EXPERIMENTS ON THE DEVELOPMENT OF THE HEAD OF THE CHICK EMBRYO

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(With Two Plates and Twenty Text-figures)

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I. INTRODUCTION

The development of the head and its associated organs has been the subject of more experimental work than that of most other parts of the embryo. The voluminous literature relating to the eye has recently been collected and reviewed by Mangold (1931). Most work has been done on the Amphibia, which are technically the easiest of the vertebrates to investigate. Less is known about the bird embryo, which is protected by its layers of shell and albumen, while the mammal still preserves nearly all its secrets inviolate within the body of its mother.

In the early years of this century, Barfurth and Dragendorff (1902) and Dragendorff (1903) made defects experiments on the eye of the chick, operating through an opening cut in the shell; and the same method has been used by other workers,

the most recent of whom is Reverberi (1929*a*, *b*). The experiments to be reported here were performed by another method, the embryo being first removed from the shell and then cultivated *in vitro* by the technique which has previously been described (Waddington, 1932). For experimentation on the head and cephalic organs, the *in vitro* method has both some advantages and some disadvantages as compared with the method of operating through the shell. The chief disadvantage is that the embryos cannot be kept alive for more than a comparatively short period. Two days is the usual upper limit for successful cultivation in the same watch-glass full of plasma-extract medium, and although this span of life may be lengthened to some extent by transplanting the embryo or particular organs into new medium, morphogenesis is usually somewhat abnormal in the transferred tissues, although histogenesis may proceed nearly normally (see Pl. II, fig. 3). On the other hand, the advantages of the method more than outweigh the difficulties due to the limited life span. They are twofold. Firstly, the embryos are operated on when they are free from yolk and are therefore much more easily visible, so that the limits of extirpated or transplanted fragments of tissue can be determined with absolute certainty; and secondly, the method enables one to carry out embryonic transplantations. The second advantage is of crucial importance; the whole progress of experimental embryology in recent years has depended on the discovery of methods of placing embryonic tissues into new situations within the embryo. Spemann (1906), working with the Amphibia, was the first to discover such a technique at the beginning of the century, and gave thereby the impulse to the whole subsequent development, which is proceeding through the techniques worked out for echinoderms by Hörstadius (1928), for birds and mammals by Waddington (1930, 1934), and just recently for fish by Luther (1935) and Oppenheimer (1934*a*, *b*).

In the present study the transplantation technique serves two purposes. One, the more important, is to allow us to study the mutual interaction of two embryonic tissues, the other is to provide a method of investigating the capacity of embryonic rudiments to differentiate in isolation. If a transplant is made into the non-embryonic area of a chick blastoderm in the somite stages, the transplant proceeds with its development, and there is no evidence that it is in any way affected by the host embryo. It is self-differentiating in a way similar to that seen when a rudiment is transplanted to the chorio-allantois of older chick embryos, with the advantage that the mechanical conditions in the vascular area of cultivated chicks seem to allow the grafts to preserve their normal morphological relations better than do grafts on the chorio-allantois.[1]

The results to be communicated here have been accumulated over a considerable period of time, in which attention has mainly been fixed on other problems concerning the development of the primitive streak stage. When for any reason a batch of eggs was allowed to develop too far to be used in these main investigations, experiments were made on the development of the head structures, and in this way

[1] The chorio-allantois, and perhaps the vascular area of the younger chicks used here, cannot be regarded as neutral as regards the determination of neural differentiation (Waddington, 1935), but this determination had already been completed in all the grafts with which we shall be concerned with in this paper.

a fairly complete range of stages has been used. While preparing the material for publication a few confirmatory experiments have been performed.

II. REGULATION OF DEFECTS IN THE PRIMITIVE STREAK AND HEAD PROCESS STAGES

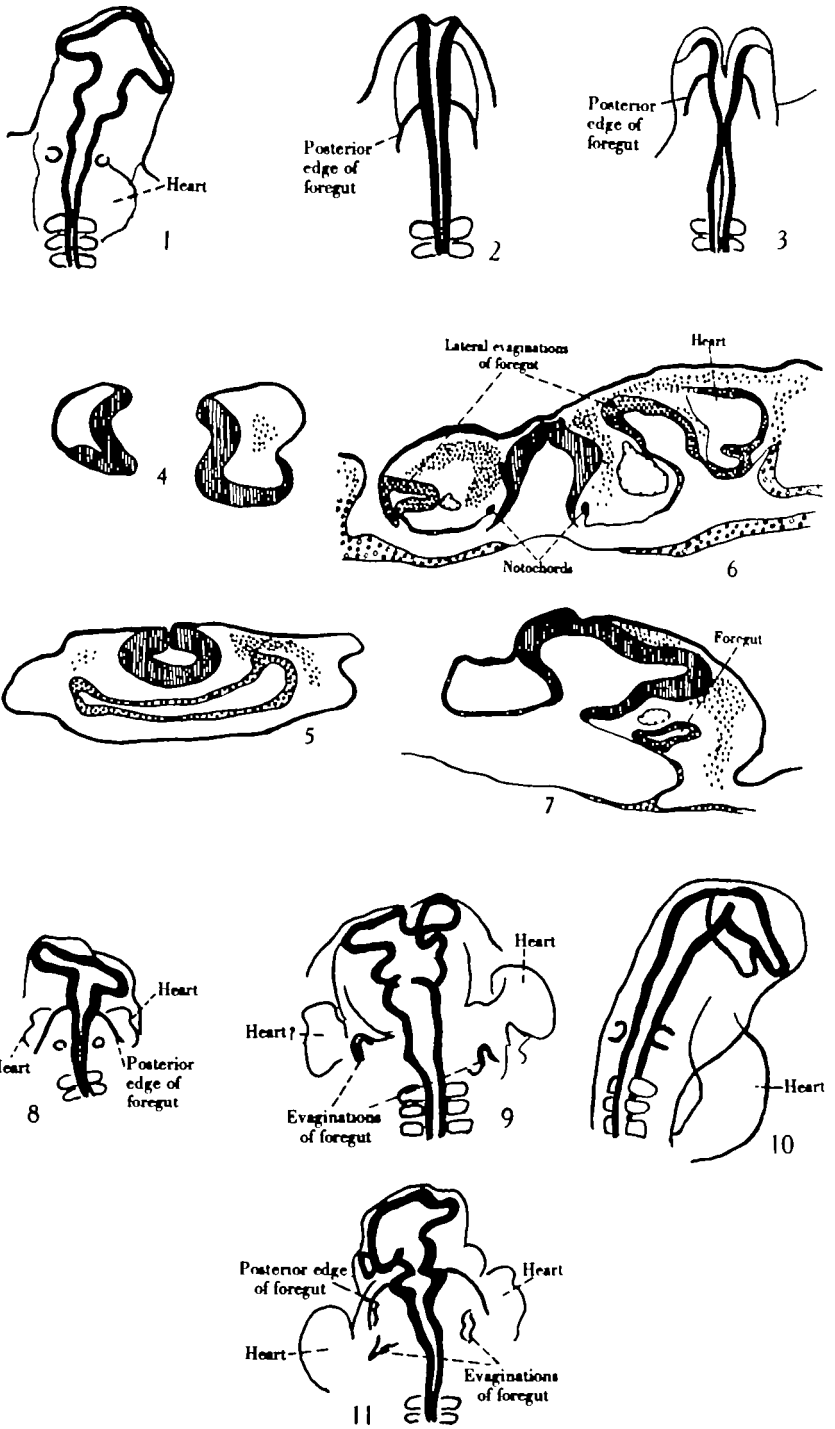
(*a*) *Neural tissue*

The defects in these stages were made by removing small pieces of tissue extending forwards from the level of the primitive pit nearly to the edge of the area pellucida. Most of the fragments were triangular in shape, with the apex at the primitive pit and the triangle symmetrically placed on the mid-line, but some lay more laterally, so that one presumptive optic rudiment was removed. The angle at the apex of the triangle varied from about 30° up to about 90°.

