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The Determination and Positioning of the Nose, Lens and Ear

II. THE ROLE OF THE ENDODERM [1]

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These studies are an attempt to define the essential tissue interactions that lead to nose, lens and ear formation in the sites these organs normally occupy. The explant experiments in the first paper of this series (Jacobson, '63) demonstrated that endoderm is involved in nose induction and to some extent in lens induction. At early neurula stages the prospective ear epidermis is underlain by prospective heart mesoderm that is inducing ear (Jacobson, '63) and is simultaneously being induced to form heart by the underlying endoderm (Jacobson, '60, '61). It is conceivable that the endoderm has at least an indirect role in ear induction. The endoderm is itself determined at very early stages. Since it is to a large extent a mosaic of future parts at early neurula stages, it would be reasonable that these already determined different regions of the endoderm may be the sources of specifically different inductor substances that would help specify that nose form anteriorly, ear posteriorly, and lens between. The defect and reversal experiments that follow provide information about this.

MATERIALS AND METHODS

The embryos were of the West Coast newt, *Taricha torosa*. Procedural details are given in the first paper of this series (Jacobson, '63) and in papers on heart studies (Jacobson, '60, '61) for which some of these experiments were originally done.

The entire endoderm can be removed cleanly at neurula through tail bud stages by dorsal, ventral or lateral approaches. All three approaches were used in these experiments. Through a cut made in the ectoderm and mesoderm with iridectomy scissors, the endoderm can be separated out with hair loops gently enough that it remains intact, not visibly injured, and with no adhering mesoderm cells. The wound in the ectoderm and mesoderm heals quickly. In some experiments, before the wound closed, parts of the endoderm were returned to the embryo, or the entire endoderm was replaced after reversing its anterior-posterior axis. Other tissues, such as neural plate and fold, were removed along with the endoderm in some cases.

Embryos were reared for 3 to 4 weeks at 17°C then fixed, serially sectioned, stained and examined.

EXPERIMENTS

Endoderm-free embryos

Endoderm is best removed from older stages through a lateral slit in the body wall. A lateral or ventral approach does not disturb the nervous system, while a mid-dorsal incision sometimes results in a bilateral replication of the nervous system. So there would be no complications from different modes of operation, the endoderm was remved through a lateral slit from embryos at stages 14 (early neurula), 19 (late neurula), and 22 (early tail-bud). Embryos were examined 3 to 4 weeks later.

Of eight cases from which the endoderm was removed at stage 14, two formed normal bilaterally situated noses, one formed a pair of noses fused at the midline, and two formed single noses. The other three cases formed no noses at all. Bilaterally

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situated lenses associated with optic cups were found in five of the eight cases, two cases formed single lenses, and one case formed no lenses at all. Seven of the eight cases formed bilaterally situated ears, the other cases formed but a single ear on the right side. Noses, lenses and ears were not well differentiated (figs. 1, 2, 3).

Of nine cases from which the endoderm was removed at stage 19, six cases formed but single noses and three cases formed noses fused at the midline, but with two nostrils. Six cases formed bilaterally situated lenses, all but one associated with optic cups. The other three cases formed a lens on one side of the head only. Six cases formed bilaterally situated ears, and three cases formed ears on one side only. Differentiation of noses, lenses and ears was somewhat better than in embryos from which the endoderm was removed at stage 14 (figs. 4, 5, 6).

Six cases from which the endoderm was removed at stage 22 formed bilaterally situated noses in all but one case in which a single nose formed; the same was true of lenses. Bilaterally situated ears formed in every case. Differentiation was better in these noses, lenses and ears than in either of the previous experiments (figs. 7, 8, 9).

Ninety-seven other embryos were deprived of their endoderm at stages ranging from stage 14 to stage 26. In these additional experiments, a lateral approach was used to remove the endoderm in embryos older than stage 20, while either a mid-ventral or a mid-dorsal incision was used for younger embryos. Results were substantially the same as in the 23 cases described above. Examples of the noses, lenses and ears that formed in the embryos from which the endoderm was removed at stage 26 are illustrated in figures 10, 11, 12.

The anterior-posterior distribution of noses, lenses and ears is proper in most cases and is likely related to neural and mesodermal inductors remaining in the embryo. In two cases of removal of the endoderm at stage 14, and in three cases at stage 19, the antero-posterior distribution of the noses, lenses and ears was not in order. All of the cases in which endoderm was removed at stage 20 or older had their placodal derivatives properly distributed along the antero-posterior axis.

