STUDIES ON REGIONAL SPECIFICITY WITHIN THE ORGANIZATION CENTRE OF URODELES

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(With Fifteen Text-figures)

INTRODUCTION

It has been known since the work of Spemann (1929, 1931) that there are regional differences in the activities of different parts of the Urodele organization centre in the young gastrula stage. Thus, presumptively, anterior regions, which are at the blastopore lip at an early stage of gastrulation, tend to induce anterior parts of the nervous system, consisting of brain and associated organs, whereas, presumptively, posterior parts tend to induce regions of the trunk or tail. Holtfreter (1936) has mapped more exactly the areas covered by these 'head-organizers' and 'trunk organizers'. Lehmann (1938, 1942, 1945) has identified similar regional differences by observing the effect of temporary exposure of gastrulating embryos to certain deleterious solutions, particularly of lithium salts. He distinguished four zones in the organizer region; the archencephalic zone in the anterior, which later underlies the forebrain, midbrain, eyes, nose, etc.; then the deuteroencephalic zone, in the region of the hindbrain; the trunk zone; and the tail zone, later characterized by the development of the dorsal and ventral tail fins. Dalcq (1947) agrees that there are two different sectors in the brain organizer, which he prefers to call the acrencephalic and chordencephalic instead of archencephalic and deuteroencephalic, as Lehmann does. Nieuwkoop (1947) presents evidence that in secondary induced embryonic axes, the brain is always fully formed up to a certain level, anterior to which it is altogether absent, and on this basis distinguishes at least seven successive zones in the brain.

The evidence for the existence of such specific regions within the organization centre comes, of necessity, from phenomena which imply a mosaic type of development; for instance, inductions in which a part of the organizer causes the appearance of only part of the embryonic axis, or susceptibility experiments in which one region is differentially affected. On the other hand, we have plenty of evidence for considerable lability in the determination of the various regions of the presumptive mesoderm in the early gastrula, for instance in the transplantation experiments of Bautzmann (1932, 1933), Töndury (1937), Raven (1938), the isolations of Holtfreter (1938), and even the original inductions of Spemann (1931), in which the grafts did not always develop into their presumptive fate; while Yamada (1937, 1939, 1940) has shown that even in the neurula the mesoderm is not finally determined. It is therefore necessary to inquire into the status of the regional differences within

the organizer from the early gastrula stage onwards. The indirect evidence would suggest that they must originally be relatively labile and only gradually become more fixed. Direct experiments on the matter are, however, surprisingly lacking up to the present. Hall (1937) found that a posterior organizer, transplanted from an old gastrula into the region of the anterior organizer of a young one, brought about a suppression of head development, the anterior part of the neural system being in the form of a narrow cylindrical tube; while, in the reciprocal experiment, young anterior organizer could be fully incorporated into the posterior of an older gastrula without causing any tendency for brain development in its neighbourhood. This is almost the only series of experiments dealing directly with changes of anterior-posterior regionality within the organization centre of Urodeles, although Bautz-mann (1932, 1933) exchanged material between the sides and the midline, and Töndury reversed median strips of tissue which, however, were long enough to bring presumptive ectoderm into the organization centre, while parts of the latter were transferred right outside its boundaries.

As regards the Anura, Waddington (1941) has published the results of reversals of the anterior-posterior axis of a median strip of the organizer, and of exchanges between anterior and posterior organizers of the early gastrula, using the eggs of *Discoglossus*. In general, it was found that the transplanted portions had a strong tendency to carry out autonomously the gastrulation movements proper to their place of origin, and that this prevented their incorporation into the main body of the embryo, on which, in consequence, they showed little sign of exerting a specific regional action. *Discoglossus*, however, is a species in which the morphogenetic movements of gastrulation are particularly vigorous and rapid, involving a very great condensation and elongation of the tissues in the dorsal plane. The evidence to be presented here demonstrates that in *Triton*, in which these movements are more gentle, translocated parts of the organizer can be much more readily controlled by the remainder of the gastrula and thus incorporated into the embryo.

The material to be described here has been accumulated over a number of years, from 1937 onwards. Except for about sixteen organizer reversals, five anterior-in-posterior, and three posterior-in-anterior grafts, which were carried out by Yao, all the operations were made by Waddington, who is also responsible for the detailed examination of the specimens and the writing up of the paper.

All experiments were made on eggs of *T. alpestris*.

EXPERIMENTAL RESULTS

(1) *Organizer reversals*

The eggs were operated at the stage when gastrulation is just beginning, the blastopore being represented by a fairly broad, shallow groove, lined by flask cells, but with as yet no definite archenteron extending inwards from the surface. The operation consisted in excising a rectangular region, bounded by the blastopore groove on the vegetative side, and extending animal-wards about to the boundary of the presumptive mesoderm; its width from side to side was rather greater than the

extent of the blastopore (see Fig. 1). In freeing this flap of tissue from the embryo it is necessary to dissect it away from the underlying cells in the immediate neighbourhood of the blastopore. In doing so, a considerable quantity of the deeper-lying presumptive prechordal mesoderm was left attached to the fragment, but the boundaries of this tissue are not clear in the living egg, and some of it was probably left *in situ*. After the fragment had been completely separated from the egg, it was rotated about an axis perpendicular to the egg surface, and replaced, so that its original animal side lay next to the blastopore, while the original blastopore lip lay near the boundary of the presumptive mesoderm.