Twenty-five embryos, operated on at various stages in the development of the primitive streak and with defects varying in magnitude, have been obtained. The degree to which the defect has been regulated varies considerably. As might be expected, regulation is better in younger than in older embryos, and the smaller the defect the greater is the probability that it will be completely compensated. Lateral defects are regulated in exactly the same way as median ones.

Observation of the embryos during their development showed that complete regulation is only possible if the hole left by the operation fills up before the neural plate appears. This filling up of the hole is carried out by a combination of two rather different processes, which can in some cases occur separately. One process is the growing together of the sides of the hole towards the mid-line, where they eventually join; the second is a growth forward from the region of Hensen's node.

The first process is characteristically found in those embryos in which a narrow triangle, with a sharply pointed apex, has been removed. It may in favourable cases lead to perfect regulation, with the formation of a complete and normal-sized head (Text-figs. 1, 2). In other specimens the regulation appears complete when the embryo is examined in surface view, but sections show that, although the sides of the neural tube are normal in shape and position, and may even have fused dorsally to form the neural crest, yet the floor of the tube is missing, or at least the two halves of the floor are not united (Text-figs. 3, 4, 5, 6). In these embryos it is probable that a small split was still open at the time when the neural plate began to differentiate. As was said above, neural tissue in these early stages seems to be unable to grow together and fuse with other neural material, and the split can therefore not be healed up. This failure of two adjacent pieces of neural tissue to fuse is commonly seen in grafting experiments with the primitive streak, where the graft and induced neural tissues are usually completely separate; in fact it is only on this account that they can be distinguished with certainty. In later stages, as we shall see, wounds in the neural tube can be healed and the two edges grow together and fuse quite frequently. The main reason for this different behaviour in the early and late stages is probably a difference in the neural tissue itself, but other factors may play a part. In the early stages of head formation, when the neural plate is



Text-figs. 1–11.

first rolling up into a tube, and the head fold is forming, the neural tube in this region is surrounded by comparatively little mesenchyme. The more plentiful mesenchyme of later stages probably holds together the two edges of a wound in the neural tube, and the failure of fusion in the early stage may be partly due to the lack of such a packing material. Further, if a cut is made through both ectoderm and endoderm in the primitive streak or head process stages neural tissue which

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Text-fig. 1. No. 188. Operated at 21¼ hours, long primitive streak stage, narrow triangle removed in front of primitive pit. Cultivated 45 hours. Complete regulation. Sketch of whole mount. (× 30.)

Text-fig. 2. No. 438. Duck embryo, operated at 25 hours, medium primitive streak stage, medium-sized triangle removed in front of Hensen's node. After 20 hours the wound was repaired and the headfold had appeared. Fixed after 28 hours. Sketch of whole mount. (× 30.)

Text-fig. 3. No. 367. Operated at 18½ hours, medium primitive streak stage, medium triangle removed. After 8½ hours the wound had been partly covered, and the head process was visible growing from Hensen's node forwards to the posterior edge of the hole. Fixed after 22¾ hours with five somites. Sketch of whole mount. (× 30.)

Text-fig. 4. No. 367. Section through most anterior end of above specimen of Text-fig. 3, showing failure of regulation. (× 65.)

Text-fig. 5. No. 367. Section slightly farther posterior in same specimen, showing complete regulation in this region. (× 65.)

Text-fig. 6. No. 169. Operated at 18 hours, long primitive streak stage, 45° triangle removed from in front of Hensen's node. Fixed after 46 hours. Section through the posterior part of the head (× 65). Notice that in this region the neural folds have united dorsally, although ventrally there is still a large gap unhealed. Near the ventral edge of the neural fold on each side is a notochord; these two strands of notochords become united farther posteriorly, near the place where the ventral gap in the neural tube is closed. Notice also the lack of ventral closure of the foregut, and the position of the two lateral evaginations. On the left the foregut is open to the exterior, the ectoderm of the headfold having united with the foregut endoderm. On the right one of the two heart vesicles can be seen.

Text-fig. 7. No. 384. Operated at 13 hours, medium primitive streak stage, narrow triangle removed. Fixed after 25 hours. Section through the anterior end. Notice the union of the ectoderm and endoderm on the left, the headfold having broken through into the foregut. The right side shows an optic vesicle, with underneath it a tubule derived from the lateral evagination of the foregut. Slightly posterior to this section the neural folds unite dorsally, but they remain separated ventrally for a considerably greater distance towards the posterior. (× 65.)

Text-fig. 8. No. 142. Operated at 20¾ hours, long primitive streak stage. Medium triangle removed from in front of primitive pit, extending right across the blastoderm to the edge of the area opaca. When the embryo was explanted, the edges of the wound were stretched away from one another, widening the angle at the apex of the triangle. Repair took place by forward growth of the material at the apex, not by inward growth of the sides of the wound. The sketch shows the head of the embryo fixed 43¼ hours after the operation. Notice the very short head, with apparently complete repair of the neural tissue, and the separated heart vesicles. (× 30.)

Text-fig. 9. No. 175. Operated at 21¼ hours, early head-process stage. The head process was removed with a narrow triangle. Fixed 24 hours later. The sketch shows the incomplete fusion of the two sides of the wound, giving rise to a contorted brain, which, however, is considerably more normal in shape than those of specimens operated at later stages in the development of the head process. The two heart rudiments were both twitching, as indeed were those in most of the embryos which were old enough at fixation; in this specimen it was particularly easy to verify that the beats are not synchronous. (× 30.)

Text-fig. 10. No. 236. Operated at 21¾ hours, long primitive streak stage, a narrow triangle was removed in front of Hensen's node but slightly to the right of the mid-line. Fixed after 43 hours. Complete repair of the right side of the brain and formation of a normal optic vesicle. (× 30.)

Text-fig. 11. No. 199. Operated at 23 hours, long primitive streak or beginning head process stage. Narrow triangle eliminated. Complete repair of neural parts of head, but foregut not closed ventrally and heart rudiments, which were beating non-synchronously, are widely separated. (× 30.)

develops near the cut is very frequently united to the endoderm along its free edge, and this may happen early enough to hinder the union of two masses of neural tissue on either side of the cut.