All endoderm removed except anterior dorso-lateral endoderm or posterior endoderm

At early neurula stage 16, the entire endoderm was removed through a slit in the dorsal midline, then the dorsolateral anterior epithelial wings of endoderm were cut from the endodermal mass and returned to the cavity from which the endoderm was removed. Of five cases, one embryo formed the usual two noses, two formed single noses and two formed no noses at all. Four of the five cases formed single lenses in embryos with single eye cups and the fifth formed two lenses in a single midline eye cup. All five cases formed the usual two ears each.

In seven cases done at early neurula stages 15 to 16, the posterior fourth of the endoderm was severed from the excised endodermal mass and returned to the cavity from which the endoderm had been removed. The operational approach was dorsal. Every case formed a large single nose. Two cases formed single lenses in single eye cups. Three cases formed two lenses each in fused eye cups, and two cases formed three lenses each, in one case in three separate eye cups, and in the other in two fused eye cups. Four cases formed two bilaterally situated ears, and three cases formed but one ear each. Wherever the posterior endoderm came into contact with the brain, necrotic cells were present. The antero-posterior order of the placodal derivatives was proper in all but one case in which the ears were anterior to the lenses.

Endoderm reversals

In these experiments, the endoderm was removed through a mid-ventral slit at early neurula stage 15, the endodermal mass was rotated 180° reversing the antero-posterior axis then returned to the cavity from which it had been removed.

Of ten cases, one formed three noses, seven formed two noses each, and two formed single noses. Nine of the ten cases formed two lenses each in optic cups, and one case formed two fused lenses in two fused optic cups. Nine cases formed two

ears each, and one case formed but a single ear. The noses, lenses and ears that formed were in proper order.

Some control or sham operations were done with this series. In four cases the endoderm was removed through a ventral slit at stage 15 then replaced without reversing the axis. All cases formed normal numbers of noses, lenses and ears properly situated.

Endoderm-free embryos from which other tissues were removed as well

In these experiments, the entire endoderm was removed by a dorsal approach from early neurulae of stages 14 to 16, and other tisuses were also removed.

In a couple of cases, the entire endoderm and the entire neural plate were removed. This left the entire epidermis and neural fold and all of the mesoderm. No noses, lenses, or ears formed.

The same experiment was done removing the entire endoderm and neural plate, but in these cases the mesoderm subjacent to the neural plate was removed as well. In eight cases, just three small doughnut shaped structures were formed that may be ears. No noses or lenses formed.

In seven cases, the brain plate, subjacent mesoderm, and adjacent neural fold were removed in addition to the entire endoderm. In one case a small lentoid formed, and in another a small ear formed.

The endoderm and the entire neural plate and fold and subjacent mesoderm were removed in six cases. This left just the epidermis and subjacent mesoderm. The only placodal derivatives to form were one dubious lens and one dubious ear.

The entire ectoderm, that is the epidermis, neural fold and plate, was isolated in four cases. These embryos lacked all endoderm and all mesoderm. When so much of the embryo is removed it becomes difficult to say which is the embryo and

TABLE 1

Summary of operations

Operation	Numbers of			
	Cases	Noses	Lenses	Ears
Endoderm-free. Stage 14	8	7	12	15
Endoderm-free. Stage 19	9	12	15	15
Endoderm-free. Stage 22	6	11	11	12
All but anterior dorso-lateral endoderm removed. Stage 16	5	4	6	10
All but posterior endoderm removed. Stages 15–16	7	7	14	11
Antero-posterior axis of the endoderm reversed. Stage 15	10	19	20	19
Sham operation endoderm removed and replaced in same orientation. Stage 15	4	8	8	8
Lack endoderm and neural plate. Stage 16	2	0	0	0
Lack endoderm and neural plate and subjacent mesoderm. Stage 16	8	0	0	3?
Lack endoderm and brain plate and subjacent mesoderm and adjacent neural fold. Stage 15	7	0	1	1
Lack endoderm and neural plate and fold and subjacent mesoderm. Stage 14	6	0	1?	1?
Lack endoderm and all mesoderm. Stage 15	4	3	1	2
Lack endoderm and all mesoderm and neural plate and fold. Stage 16	4	0	0	0

which is the explant. One of the four cases had two noses, one had one nose and two had none. One case formed one lens and three had none. Two cases had single ears and two had none. Differentiation was poor (figs. 13, 14, 15).

