. Together the two sets of experiments show the alternate inductive pathways that are possible, but it should be emphasized that the structures obtained were never as well differentiated as under normal circumstances when all inductors act synergistically.

SUMMARY

1. The relative roles of various inductors in the determination and positioning of the nose, lens and ear were studied in the newt, *Taricha torosa*.

2. Belly epidermis of an early neurula implanted in place of the prospective placodal epidermis of a host early neurula forms normal noses, lenses and ears indicating there is sufficient inductive activity following early neurula stages to elicit these organs in normal positions.

3. Prospective nose, lens and ear epidermis of the early neurula is not sufficiently determined to form these organs when it is explanted by itself.

4. Noses formed in the prospective placodal epidermal strip when either anterior endoderm or neural plate and fold were present in the explant.

5. Lenses formed in the epidermal strip when the explant included prospective heart mesoderm or neural plate and fold. A small lens also developed in an explant that included anterior endoderm and neural fold.

6. Ears formed in explants that included adjacent neural plate and fold or heart mesoderm. Presence in the explant of

neural plate and fold from regions posterior to the ear site also favored ear formation.

7. The sites of formation of the noses, lenses and ears in the explants varied widely and were often neither in an order appropriate for the position the epidermis had occupied in the embryo, nor in proper relationship to each other. Only explants consisting of most of the normally associated tissues (of prospective placodal epidermis, adjacent neural fold and plate, and subjacent mesoderm and endoderm) were able to form normal numbers of noses, lenses and ears in normal sites.

8. When the nose, lens and ear epidermal areas were explanted separately, in each case with their respective adjacent areas of neural fold and plate, the epidermal portions formed the organ expected of them in many cases, and in fewer cases formed also the adjacent organ types.

9. The extent of differentiation of induced noses, lenses, and ears was greater the more normal inductors there were in the explants. Best differentiation occurred when neural plate and mesoderm and endoderm were included in the explant with the prospective placodal epidermis. Neural plate alone led to somewhat poorer differentiation, while endoderm and mesoderm with no neural plate elicited organs that differentiated even more poorly.

10. Considerable regulation is possible in both the epidermis and the inductor tissues as shown by the donors of the explanted tissues that often formed noses, lenses and ears even though their prospective placodal epidermis was removed, and in many cases their normal inductor tissues as well.

11. These experiments reaffirm that nose, lens, and ear are each induced by a sequence of inductors, and determination occurs over an extended period of time. Correct positioning and complete differentiation occurs only when all normal inductors are present in their usual positions.

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PLATE 1

EXPLANATION OF FIGURES

7 Nose formed in an explant of prospective placodal epidermis and subjacent heart mesoderm and pharyngeal endoderm (operation 1d). All photographs in figures 7–21 are taken at the same magnification to facilitate direct comparison of the size and differentiation of the noses, lenses, and ears.

8 Lens formed in the same explant (operation 1d) as figure 7. The lens (L) lies just below the nose (N).

9 Ear formed in the same explant (operation 1d) as figures 7 and 8.

10 Nose formed in an explant of prospective placodal epidermis and adjacent neural fold with subjacent pharyngeal endoderm (operation 2b).

11 A lentoid formed in another explant with the same tissues as figure 10 (operation 2b).

12 Ear formed in an explant of prospective placodal epidermis with neural plate and fold from a position posterior to the ear site (operation 1h).

13 Nose formed in an explant of prospective placodal epidermis, adjacent neural fold and the more lateral portions of the adjacent neural plate (operation 3a).

14 Lens formed in another explant with the same tissues as figure 13 (operation 3a).

15 Ears formed in same explant as figure 14 (operation 3a).

16 Nose formed in explant of prospective placodal epidermis, neural fold and plate, and prospective heart mesoderm (operation 3b).