The second process, growth forward from Hensen's node, occurs in specimens in which a wide-angled triangle has been removed. The regulation achieved depends mainly on the exact position of the apex of the triangle. If the primitive pit is left uninjured, the apex lying slightly anterior to it, forward growth may proceed along the whole breadth of the hole and a whole head may be formed, usually relatively very short in comparison with the normal head but nevertheless complete in transverse section (Text-fig. 8). If, on the other hand, the primitive pit is injured, growth takes place on each side and the floor of the neural tube is missing in the mid-line. In the latter case it is difficult to be sure how much regulation has occurred. The neural material on each side folds into a curve which is usually more than half a cylinder, but it only very rarely forms a completely closed circle in cross-section. Each side also produces an optic evagination, but it has never been observed that such a half-neural tube rounds itself up to a complete cylinder and produces two optic vesicles. The power of the half-heads to complete themselves seems therefore to be comparatively slight (Text-figs. 7, 9; Pl. I, fig. 1).

Comparatively small injuries to the primitive pit will cause the floor of the neural groove to be absent over a considerable length of the embryo, extending well into the region of the somites. This is an expression of the fact that the presumptive material for the floor of the tube is concentrated anteriorly in the primitive streak stage and moves back later to its final position (Wetzel, 1929; Waddington, 1932). In embryos which are split through the lack of the floor material, the sides of the neural tube become united to the endoderm below. The notochord is usually missing in the region of the split, though it may be present in more posterior regions. This would seem to indicate that the presumptive notochord material is located very near the presumptive floor material and tends to be removed with it. In one specimen, which is split for only a short distance, two notochords are present just anterior to the point where the sides of the neural tube become united (Text-fig. 6). Each lies nearly under the free ventral edge of the piece of neural tube with which it is associated, showing that there has been little tendency for the half-neural tubes to complete themselves to laterally symmetrical structures. The two notochords fuse slightly posterior to the point of fusion of the two branches of the neural plate.

In the majority of the operated embryos, the attempt at regulation seems to have involved both forward growth and growth from the sides. The resulting heads consist fundamentally of two separate half-heads more or less fused along the mid-line. If the operation is made at a slightly later stage, when the head process is just appearing, both processes seem to proceed very much less readily (Text-fig. 9), and in older head-process stages are absent altogether. The head-process stage seems in fact to be a period of minimal regulatory power for median defects. The embryos which have been obtained show a complete lack of fusion in the mid-line, and in the confused masses of neural tissue which appear on each side no tendency to form complete heads can be discerned.

It seems, then, that regulation of the head neural tissue in the early stages occurs only before the onset of differentiation indicated by the appearance of the head process. It is confined to the period in which the material out of which the head will be formed is being prepared, and does not occur during the actual process of head formation.

(*b*) *The heart and foregut*

In all the operated embryos, the ventral fusion of the endoderm to form the foregut, and the union of the two heart rudiments are very much retarded. Even in specimens in which complete regulation has occurred, the heart rudiments may remain widely separated at least until the ten-somite stage. The foregut also tends to be open ventrally. This failure of the walls of the foregut to fold in towards the mid-line is not easy to understand. If the folding were due to forces acting from the lateral parts of the blastoderm towards the mid-line, one would expect median defects to aid the process rather than to hinder it. One must suppose that, on the contrary, the sides of the foregut are drawn together by influences situated centrally, and that these influences have been weakened by the median defects.

A similar though slighter failure of the ventral fusion is not uncommonly seen in unoperated embryos grown *in vitro*. It seems to be a purely mechanical phenomenon affecting only morphogenesis. At least it has no apparent effect on histogenesis, since the separated heart rudiments may begin contraction at the appropriate stage of development in relation to the stage attained by the embryo as a whole. Rudnick (1935) has recently drawn attention to similar phenomena which she describes as being due to differential inhibition of the upper or the lower surface of the blastoderm, according to which is placed against the clot. In her table she lists examples of very considerable disparity in the stages of development attained by the neural and mesodermal organs in her embryos, and it may be that there has been a real differential inhibition affecting histogenesis as well as morphogenesis, since the technique she employed seems likely to lead to comparatively rapid drying of the surface of the culture medium. In my specimens, however, no marked difference can be found between the heart rudiments which have been mechanically separated by operations in the mid-line of the embryos, and heart rudiments which remain separate through a failure of morphogenesis in unoperated embryos; neither show any differential inhibition of histogenesis.

The separated heart vesicles are roughly mirror images of one another, each developing a fold of its outer lateral surface inwards towards the mid-line of the embryo (Text-fig. 9, etc.). The left vesicle therefore acquires the curvature corresponding to the normal curvature of the complete heart. It has been impossible, however, to cultivate the embryos long enough for anything like complete morphogenesis of the heart rudiments, and the symmetry relations of the half-hearts cannot therefore be determined with any certainty. As far as the first simple curvature is concerned, the conditions seem to be similar to those described in the Amphibia by Ekman (1925). The separate heart rudiments never acquire any

functional connection with the developing blood sinuses, and no circulation occurs in these embryos.

Although the ventral closure of the foregut can, as we have seen, be easily disturbed, its lateral evaginations show considerable power to develop in atypical situations. Even if the dorsal roof of the foregut is missing or interrupted as a result of the defect, the lateral evaginations occur. They may under such circumstances lie at right angles to the surface of the blastoderm, or even be reversed, pushing inwards from the sides towards the mid-line (Text-fig. 6). This is probably a response to mechanical conditions occasioned by the abnormal morphogenesis of the main embryonic axis. Towards their anterior end these portions of the foregut tend to become rounded into complete tubes with a closed cross-section, but whether this regulation affects more than the gross morphology cannot be determined at the comparatively young stage at which the embryo have to be killed.

The formation of the headfold proceeds independently of the folding which forms the foregut. When, therefore, the foregut remains widely open ventrally, the posterior part of the headfold comes in contact with the anterior part of the foregut, and the tissues may break through so that the spaces within the folds become continuous. In such cases endoderm which should be part of the roof of the foregut becomes continuous with the ectoderm of the head, and therefore forms a part of the ventral or ventro-lateral surface of the head (Text-figs. 6, 7). At the same time, the ventral surface of the headfold becomes continuous with part of the ventral, but unclosed, surface of the foregut. The endoderm retains its histological character for some time even in such close connection with the ectoderm, from which it can be distinguished by its comparative lack of intracellular vacuoles.

