ON THE EXISTENCE OF REGIONALLY SPECIFIC EVOCATORS

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Spemann (1931) pointed out that different parts of the organization centre in the amphibian gastrula have different properties, in that the more anterior regions tend, other things being equal, to induce the formation of more anterior parts of the neural system, such as the brain and associated sense organs, while the posterior parts tend to induce the neural tube of the trunk or tail. Living organizer grafts frequently become more or less fused with the induced tissues, so that if a definite organ is finally produced, the individuation of it involves both inducing and induced materials, the former playing the leading part. When dead pieces of the organizer are used as grafts, they cannot be similarly incorporated, and any individuation which may occur must have arisen entirely within the reacting material. It might still be possible, however, that the dead organizer was able to specify which region of the embryonic axis was represented by the induction, and thus guide the individuating processes. It has been known, however, since the work of Holtfreter (1934a) that organizer material after being killed loses its power to induce mesodermal tissues. It evocates only the appearance of neural tissue. If this becomes individuated into any recognizable organ, it is usually found to represent some region of the brain or eye, while the organs resulting from secondary inductive effects of the brain (e.g. nose or ear) are also frequently found. It is certainly not impossible to find a dead material which can induce more posterior regions of the axis, since Holtfreter (1934b), Chuang (1938) and Toivonen (1949, 1950) have demonstrated such activity on the part of dead adult tissues or extracts from them. No phenomena of the kind have, however, been described as resulting from the action of dead parts of the gastrula organizer, and it has been usual to consider that this material loses its trunk-inducing powers, and becomes solely a specific head-organizer, when killed.

The experiments to be described in this communication were carried out with the intention of discovering whether a very mild heat-treatment of organizer tissues would leave any posterior-inducing capacity still verifiable in them; and further to investigate the steps which led up to the formation of any specific organs which might be found. Experiments to this end have been carried out in a desultory way for some years, and more recently a fairly large series has been undertaken.

The material used was the newt, in most cases *Triturus alpestris*, although a few experiments have also been made with *T. palmatus*. The inducing material was taken from stages somewhat later than the gastrula, since it was thought that the regional differences would become stronger as development proceeds; it was taken from a number of stages between that of the open neural plate and that of the late tail bud. The donor embryos were immersed for a few seconds (not more than five) in water at about 90° C., and then transferred to cold water in which the anterior and posterior parts of the axis were removed to be used as inducers. These fragments of coagulated tissue were wrapped in flaps of ectoderm from young gastrulae with sickle-shaped blastopores, and the 'sandwiches' cultivated in Holtfreter solution in the usual way.

RESULTS

No sign of the induction of axial mesoderm was seen in any of the cultures.

Large masses of neural tissue were formed in nearly every case in which the implant was retained within the ectodermal flaps. It usually requires a good deal of imagination to assign such masses to any particular part of the normal neural axis. The eye is the organ which can, when it is well formed, be recognized with the greatest certainty. Eyes were produced with considerable frequency (about 30 %) by the explants containing anterior organizer regions, and an even higher frequency might perhaps have been found if the cultures, instead of being fixed at times suitable to exhibit the stages of individuation, had been cultured for longer till histological differentiation was more advanced. From the explants containing posterior pieces of the organizer, eyes were not produced at all in these experiments. Although in the literature there are many cases of the induction of eyes by implants of dead posterior organizers into whole embryos (e.g. Holtfreter, 1934 *a*, table 10), or pieces of dead anterior organizer isolated in ectodermal sandwiches (e.g. Okada & Hama, 1944; Lopaschov, 1935, 1936), there appears to be no record of the induction of an eye by a dead posterior organizer in isolated ectoderm. It may be that this really does not occur, but we shall see later that it may not be the direct indication of a specific difference between anterior and posterior dead organizers which it seems to be at first sight.

It seems better merely to describe the types of inductions which occur at stages of differentiation corresponding to the late tail bud or early swimming larva, rather than to attempt to assign them to particular parts of the nervous system.

Type 1. These are large neural vesicles, with a rather extensive lumen, and walls varying from thin to moderately thick. They are the most brain-like in appearance, and indeed in many cases there is no doubt that they do actually represent some part of the brain. An epiphysis may sometimes be found attached to them. They may be accompanied by eyes, such as those described above, and in other cases there may be placodes or other neuralized thickenings still attached to the overlying epidermis in their neighbourhood. Some of these placodes undoubtedly represent nasal pits. There are other examples, however, in which such secondary

structures are absent, and it is only the morphology of the neural vesicle itself which reminds one of the brain (Pl. 20, figs. 3, 4).*

Type 2. The neural structures may take the form of a mass of contorted, thin-walled tubules (Pl. 20, fig. 5).

