THE ORIGIN OF COMPETENCE FOR LENS FORMATION IN THE AMPHIBIA

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(With Two Plates.)

INTRODUCTION.

In normal development one process of induction follows another in regular sequence. The neural plate is induced by the axial mesoderm, and after a certain lapse of time the lens is induced by the eye-cup. The result of each process of induction depends upon the properties both of the inducing agent and of the reacting material. Material which is capable of reacting to a given inducing stimulus is said to be "competent" for that process of induction (Waddington, 1932). It is easy to set an upper limit to the period of competence, since we can determine experimentally the time after which the tissue will no longer react. It is much more difficult to make precise, either experimentally or theoretically, what is meant by the lower limit of the period of competence. A competent tissue should be thought of as an unstable system which has two or more ways of change open to it, the decision as to which way it actually follows being taken by the relevant organiser. Now if we consider any particular process of induction, such as the induction of a lens, it is almost certain that an instability as regards lens formation is not present till comparatively shortly before the lens induction normally occurs. Blastula ectoderm for instance can scarcely be competent for lens formation in this sense. But the only way in which this non-competence could be demonstrated would be to place a lens-inducer in contact with ectoderm for a certain length of time and then remove it; if no lens was induced, even after an application of the stimulus long enough to perform an induction on ordinary competent ectoderm, we might conclude that the failure was due to a lack of competence in the ectoderm tested. But, quite apart from the manipulative difficulties involved in such an experiment, we have every reason to believe that some, if not all, inductions are due to the diffusion of active chemical substances into the reacting tissue. It is therefore possible that an active substance might diffuse into a tissue before that tissue showed the particular instability which was being searched for, and that at a later period, after the organiser had been removed, the instability might arise and the active substance, already within the tissue, perform an induction. In such a case it would be impossible to demonstrate experi-

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mentally the lower limit of the period in which the instability, that is, the competence, exists.

Although it is difficult to make precise the time at which competence arises, we can investigate the conditions on which its origin depends. The object of the present paper is to discover whether, and to what extent, the competence for lens formation in the newt depends on the main mesodermal organisation centre of the gastrula. In the newt the competence is only realised when the stimulus of the eye-cup is applied, but in other Urodeles the lens can be formed without a definite inducing stimulus.

The arising of lens competence might be supposed to be dependent on the previous induction of a neural plate in the mass of ectoderm, or on some process controlled by the side-plate mesoderm or head mesectoderm, or finally on some process controlled by any kind of mesoderm.

EXPERIMENTAL WORK.

The question was investigated by making implants of lens-inducing tissue into ectoderm isolated from the gastrula. A set of twenty isolates were made, of which nineteen survived to be investigated. The isolates consisted of presumptive neural plate and epidermis taken from young gastrulae of *Triton alpestris*. They were cultivated in Holtfreter solution at 25° C. Gastrulae of the same age were placed in a dish just beside them. The cultivation lasted just over 24 hours, when the unoperated gastrulae had developed open neural plates with well-defined neural ridges. From these gastrulae, presumptive eye tissue was removed together with some of the underlying mesoderm, and this tissue was then implanted into the isolated masses of ectoderm. In order to obtain the eye implants, the open neural plates were split down the mid-dorsal line, and the presumptive eye material from each side then cut across transversely, so that four implants were obtained from each embryo. These implants did not contain any of the neural crest tissue, which was carefully removed, nor any prechordal mesoderm, though some of the archenteron roof was left attached to the under-surface of some of them. The isolated ectoderm fragments had all of them rolled up so that no free cut edges could be seen. In most of them there was a slight space between the upper and lower sheets of tissue, as has been described by Holtfreter (1933) in the ectodermal parts of exogastrulated embryos. In a few this space was quite large, forming a small hollow vesicle, and in these explants the eye tissue could be easily inserted into the vesicles. In the majority of the explants, the graft was made by dissecting a hole in the upper layer into which the eye tissue fitted, lying still on the surface.

The explants, with the grafts attached, remained in Holtfreter solution for a further 4 days at 25° C. They were then fixed and sectioned.