Some thirty embryos operated in this way are available for examination; most of them were fixed in late tailbud stages. Of these, nineteen appear to be completely normal in every way. In them regulatory processes originating from the main body of the embryo must have completely overcome the initial disorganization caused by the reversal of the median strip of the organizer.
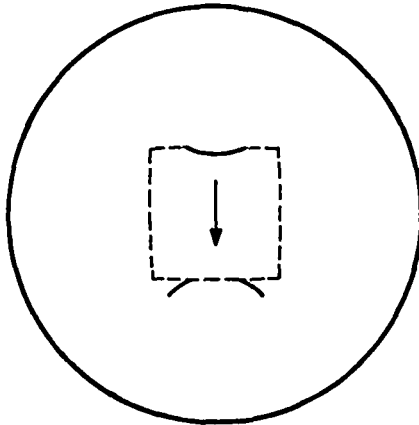

Fig. 1. Diagram of operation, reversal of organizer.

In another group of four embryos, there is some disturbance of the notochord. Usually this takes the form of an enlargement of this organ in the anterior region or a reduction of it in the posterior, or both these effects may occur together. This is probably due to a tendency for the displaced blastopore lip to begin invaginating in its new, more anterior position; if material invaginating in this position were then joined by other tissues proceeding from the blastopore lip in the usual way and carried forward with them, one might expect to find an excess of material in the anterior part of the archenteron roof, while if the remainder of the embryo failed to compensate completely to such a process, a deficiency in more posterior regions would result.

In another small group of four or five embryos (partly overlapping with the last group) there is greater or lesser degree of spina bifida posterior. In these embryos it is clear that the reversed section has acted as an impediment to the normal gastrulation movements of the remainder of the egg, so that the latter have become split into two streams, each of which has given rise to a half embryo. In most cases, it is probable that the impediment has occured because the displaced blastopore material

has attempted to invaginate in its normal direction (which, in its reversed position, would be towards the blastopore) but has been prevented in its movement by the invagination proceeding in the unaltered lateral parts of the germ, and has thereby been reduced to immobility. In similar experiments on the Anuran *Discoglossus* (Waddington, 1941) such an autonomous invagination of the reversed piece usually succeeds very much more completely, possibly because the gastrulation movements in that form are much more vigorous and rapid than they are in *Triton*. Only one case of a partially successful autonomous invagination of the reversed section has been found in the latter. In it (embryo no. OR 57, Fig. 2) the reversed graft formed

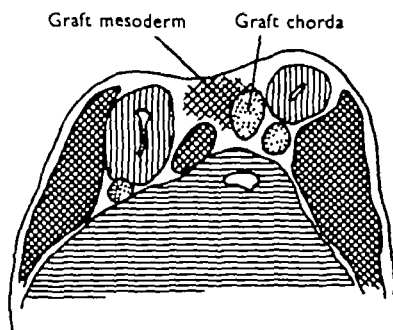

Graft mesoderm   Graft chorda

Fig. 2. No. OR 57. Section through mid-trunk region. The independent invagination of the graft has split the host axis into two halves, each of which has undergone considerable regulation.*
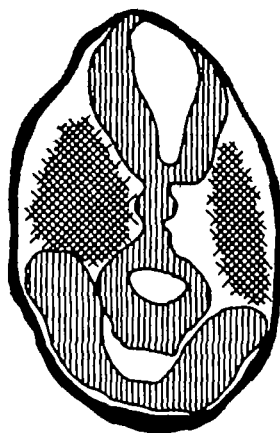
Fig. 3. No. K 6b-3. Organizer reversal. Section through eye region, exhibiting microcephaly.

a knob-like outgrowth which was originally seen protruding from the centre of the neural plate. In the later stages it was visible, caught between the two sides of the neural groove, which had folded together pinching it between them. In sections, this protuberance is found to contain a number of axial tissues, including chorda, somitic mesoderm and probably neural tissue, which, as is well known (Holtfreter, 1938) can be developed by isolated fragments of the presumptive mesoderm. The presence of this mass of extraneous tissue causes the main neural system of the embryo to have the appearance of a spina bifida; and it is noteworthy that in this case each of the halves of the neural tube has to a considerable extent become regulated into a bilaterally symmetrical cross-section, so that we have almost a complete doubling of the axis. In other spina bifidas described in this paper, the two half-axes are usually widely separated by an expanse of uncovered endoderm, from which the graft tissues have fallen away; and in these cases there is little tendency for the regulative restoration of their bilateral structure. Regulation leading to complete doubling of the axis has, however, been described by Bautzmann (1932) in

* In all the diagrammatic drawings of sections, the neural tissue is lined vertically, the endoderm lined horizontally, the mesoderm cross-hatched and the notochord dotted.

other experiments, in which the invagination stream was divided into two without complete loss of tissue continuity between the two sides. A similar phenomenon has been demonstrated in the chick embryo (Abercrombie, 1950; Abercrombie & Morgan, 1950).

Finally, some three or four embryos show a slight under-development of the head (Fig. 3). This microcephaly is almost certainly due, not to a direct action of the reversed section, but to a partial inhibition of invagination, leading to deficiency in the anterior archenteron roof, caused by the antagonistic action of the graft on the normal movements of the remainder.

(2) *Exchanges of anterior and posterior organizer*

In these experiments, rectangular flaps of tissue were excised, containing either the presumptive anterior or posterior median regions of the organization centre (Fig. 4). The anterior region being removed from one egg, and the posterior from
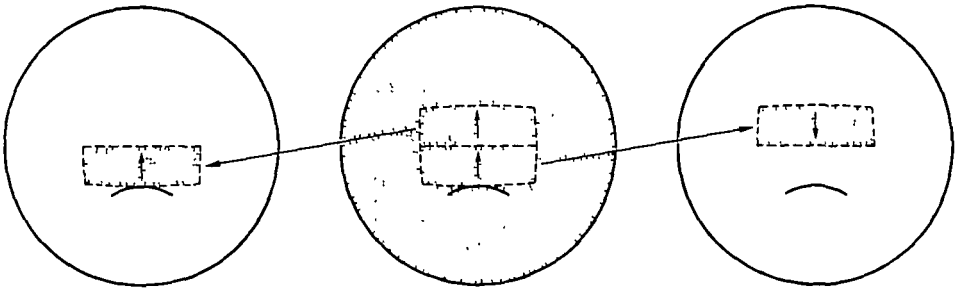

Fig. 4. Diagram of operation, exchanges of anterior and posterior organizers. Centre: donor; on left: post. in ant. *aa*; on right: ant. in post. *ap*.

another, the two pieces were exchanged, so that one embryo received a posterior-in-anterior graft and the other an anterior-in-posterior. The grafts were made either in their original orientation, i.e. with the originally anterior material still towards the anterior (a so-called *aa* graft) or with the anterior-posterior axis reversed (an *ap* graft). Some of the donor embryos were vitally stained with Nile blue, so that the graft could be recognized among the host tissues.