Type 3. Masses of well-developed neural tissue, with a narrow lumen, and walls ranging from moderate to excessive in thickness (Pl. 20, fig. 6).

Type 4. Thick solid masses of neural tissue with no lumen (Pl. 21, fig. 1).*

Type 5. Whole mass neuralized, there being no epidermal covering (Pl. 21, fig. 2).

Type 6. Small neural tubes embedded in a mass of neuralized ectoderm (Pl. 21, fig. 3).

Type 7. Similar small tubes, but lying in a projection with a well-formed epidermis covering them (Pl. 21, fig. 4). Such tubes often pass into a mass of neural tissue which retains its continuity with the superficial epidermis, and the base of the projection containing them may also be filled with a similar neuralized tissue (Pl. 21, fig. 5).

Type 8. Masses of neuralized cells in which no well-defined tubules are formed. They may be clothed in a definite epidermis, or pass gradually into a solid mass of non-neuralized ectoderm.

Before attempting any further evaluation of these inductions, it is as well to examine the appearances presented by similar experiments at earlier stages. In general, the earlier the stage, the more chaotic the induced tissues are likely to be. We frequently find the whole interior of the explant full of a complex of neural vesicles and tubes, with larger plate-like areas lying against the more extended surfaces of the dead implant (Pl. 20, figs. 1 and 2). At very early stages one can sometimes find a symmetrically folded neural groove, but in the immediately succeeding stages it is rare to find any formation which bears much resemblance to the normal neural system of similar age.

DISCUSSION

The chaotic arrangement of the induced neural tissue in its early stages, and the great variability of its appearance later, suggests that in these inductions by dead parts of the organizer we are not in fact dealing with any sort of specific regional influences at all. It seems more likely that the implant merely evocates masses of neural tissue, but that if some region of the mass happens to have a shape roughly like that of some part of the neural system, forces arise within it which cause it gradually to approach that part in its development. This would amount to the spontaneous self-individuation of particular organs, arising on the basis of chance approximations to some normal arrangement.

There is a good deal of indirect evidence which makes it plausible to suggest that such self-individuation can occur. It would, of course, be a phenomenon related to the usual developmental tendency to 'regulate' defects, and thus not unexpected on general grounds. It is probably significant also that on looking

* For Pls. 20 and 21 see following article.

through a series of sections of such inductions, one can frequently find places where the appearances strongly resemble the cross-section characteristic of some part of the neural tube; but the neighbouring sections usually show that the shape is not normally extended longitudinally, and that one is dealing with a vesicle rather than a tube. The fact that the characteristic cross-sectional patterns are produced at all under these circumstances indicates that they are in some way equilibrium forms, and suggests that complete organ shapes may similarly be shapes to which any not too abnormally formed neural mass of appropriate size will tend.

Essentially this conclusion was reached by Barth & Graff (1943), who studied the effects of freeze-dried anterior and posterior parts of the axis of the neurula when grafted into explants of isolated ectoderm. They also found that no mesodermal induction took place, and that the neural tubes or neural masses induced had no definite structure. They did not cultivate their explants very long, and perhaps for that reason do not seem to have seen any specific organs such as eyes.

If such an interpretation is adopted, it is still necessary to explain why the self-individuation to brain regions occurs more often than to shapes characteristic of the trunk, and why these induced brains are produced more often by anterior than by posterior implants. With regard to the first question, there would seem to be two factors to take into account. One is the shape of the inducing implant, which presumably controls the original shape of the evocated neural rudiment. Most of the implants were either roughly square, or had a cubical or thick-cylindrical form. They had no part which would seem likely to induce a long narrow band of neural tissue, from which an elongated neural tube might have been expected to arise. Secondly, there seems no doubt that the characteristic shape of the trunk neural tube is causally connected in some way with the presence of mesoderm; those inducers which produce posterior inductions (e.g. in the experiments of Chuang and Toivonen) appear always to call forth mesoderm as well as neural tissue. It seems likely that the mesoderm is essentially involved in the elongation of the neural system, and that this in its turn is decisive for the assumption of the characteristic neural tube shape. Yamada (1950) has been led by such considerations to suggest that posterior induction always involves a structural factor in the inducer, and although it seems difficult to make his hypothesis in its original form cover the induction of tails by coagulated embryo extract (Holtfreter, 1934*b*), there seems no doubt that dynamic factors are more important for the morphogenesis of the posterior than for that of the anterior part of the body. In the absence of either an elongated inducing substratum or of the appropriate dynamic tendencies, the formation of neural vesicles with a cross-section similar to that of the neural tube may be as close an approximation to the trunk neural system as we have any right to expect. There is no clear evidence that such neural vesicles are induced more often by posterior than by anterior implants.