III. REGULATION OF DEFECTS TO THE BRAIN IN EARLY SOMITE STAGES

Defect experiments have been made on the heads of embryos with from four to twenty-five somites. The main experiment on the regeneration of the brain was performed as follows: the whole head was split down the mid-line from the anterior to a point just posterior to the forebrain, and the right[1] half of the forebrain was then removed completely by a transverse cut, taking with it the overlying epidermis and mesenchyme. The regulation of the brain after this defect is nearly complete, even in the oldest stages operated (Text-figs. 13, 15, 16). In some specimens the cut edges of the brain become joined to the overlying epidermis, and the two layers grow over the wound and eventually close it completely. Text-fig. 13 shows the final healing of the wound in such a specimen. In Pl. I, fig. 2, the epidermis has

[1] Nearly all the experiments have been made on the right eye, since it was found that the left eye, which comes, through the rotation of the embryo, to lie against the plasma clot, tends to die and disintegrate at a stage when the right eye is still perfectly healthy. It does not appear that the development of the left eye is gradually inhibited; its differentiation probably proceeds at the normal rate until the tissues become necrotic. The cause of the necrosis might be a lack of oxygen or the accumulation of waste products; the evidence does not yet allow one to decide between these two possibilities.

covered the wound completely before the two edges of the brain have extended quite far enough to reach one another. In other specimens the epidermis may be still more in advance of the neural tissue (*e.g.* Text-fig. 14), and in these it seems that the cut edges of the brain and of the epidermis have never healed together but have grown separately across the wound. The mesenchyme appears to grow with the epidermis and to form a space of the appropriate shape and size, along the inside of which the neural tissue gradually extends. Pl. I, fig. 3, shows the healing together of the two edges of neural tissue in such a case. In all these types of repair, the sheet of neural tissue spreading over the wound tends to become very thin. It should be noticed that in these embryos two masses of neural tissue have no difficulty in healing together, although at an earlier stage this seems to be impossible, as we saw above.

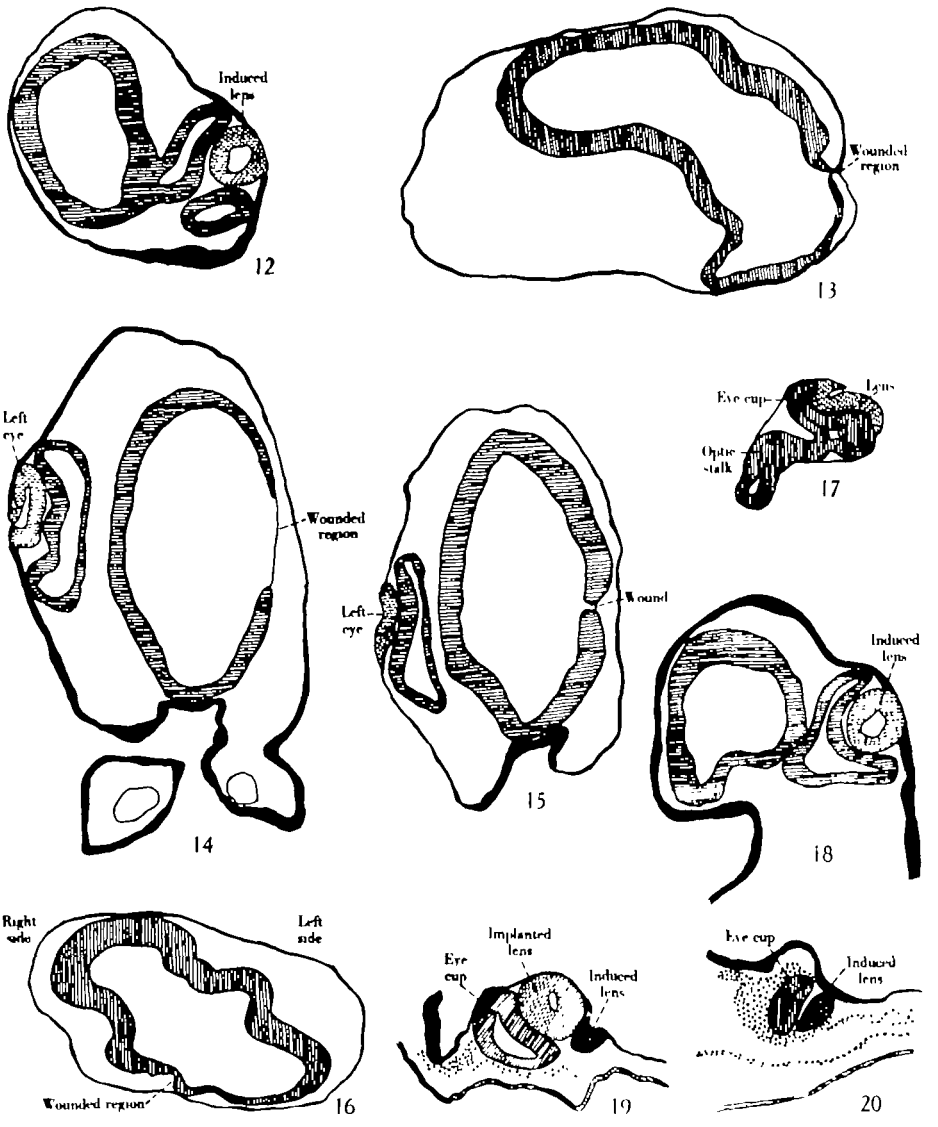
The tissue which covers the wound in these operated embryos assumes the shape required to complete the brain. But the entire head is not completed, since there remains always one important feature which is not restored. The operation, as well as removing half the forebrain, removes the entire right optic rudiment, and in the repaired heads no trace of the right eye develops. It will be shown later that parts of the eye rudiment can regulate themselves to complete eyes, but it is clear that after the removal of the complete rudiment, a new eye cannot be formed from the brain.

IV. INDUCTION OF A NASAL PLACODE BY THE REGULATED BRAIN

The epidermis, mesenchyme and neural tissue which close over the wound assume, as has been said, the shape of the right half of the head. This assumption of the appropriate shape is more than a mere morphological process, since the regulated tissues also begin to fulfil their appropriate developmental functions.

Among the tissues which are removed by the operation is the presumptive epidermis for the right nasal placode. The exact location of this presumptive epidermis has never been described, but there can be no doubt that it lies somewhere above the right half of the forebrain. In the repaired heads, however, there is to be found in some cases a nasal placode on the right side as well as on the left (Pl. I, figs. 4, 5). There appears to be only one way to account for this placode; one must assume that the non-presumptive nasal epidermis which grows over the wound finds itself in the physiological situation which normally causes the appearance of a nasal placode, and that it therefore responds in that way. This physiological situation must expose the epidermis to some inducing stimulus, which may either proceed from a definite part of the forebrain, or may, perhaps more probably, be a general "Situation stimulus" of the kind which Spemann and Schotte (1932) showed to be responsible for the induction of the mouth in the Amphibia. In any case one must assume that the formation of the nasal placode is induced by the underlying tissues, which are themselves part of the wound covering, and in performing the induction these tissues demonstrate that they are repairing the

wound not only morphologically but also physiologically. Zwilling (1934) has shown that in the Amphibia the nasal placode is induced by the forebrain.



Text-figs. 12–20.

V. REPAIR AND SELF-DIFFERENTIATION OF THE EYE-CUP

Defect experiments on the eye-cup of the chick, performed on the embryo still enclosed in the shell, have been made as long ago as 1902 by Barfurth and Dragendorff. More recently Reverberi (1929*a*, *b*) has described similar experiments on the same material. These authors showed that the eye-cup is not regenerated from the brain after complete removal, and are thus in agreement with the results

described above. Reverberi also describes the regulation of small fragments of the eye-cup of seven-somite embryos to form complete eyes. This observation has been confirmed in the *in vitro* experiments for embryos of similar or greater age (see Text-fig. 12). The parts of the optic vesicle which were removed from these embryos were in a few cases inserted between the ectoderm and endoderm of the area pellucida of the same embryo and cultivated with it. Such fragments of the optic vesicle can pursue their normal development and form apparently complete eyes with some optic stalk attached, as Reverberi also demonstrated for fragments isolated within the egg (Text-fig. 17).