(a) *Posterior-in-anterior grafts.* There are thirty-eight specimens in which this operation was performed on the young gastrula in which the blastopore is still an open groove. In nine of these the anterior-posterior axis was reversed.

Ten of these embryos appear perfectly normal in every way. Regulation can, therefore, be quite complete in favourable cases.

The remaining embryos show various types of abnormality. The commonest of these are: microcephaly, which in extreme cases takes the form of cyclopia, or even the total absence of eyes; and spina bifida. The explanation of the latter condition is fairly obvious; the relatively inactive posterior region has failed to invaginate, or has invaginated less rapidly than the lateral parts of the presumptive mesoderm, and has therefore acted as a block, splitting the archenteron roof in two. In the weaker grades of this, one finds a doubling of the chorda, and perhaps some doubleness

in the neural tube. There are also cases in which the only abnormality is a slight enlargement of the notochord, usually in the mid-trunk region (Fig. 5). These are probably to be attributed to a certain regulatory production of extra chorda by each lateral part of the host, during an early stage at which the graft is still unassimilated, followed by fusion between these host chorda masses and the chorda derived from the graft itself; they can therefore be regarded as similar in nature to the spina bifidas, which differ in that the later fusion fails to take place.
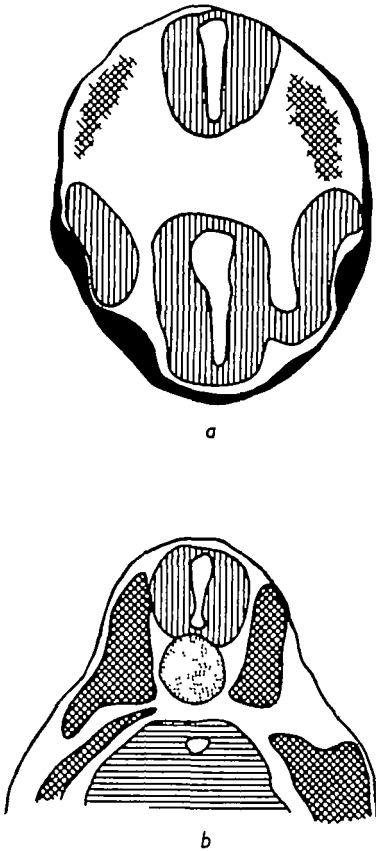

a

b

Fig. 5. No. E 106 b–7. Post. in ant. *aa.* Normal embryo, except for enlargement of chorda in trunk region, shown in *b.*
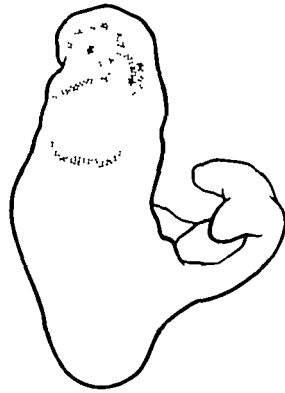

Fig. 6. No. K 8 b–2. Post. in ant. *aa.* Spina bifida.

In higher grades of spina bifida, the embryo is completely split up the back. Usually, in such cases, the graft tissue finally separates itself completely from its host, drops off and is lost. The mid-dorsal region is then occupied by a broad expanse of naked endoderm, on each side of which runs a half-axis, which often curls up dorsally as a bifid 'tail' (see Fig. 6). There is usually little tendency for these separate half-axes to regulate to a bilaterally symmetrical cross-section.

The microcephaly can be brought about in a number of different ways. The least specific of these is by a general inhibition of gastrulation movements, probably

leading to a deficient development of archenteron roof in the anterior. In experiments in which the graft was vitally stained, it could in a number of cases be seen that the grafted posterior organizer had not moved forward far enough to underlie the head, as it should have done if it had behaved entirely in accordance with the position in which it had been placed. Presumably this material, which had not yet reached the stage of invagination at the time it was removed from the donor, is at first relatively passive; we know that the surroundings of the blastopore lip can induce a tendency to invagination in indifferent material placed among them (Raven, 1938; Töndury, 1937); but this would undoubtedly take some time to affect the rather large pieces involved here, and it is not unexpected to find that some of these posterior organizer grafts lag behind and fail to reach the front end of the archenteron. However, even when the graft only reaches the anterior region of the trunk, there is an archenteron roof farther forward than this, formed from presumptive mesoderm from the main body of the embryo. The relative size of this anterior part of the archenteron roof has not been directly measured in such embryos, but in such situations the brain may be reduced in size, leading to microcephaly, cyclopia, etc., and it may be presumed that this is a consequence of a deficiency in the anterior mesoderm.