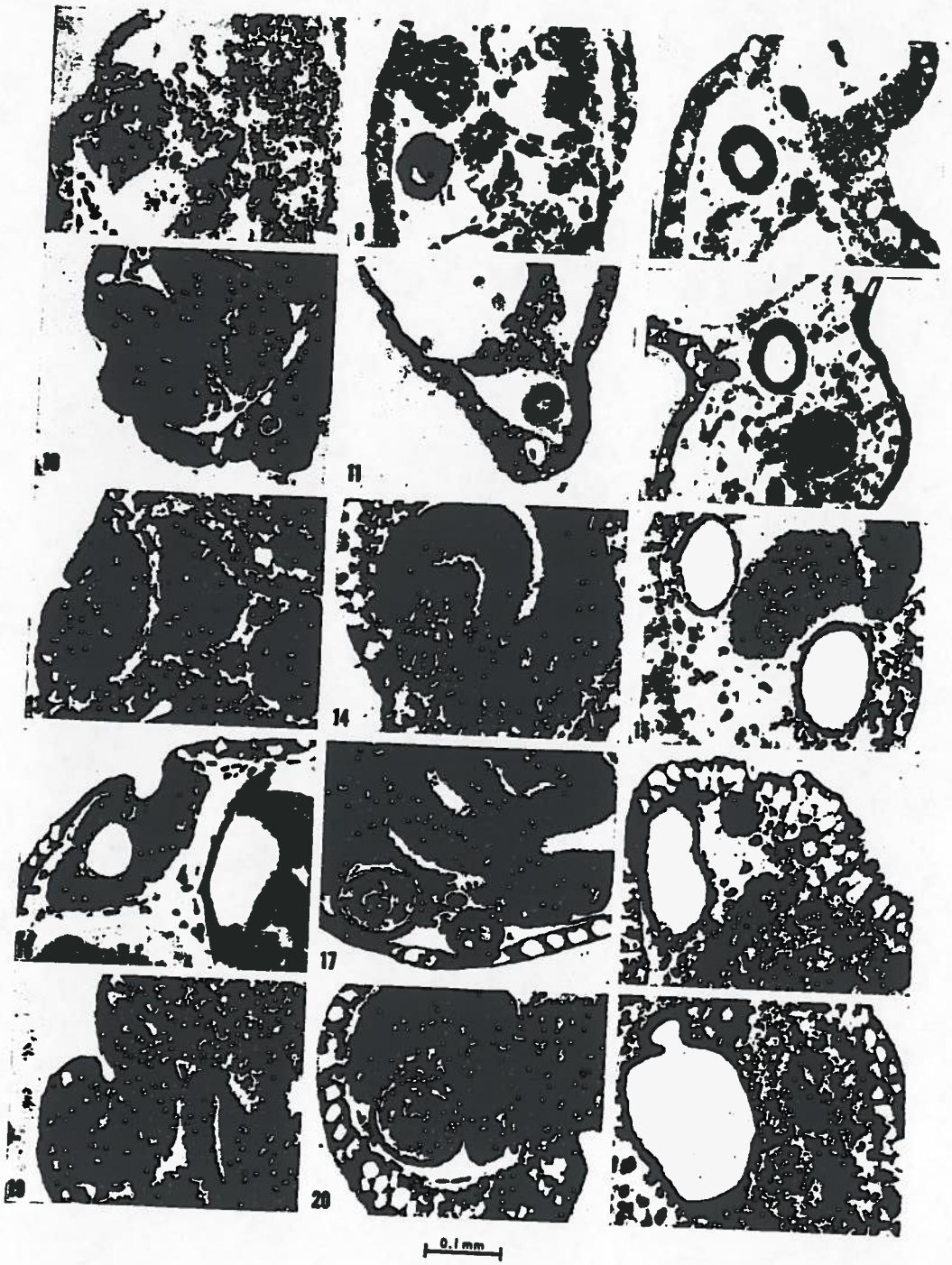
17 Lens formed in same explant as figure 16 (operation 3b).

18 Ear formed in same explant as figures 16 and 17 (operation 3b).

19 Nose formed in explant of prospective placodal epidermis, neural fold and plate, and subjacent mesoderm and endoderm (operation 3c).

20 Lens formed in another explant with same tissues as figure 19 (operation 3c).

21 Ear formed in another explant with same tissues as figures 19 and 20 (operation 3c).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