VI. INDUCTION OF THE LENS

The part of the optic vesicle left in place after partial extirpation of the rudiment induces the formation of a lens from the epidermis which grows over the wound (Text-figs. 12, 13, 18). This result, which was already known through the work of

Text-fig. 12. No. 231. Operated at 47 hours, 15 somites. Right optic vesicle partially eliminated, the overlying ectoderm also being removed. The section shows the regulated eye with a new lens induced from the skin which covers the wound. Notice the choroid fissure. Fixed 21 hours after operation.

Text-fig. 13. No. 521. Operated at 42½ hours, 10 somites. Entire right half of forebrain, with overlying epidermis, etc., removed. Fixed 24 hours after operation. Almost complete repair of the brain, the neural tissue and epidermis having united at the cut edge and then grown together over the wound. Notice the thinness of the neural layer taking part in the repair.

Text-fig. 14. No. 792. Operated at ? hours, fifteen somites. Entire right half of forebrain removed, with overlying tissues. Fixed 21 hours after operation. Repair has occurred first by growth of epidermis and mesenchyme, which leaves a space along the inside of which the neural tissue is extending.

Text-fig. 15. No. 694. Operated at 46 hours, twenty-two somites. Entire right half of forebrain removed. Fixed 19 hours after operation. Repair of brain by growth of neural tissue along the inside of space left by epidermis and mesenchyme. (See Pl. I, fig. 3.)

Text-fig. 16. No. 255. Operated at 49 hours, eleven somites. Nearly the entire right half of forebrain removed. Fixed 24 hours after operation. Complete repair of brain with a thin wall of neural tissue. The embryo is turning so as to bring the right side of the head against the clot instead of the left. There is only very slight trace of an optic vesicle on the right side.

Text-fig. 17. No. 255. Operated as above, the part removed from the forebrain being cultivated as a graft in the area pellucida to the left of the head. It has differentiated to a piece of optic stalk, an optic cup and lens. No choroid fissure can be found.

Text-fig. 18. No. 207. Operated at 50 hours, five somites. The ectoderm from the right side of the head, including the presumptive lens ectoderm, was removed together with the underlying mesenchyme, leaving the neural tissue of head untouched. Fixed 24½ hours after operation. Induction of a new lens from the epidermis which grows over the wound.

Text-fig. 19. No. 691. Operated at 45½ hours, twenty somites. The right optic vesicle was removed, with the overlying ectoderm, etc., and grafted into the side of the area pellucida. It has differentiated into an eye-cup and lens, and there is also a small patch of lens tissue which is separate from the main lens transplanted with the eye-cup but is connected with the host ectoderm, from which it is being folded off; it is clearly induced by the graft. Fixed 19½ hours after operation.

Text-fig. 20. No. 32–118. Operated at three somites . Right neural fold eliminated in eye region, and grafted under the ectoderm to left of the first somite. The presumptive lens epidermis was also eliminated in the eye region, but was cleaned away from the neural tissue before the latter was grafted. Fixed 23½ hours after operation. Repair in the head region is bad. The graft neural tissue forms an extensive irregularly shaped mass, the thickness of which raises the host ectoderm above it into a slight swelling. At the left anterior (illustrated in figure) and right posterior corners of the swelling there are deep pit-like folds of the ectoderm, with a lumen very narrow in proportion to the thickness of the walls, which are composed of cells intermediate in type between early lens cells and the characteristic vacuolated epidermis.

Epidermis black, *neural tissue* hatched, *mesoderm* omitted from all figures except 19 and 20. (All × 65.)

Dragendorff (1903) and Reverberi (1929*a*, *b*), shows that the potentiality to react to the lens inducing stimulus is not confined to the presumptive lens region but is found at least within a fairly narrow area around it. How much farther it extends cannot be determined from the work reported in the literature, and in fact could hardly be discovered by any method other than the *in vitro* technique which allows grafts to be made into any part of the blastoderm. Even with the aid of this technique, however, it is not easy to make grafts suitable for obtaining lens inductions. It is extremely difficult to strip the lens epidermis away from the optic vesicle without leaving small fragments of it attached. In fact, this can only be done in early stages, of about four to five somites, when there is still a space between the optic neural fold and the lens epidermis with which it will eventually come in contact. From these embryos presumptive optic cup can be obtained which is certainly not accompanied by presumptive lens epidermis, and the problem then arises of placing this neural tissue in contact with ectoderm in which it can induce a lens. If the implantation is made into embryos in any of the somite stages, it is in the first place difficult to separate the ectoderm and endoderm sufficiently to place the graft between them, and secondly, even if this has been done, it is usually found that the graft lies in the coelom between the two layers of mesoderm, and is therefore not in contact with the ectoderm at all. Only in one such case (in which lens epidermis was also included in the graft, Text-fig. 19) has a small lens been induced in an embryo operated at twenty somites. One can more easily graft the neural tissues into primitive streak blastoderms, but it is then difficult to keep the cultures alive long enough for a lens to develop. This has, however, been done in several cases, the embryos being transferred on the second day to new medium. No fully formed lenses of the normal size have been induced, but in three cases there are small induced thickenings of the ectoderm which are apparently not neural in nature and must be regarded as induced lentoids (Pl. II, fig. 1). In some other specimens, the grafts, particularly if they included presumptive brain tissue, have performed neural inductions.

These experiments prove definitely that a lens may be induced in ectoderm which lies a long way from the normal presumptive lens epidermis, in fact, even in extra-embryonic ectoderm. This has already been suggested by Danchakoff (1924, 1926) and Hoadley (1926), who found that in chorio-allantoic grafts of the eye-cup, the optic tissue often assumed a quite irregular shape, and that wherever it came in contact with epidermis it induced a lens. However, it is clear that in these experiments the provenance of the epidermis could not be determined with any certainty, whereas in the *in vitro* grafts the position of the inductions is immediately obvious.

In some cases grafts of optic neural tissue have induced very deep pit-like infoldings of the ectoderm (Text-fig. 20). The nature of these folds is not clear. They differ in several ways from lenses; the ectoderm is very little thickened, and the foldings are much deeper, with a narrower lumen. On the other hand, the cells do not remain typical epidermis cells. They lose their vacuoles, and are filled with cytoplasm which stains diffusely in haematoxylin, but they are larger than typical

lens cells of this stage, so that the nuclei are less closely packed. The foldings may perhaps represent some attempt at a lens-like reaction on the part of the ectoderm, but they are more probably related to the slight foldings which are nearly always found along the edges of an area of ectoderm beneath which a potentially inducing graft lies.