The implanted tissue has, in all cases except one, been covered up by the ectoderm, and has differentiated to well-recognisable masses of neural tissue and notochord. Although the implants contained only one lateral half of the neural plate, they have produced laterally symmetrical portions of the brain, a very good example of which is figured in Pl. I, fig. 4 (embryo No. D 59–*g*). This power of lateral regulation

does not extend however to the production of two eyes. Each portion of brain is accompanied by one large eye-cup, placed underneath it like a cyclopic eye. But it is probable that the eyes are not really cyclopic, that is, formed from two rudiments which have secondarily fused, but they are more likely developed from the single implanted rudiment, the regulated brain never having produced a second rudiment.

The condition of the ectoderm is very different in different specimens. In some it forms a more or less closely packed mass of cuboidal cells surrounding the implant (Pl. II, fig. 7). This is the normal appearance of gastrula ectoderm cultivated in isolation from the mesoderm. In other specimens the implant lies in a larger or smaller hollow space within the ectoderm, which may in some cases be blown out into a thin-walled vesicle. Such vesicles are normally formed by ectoderm when it is accompanied by some mesenchyme (Holtfreter, 1934). In some of our specimens a little mesenchymous tissue is present (*e.g.* Pl. II, fig. 8), derived presumably from the implant, but the tissue is always very sparse and in most cases is entirely absent (*e.g.* Pl. I, figs. 1 and 2), and in these specimens the vesicle formation is probably a direct reaction to the presence of the implanted neural tissue. The walls of the vesicle may be formed of a two-layered epidermis (Pl. II, fig. 8) indistinguishable at this stage from the epidermis of a normal embryo of the same age. In other specimens, however, the inner layer of the epidermis is thickened and has elongated nuclei arranged in a "palisade", presenting the appearance of the thickened sensory epithelium of the mouth region of the normal larva (Pl. I, fig. 1). This sensorisation of the inner layer may occur in specimens in which the vesicle wall is thick and many layered. It can probably be taken as a weak reaction of the same type as that which leads to neural differentiation, since very similar tissues are formed when weakly inducing substances are implanted into the blastocoele of young gastrulae (Needham, Waddington and Needham, 1934). In some specimens an actual neural reaction occurs, and in these specimens it seems that the sensorised layer has rolled up into a tube and proceeded further with a typical neural differentiation (Pl. I, fig. 2 and Pl. II, fig. 5). These induced neural tubes can be distinguished from implanted neural tissue by their earlier stage of differentiation, since, although the tissues are of the same actual age, the implanted neural tissue had begun developing in the neural direction some time before it induced the formation of the secondary tubes.

The occurrence of sensorised epithelium and still more that of induced neural tissue shows that the competence of the isolated ectoderm to react to the neural-inducing stimulus does not disappear so rapidly as it does in normal development. This conclusion has also been demonstrated by implanting pure mesoderm into isolates under similar conditions, when there can be no question of confusing implanted with induced neural tubes (Waddington, 1935).

In seven of the specimens, a lens has been induced by the eye-cup where it touches the ectoderm. It is clear, then, that the competence for lens formation is not formed as a reaction to any specific stimulus exerted by the non-axial mesoderm, since none of this mesoderm has ever been in contact with the ectoderm in these isolates. One of the induced lenses has been formed in a vesicle in which neural tissue has been induced (D 59–*a*, Pl. I, figs. 1 and 2), and one in a vesicle with well-

defined sensorisation (D 59–*k*) but some of the others, particularly D 59–*f* (Pl. I, fig. 3) and D 59–*p* (Pl. II, fig. 8), occur in vesicles in which there is no induced neural tissue and extremely little sign of any sensorisation of the inner layer of the ectoderm. These two last specimens demonstrate that the competence for lens formation is not dependent on the previous occurrence of any grade of neural induction. The competence must in these cases have developed in the ectoderm either entirely independently of any outside stimulus, or in consequence of a reaction between the ectoderm and the implant quite different from the reaction which leads to neural induction. The specimens in which no lens has been induced make it probable that a reaction of this special kind does exist. In some cases the failure of lens induction is clearly due to lack of contact between the eye-cup and the ectoderm, *e.g.* D 59–*g* (Pl. I, fig. 4), but in at least five specimens the eye-cup is in contact with the ectoderm and yet no reaction has occurred. These are all specimens in which the ectoderm has not formed a vesicle but remains a compact mass such as isolated ectoderm normally forms *in vitro*. D 59–*l* (Pl. II, fig. 7) is an example. The regularity of the result allows one to conclude that the competence for lens formation does not arise in the ectoderm unless it develops into a thin-walled vesicle. In the same degree as vesicle formation is dependent on the presence of the implanted tissue, so also is the competence for lens formation.