Reduction of the head also occurs in embryos in which the graft material invaginated more rapidly and reached the anterior of the archenteron roof. Under these circumstances, the brain will be underlain by tissue which was presumptively posterior organizer; and unless this tissue is endowed with an anterior character by regulatory processes within the sheet of mesoderm, it will tend to induce trunk rather than brain structures. Phenomena of this kind were described by Hall (1937), when he grafted fragments of posterior organizer taken from later gastrulae. This can be confirmed from the present material (see below). Similar effects may be produced when the grafts are taken from younger stages, but in that case they are much less frequent than they were in Hall's material. It is not possible to give an exact figure for the frequency of their occurrence, since it is not always possible to distinguish them clearly from cases of microcephaly produced by inhibition of gastrulation; but whereas Hall found twenty cases of complete absence of brain and only four in which the brain was partly developed, in our series of grafts from the early gastrula there are ten completely normal embryos and eight with microcephaly (apart from several which combine microcephaly with spina bifida). Moreover, in the present series, the microcephaly is much less extreme than that described by Hall as typical. Whereas in his cases the brain was reduced to a simple tube with narrow lumen, in ours the microcephaly usually amounts to no more than a reduction in size, an under-development or complete failure of the optic lobes, and a smoothing out of the divisions between the lobes of the brain (Fig. 7). The most extreme cases of microcephaly found in the young gastrula grafts have been in embryos in which the anterior-posterior axis of the graft was reversed (*ap* grafts), several of which have led to a condition approaching, though not attaining, that described by Hall.

One can conclude that the presumptive posterior region of the organizer at the early gastrula stage can be completely converted, in these grafts, so as to develop into anterior tissues and so as to induce the anterior parts of the neural system. This

conversion is perhaps less easy when the graft is made with a reversed orientation, as is evidenced by the more extreme microcephaly which may be produced in such cases, but even among them some instances of perfect regulation occur.

The difference of behaviour of these early grafts and those in which the posterior organizer is taken from embryos in later gastrula stages has been directly confirmed in a special series of operations from which sixteen specimens are available. The dorsal blastopore lips, including both the superficial and the already invaginated layers, were removed from mid-gastrulae with smallish yolk plugs and grafted into the anterior organizer region of young gastrulae, in the manner described by Hall. The donors were somewhat younger than Hall's, being nearer Harrison's stage 12 than 13. The results were more or less intermediate between those in the earlier
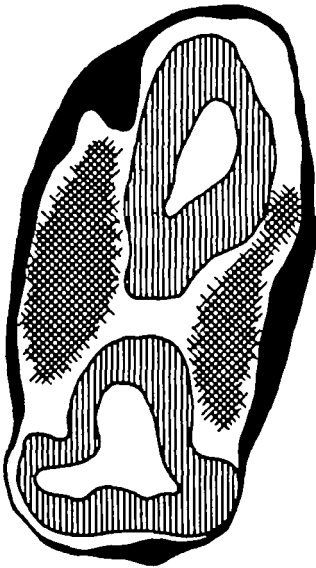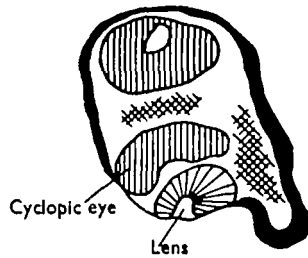

Fig. 8. No. H 19–2. Post. of small yolk-plug gastrula in ant. of young gastrula. Section of anterior end of head, late tailbud stage, showing extreme microcephaly and cyclopia.

Fig. 7. No. E 107 *b*–3. Post. in ant. *ap.* Section through anterior of mid-tailbud stage, exhibiting moderate microcephaly.

grafts described above and those recorded by Hall. No perfect embryos were obtained when the graft came from an embryo later than the horse-shoe blastopore stage. In all the others, the head was considerably reduced, with either two very small eyes, or, more usually, one small cyclopic eye (Fig. 8). The formation of extra notochord in more posterior regions was also common, but complete spina bifida was rather rarely found, probably because in these grafts the posterior organizer was already in process of invagination and could therefore more easily keep pace with the movements of the host.

(*b*) *Anterior-in-posterior grafts.* Thirty-four embryos are available, from experiments in which an anterior organizer was placed in the position of the posterior organizer of a young gastrula. Of these thirteen are apparently quite perfect, two

of them from the small group of seven in which the anterior-posterior axis had been reversed.

The others show, to a greater or less extent, the same type of abnormalities due to disturbances of gastrulation which have been described above, namely enlargement or doubling of the notochord, spina bifida and microcephaly. In several cases the spina bifida is quite slight, as in Fig. 9. The microcephaly is also usually not
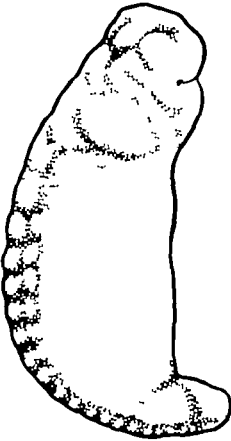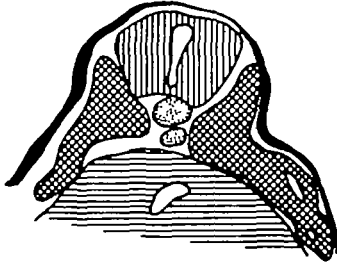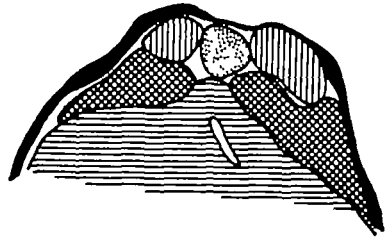

Fig. 9b.

Fig. 9c.

Fig. 9a.