Technical difficulties, which have been described above, have prevented the collection of a large series of inductions, but it is perhaps significant that the lenses induced at a distance from the normal position are much worse developed than those which are induced in the immediately neighbouring epidermis which covers a wounded eye-cup. We know several cases in the Amphibia (see Mangold's review, 1931) in which the neighbouring epidermis can react to the lens-inducing stimulus, while that farther removed from the normal lens position cannot; perhaps conditions are similar in the chick, the neighbouring epidermis reacting easily to the stimulus, with the formation of a normal lens, while the extra-embryonic epidermis forms only a lentoid.

VII. SELF-DIFFERENTIATION OF THE LENS

It is possible, before the optic cup touches the lens epidermis, to remove the former in such a way that the lens epidermis is left uninjured. Since, as we have seen above, the lens is induced by the optic cup, it might be expected that the lens epidermis from which the optic cup has been removed in this way would not succeed in differentiating into a lens. This expectation is only partly fulfilled. The epidermis does not form a normal lens, but it does form a small thickening which must be regarded as an indication of a tendency to self-differentiate to a lens (Pl. II, figs. 2, 3, 4). A similar tendency to self-differentiation of the presumptive lens epidermis is found in some Amphibia (see Mangold), particularly in those in which the ectoderm near the normal lens position reacts easily to the lens inducing stimulus, while that which lies farther away reacts less easily. The chick appears to be comparable to species such as *Bombinator pachypus* and *Amblystoma punctatum*, in which lentoids are formed from the presumptive lens epidermis after removal of the eye-cup, and in which the epidermis farthest removed from the lens cannot be induced to form a lens at all.

VIII. DISCUSSION

(*a*) *The period of minimal regulation*

Most of the points which have arisen in the course of this investigation have been discussed in their own contexts above. Only a few general considerations require further mention.

One is immediately struck by the fact that the amount of regulation which is possible is different at different ages. The only stage in which there appears to be no limit to the possibilities is in the primitive streak stage before the neural plate appears, when it seems that any defect can be regulated if only there is time to complete the repair before actual histological differentiation begins. As soon as the

head process appears and the neural plate begins to become a definite entity, the capacity for regulation disappears almost completely, to return again later when the determination of the neural tissues as such is finished and histogenesis is well under way. This phenomenon of a minimal capacity for regulation during the crucial period of determination and the beginning of differentiation has recently been fully investigated by Svetlov (1934) in the tailbuds of Anura, and if one considers longer intervals of time it is fairly usual to find that an embryonic organ passes through a period of mosaic development only to become more plastic at some later stage in its history. The most striking example is in the ascidians, where the egg possesses rather rigidly determined "organ-forming substances", although the adult is equally remarkable for its highly developed regulatory and regenerative capacities. Harrison (1933) has urged that these phenomena go far to invalidate the whole conception of determination, a conclusion which would leave experimental embryology with hardly a single well-defined concept still standing. But in this he may be too pessimistic. The theoretical difficulty can be avoided if we take the concept of determination to apply always to a single process of differentiation. We can as yet in practice give no definite meaning to the phrase "a single process of differentiation", but it is surely indubitable that the processes by which a given part of the cytoplasm of an ascidian egg become converted into some organ of the adult differ from the processes by which that organ may be formed from some other part of the body during regeneration. If that be so, we can still retain a precise definition of determination; that a tissue is determined to undergo a certain process of differentiation when it is found to undergo that process under any conditions in which it is able to differentiate at all. Then the statement that a certain part of the cytoplasm of an egg is determined in respect to a given process need carry with it no corollaries as to the behaviour of a much later derivative of part of the egg with respect to quite other processes, even if both sets of processes lead to the formation of the same tissues. Similarly, to return to our own case, the repair of a defect in the brain at the head-process stage involves the lateral growth of not yet differentiated neural tissue, which may well be a quite different process from repair by lateral growth of already differentiated neural tissue. There is no difficulty in imagining that one sort of repair is possible and the other not. But if we adopt such a point of view, we still have to explain why the defect which is unrepaired in the head-process stage does not immediately become regulated when the embryo arrives at the somite stages in which repair is feasible. A similar phenomenon was found by Svetlov, who discovered that the defects made to the tailbud in earlier neurula stages persisted throughout the life of the tadpole. Svetlov found, however, that these defects were repaired in later stages if the edges of the wound were cut again, giving rise to free edges of tissue. It may be that the presence of a free edge is in some way necessary to allow the tissue growth to take place, or possibly specific wound hormones are required to start the process of healing. A similar state of affairs may well exist in the chick, but no experiments have been made to ascertain whether healing of unrepaired defects can be induced in later stages by secondary wounding.

(*b*) *Fields and districts*

We have seen above that after the head-process stage is passed the forebrain and the optic vesicle can each separately regulate back to the normal shape after injury, but that a new optic vesicle cannot be formed from the forebrain, and we may probably also assume that a new brain cannot be formed from the optic vesicle, though this last point has not been conclusively tested. The brain and optic vesicle clearly constitute different systems as regards regulation. Such closed systems within which regulation or regeneration takes place are known also in older animals; Guyenot (1927) has described them in the adult newt as regeneration fields. They are regions in which there must occur some set of processes which normally give rise to a series of states which constitutes normal development, and which bring about a return, after disturbance by the removal of tissue, to the same series of states, which can therefore be regarded as in some way a series of equilibria. Such a region may be legitimately spoken of as a field, since we can define the field activity, at least by describing the end-product which it produces; in our case, for instance, we can say that there are two fields, the activity of one of which is such as to produce a single complete forebrain, while that of the other produces a single complete eye. In order to avoid confusion with other uses of the word field, such a region in which there is a tendency to build up a single unit has been called an individuation field (Waddington and Schmidt, 1933).

It is possible to go one step farther in discussing the eye individuation field and specify one of the processes concerned in determining the equilibrium series of states. The completion of the eye by the formation of the lens is performed, in the early stages, by the induction of a lens from the overlying epidermis. It is uncertain how long this mechanism persists, but there is presumably a maximum age after which lenses cannot be induced from epidermis; it is possible that in later stages other processes may occur to restore a disturbed equilibrium, such as the Wolffian regeneration of lens from the iris which occurs in the Amphibia.

Once the lens induction has occurred, the activity which forms a vesicle of a definite shape, and then causes the thickening of one side and the formation of fibres, etc., may perhaps have a certain autonomy over against the remainder of the forces concerned in the eye field. The independence, if any, is probably not very well marked, since Kirby (1927) found that the lens fibres did not appear in lenses cultivated *in vitro* after isolation from the eye, and Danchakoff (1926) found that continued connection with the eye-cup is necessary for the formation of a normal lens. In the preliminary stages of eye development, an independence of the lens field from the rest of the eye field would appear as a capacity for the self-differentiation of the lens after removal of the eye-cup before the induction had taken place. As we have seen, a capacity for this self-differentiation is present though rather feeble. The evidence, as far as it goes, suggests that the equilibrium states which are the stages in the normal development of the lens all depend to quite a large extent on factors located within the rest of the eye, but the weak capacity for self-differentiation shows that one at least of the minor processes concerned in the lens field is

present in the epidermis. The region of epidermis within which the capacity is present may be called the self-differentiating lens district.