DISCUSSION.

It seems, then, that the competence for lens formation only arises in thin sheets of ectoderm, either in the epidermis of the neurula or in the thin walls of the isolated vesicles. These sheets of ectoderm are in contact with different tissues in the two cases; in the neurula with mesenchyme or side-plate mesoderm, in the vesicles with neural tissue or axial mesoderm. It hardly seems probable that the function of these various neighbouring tissues is to exert some specific chemical influence on the processes which give rise to the competence. One can more easily suppose that the competence can be independently produced by the ectoderm provided only that the mechanical conditions are favourable, *i.e.* that the ectoderm is in the form of a thin layer. The formation of a thin-walled vesicle may itself be dependent on purely mechanical factors, as Holtfreter has suggested, since ectoderm growing on a hard surface may spread out in thin sheets; however, the amount of mesenchyme included in some vesicles is very small, and it may be that osmotic phenomena are of more importance in distending the vesicle than actual mechanical rigidity.

As yet few investigations have been made of the ways in which the various competences arise in embryological development. Waddington (1934) has investigated the origin of the competence of the ectoderm of the chick blastoderm to form neural tissue, and found it to be independent of the last previous induction which has occurred in the embryo, namely that of primitive streak by endoderm. The formation of lens competence in the case we have examined here is, as we have seen, relatively independent of the mesoderm organiser. It is possible that future investigation will show that this result can be generalised, and that the previous

inductions provide only the general conditions (such as the arrangement of the ectoderm in a thin layer) in which each cell then produces a competence, *i.e.* becomes unstable in a particular way, through the independent action of internal factors. These internal factors may well be related in a very immediate way to the genes.

SUMMARY.

1. Presumptive ectoderm of *Triton alpestris* was removed from the young gastrula and cultivated in Holtfreter solution at 25° C. until control embryos had developed open neural plates.

2. Presumptive eye material from neural plate embryos, with some attached archenteron roof, was then implanted into the isolated fragments of ectoderm.

3. The grafted tissue formed single complete eyes, and bilaterally symmetrical portions of the brain, although the implant contained asymmetrical portions of the neural plate.

4. In some of the explants the competence for neural differentiation was retained even to this late stage, and neural tubes were induced. In other specimens the inner layer of ectoderm consists of long, cylindrical, regularly arranged cells, like those of the sensory inner layer of ectoderm in the mouth region of a normal larva. Still other specimens formed thin-walled vesicles with no sensorisation, and others again differentiated into the compact masses of tissue normally formed by isolated gastrula ectoderm.

5. Lenses were induced in all types of explant mentioned above except the last.

6. It is concluded that the formation of lens competence is not dependent on the presence of non-axial mesoderm or on the previous occurrence of a process of neural induction, but is dependent on the differentiation of the ectoderm into a thin layer, which differentiation may be brought about in various ways, and perhaps purely mechanically. The formation of a thin layer of ectoderm is probably a sufficient as well as a necessary condition for the origin of lens competence.

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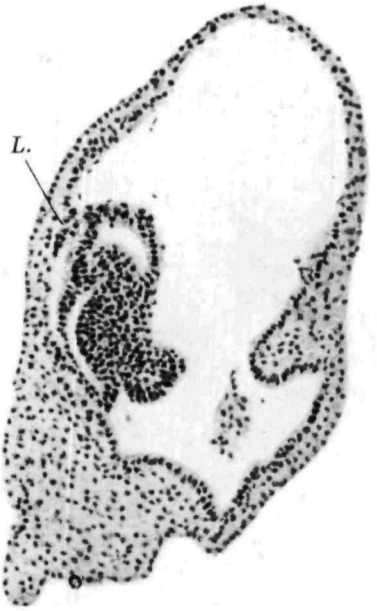
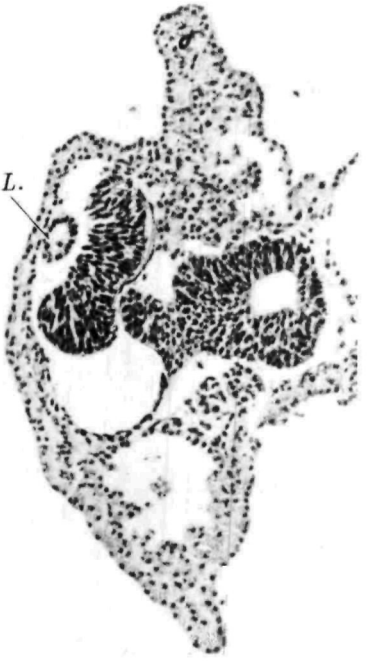


Fig. 1.