Fig. 9. No. K 8b–4. Ant. in post. aa. a, tailbud stage, with slight microcephaly; b, double notochord in neck region; c, spina bifida in trunk.
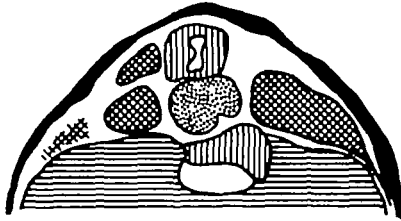

Fig. 10. No. 107b–13. Ant. in post. ap. Enlarged notochord in posterior trunk region. Under the notochord is a mass of neural tissue derived from the graft, which has been carried forward with the invaginating host mesoderm.

pronounced. It must certainly be attributed to a partial inhibition of gastrulation, leading to a reduction in the mass of the anterior archenteron roof. In the aa grafts the mechanism of this reduction is rather obscure; it may be simply a consequence of the damage done during the operation, and the difficulty of obtaining satisfactory healing of the rapidly elongating anterior organizer into a region which is itself undergoing active gastrulation movements. In the ap grafts, it is easy to see that there would be a tendency for an invagination stream to proceed from the graft in a direction opposite to that of the main mass of mesoderm moving forward from the host blastopore. In Discoglossus these two streams usually remain quite distinct and come into direct conflict, when the graft stream may be bodily bent round and

carried forward embedded in the host mesoderm (Waddington, 1941). Only one example of this has been found in the *Triton* material, but there are several other cases in the *ap* series in which the chorda is greatly enlarged in the posterior trunk region (Fig. 10), and reduced or altogether absent in the anterior, which also exhibits microcephaly, and these can also be accounted for by a partially successful attempt of the anterior graft to invaginate according to its presumptive fate.

A few specimens are available (five in sections, a similar number observed *in toto* but not sectioned) in which the anterior organizer of a young gastrula was substituted for the posterior organizer of an older host, in Harrison's stage 12. Hall has shown that such grafts may be perfectly assimilated in even later stages, and perfect assimilation was, as might be expected, also found in many cases here. However, in *ap* grafts of reversed orientation regulation was not always perfect, and extra masses of chorda could be found in the posterior trunk region.
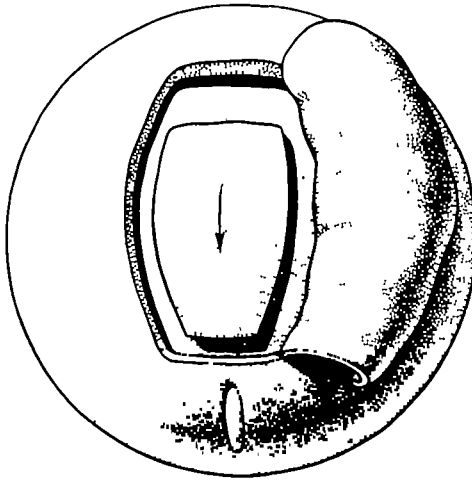

Fig. 11. Diagram of operation. Extra archenteron roof added, in reversed orientation, under the presumptive neural plate of a late gastrula. The anterior-posterior extent of the added material was actually greater than shown in the diagram.

(3) *Addition of extra archenteron roof*

We have seen that regulation may be perfect in the early gastrula stage; Hall has shown that the posterior organizer of a late gastrula has a regional character which cannot be overcome by the presumptively anterior surroundings of a young gastrula; and one wonders whether this character is so firmly fixed that it cannot be altered by influences present in the late gastrula. A small series of experiments was made to test this. In a late gastrula in which the yolk plug had just become slit-like (Harrison's stage 13 or slightly later), the whole presumptive neural plate was freed along one side and round the anterior, and a second archenteron roof, removed from another similar gastrula, placed with reversed orientation on top of the normal roof, the neural ectoderm then being carefully folded back and allowed to heal over the double layer of mesoderm (Fig. 11). It is impossible to carry out this operation as perfectly

as the description would suggest; there is a strong tendency for the second roof to roll up, and some damage is usually done to it while spreading it out flat over the roof of the host, and although the reversal of its anterior-posterior axis presents no difficulty, it is difficult to recognize with certainty its midline and still more difficult to be sure that after the ectodermal covering is restored this midline is accurately above that of the host. In some cases, in fact, the whole added archenteron roof has shifted to one side of the host axis, and has there produced an induction of normal type.

Only about seven embryos are available in which the operation was tolerably successful. This is not nearly enough to elucidate all the consequences which may follow such an addition to the mesoderm layer. There are, however, three instances in which a relatively perfect and complete embryo has been formed; embryos, that is to say, which differ from normal only in the exaggerated thickness of their axial mesoderm (somites and chorda) (Fig. 12). And another embryo is fairly normal
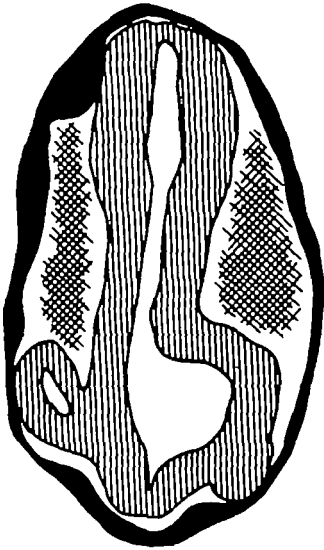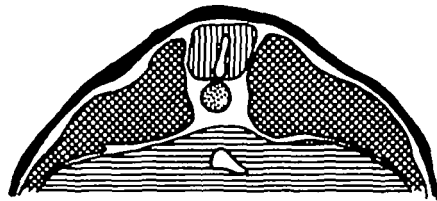

Fig. 12 b.

Fig. 12 a.

Fig. 12. No. E 128b–2. Extra archenteron roof added, ap. a shows a relatively normal head; b, the trunk region with unusually thick mesoderm.

except that one side of the neural system is over-developed and larger than the other. These suffice to demonstrate that regulation of anterior-posterior quality is still possible in the late gastrula stage, provided suitable influences can be brought to bear.