Another set of factors which must be concerned in the lens field arises from the lens competence. The lens competence is a condition of instability between two sets of factors, one of which, if activated by lens induction, will cause the tissue to develop into a lens, while the other will produce a development into ordinary epidermis. This instability is manifest as a capacity to react to the lens-inducing stimulus, and wherever this reactivity is to be found, there must be a set of forces which could make up part of a lens field should such a field be induced. This set of forces may be very similar in nature, though weaker in intensity, to the forces which cause the labile determination of the lens before induction. But the reactivity to the lens-inducing stimulus is found over a considerably wider area than the capacity for self-differentiation, and we must take this reactivity to define its appropriate lens-reactive district.

One can in this way take any process in which one is interested and call the region in which it can occur a district. It seems unwise to speak of such regions as fields, since we can neither specify the forces which they are fields of, nor does the whole region develop towards a definite equilibrium state. There is, however, often a clearly marked variation in intensity of the defining process within the limits of a region, such as the probable variation in intensity of reactivity to the lens-inducing stimulus within the lens-reactive field, where the variation seems to affect both the sensitivity to minimal stimuli, and the capacity for producing a fully formed lens as a result of the reaction. These variations in intensity may eventually turn out to be determined by the field of some definite force, for instance the diffusion field of some substance, and when we have discovered this mechanism we could substitute the expression "the field of diffusion of such and such a substance" for "lens-reactive district". But until the hypothetical force is discovered, the introduction of the word field in this context only tends to rob that useful term of any definite meaning.

IX. SUMMARY

1. Experiments were made on the development of the head of chicken embryos cultivated *in vitro*.

2. Defects in the presumptive head region of primitive streak embryos are regulated completely if the wound fills up before the histogenesis of neural tissue begins in the head-process stage. Different methods by which the hole is filled are described.

3. No repair occurs in the head-process and head-fold stages, and in this period two masses of neural tissue cannot heal together.

4. Median defects, even if repaired as regards neural tissue, cause a failure of the ventral closure of the foregut. The lateral evaginations of the gut develop typically in atypical situations. The headfold may break through and join up with the endoderm in such a way that the gut acquires an anterior opening.

5. The paired heart rudiments may develop separately. The separate vesicles begin to contract at a time appropriate to the development of the embryo as a whole.

The two hearts are mirror images, the left one having the normal curvature, but the embryos do not survive long enough for the hearts to acquire a very definite shape.

6. The forebrain has a considerable capacity for repair in the early somite stages (five to twenty-five somites). One-half of the forebrain can remodel itself into a complete forebrain. In some cases the neural plate and epidermis grow together over the wound, in others the epidermis and mesenchyme make the first covering, leaving a space along the inside of which the neural tissue grows. The neural tissue may become a very thin sheet.

7. The repaired forebrain may induce the formation of a nasal placode from the non-presumptive nasal epidermis which covers the wound.

8. If the optic vesicle is entirely removed, a new one is not formed, but parts of the vesicle can regulate to complete eye-cups, either when still attached to the forebrain or after being isolated in the extra-embryonic regions of another embryo.

9. Injured optic vesicles induce lenses from the non-presumptive epidermis which grows over the wound. Transplanted optic neural tissue from embryos of about five somites induces the formation of lentoids from extra-embryonic ectoderm, but only in a small proportion of cases.

10. The presumptive lens epidermis can produce a slight thickening even when contact with the optic cup is prevented.

11. The significance of periods of minimum regulatory power for the concept of determination is discussed.

12. The data concerning lens formation are discussed in terms of the field concept.

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EXPLANATION OF PLATES

PLATE I

Fig. 1. No. 170. Operated at 18¾ hours, long primitive streak stage. A wide (90°) triangle removed in front of Hensen's node, and a piece of epiblast from another embryo put into the hole. The graft did not heal with the host, but has prevented any approximation of the lateral edges of the wound. Fixed 47¼ hours after the operation. It will be seen that the two halves of the head have each produced an optic vesicle but that they have manifested very little capacity to regulate themselves to bilaterally symmetrical heads. Notice the distinct folds of the lateral evaginations of the gut, and the two hearts.

Fig. 2. No. 790. Operated at 48 hours, twelve somites. Right half of forebrain completely eliminated together with overlying tissues. The brain is almost completely repaired. The section shows the healing of the two edges which have closed over the wound. Fixed 43 hours after operation.

Fig. 3. No. 694. Operated at 46 hours, twenty-two somites. Right half of forebrain entirely eliminated. Healing of the wound (see Text-fig. 15).

Fig. 4. No. 693. Operated at 45¾ hours, fifteen somites. Right half of forebrain completely eliminated, together with overlying tissues. The brain is completely repaired. The section shows the right nasal placode, which has been induced in the epidermis which has grown over the wound. It is at nearly the same stage of development as the left placode. Fixed 23¾ hours after the operation.

Fig. 5. No. 702. Operated at 46 hours, about fifteen somites. The right half of the forebrain was completely removed, with overlying tissues. The section shows the right nasal placode, and under it the healing of two edges of the repaired brain.

PLATE II

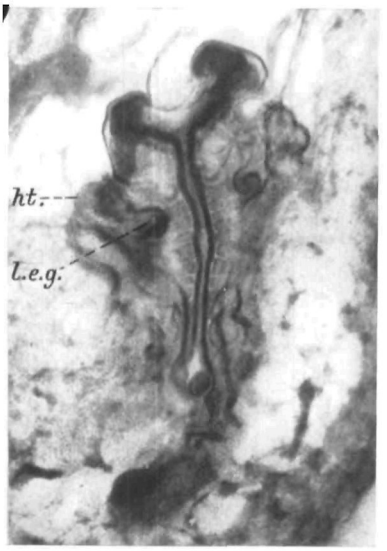
Fig. 1. No. 33–053. Operated in long primitive streak stage. Optic neural plate tissue from a head process stage embryo, with some underlying mesoderm, was grafted between the ectoderm and endoderm to the left of the anterior part of the streak. 26¼ hours later the entire embryo was removed from its original clot and transferred to a new culture vessel. The embryo was finally fixed after a total of 47¼ hours' cultivation. The host embryo has continued its development at about the normal speed, but the neural plate is flat and unfolded in the head region. Above the graft there is a thickening of the ectoderm which is non-neural in nature and probably represents a small induced lentoid.

Fig. 2. No. 33–051. Operated at 25¾ hours, five somites. The right optic neural tissue was removed, the presumptive lens ectoderm being left uninjured. Fixed 27 hours after the operation. The removal of the optic tissue does not seem to have been absolutely complete, since a small evagination of the neural tube has developed, probably representing an attempt to form an optic vesicle. This evagination does not reach the superficial epidermis, which, in spite of this lack of contact, is thickened in the place where the lens might have been expected to appear.