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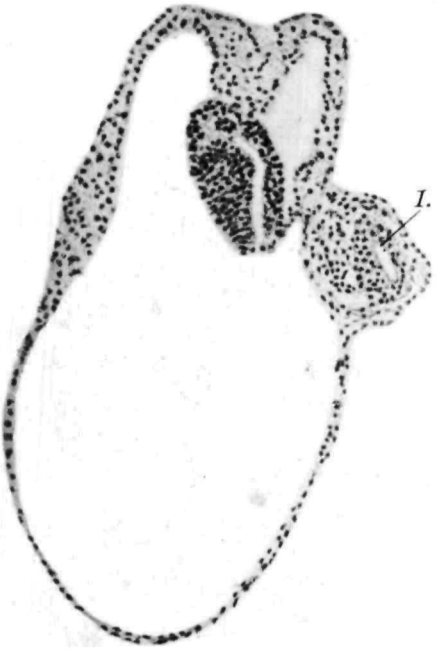

Fig. 2.

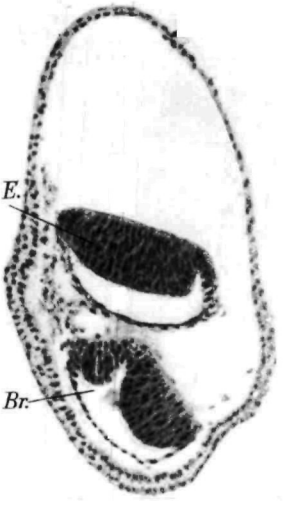

Fig. 4.

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Fig. 5.

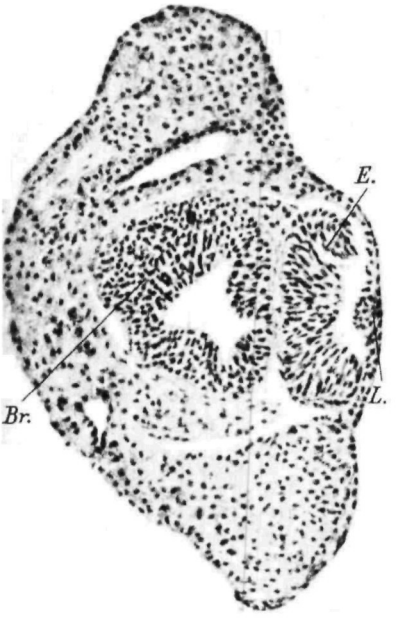

Fig. 6.

Fig. 8

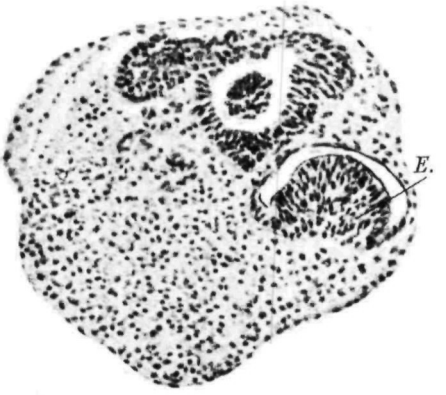

Fig. 7.

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EXPLANATION OF PLATES.

PLATE I.

Fig. 1. (No. D 59–*a*.) Sensorisation of inner layer of vesicle; the section cuts the edge of the induced lens (*L*).
Fig. 2. (No. D 59–*a*.) Same specimen, induced neural tissue formed into a tube (*I*).
Fig. 3. (No. D 59–*f*.) Induced lens (*L*) in explant with little sensorisation.
Fig. 4. (No. D 59–*g*.) Symmetrical brain and eye-cup.

PLATE II.

Fig. 5. (No. D 59–*g*.) Same specimen, induced neural tube (*I*).
Fig. 6. (No. D 59–*h*.) Symmetrical brain and single eye; small induced lens (*L*).
Fig. 7. (No. D 59–*l*.) Failure of induction in compact mass of ectoderm.
Fig. 8. (No. D 59–*p*.) Induced lens (*L*) in explant with no sensorisation.

Br. brain, *E*. eye, *I*. induced neural tissue, *L*. induced lens.