In all the other three abnormal embryos the notochord is lacking in large sections of the trunk, its place being occupied by a thick layer of apparently somitic mesoderm (Fig. 13); in two of them the head is very badly developed, with no proper brain or eyes. A much larger series would be necessary to reach a full understanding of the various abnormalities, but it may be surmised that in these cases the midline of the added archenteron roof did not coincide with that of the host, so that axial mesoderm

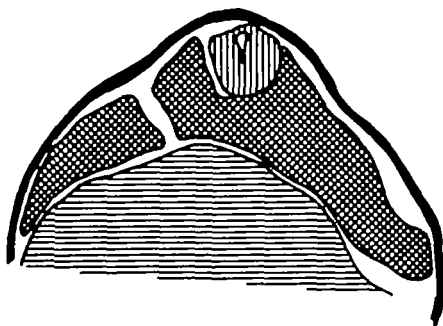was brought into contact with more lateral material, and altered in its development (cf. Yamada, 1937–40).

Fig. 13. No. E. 89*b*–3. Extra archenteron roof added, *ap*. Section through trunk region, showing absence of notochord and thick somitic mesoderm.

DISCUSSION

The evidence presented above makes it clear that a fully normal embryo may be formed after the reversal of the organizer, or the exchange of its anterior and posterior parts, when the operations are made in early gastrula. Such a result involves the complete regulation of the topographically altered organization centre. As Raven (1948) points out, 'regulation' is an 'unclear and heavily charged word', which it is in many ways desirable to supersede by more precise expressions. According to his recommendations, one should classify the phenomena in question (in terms originally due to Dalcq) as a 'teleogenetic paragenesis', i.e. the production of a normal end-result by means of an abnormal process of development. Another way of describing the same situation is to use a term originally introduced by Waddington & Schmidt (1933), and say that the individuation field of the primary organizer can restore its normal configuration after being disturbed by the reversals or exchanges in question.

It was pointed out in the Introduction that this differs sharply from the result of similar experiments in the Anuran *Discoglossus* (Waddington, 1941), and it was hinted that a cause of this difference may be sought in the different tempo and extent of the gastrulation movements in the two species. At present this seems to remain the most plausible suggestion. Certainly the inherent tendencies to perform specific types of movement, which were the most interesting and as yet inexplicable feature of the *Discoglossus* experiments, are very much less marked in *Triton*. They are, however, expressed in certain ways, for instance, after organizer reversals; but in general the power of inducing appropriate movements, which the main bulk of the organization centre is known to possess (Raven, 1938; Töndury, 1937) usually succeeds in overcoming the autonomous tendencies of the graft. The nature of the inductive influence which has such effects on tissue movements is one of the most fascinating and obscure problems of physico-chemical embryology.

In the chick embryo, the possibilities of regulation seem rather similar to those in *Triton*, since Abercrombie (1950) has demonstrated complete regulation following

the reversal of various median portions of the organization centre, comprising parts of the primitive streak.

There is evidence in the present experiments for an increasing fixity of regional character during the progress of gastrulation in *Triton*. Thus when a young posterior organizer is placed in the anterior region of an early gastrula, a normal embryo may be formed; but when, in an exactly similar experiment, the graft is from an older stage, it cannot be fully converted, and some degree of microcephaly results, which reaches an extreme form when the graft is from the still older stage used by Hall. Nevertheless, the regional character is even then by no means irrevocable, as is shown by the formation of normal embryos following the addition of an extra archenteron roof, in reversed orientation, to a late gastrula of about the stage Hall employed. There are, perhaps, two main respects in which this experiment differs from the earlier one. In the first place, the host embryo is here an old gastrula, whereas in Hall's experiments the posterior graft was made into a young stage. At this greater age the regional structure within the host would be more intensely developed, and may be able to exert a stronger influence on the graft. Secondly, the added archenteron was placed inside the gastrula, lying with its whole surface against that of the normal archenteron, and it seems reasonable to suppose that in this situation it is more accessible to influences from the host than it would be when only attached to the sheet of host mesoderm along its edges, and with the necessity to undergo the movements of gastrulation. In any case, a lability of regional determination at this stage cannot be considered surprising in view of Yamada's demonstration that even later, in the neurula, the histogenetic fate of the various types of mesoderm is not finally fixed.

Considerable discussion has taken place in recent years as to the nature of the regional differences within the organization centre. The main debate has been between those, like Dalcq & Pasteels (1937, and later), who argue that these are primarily quantitative, and others, of whom Lehmann (1938, and later) is one of the most prominent, who urge the importance of qualitative differences. It is essential, of course, to specify precisely the stage of development to which one is referring. It is certain that there must eventually be qualitative differences in the chemical constitution of the different parts of the axis; the question is at what stage they arise, and whether they are preceded by a field of quantitative differences.

As regards the situation during the phase of gastrulation, before the appearance of the neural plate, there seems now general agreement as to the facts which have to be explained. It is conceded by both sides that chordal part of the archenteron roof behaves in most ways as a single unified region, while the pre-chordal plate acts as though it were to some extent independent. Dalcq (1947), in consequence, supports the view that 'quantitative relations are fundamental', but makes a 'reservation for the relative specificity of the pre-chordal region', and considerably more hesitantly for the parachordal mesoderm. Lehmann (1945, p. 318) emphasizes the special position of the pharyngo-dorsal blastema (which corresponds to Dalcq's pre-chordal plate), but admits that there is a good deal of evidence for mainly quantitative differences within the chorda-mesodermal region.

Both these alternatives seem to be somewhat too simply posed. There are other possibilities to be considered. Five of them were already listed at an early stage in the discussion (Waddington, 1937).

(1) Different regions of the gastrular organizer might contain different evocator substances, each specific for a particular region of the axis. The evidence, from extraction experiments and studies on regulation, is all against this hypothesis.

(2) There may be a basic evocator substance, which, in different regions, is 'modulated' by other additional substances or slight changes in its structure (for instance, if the basic evocator is a protein). It can now be taken as almost certain that there is a basic evocator; and the eventual development of specific local chemical differences in stages later than the gastrula could be considered modulations of it; but whether such modulation has already occurred during gastrulation is still open for discussion.