The reason for the greater frequency of brain inductions by anterior implants is probably to be found also in the influence of the shape of the implant on that of the induced neural tissue. The anterior implants frequently consisted of one half

of the forebrain, and thus had a smooth surface of comparatively large area, which one might expect to induce a single broad neural plate which would start with a certain resemblance to the brain region at a similar stage. The more equi-dimensional shape of the posterior implants (except those taken from open neural plate stages) would be more likely to induce a number of separate smaller rudiments.

The tendency for more definite organs to be induced by implants grafted into whole embryos than by those enclosed in isolated flaps of ectoderm is probably also connected with the fact that in the former the area of contact between the inducer and the reacting tissue is more regular than it can be when the ectoderm is free to contract into random folds, as it does in explants.

The evidence described in this paper does not, of course, suffice to disprove the hypothesis that there may exist evocators which specifically induce some definite part of the embryonic axis. All that has been shown is that in the fairly early larval stages such specific influences are not strongly developed, or are destroyed by slightly elevated temperatures, and that the facts can be adequately explained without postulating them. The most apparently convincing evidence for the existence of such regionally specific substances (or 'modulators' as I have (1937) proposed calling them) is that derived by Chuang and Toivonen from implantations of dead adult tissues of various kinds. Both these authors have shown how the regional properties of such implants change after the application of various treatments, and Toivonen (1950) and Kuusi (1951) have carried the chemical study of them some distance. It is notable, however, that evocators which produce posterior structures always also produce considerable amounts of mesoderm. In fact, as far as one can judge from the published descriptions, the posterior quality is strictly proportional to the quantity of mesoderm; with much mesoderm the induced axis becomes a tail or trunk, with less it forms the hind-brain accompanied by ears, with none the archencephalic region of the brain. It seems quite possible, therefore, that the primary action of all these tissues and extracts is to evoke merely a mass of tissue with a certain proportion of mesoderm and neural plate, and that the individuation of this mass, so that it develops into a definite organ, is always a self-individuation, taking place within the evoked material in a manner determined by the mesodermal-to-neural ratio. If this were so, it would strictly be incorrect to speak of regionally specific evocators at all. We should be dealing only with mixtures of neural and mesodermal evocation. Only the future can tell, however, whether this suggestion, which now appears to be the simplest admissible, will prove to be correct.

SUMMARY

1. Pieces of the embryonic axis, taken from the anterior and posterior regions of embryos from the open neural plate to the late tail-bud stages which had been coagulated by a few seconds' immersion in water at 90° C., were inserted into flaps of gastrula ectoderm which were then cultivated in Holtfreter solution. No

induction of mesoderm occurred, but neural tissue was evoked in a high percentage of cases.

2. In early stages the neural tissue usually formed a more or less chaotic tangle of tubes and rods. At later stages it assumed a variety of forms, some of which were similar to parts of the brain, and such brain-parts might be accompanied by secondary structures such as eyes, nasal pits, ears, etc. No elongated tubes resembling the trunk neural tube were seen, although certain neural vesicles may have a cross-section very like that of the neural tube.

3. The induction of recognizable brain or eye was not uncommon when anterior implants were used, but was not seen at all with posterior implants. There was no other difference between the two sets of experiments.

4. It is suggested that the appearance of such organs is not due to the direct action of a regionally specific inducing factor, but rather that all such definite forms arise by a process of self-individuation which occurs within the induced mass of neural tissue. The direction this self-individuation takes, and thus the nature of the organ finally formed, is supposed to depend on chance resemblances between the mass and shape of parts of the original chaotic mass and some part of the normal embryo. It is argued that this could account for the apparently specific effect of the anterior implants.

5. In other experiments in which mesodermal tissues are also induced (e.g. with implants of adult tissues) it is likely that these take part in the self-individuation processes and tend to direct these towards the formation of posterior organs such as trunk and tail.

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