Fig. 3. No. 33–054. Operated at 26¼ hours, four somites. The neural optic tissue was removed and the lens ectoderm left uninjured. After 25¾ hours the head of the embryo was cut off just posterior to the heart and was transferred to a new culture vessel. It was fixed after a total of 70 hours' cultivation. The head has become very flat and much of its structure is partially lost, but histogenesis seems to have continued more or less uninterruptedly. The figure shows the left, normal, optic rudiment. The eye-cup has become merged in the optic stalk and appears in the section as an elongated tube. The lens is unfolded and still connected with the epidermis. The development of the lens fibres is well advanced.

Fig. 4. No. 33–054. The section shows the region of the right optic rudiment of the same embryo. There is no trace either of eye-cup or optic stalk. Over the position in which they might be expected, the epidermis is folded and slightly thickened. The thickened tissue probably is the result of the self-differentiation of the lens rudiment. It will be seen that the differentiation has proceeded much less far than that of the normal lens.

br. brain; *epi.* epidermis; *g.* graft; *ht.* heart; *l.* lens; *l.e.g.* left evagination of foregut; *n.pl.* nasal placode; *n.t.* neural tissue.



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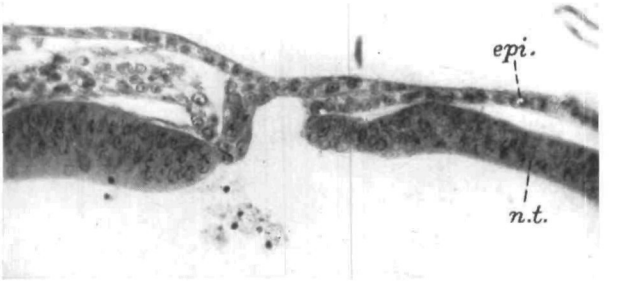

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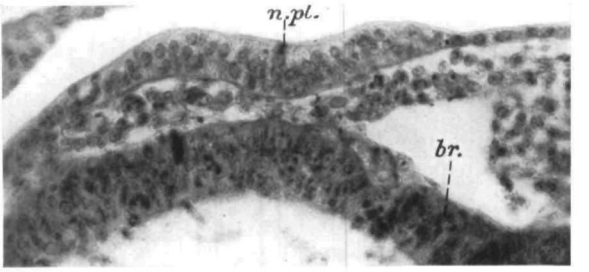

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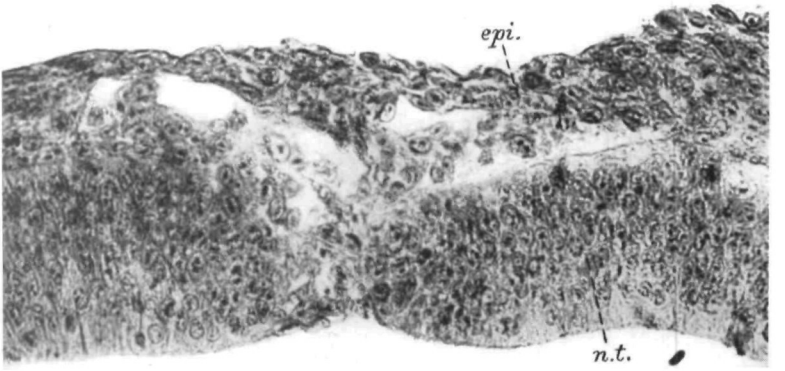

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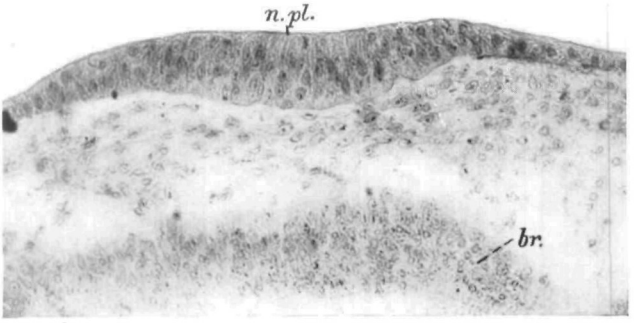

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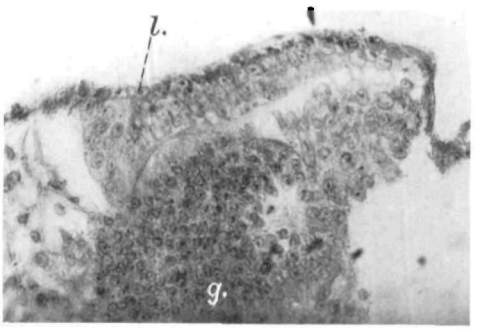

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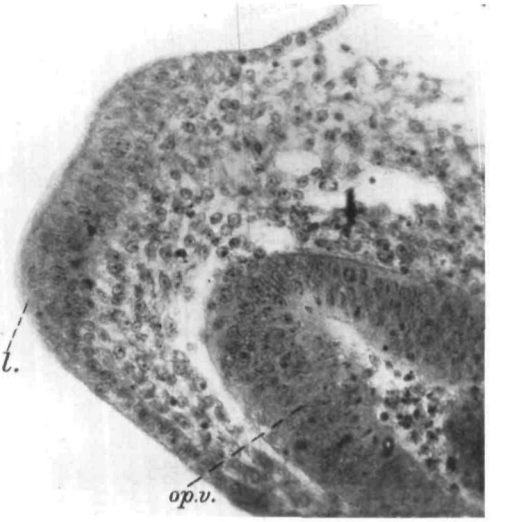

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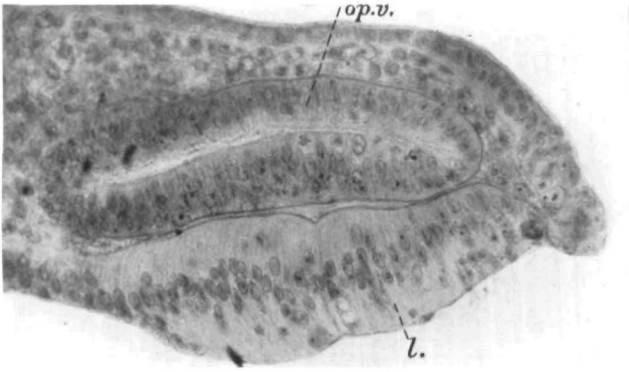

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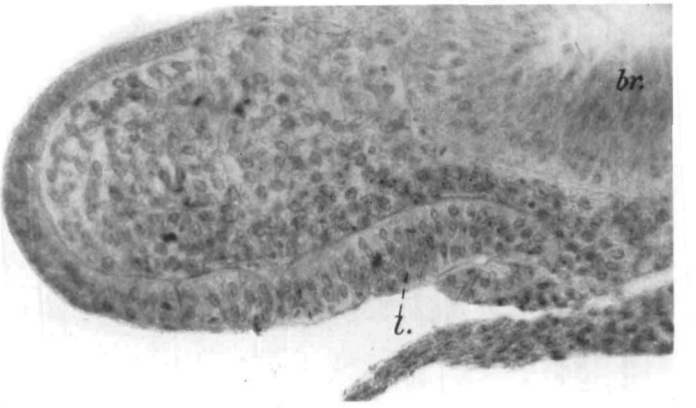

Fig. 4.