(3) The regional differences could be quantitative, particular regions being induced by particular absolute concentrations of evocator. This would seem some-what unlikely, both on account of the regulative phenomena encountered, and because it would lead one to expect more evidence of regional specificity in experi-ments with dead organizers, evocator extracts, etc., than is actually found.

(4) The regional quality could depend on relative concentrations, i.e. on quanti-tative gradients. This would be the simplest explanation of regulation, but it would be difficult on such a basis to account for those cases where regional specificity has been shown by abnormal evocators, as in Chuang's (1938 and 1940) and Toivonen's (1938) experiments.

(5) The possibility was suggested (Waddington, 1937) that specificity might depend on some 'specific spatial properties', those mentioned as examples being related to the micro-structure, such as the formation of partially oriented aggregates, resulting in liquid crystals, specific surface properties, etc. According to the fashion of the day, hypotheses such as this tend, perhaps, to be rather too easily accepted in the capacity of *deus ex machina*, and it seems advisable to hold such modes of explanation in reserve until there is some positive evidence in favour of them, or at least till all more readily verifiable alternatives are exhausted.

No one of these five possibilities (including the extreme qualitative hypothesis, no. 1, and the extreme quantitative ones, nos. 3 and 4) seems fully satisfactory. It is more probable that the actual situation corresponds to some combination of them. The existence of regionally specific inductors in dead adult tissues, demonstrated by Chuang and Toivonen, and the hints that similar effects can be produced by dead material from later embryos (see Lopashov, 1936; Waddington, 1937) strongly suggest that something comparable to the postulated modulation of the basic evocator does occur after the stage of gastrulation; while the ease of regulation at the earlier stage, new evidence of which has been brought forward in this paper, is most easily accounted for in terms of a quantitative hypothesis such as no. 4 above. Moreover, if attention is paid to the actual events which are occurring while the evocator is being produced, it is easy to see ways in which it may plausibly be supposed that such quantitative differences arise. Indeed, the phenomena of gastru-

lation invite our attention to two further types of factor, one concerning the time relations of the various processes, and the other to spatial considerations, not on the scale of 'intimate structure' originally contemplated in hypothesis no. 5, but on the more straightforward scale of the gastrulation movements themselves.

Attention has earlier been drawn (Waddington, 1940) to some of the phenomena which would almost inevitably follow from the mere physical events of gastrulation. The neural evocator is liberated at the blastopore, in the stream of mesoderm which moves forward as the archenteron roof. From this it diffuses into the overlying tissue, and also presumably laterally from the midline towards the lateral mesoderm. One can probably also presume that the liberation is not a sudden
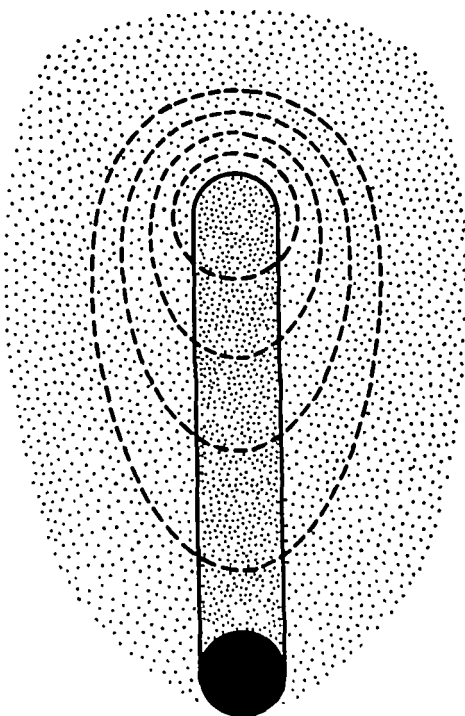

Fig. 14. Diagram showing the yolkplug blastopore (black) from which there extends forwards the layer of mesoderm (dotted). The evocator may be supposed to be liberated in the archenteron roof (close dots), and then is distributed in accordance with the concentration contours shown.

process, complete in the tissue immediately after it has invaginated, but that it continues for some time during the forward movement of the mesoderm sheet. A process of this kind would give rise to some sort of pear-shaped distribution of evocator within the mesoderm (Fig. 14), but we know too little about the exact directions of movement within the mesoderm, the initial concentrations of precursor and rates of liberation, diffusion, etc., for it to be worthwhile attempting to calculate a more precise shape. The pear-shaped region would, however, increase in area as it moved forward, and probably the concentration in the centre of it would increase. Nieuwkoop (1947) has drawn a figure of a rather similar 'mesodermal gradient',

but instead of deriving it from considerations of the production and diffusion of the evocator, he merely postulated that it already exists in the presumptive mesoderm of the early gastrula, waiting to be passively folded in to form the archenteron roof.

Nieuwkoop suggests that this mesodermal gradient system reacts with an ectoderm which is also characterized by a field of quantitative gradients. There is, however, no independent evidence for the existence of such a system; in fact, what evidence there is (e.g. Holtfreter, 1933 a) is against it, and Nieuwkoop can do no better than argue that the experimental analyses made up to the present have been too crude to reveal it. There are, however, two other major factors which must be involved and which may have an influence; and a consideration of these suggests that all the
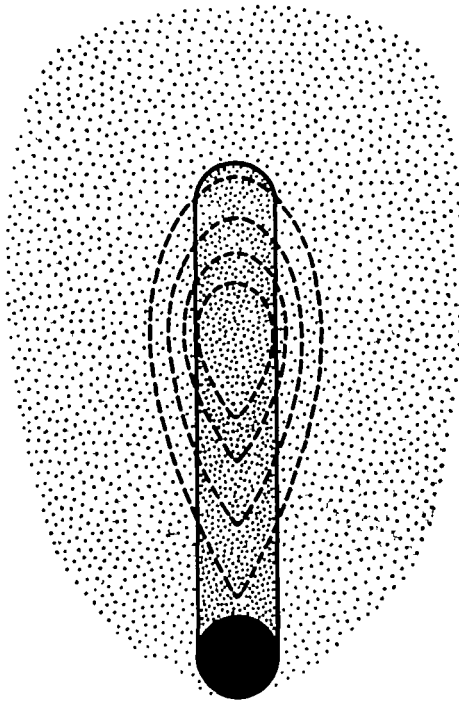

Fig. 15. Concentration contours in the ectoderm, which moves down over the mesoderm towards the blastopore.

known facts may be explained without calling on such an insecurely founded entity as an ectodermal field.