Finally, the epidermis was isolated by itself in four cases. These isolates lacked all endoderm, mesoderm, and neural plate and fold. No noses, lenses, or ears formed.

Table 1 summarizes all the experiments in this paper.

DISCUSSION

Removal of the endoderm has only minor repercussions on production of noses, lenses and ears provided the neural plate is present. On the other hand, removal of the anterior neural plate or the entire neural plate at the early neurula stage (Jacobson, '58, '63) more seriously decreases production of noses, lenses and ears. In these cases, with the endoderm present but the neural plate gone, noses formed in 14 to 17% of the cases, lenses in 26 to 43% of the cases, and ears in 86 to 100% of the cases.

The experiments in this paper indicate that removal of the endoderm reduces nose production more than lens or ear production. Removal of the endoderm and all of the mesoderm reduces lens and ear production more than it does nose production. These results are in accord with the results of the explant experiments in the first paper of this series (Jacobson, '63). These experiments lead to the same conclusions as in that paper. Endoderm is an important inducer of nose and to a lesser extent of lens. Mesoderm is an important inducer of ear and lens. The neural system is a principal inducer of all three organs. Noses, lenses and ears that form in the absence of one or another of their normal inducers are seldom as well differentiated as organs in intact embryos. These various inducers act together over an extended period of development.

The experiments in which anterior or posterior endoderm was left in otherwise endoderm-free embryos, or in which the endoderm was removed then replaced with its antero-posterior axis reversed, suggest that if regionally specific inductor substances do emanate from the endoderm, their lack or their misplacement can be overcome by other tissues, particularly nervous tissue.

Noses, lenses and ears form even though the endoderm is removed in neurula or later stages. However, differentiation of all three organs is better the longer the endoderm remains in the embryo so there is a continuing influence of the endoderm on the formation of these organs throughout neurulation. This is true, also, of the central nervous system which will be analyzed further in a later paper. These experiments do not tell us the extent to which endoderm has functioned as a placode inductor prior to neurula stages. The next paper in this series will show that the placodal structures are already determined to some extent by the inductive activity of the endoderm and mesoderm prior to neurula stages.

There is no doubt that the endoderm is a primary inductor of a number of organs including the nose and lens as shown in these papers, and the heart as shown by Bacon ('45) and Jacobson ('60, '61).

SUMMARY

1. The particular role of the endoderm in nose, lens and ear determination was studied in the newt, *Taricha torosa*.

2. In general, removal of the endoderm at neurula stages has the greatest effect on nose production, a somewhat lesser effect on lens production, and little effect on ear production. Fewer of these organs form in the absence of the endoderm.

3. When these organs form in the absence of the endoderm, their differentiation is better the older the embryos were when they were deprived of their endoderm.

4. Experimental cases in which some anterior or some posterior endoderm was left in otherwise endoderm-free embryos, and others in which the endoderm was removed and returned with the anterior-posterior axis reversed, formed noses, lenses and ears in a nearly normal fashion.

5. Experiments in which other tissues as well as the endoderm were removed showed that, in the absence of the endo-

derm, the neural system is the principal inductor of nose, lens and ear and that mesoderm participates in lens and ear induction. There was no obvious role of the neural fold.

6. These experiments support the conclusion that endoderm has a role in nose and lens induction, and acts together with neural inductors to lead to normal nose and lens differentiation. If regionally specific inductor substances emanate from the endoderm, their lack or their misplacement can be overcome by other tissues, particularly nervous tissue.

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PLATE 1

EXPLANATION OF FIGURES

1– 3 Nose, lens, and ear, respectively, formed in embryos deprived of their endoderm at early neurula stage 14. Figures 1–15 are all at the same magnification to facilitate comparison of size and differentiation of the organs.

4– 6 Nose, lens, and ear, respectively, formed in embryos deprived of their endoderm at late neurula stage 19.

7– 9 Nose, lens, and ear, respectively, formed in embryos deprived of their endoderm at early tail-bud stage 22.

10–12 Nose, lens, and ear, respectively, formed in embryos deprived of their endoderm at later tail-bud stage 26.

13–15 Nose, lens, and ear, respectively, formed in embryos deprived of their endoderm and mesoderm at early neurula stage 16.

PLATE 1