The first of these factors is that of time. During the period when the evocator must be supposed to be diffusing into the presumptive neural plate, the latter is moving towards the blastopore, passing over the sheet of mesoderm which is flowing the opposite way. Again we know too little of the details to make it worth attempting an exact calculation of the consequences of this, but it is easy to see in general terms that the concentration contours in the ectoderm will differ from those in the mesoderm somewhat in the manner indicated in Fig. 15. Moreover, there is a

second variable to be considered: these concentrations will have been accumulated over different periods of time; the most anterior region of the neural plate will have been underlain by evocating mesoderm for a shorter time than some of the more lateral and posterior tissue which may lie within the same concentration contour.

Some evidence for the existence of such concentration gradients is already available. In the uninvaginated mesoderm, Bautzmann (1926) showed that the organization centre was relatively sharply bounded as against the more lateral mesoderm which showed no inducing capacity; in the neurula, on the other hand, Holtfreter (1933 b) found that lateral plate was almost as powerful an inductor as axial mesoderm. It is, however, the stage between these two, that of gastrulation, in which we are interested, and at that stage the existence of an inductive capacity in the lateral plate, definite but weaker than that of the archenteron roof, has been demonstrated by Waddington (1936). More recently, Raven & Kloos (1945) have presented evidence for a similar lateral gradient within the axial mesoderm. Gallera (1947) and Damas (1947) (see also Dalcq, 1947) have also found a cranio-caudal decrease in the 'differentiation potential' of the ectoderm of the neural plate. As yet, comparatively little is known about such gradients. What information we have suggests that they are similar, in that in both of them regions at the 'high' end induce or develop into well-formed neural organs while the 'low' end forms only neural crest. This suggests that the medio-lateral gradient, which would involve only concentration, is effectively similar to the antero-posterior one, which would also involve the length of time over which the concentration had been accumulated. But further work may show that this time factor cannot be left out of account. In particular, the distinction between the pre-chordal plate and the remainder of the archenteron roof may prove to correspond with the difference between the gradient in front of the region of highest concentration, in which the evocator content has been rapidly built up, and that in the more posterior and lateral regions in which it has been slowly accumulated.

Thus the conditions for the production and diffusion of an evocator within the dynamic topographical situation of the gastrula are sufficient to suggest an extremely flexible hypothesis which, by suitable assumptions as to the relative rates of the various component processes, could be made to fit many different facts; but it is worth noting that there is no reason why these assumptions should not be open to independent testing.

There is, however, one class of facts which cannot be explained by the considerations which have so far been advanced. We have seen in the experimental part of this paper, in confirmation of Hall's earlier results, that the posterior organizer of the mid-gastrula is already different to the anterior organizer; but at this time it still lies on the surface of the embryo; it cannot therefore differ merely in a lower content of free evocator, since according to the views so far advanced no evocator would have yet been produced within it. Moreover, when it is transplanted to the blastopore lip of a young gastrula and from there invaginates so as to reach the anterior end of the archenteron roof, we have as yet no way of understanding why it does not develop a full quota of evocator and thus act as an anterior organizer. There is,

however, another factor which might come into play here. The posterior regions of the organizer, during their movement towards the blastopore, are themselves for some time underlain by active inducing material. It is conceivable that this preliminary submission to the diffusing evocator, as well as the evocator liberation after invagination, is connected with the determination of regional properties.

A careful consideration of the actual events of gastrulation seems therefore to force on our attention a number of variables in the spatio-temporal conditions of evocator production and diffusion which might go far to explain the facts about regional determination at that stage as far as they are yet known. These factors urgently demand investigation. The same is true of the closely allied question of why it is that a piece of ectoderm grafted so as to remain on the surface above archenteron roof becomes neuralized, whereas a similar piece which passes below the surface into the archenteron roof becomes mesoderm.

SUMMARY

1. In young gastrulae of *Triton alpestris* the median part of the organization centre immediately in front of the blastopore was excised and replaced after reversal of its anterior-posterior axis. Completely normal embryos developed in many cases, but in others there was some degree of microcephaly or spina bifida.

2. Similar normal embryos can develop after exchange of the anterior and posterior regions of the organizer, either with normal or reversed orientation.

3. The spina bifida which frequently appears is a consequence of the impediment offered by the graft to the normal gastrulation movements.

4. Microcephaly may also result if the presence of the graft prevents the proper development of the anterior part of the archenteron roof.

5. It may also be caused when the posterior organizer region is brought into the anterior region, if the host fails to convert it into anterior material. This occurs the more frequently the older the grafted posterior material is.

6. Even at the end of gastrulation (slit yolk-plug stage) the regional character of the archenteron roof is not finally determined. A fairly normal embryo (with over-thick mesoderm) may develop if an extra archenteron roof is added with reversed orientation between the normal archenteron roof and the presumptive neural plate.

7. The determination of the regional structure of the archenteron roof and the neural plate is discussed. Attention is drawn to the spatio-temporal factors involved in the production and diffusion of the evocator with the dynamic system of the gastrulating egg.

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